

Original article

Frequency distribution of trichomoniasis in pregnant women referred to health centers of Ardakan, Meibod and Yazd, Iran

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Abstract

Introduction and objective: *Trichomonas vaginalis*, a flagellate's pathogen protozoon, that is the cause of most common types of vaginitis may serve as a cofactor in HIV, associated with adverse pregnancy outcomes and predispose pregnant women to premature rupture of membranes and early labour. The prevalence range of disease is from 5% to more than 50% in different populations. In this study, we documented the prevalence of it in the enrolled population and determined the frequency distribution of trichomoniasis in health centers of Ardakan, Meibod, Yazd cities, and evaluation of diagnosis methods in 2006-2008.

Materials and methods: A total of 551 pregnant women were studied in health centers of Ardakan, Meibod and Yazd cities. Two sterile swabs were used to collect vaginal samples from each subject. The first one was used for making smear for Giemsa staining method and the second swab was specifically meant for culture. For obtaining some demographic information about the age, gender and marital status of the patients, structured questionnaires were administered to all the subjects examined, while in depth interviews were conducted on some subjects where questionnaires were not helpful. Data obtained were analyzed statistically by using chi-squared test (χ^2) and students' T-test.

Results: Of 270 subjects studied in Ardakan, 16 cases (5.9%) had infection. In addition, of 181 subjects studied in Meibod, nine cases (5%), showed infection, and of 100 subjects studied in Yazd, two cases (2%) had infection. Clinically, 307 cases out of 551 subjects (55.7%) lacked any type of clinical symptoms. The rest of the patients showed clinical demonstration of whom 244 cases (44.3%) had vaginal discharge. There was no statistically significant correlation between trichomoniasis and factors such as gender, level of literacy, and number of pregnancies (P value=0.05). Most of the subjects belonged to the age group 21-25 year, this being consistent with more sexual activity. In addition all of the studied cases were at pregnancy age that, the incidence of infection is naturally insignificant for those at the middle years of pregnancy age range.

Conclusion: Mere microscopic diagnosis should be avoided since inexperienced pathologists readily mistake white or colorless vaginal discharge for semen. Additionally, obstetricians and midwives should instruct their patients in this regard and notify the sexuality transmitted disease pathogens to medical lab personnel.

Keywords: Trichomoniasis, Pregnant women, *Trichomonas vaginalis*, Yazd

Introduction

Trichomonas vaginalis is a flagellated protozoan and was first described as a vaginal pathogen during the early years of the 20th century, which affects at least 180 million individuals globally, making it the most prevalent non-viral sexually transmitted pathogen worldwide. It is the only parasitic sexually transmitted disease (STD) which may increase the risk of transmission of HIV and predispose pregnant women to premature rupture of membranes and early labour has a worldwide distribution, and it has different prevalence in the different points of the world [1-4]. The prevalence ranges from less than 1% to more than 50% in different populations [5-8].

The factors associated with high prevalence are the same as those of sexually transmitted diseases; poor personal hygiene, multiple sexual partners, and low

socio/economic status. There is a 50% chance that sexual partners will eventually exchange the parasite, a strictly anaerobic organism that survives at pH 3.5-8.0. The parasite is sensitive to drying effect and to atmospheric oxygen. Therefore, once having left the body, it will not survive beyond a few hours [5-8].

Typically, a patient with trichomoniasis presents intense frothy yellow-greenish vaginal discharge, irritation and pain in the vulva, perineum and thighs, and dyspareunia and dysuria. The common vaginal infection caused by *T. vaginalis* seems to play a significant role in infertility [9]. Pitfalls in diagnosing trichomonal vaginal infection frequently leave the condition. During an infertility workup, a concerted effort should be made to exclude the presence of this parasite. Figures 1-3 shows *T. vaginalis* in different staining methods.

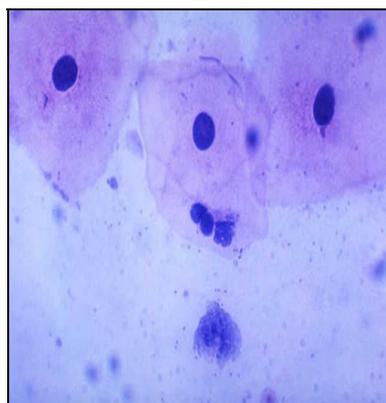


Fig. 1: *T. vaginalis* trophozoite (Giemsa staining) [10]

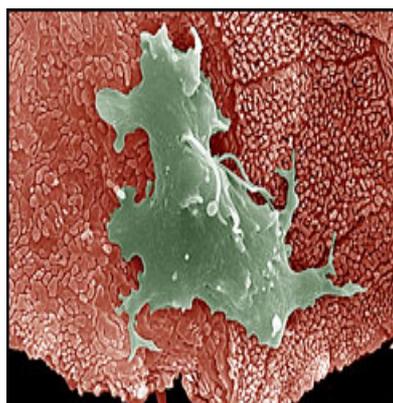


Fig. 2: *T. vaginalis* trophozoite on epithelial cells of vagina [10]

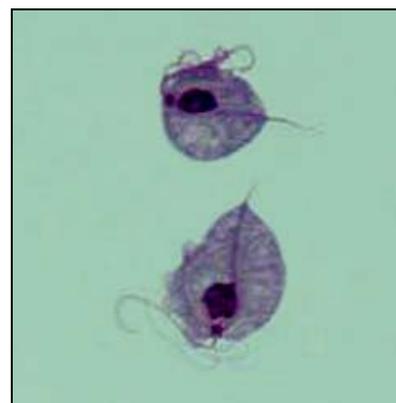


Fig. 3: *T. vaginalis* trophozoites obtained from *in vitro* culture [10]

Since the organism is present in approximately 20% of the infertile population, it is important to mention several key issues that bear relevance on the reproductive process. A poor postcoital test will turn favorable following drug therapy. By virtue of their mobility, *Trichomonas* not only infect the cervical canal, but simultaneous endometrial infection is commonly present. When endometrium measured in luteal phase on sonography is less than 10mm in diameter and shows poor structural development, a search for bacterial and trichomonal infections is mandatory.

Over 50% of women with *T. vaginalis* are asymptomatic. Very few will exhibit the typical greenish, "frothy" vaginal discharge with the bright or dark red edematous appearance of the cervix. Still, *T. vaginalis* is believed to be an infrequent cause of the so-called non-specific urethritis and documenting it in the urethral smear does not prove that this organism is the causative agent. It is unusual to see cases involving the epididymis and the prostate [11-13].

In this study, we documented the frequency of the most common non-viral genital infections in the enrolled population and determined the frequency distribution of trichomoniasis in Ardakan, Meibod and Yazd health centers and evaluated the diagnostic methods in 2006-2008.

Materials and methods

In the present study 551 subjects were sampled during 2006-2008 in Ardakan, Meibod and Yazd cities. Two sterile swabs were utilized to collect vaginal samples from each subject. The first swab was used for Giemsa staining method (8380091 Farzan Pajoo Teb Tehran, Iran) and saline wet preparation [14-17]. While the second swab was specifically meant for Kupferberg medium (2331 Dandurand Montcal; Quebec Canada G2G 3C5), with human serum substituted with bovine serum [18,19-22].

Saline wet preparation of each swab was examined using $\times 10$ and $\times 40$ objectives of the microscope for motile flagellates. Differential staining of the trophozoites cytoplasm and flagella were used as guide for the identification of the stained parasite under the microscope. In addition, turbid appearance of the cultured specimen after incubation indicated the presence of the parasite. To obtain some demographic information about the age, gender and marital status of the subjects, structured questionnaires were administered to all the subjects examined. In depth interviews were conducted on some subjects where questionnaires were not helpful. Data obtained were analyzed statistically by using chi-squared test (χ^2) and students' T-test [23,24].

Results

Overall, a total of 551 females being at different months of pregnancy to delivery were studied at Yazd, Meibod, and Ardakan health centers. Of 270 subjects studied in Ardakan, 16 cases (5.9%) had infection. In addition, of 181 subjects studied in Meibod, nine cases, (5%) showed infection, and of 100 subjects studied in Yazd, two cases (2%) had infection.

The subjects' age ranged from 18-45 years with a mean of 25 ± 5.5 . Most of the subjects (260 cases, 47.18%) belonged to the age group 18-24, followed by 186 cases (33.91%) to 25-34 year and 105 cases (18.91%) to 35-45 year. Most of them (280 cases, 50.81%) were in the third trimester of pregnancy. Regarding literacy, most of the subjects 243, (44.1%) had diploma degrees, 172 (31.21%) had primary or secondary school certificate, 84 cases (15.24%) had university degrees, and the least of them, 52 cases (9.43%) were illiterate. Husbands of the studied women were in the age group 20-54 years, mostly being craftsmen, employed and literate.

The number of pregnancies of the study women varied from 1-6 years. Most of them, 265 cases (48.09%) were gravida I, 127 cases (23.04%) were gravida II, 79 cases (17.33%) were gravida III, 49 cases (8.89%) were gravida IV, 22 cases (3.99%) were gravida V, and 9 cases (1.63%) were gravida VI. In addition, 67 cases (12.15%) had abortion in their medical history. The highest abortion frequency belonged to single abortion women (45 cases, 67.16%) followed by two abortion women (13 cases, 17.89%), three abortion women (9 cases, 13.46%) and four abortion women (1 case, 1.49%).

Clinically, 307 cases out of 551 subjects (55.71%) lacked any type of clinical symptoms. The rest of the subjects showed clinical demonstration of which 244 cases (44.28%) had vaginal discharge. Since this discharge lacked microscopically visible pathogens, this group was also added to the group of healthy pregnant subjects. Hence, there were totally 524 healthy subjects. In 27 subjects (8.72%), a positive test result for infection was added to clinical topical symptoms such as inflammation, discharge, itching, fetid smell, and strawberries vulvovaginitis (Table 1). The relationship between this pathogen with different variables was examined. There was no statistically significant correlation between trichomoniasis and factors such as gender, level of literacy, and number of pregnancies (P value = 0.05).

Most of the patients with trichomoniasis aged 21-25, had high school diploma and were experiencing their first pregnancy. They were referred to the health centre for family control. There was a statistically significant difference between trichomoniasis and vaginal pH (P value = 0.001) in that 74.4% of those infected by parasite had a pH of 6-7 and 25.6% in pH up-8. Using the light microscopy at 40x power, we found a statistically significant

relation the number of leukocytes and trichomoniasis (P value <0.05).

Table 1: Clinical features of trichomoniasis

Clinical observation	N	%
1-Negative:	524	91.28
Asymptomatic	307	55.71
Symptomatic	244	24.28
2-Positive:	27	8.72
Inflammation	24	88.89
Vaginal discharge	25	92.60
Irritation & itching	22	81.48
Unpleasant odor	26	96.29
Strawberry erythema	19	70.37

Finally, there was a statistically significant correlation between trichomoniasis and its diagnostic method (P value <0.05) in that 94% of the cases with clinical symptoms whose vaginal discharge was stained and directly tested, the test result was positive in the Dorsae culture medium (Table 2).

Table 2: Results of testing for *T. vaginalis*

Positive specimens	Direct methods		Culture method
	Wet mount	Giemsa stain	
17	+	+	+
3	-	+	+
7	-	-	+
Positive ^a	17/551 (3.08%)	20/551 (3.62%)	27/551 (4.90%)

^a of 551 tested specimens, 27 were positive by all methods

Discussion

The findings with pregnant women indicate that there are significant changes due to trichomoniasis in them. During pregnancy, due to wide changes in hormone level, immunologic and physiologic changes, and also pH imbalance and natural intravaginal condition, a favorable environment for parasite replacement and growth is developed. The adverse consequences of trichomoniasis, especially its metabolites,

can lead to infertility, premature delivery, and low-weight offspring [4,25]. Most of the subjects were aged between 21 and 25, this being consistent with more sexual activity in this age group.

Yet, as all of the studied cases were at pregnancy age, the incidence of infection is naturally insignificant for those at the middle years of pregnancy age range. Social customs of marriage and birth are culturally different in communities. Additionally, data related to all studied age groups with regard to incidence of trichomoniasis are consistent with those of reference textbooks [26]. Naturally, there is no significant correlation between literacy, number of pregnancies, and the degree of trichomoniasis incidence in the community since the health services and hygienic information are equally available to all people.

Shobeiri *et al.* [27] reported, the majority of the women in the case and control groups, had an age range of 15-20 years at marriage. Hart [28] studying 5,365 women independent of their clinical condition, in 1988-1991, found trichomoniasis in 1.8%, bacterial vaginosis in 13.7%, and candidiasis as the main agent in 17.6% of patients. Toloï and Franceschini [29] analyzed 133 patients and found *Candida* in 26%, *Gardnerella* in 8%, and *Trichomonas* in 0%.

Akbarian *et al.* [30] reported that the low level of *T. vaginalis* infection in pregnant women, in comparison with those of free sexual relationship countries is essentially due to their culture, family ethics and constitutional believe. They also showed that since the vaginal cavity is an eco-type and natural biological living orifice; therefore, the mere presence of *T. vaginalis* cannot cause sterility in women unless under non-optimum or non-physiologic conditions.

One of the basic conditions for replacement and growth of parasites on the

reproductive tract mucus is the presence of abundant glycogen and a pH of 3-5.5. Naturally, as the pH increases toward alkalinity, the milieu will be more favorable for parasites. Jack *et al.* [31] also mentioned an increasing vaginal pH as the necessary conditions for growth and multiplication of parasites. They also reported that culture techniques are highly sensitive (95%) and should be considered in patients with elevated vaginal pH, increased numbers of polymorph nuclear leukocytes, and absence of motile trichomonads and clue cells or when microscopy is unavailable or yields unreliable results.

In the present study, an increase in leukocytes specially polymorph nuclear leukocytes, meant an increase in the trichomoniasis infection and had a direct correlation with it. It is advisable to suspect trichomoniasis in the presence of white blood cells even in the absence of motile trophozoite or clue cells. Clue cells were found to be an independent risk factor for trichomoniasis among 249 South African women attending family planning clinics; however, these characteristics are neither sensitive nor specific. The clinician's ability to predict vaginal infection with *T. vaginalis* based solely on physical examination was shown to have a positive predictive value of 47% among commercial sex workers in Cameroon [32].

According to Fattahi Bafghi *et al.* [33], the precise clinical and laboratory examination of the pregnant women and their vaginal discharges are important since they may be wrongly identified as vaginal secretion or semen liquid with pathogenic materials, particularly by an inexperienced gynecologist. Finally, it was shown that the diagnostic method using parasite culture is a very sensitive procedure (95%) and infection with parasite is correlated with the diagnostic method.

Hobbs *et al.* [11] showed that when PCR is used to detect *T. vaginalis*, fewer

cases are missed if a single specimen would be used. Nevertheless, PCR detection from both urine and semen specimens increased the number of cases by 16% compared to urine specimen alone. However, the study design did not permit PCR testing from urethral swabs, and this was the most sensitive sample type for culture. Thus, it is possible that urethral swab PCR may have eliminated the observed benefits of testing multiple specimens.

Fattahi Bafghi *et al.* [33] reported that, there was a significant difference between microscopical and clinical diagnosis of trichomoniasis. Culture of the organism is more sensitive than immediate examination of slide wet mount preparations. Culture is not widely used in routine office or clinic diagnosis of trichomoniasis because it is an inconvenient procedure. Compared with wet mounts, culture requires more time and effort, and because the medium is complex, it is more expensive, may not be widely available, and generally has a limited shelf life. Thus, there is a need for a technically simple, self-contained, and accurate culture method that provides dry ingredients with a long shelf life [33].

Changes due to trichomoniasis infection, the most common cause of vaginitis especially during pregnancy, should not be neglected as they may lead to infertility, premature birth, and low weight offspring. Sampling the vaginal discharge, especially if voluminous and fetid, is necessary for studying trichomoniasis. Mere microscopic diagnosis should be avoided since inexperienced pathologists readily mistake white or colorless vaginal discharge for semen. Additionally, obstetricians and midwives should instruct their patients in this regard and notify the sexually transmitted disease pathogens to medical lab personnel.

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