

Original Article

Prevalence of *Trichomonas vaginalis* infection in symptomatic and asymptomatic women in Central Vietnam

Phuong Anh Ton Nu¹, Vu Quoc Huy Nguyen², Ngoc Thanh Cao², Daniele Dessi³, Paola Rappelli³, Pier Luigi Fiori³

¹ Department of Parasitology, Hue University of Medicine and Pharmacy, Hue City, Vietnam

² Department of Obstetrics and Gynecology, Hue University of Medicine and Pharmacy, Hue City, Vietnam

³ Division of Clinical and Experimental Microbiology, Department of Biomedical Sciences, University of Sassari, Sassari, Italy

Abstract

Introduction: The diffusion of trichomoniasis in Vietnam has been scarcely studied. The aim of this study was to investigate the prevalence of trichomoniasis in a group of symptomatic and asymptomatic women in Central Vietnam. Relationships between education, socioeconomic and marital status, and sexual behavior with infection have also been investigated.

Methodology: 249 symptomatic and 534 asymptomatic women from Hue City, Vietnam, were enrolled in this study. All women were interviewed about socioeconomic and behavioral status. They underwent clinical examination, and vaginal swabs were taken to assess *T. vaginalis* infection by wet mount microscopy examination. In addition, an ELISA test to detect antibodies to *T. vaginalis* in patients' sera was used.

Results: The overall prevalence of trichomoniasis assessed by microscopic examination was 6.6%. A significant difference between symptomatic and asymptomatic groups was observed, resulting in 19.3% and 0.7%, respectively. Anti-*T. vaginalis* antibodies were detected in 31.3% of symptomatic and in 13.3% of asymptomatic women. High-risk sexual behaviour, residence in urban areas, and low level of education were positively associated with infection.

Conclusion: This is the first report on the diffusion of trichomoniasis in Central Vietnam on symptomatic and asymptomatic subjects. Data demonstrated that *T. vaginalis* is a common cause of vaginal infection in the Hue province. The prevalence detected by microscopic examination was high in symptomatic subjects, while serological ELISA test detected infection also in asymptomatic patients, who tested negative by microscopy. The ELISA test may be useful to detect infection, especially in asymptomatic population.

Key words: *Trichomonas vaginalis*; Vietnam; seroepidemiology; diagnosis; sexually transmitted disease.

J Infect Dev Ctries 2015; 9(6):655-660. doi:10.3855/jidc.7190

(Received 25 May 2015 – Accepted 29 May 2015)

Copyright © 2015 Ton Nu *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The flagellate protist *Trichomonas vaginalis* is the causative agent of the most common non-viral sexually transmitted infection, affecting more than 270 million people worldwide. The World Health Organization (WHO) estimates that nearly 90% of infections occur among people living in resource-limited settings [1]. Trichomoniasis is positively associated with increased risk for preterm delivery, pelvic inflammatory disease, invasive cervical and prostatic cancer, and a higher susceptibility to HIV transmission [2-5]. The infection in women includes a broad range of symptoms, ranging from severe vaginal inflammation accompanied by a frothy malodorous discharge and dyspareunia to an asymptomatic carrier state, which affects up to 50% of cases. The protozoon induces a local inflammation exerting a cytopathic

effect on vaginal cells, mediated by pore forming proteins [6]. Most *T. vaginalis* isolates establish a symbiotic relationship with the bacterium *Mycoplasma hominis*, which influences the protozoan metabolism and up-regulates local inflammation [7-9].

Diagnosis of trichomoniasis is usually based on direct microscopic examination of wet mount preparations and/or on cultivation on specific media. Microscopic examination is inexpensive and rapid, but it is characterized by low sensitivity, and strongly depends on operator's experience and on protozoa viability [10]. Cultivation is more sensitive than microscopy and is considered the gold standard for the diagnosis of trichomoniasis, but it is rarely included in routine testing especially in resource-limited countries since it requires a completely equipped cell culture laboratory for *T. vaginalis* identification. Recently,

molecular protocols for *T. vaginalis* detection have been proposed, but they have not been included in routine diagnosis yet, especially in developing countries [11].

Serological diagnosis represents an alternative to direct detection of the protozoan, as demonstrated by Mason *et al.* [12] who described a reliable enzyme immunoassay to detect specific antibodies in sera collected in Zimbabwe. The recent description of the kinetics of humoral anti-*T. vaginalis* response during infection may facilitate the interpretation of results in serological analysis [13].

Although trichomoniasis is diffused worldwide, its prevalence greatly varies among different populations. Trichomoniasis is not a reportable infection, despite its high impact on public health, so data regarding diffusion of *T. vaginalis* are scarce and incomplete in most countries. In many cases, especially in asymptomatic patients, the infection is not recognized and its prevalence is therefore generally underestimated. Until now, only a limited number of epidemiological investigations describing the prevalence of trichomonad infection in Vietnam have been reported in literature. Lan *et al.* [14] showed that the prevalence of *Trichomonas vaginalis* infection in a group of married women in the Bavi district of North Vietnam was 1%. Similar results were obtained in a group of pregnant women in Hanoi, Vietnam [15]. As expected, prevalence was higher in women at high risk for sexually transmitted infection, as shown in a group of female sex workers in South Vietnam, showing a prevalence of 8.9% [16]. Since these studies used only the direct microscopy to diagnose *T. vaginalis*, the diffusion of the protozoan may be underestimated. Moreover, no epidemiological analysis conducted so far in Vietnam correlated the presence of *T. vaginalis* with specific symptoms.

The aim of this study was to estimate the prevalence of *T. vaginalis* in a large group of women from Hue City, Vietnam, complaining of symptoms of vaginitis, and to compare results with a matching group of asymptomatic subjects. Moreover, in order to highlight sub-clinical infections, the classical microscopic examination was complemented with the more reliable and sensitive serological analysis by ELISA.

Methodology

Patients / Study population

A cross-sectional study was conducted between September 2010 and June 2012 in Hue city, Vietnam, to determine the prevalence of trichomoniasis among

symptomatic and asymptomatic women. A total of 249 women attending the Gynecological Clinic of Hue University Hospital, presenting symptoms of vaginitis and 534 asymptomatic women, attending the Family Planning Program of the University Hospital, were enrolled in the study.

Each woman was interviewed by a physician, and a structured questionnaire was filled. Questions included place of residence, socioeconomic and marital status, sexual behavior and education.

Depending on marital status and sexual behavior, patients were sub-divided in high and low risk for sexually transmitted infections. The high-risk group included women with at least two sexual partners in the last year, while the low-risk group included subjects declaring no more than one partner in the same period. Based on educational level attained, participants were divided in high-level (high school degree or higher) and low-level (illiterate, primary, or secondary school education).

All patients underwent gynecological examination, and signs and symptoms were recorded. After clinical examination, a vaginal swab and a blood sample were collected for further investigations. Vaginal samples and sera were labeled with a reference code, and microbiological and serological tests were conducted anonymously. Informed consent was obtained from all subjects. The collection of samples was approved by the Bioethics Committee of the Hue University of Medicine and Pharmacy, Vietnam (21.10.2010).

Laboratory methods

Vaginal samples were examined immediately after collection. Each cotton swab was put in a sterile vial containing 0.5 ml of 0.9% saline and gently agitated, and the suspension was used to prepare wet mount slides. Slides were carefully observed under microscope for the presence of *T. vaginalis* by a trained microbiologist.

Blood specimens (1 ml) of 777 out of 783 subjects (six symptomatic patients refused to undergo the blood test) were collected in sterilized vials, then sera were separated by centrifugation and stored at - 80°C until use.

Serological analysis was performed by ELISA test according to a previously described method [17], with minor modifications. Briefly, antigen coated plates were prepared using the *Mycoplasma hominis*-free *T. vaginalis* reference strain G3: protozoa in logarithmic growth phase were harvested, washed three times in phosphate buffered saline (PBS) and suspended at 1×10^6 cells/ml, then 50 μ l aliquots were added to each

well of a microtiter plate. Plates were air-dried overnight, and then fixed with ice-cold 95% ethanol for 10 minutes at room temperature. Wells were finally blocked with 1% bovine serum albumin (Sigma Aldrich) in PBS for 2 hours. After extensive washing with distilled water, plates were air dried and stored at 4°C until use.

Patients sera were diluted 1:100 in PBS plus 5% bovine serum albumin, then added to each well of the coated plates and incubated for 37°C for 1 hour. Alkaline phosphatase-labeled anti-human IgG was used as secondary antibody. Plates were read at 405 nm. All sera were tested in duplicate. Cut-off value was calculated as described elsewhere [13] using sera from 50 healthy Vietnamese children as negative controls.

Data analysis

Statistical analysis was performed using Microsoft Excel 2010 and MedCalc statistical software. Comparison of proportions between two rates were calculated by Chi-square test. Comparison of two means were calculated by Independent Sample T-test. *P*-values < 0.05 were regarded as statistically significant.

Results

A total of 783 women from central Vietnam (Hue province) were enrolled in this study in order to investigate the prevalence of trichomoniasis in this

geographical area. Among them, 249 showed symptoms of vaginal inflammation, while 534 were asymptomatic. The mean age was comparable between the two groups (age ranging from 20 to 60 years, mean 38+/-10), with 91.3% of subjects in reproductive age. Based on sexual behaviors, 46.9% of symptomatic and 90.8% of asymptomatic women were classified to be at a low-risk for sexually transmitted diseases. Sociodemographic characteristics of each group are reported in Table 1.

Vaginal specimens from symptomatic and asymptomatic women were analyzed by microscopic examination, immediately after sample collection. Trichomoniasis has been diagnosed in a total of 52 women (overall prevalence 6.6%). Infection was considerably more common in symptomatic than in asymptomatic women, involving 19.3% and 0.7% of subjects, respectively (*p* < 0.0001). As reported in Table 2, significant differences in the distribution of trichomonad infection based on residence, educational levels and sexual behaviors were observed. Data obtained clearly demonstrated that trichomoniasis is more common among women living in urban areas and in the group of lower level of education. Moreover, as expected, the infection is more diffused in patients at a high-risk than in patients at a low risk sexual behavior (*p* < 0.0001).

All patients underwent gynecological examination: the most frequent signs and symptoms of women who tested positive for trichomoniasis by microscopic

Table 1. Socio-demographic characteristics of patients under study.

		Symptomatic Group n = 249	Asymptomatic Group n = 534
Residence	Urban	52.3%	28.3%
	Rural	47.7%	71.7%
Education	High level	39.1%	24.3%
	Low level	61.9%	75.7%
Sexual behaviour	Safe	46.9%	90.8%
	Unsafe	53.1%	9.2%

Table 2. Prevalence of *T. vaginalis* infection detected by microscopic examination

Subgroups		Number of positive patients (%)	<i>P</i>	χ^2
Symptoms	Symptomatic women	48/249 (19.3)	< 0.0001	92.117
	Asymptomatic women	4/534 (0.7)		
Residence	Urban	30/283 (10.6)	0.0014	10.227
	Rural	22/500 (4.4)		
Education	Low level	31/228 (13.6)	< 0.0001	23.437
	High level	21/555 (3.8)		
Sexual behavior	Low-Risk	19/603 (3.2)	< 0.0001	48.368
	High-risk	33/180 (18.3)		

examination were vaginal erythema (80.4%), malodorous (73.9%), profuse (60.9%) and yellowish-green vaginal discharge (54.3). Strawberry cervix was observed in 30.4% of positive patients.

The presence of specific antibodies to *T. vaginalis* was investigated by ELISA in sera from 777 out of 783 women. As shown in Table 3, positivity to *T. vaginalis* was detected in 147 sera, with an overall prevalence of 18.9%. Specific anti-*T. vaginalis* IgG were detected in sera of all 52 women who tested positive by microscopy, confirming the reliability of ELISA test.

As expected, symptomatic patients showed a higher reactivity (31.3%) compared to asymptomatic women (13.3%); data also confirmed that unsafe sexual behaviors are a risk factor for trichomoniasis (22.7% vs 14.0%), while a role of geographical area and educational levels was not confirmed by serology.

As expected the prevalence of trichomoniasis calculated on the basis of specific circulating antibody detection is higher than that obtained by direct microscopic examination, being 31.3% vs.19.3% in the symptomatic group, and 13.3% compared to 0.7% in the asymptomatic one. The difference in sensitivity of the two techniques is higher among the asymptomatic patients: in a total of 534 women without vaginal symptoms, only 4 tested positive by microscopy, while 71 tested positive by the ELISA test.

Discussion

The diffusion of *T. vaginalis* infection in South East Asia varies among countries [1]. Different habits, sexual behaviors and symptomatology of the population under study can greatly influence results. In Vietnam, data on the prevalence of trichomoniasis are limited to a few studies, showing a very low prevalence of infection (range 1.0-1.4%) among women at low-risk for sexually transmitted infections [14-15]. Only one study was conducted on population

at high-risk, and showed that 8.9% of 406 female sex workers in the Soc Trang province of Vietnam tested positive for *Trichomonas vaginalis* [16]. None of the studies conducted so far in Vietnam put the presence of *T.vaginalis* in relation with vaginal symptoms.

To our knowledge, this is the first study reporting the prevalence of trichomoniasis in a group of symptomatic female patients in Vietnam, and comparing results with those obtained with matching asymptomatic women.

All previous studies on the diffusion of trichomoniasis in Vietnam solely utilized the direct microscopy as diagnostic tool. Even though direct microscopy is the most common method to diagnose *T. vaginalis* infection in women, its sensitivity can be extremely low. False negative results are common especially in sub-clinical infections.

In the present work *T. vaginalis* infection was detected using both microscopy and ELISA. We have chosen to perform microscopic examination as it is the most diffused technique, especially in developing countries, and in order to render our results comparable with previous studies on the diffusion of trichomoniasis in Vietnam. In addition, we chose to use a specific ELISA test to overcome the microscopy low sensitivity. In fact, serodiagnosis of trichomoniasis represents a reliable alternative method in epidemiologic studies on trichomoniasis [12]. Moreover, the recent description of the kinetics of circulating IgG antibody response in trichomonad patients can facilitate the interpretation of serological results, rendering serodiagnosis an extremely valid diagnostic tool, especially in asymptomatic infections which are most frequently missed by microscopic examination [13].

A group of 783 women was enrolled between September 2010 and June 2012 from the Hue province of Central Vietnam. Among them, 249 reported symptoms of vaginitis and 534 were asymptomatic. The overall prevalence of trichomoniasis diagnosed by

Table 3. Seroprevalence of *T. vaginalis* infection detected by ELISA

Subgroups		Number of positive patients (%)	P	χ^2
Symptoms	Symptomatic women	76/243 (31.3)	< 0.0001	92.117
	Asymptomatic women	71/534 (13.3)		
Residence	Urban	73/499 (14.6)	0.0014	10.227
	Rural	74/278 (26.6)		
Education	Low level	97/552 (17.6)	< 0.0001	23.437
	High level	50/225 (22.2)		
Sexual behavior	Low-Risk	84/599 (14.0)	< 0.0001	48.368
	High-risk	63/278 (22.7)		

microscopic examination was 6.6%, with significant differences between asymptomatic and symptomatic women (0.7% and 19.3%, respectively). Data obtained on the asymptomatic group are consistent with results described in previous works in Vietnam [14,15]. In these studies, in fact, women were enrolled based only on their status of pregnancy or marriage not mentioning vaginal symptoms, and therefore might have been considered asymptomatic. Interestingly, our results showed a consistently higher prevalence of trichomonad infection in symptomatic patients (19.3%).

The high difference observed between the two groups may also reflect the well-known difficulties in detecting *T. vaginalis* by microscopy in samples from asymptomatic patients, where the infection is characterized by a very low number of protozoa, often under the detection limit of microscopic examination. Results obtained by ELISA seem to confirm this hypothesis. In fact, specific anti-*T. vaginalis* IgG antibodies were detected in 18.9% of total population, with a seropositivity of 31.3% in symptomatic women (compared to 19.3% by microscopic examination), and of 13.3% in asymptomatic women (compared to 0.7%).

Asymptomatic *T. vaginalis* infections represent an important risk factor for local tumor initiation, HIV acquisition, and for pre-term labor and low birth weight [18]. The diagnosis of subclinical infections based on seroreactivity against *T. vaginalis* may therefore represent a useful and effective strategy to identify patients unaware of harboring the parasite, contributing to reduce complications and to limit the spread of infection.

Quote

“A journey, after all, neither begins in the instant we set out, nor ends when we have reached our doorstep once again. It starts much earlier and is really never over, because the film of memory continues running on inside of us long after we have come to a physical standstill. Indeed, there exists something like a contagion of travel, and the disease is essentially incurable”.

Ryszard Kapuściński, *Travels with Herodotus*.

Acknowledgements

This work is dedicated to our friend and mentor Piero Cappuccinelli.

This work was supported by Regione Autonoma della Sardegna (L.R. 7/2007, grant N° CRT25578).

References

- World Health Organization (2012) Global incidence and prevalence of selected curable sexually transmitted infections-2008. Geneva, WHO Press. Available at: http://apps.who.int/iris/bitstream/10665/75181/1/9789241503839_eng.pdf. Last accessed on September 7, 2014.
- Cotch MF, Pastorek JG 2nd, Nugent RP, Hillier SL, Gibbs RS, Martin DH, Eschenbach DA, Edelman R, Carey JC, Regan JA, Krohn MA, Klebanoff MA, Rao AV, Rhoads GG (1997) *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 24: 353-360
- Stark JR, Judson G, Alderete JF, Mundodi V, Kucknoor AS, Giovannucci EL, Platz EA, Sutcliffe S, Fall K, Kurth T, Ma J, Stampfer MJ, Mucci LA (2009) Prospective study of *Trichomonas vaginalis* infection and prostate cancer incidence and mortality: Physicians' Health Study. J Natl Cancer Inst 101: 1406-1411.
- Twu O, Dessì D, Vu A, Mercer F, Stevens GC, de Miguel N, Rappelli P, Cocco AR, Clubb RT, Fiori PL, Johnson PJ (2014) *Trichomonas vaginalis* homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc Natl Acad Sci USA. 111: 8179-8184.
- Galvin SR, Cohen MS (2004) The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol 2: 33-42
- Fiori PL, Rappelli P, Addis MF (1999) The flagellated parasite *Trichomonas vaginalis*: new insights into cytopathogenicity mechanisms Microbes Infect 1:149-156
- Dessì D, Delogu G, Emonte E, Catania MG, Fiori PL, Rappelli P (2005) Long-term survival and intracellular replication of *Mycoplasma hominis* in *Trichomonas vaginalis* cells: a potential role of the protozoon in transmitting bacterial infection Infect Immun 73: 1180-1186
- Morada M, Manzur M, Lam B, Tan C, Tachezy J, Rappelli P, Dessì D, Fiori PL, Yarlett N (2010) Arginine metabolism in *Trichomonas vaginalis* infected with *Mycoplasma hominis*. Microbiology, 156: 3734 – 3743
- Fiori PL, Diaz N, Cocco AR, Rappelli P, Dessì D (2013) Association of *Trichomonas vaginalis* with its symbiont *Mycoplasma hominis* synergistically upregulates the in vitro proinflammatory response of human monocytes Sex Transm Infect. 89: 449-454
- Garber GE (2005) The laboratory diagnosis of *Trichomonas vaginalis*. Can J Infect Dis Med Microbiol 16: 35-38.
- Hobbs MM, Sena AC (2013) Modern diagnosis of *Trichomonas vaginalis* infections. Sex Transm Infect 89: 434-438.
- Mason PR, Fiori PL, Cappuccinelli P, Rappelli P, Gregson S (2005) Seroepidemiology of *Trichomonas vaginalis* in Rural Women in Zimbabwe and Patterns of Association with HIV Infection. Epidemiol Infect 133: 315-323
- Ton Nu PA, Rappelli P, Dessì D, Nguyen VQ, Fiori PL (2015) Kinetics of circulating antibody response to *Trichomonas vaginalis*: clinical and diagnostic implications. Sex Transm Infect Published Online First: [April 17, 2015] doi:10.1136/sxtrans2014-051839
- Lan PT, Lundborg CS, Phuc HD, Sihavong A, Unemo M, Chuc NT, Khang TH, Mogren I (2008) Reproductive tract infections including sexually transmitted infections: a population-based study of women of reproductive age in a rural district of Vietnam. Sex Transm Infect 84: 126-132.

15. Goto A, Nguyen QV, Pham NM, Kato K, Cao TP, Le TH, Hoang QK, Le TQ, Nguyen BT, Katsube M, Ishii S, Yasumura S (2005) Prevalence of and factors associated with reproductive tract infections among pregnant women in ten communes in Nghe An Province, Vietnam. *J Epidemiol* 15: 163-172.
16. Nguyen TV, Van Khuu N, Thi Le TT, Nguyen AP, Cao V, Tham DC, Detels R (2008) Sexually transmitted infections and risk factors for gonorrhoea and chlamydia in female sex workers in Soc Trang, Vietnam. *Sex Transm Dis* 35: 935–940
17. Mason PR, Gregson S, Gwanzura L, Cappuccinelli P, Rappelli P, Fiori PL (2001) Enzyme immunoassay for urogenital trichomoniasis as a marker of unsafe sexual behavior. *Epidemiol Infect* 126: 103-109
18. Petrin D, Delgaty K, Bhatt R, Garber G (1998) Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clin Microbiol Rev* 11: 300–317

Corresponding author

Paola Rappelli
Department of Biomedical Sciences, University of Sassari,
Viale S. Pietro 43/B, 07100 Sassari, Italy
Phone: + 39079228299
Fax: + 39079212345
Email: rappelli@uniss.it

Conflict of interests: No conflict of interests is declared.