# Trichomonas Vaginalis as A Risk Factor for Human Papillomavirus: A Study With Women Undergoing Cervical Cancer Screening

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# Abstract

Background

Human papillomavirus (HPV) and Trichomonas vaginalis (TV) infections are the most common STIs. The latter has contributed to a variety of adverse outcomes for both sexes. Moreover, in Brazil, epidemiological studies on patients with STIs are limited. Therefore, this study aimed to determine the prevalence of TV and its association with HPV in women undergoing cervical cancer screening.

Methods

Women with a normal cervix were recruited from a community-based cervical cancer screening program. Gynecological examinations were conducted and questionnaires were provided. Vaginal canal and uterine cervix samples were collected and tested for the presence of TV and HPV DNA.

Results

The overall prevalence of HPV DNA was 45.68%; among these, 27.1% had a co-infection with TV (p = 0.001). The presence of TV was associated with an increased risk of HPV (p = 0.0001) and previously identified cytological changes (p = 0.0001).

Conclusions

We concluded that a TV infection is associated with an HPV infection of the cervix as well as with the cervical cytological abnormalities. Further studies could reveal the mechanisms by which these two organisms interact at the cellular level, with control for shared behavioral risk factors. This research is in agreement with Resolution No. 466/2012 of the National Health Council and has the Research Ethics Committee of the Hospital Universitário Presidente Dutra, from Universidade Federal do Maranhão, under opinion number 76328917.5.0000.5086.


# Background

Sexually transmitted infections (STIs) are a group of infections acquired through sexual contact that affect the health of people worldwide [1]. In 2016, an estimation of 376 million new infections (more than 1 million per day) was reported in persons aged between 15 and 49 years [2]. *Trichomonas vaginalis* (TV) and Human Papillomavirus (HPV) infections are among the most common STIs; however, the prevalence of these STIs varies significantly globally, with a higher prevalence in countries with low socioeconomic indices [3].

These infections can lead to a variety of complications, especially in women, including genitalia-related issues (cervicitis, urethritis, vaginitis, and genital ulceration), complications during pregnancy, infertility, increased risk of acquisition and transmission of human immunodeficiency virus, and cancer [3–5].

HPV is the most common viral infection and is associated with the development of several types of cancer, including cervical and penile cancer [6]. The incidence of HPV is greater than 528,000 cases per year, with more than 270,000 deaths caused by cervical cancer [1]. The global prevalence of HPV in women with normal cytology is estimated at 11.7%, and in Brazil, it can reach 55.4% [1, 7].

Persistent infection by viral types of high oncogenic risk, mainly by HPV types 16 and 18, is one of the main factors for the development of cervical cancer [8]. Understanding that infection with high-risk HPVs is an essential, but not sufficient, factor for the progression of cervical cancer has led to the study of cofactors, such as biological, behavioral, and environmental, in cervical carcinogenesis [9, 10, 11].

*Trichomonas vaginalis* is a flagellated, facultatively anaerobic protozoan of the human genital tract [12]. It is the most common, non-viral, sexually transmitted agent worldwide, responsible for 143 million cases in 2012 and 110.4 million in 2018 [2]. Epidemiological studies have shown that TV infection can lead to an increased risk of cervical cancer [13–16]. The interaction between cervical cancer and TV is not yet fully elucidated, but it is believed that the inflammatory process caused by this protozoan predisposes the epithelium to carcinogenesis [12, 17–19]. The rupturing of the cervical epithelium due to inflammation is associated with facilitating the entry of HPV into the basal layer of the epithelium, supporting the integration of the viral DNA into the host DNA, and overexpression of the viral oncogenes that contribute to the activation of carcinogenic mechanisms [17, 19, 20].

In this perspective, the objective of this study was to determine the prevalence of TV and its association with HPV in women who sought to undergo cervical cancer screening in Northeastern Brazil.

# Methods

Study population

This cross-sectional and non-interventional study was conducted with 562 women who were patients at the public health units of gynecological care in São Luís, Maranhão, Brazil. The study was initiated after approval by the Federal University of Maranhão Ethics Committee (number 2.383.604) and written informed consent was obtained from all patients. The sample size was calculated considering a prevalence of 11.2% of women with TV [2], a power of 90% and a 5% significance level. : “The calculated sample was 384 women, however, 562 women were included in the study.”

A semi-structured questionnaire containing sociodemographic variables (age, ethnicity, education, family income, professional activity, marital status, sexual behavior, alcohol consumption, smoking status, and reproductive health and barrier methods used) was completed by these women. Women who were menstruating, underwent hysterectomy, were virgins, or pregnant for less than 45 days postpartum were excluded from this study.

Cervical cytology

Conventional cytological smears were obtained with Ayres spatula (ectocervical sample) and endocervical brush (endocervical sample), fixed on a glass slide with ethanol, and stained using the Pap technique. Cytological examinations of Pap smear were reported using the 2001 Bethesda Reporting System.

Specimen collection and DNA extraction

For HPV DNA isolation, samples were collected and placed in the HC2 DNA Collection buffer (QIAGEN, CA, USA), and stored at -20°C until processed. HPV DNA extraction was performed using the QIAamp DNA Mini and Blood kit (QIAGEN, CA, USA), according to the manufacturer instructions. Total DNA was isolated, eluted in 100 mL AE buffer, and stored at -20°C. Extracted DNA was quantified using a NanoVue unit (GE Healthcare Life Sciences, Little Chalfont, UK).

HPV and TV detection

Presence of HPV DNA was detected using nested polymerase chain reaction (PCR) with the primer sets PGMY09/11 (first round of PCR) and GP + 5/GP + 6 (second round of PCR) [21]. Presence of TV was detected using conventional PCR with the primers TVA5/6 [22].

Amplification products were evaluated using electrophoresis with a 1.5% agarose gel in 1 × TBE buffer for 30 minutes at 5 V/cm in a horizontal unit (Life Technologies, Carlsbad, CA, USA). Bands were stained with 0.1% Gel Red (Invitrogen) and visualized using an ultraviolet transilluminator (BioRad Laboratories, Hercules, CA, USA).

# Data analysis

Statistical analysis was performed using IBM SPSS® software version 23. Data were initially subjected to descriptive analysis along with mean and standard deviation. The Kolmogorov–Smirnov test was used to verify the normality of data.

The chi-square or Fisher's exact test was used to assess the association between TV infection and sociodemographic and clinical factors (confidence interval: 95%), with $p \leq 0.05$ considered statistically significant. For multivariate analysis of the data, a hierarchical model of binary logistic regression in six levels was built, in which all variables were forced into the equation. Level 1 included sociodemographic variables (age, color, marital status and education); level 2, lifestyle (smoking status, alcohol consumption); level 3, reproductive history (age at first period and sexual intercourse, number of pregnancies, abortions and sexual partners); level 4, contraceptive methods and sexual habits; level 5, occurrence of STIs; and level 6, occurrence of HPV.

# Results

Cytological smears from 562 women were processed from June 2017 to July 2019. The HPV DNA was present in 45.68% (254) of women; among these, 27.1% (69) had a co-infection with TV ($p = 0.001$)

(Table 1).

Table 1
Characteristics of the participants with *Trichomonas vaginalis* infections

| | | Total (N = 562) | | *Trichomonas vaginalis* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Yes (N = 107) | | No (N = 455) | | P-value |
| | | N | % | N | % | N | % | |
| Age | < 29 years | 155 | 27,58 | 30 | 19,35 | 125 | 80,65 | 0.473 |
| | 30–49 years | 272 | 48,40 | 56 | 20,59 | 216 | 79,41 | |
| | 50 + years | 135 | 24,02 | 21 | 15,56 | 114 | 84,44 | |
| Skin color | White | 56 | 9,96 | 5 | 8,93 | 51 | 91,07 | 0.042 |
| | Non-white | 506 | 90,04 | 102 | 20,16 | 404 | 79,84 | |
| Relationship status | With partner | 267 | 47,51 | 51 | 19,10 | 216 | 80,90 | 0.972 |
| | No partner | 295 | 52,49 | 56 | 18,98 | 239 | 81,02 | |
| Education level | Elementary school | 196 | 34,88 | 41 | 20,92 | 155 | 79,08 | 0.707 |
| | Secondary school | 295 | 52,49 | 53 | 17,97 | 242 | 82,03 | |
| | Graduate school | 71 | 12,63 | 13 | 18,31 | 58 | 81,69 | |
| Smoking status | No | 508 | 90,39 | 95 | 18,70 | 413 | 81,30 | 0.531 |
| | Yes | 54 | 9,61 | 12 | 22,22 | 42 | 77,78 | |
| Alcohol consumption | No | 349 | 62,10 | 65 | 18,62 | 284 | 81,38 | 0.749 |
| | Yes | 213 | 37,90 | 42 | 19,72 | 171 | 80,28 | |
| Oral contraceptive use | No | 539 | 95,91 | 99 | 18,37 | 440 | 81,63 | 0.001 |
| | Yes | 23 | 4,09 | 8 | 34,78 | 15 | 65,22 | |
| Condom use | No | 433 | 77,05 | 73 | 16,86 | 360 | 83,14 | 0.050 |
| | Yes | 129 | 22,95 | 34 | 26,36 | 95 | 73,64 | |

Women aged between 30 and 49 years (48.40%), self-declared women of color (90.03%), married women/with a partner (52.49%), and women with high-school level education (52.42%) were predominant. Regarding lifestyle, women were predominantly non-smokers (90.39%) and non-alcoholics (60.09%), and regarding reproductive history, majority did not use oral contraceptives (95.90%) and

condoms (77.04%). Women also reported anal sex (67.97%), oral sex (95.60%), absence of previous STI (79%), and presence of cytological changes previously detected (67.08%) (Tables 2 and 3).

Table 2
Sexual behaviors of the participants with *Trichomonas vaginalis* infections

| | | Total (N = 562) | | *Trichomonas vaginalis* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | **Yes (N = 107)** | | **No (N = 455)** | | **P-value** |
| | | **N** | **%** | **N** | **%** | **N** | **%** | |
| Anal sex | No | 180 | 32,03 | 38 | 21,11 | 142 | 78,89 | 0.390 |
| | Yes | 382 | 67,97 | 69 | 18,06 | 313 | 81,94 | |
| Vaginal sex | No | 5 | 0,89 | 0 | 0,00 | 5 | 100,00 | 0.276 |
| | Yes | 557 | 99,11 | 107 | 19,21 | 450 | 80,79 | |
| Oral sex | No | 25 | 4,45 | 3 | 12,00 | 22 | 88,00 | 0.359 |
| | Yes | 537 | 95,55 | 104 | 19,37 | 433 | 80,63 | |
| Previous sexually transmitted infection | No | 342 | 60,85 | 63 | 18,42 | 279 | 81,58 | 0.699 |
| | Yes | 220 | 39,15 | 44 | 20,00 | 176 | 80,00 | |
| Cytological abnormality | No | 185 | 32,92 | 24 | 12,97 | 161 | 87,03 | 0.010 |
| | Yes | 377 | 67,08 | 83 | 22,02 | 294 | 77,98 | |

Table 3
Human papillomavirus status of the participants and the presence of *Trichomonas vaginalis*

| | | Total (N = 556) | | *Trichomonas vaginalis* | | | | P-value |
|---|---|---|---|---|---|---|---|---|
| | | | | **Yes (N = 106)** | | **No (N = 450)** | | |
| | | **N** | **%** | **N** | **%** | **N** | **%** | |
| HPV | No | 302 | 54,32 | 37 | 12,25 | 265 | 87,75 | 0.001 |
| | Yes | 254 | 45,68 | 69 | 27,17 | 185 | 72,83 | |

Sociodemographic characteristics, lifestyle, and reproductive history were assessed in women with TV (Table 2). Infection with TV was associated with skin color (p = 0.042), use of oral contraceptives (p = 0.001), use of condoms (p = 0.016), and the presence of previously identified cytological changes (p = 0.010).

The multivariate analysis was performed to verify the variables associated with HPV infection. When adjusted for sociodemographic characteristics, none of the variables showed a statistically significant

association ($p > 0.005$). However, among characteristics associated with sexual behaviors and lifestyle, the use of oral contraceptives ($p = 0.044$ / 95% CI = 1.028–6.964), the use of condoms ($p = 0.041$ / 95% CI = 1.021–2.828), and the presence of TV ($p = 0.001$ / 95% CI = 1.386–3.630) were statistically associated with HPV infections (Table 4).

Table 4
Multivariate analysis regarding sociodemographic characteristics, lifestyle, reproductive history, and the presence of *Trichomonas vaginalis*

| | | P-value | Odds ratio | 95% Confidence interval |
|---|---|---|---|---|
| Age | < 29 years | Ref | | |
| | 30–49 years | 0,425 | 1,285 | 0,694–2,378 |
| | 50 + years | 0,848 | 0,920 | 0,391–2,165 |
| Skin color | White | Ref | | |
| | Non-white | 0,105 | 2,294 | 0,842–6,254 |
| Relationship status | No partner | Ref | | |
| | With partner | 0,777 | 0,937 | 0,599–1,466 |
| Education level | Elementary school (complete or incomplete) | Ref | | |
| | Secondary school (complete or incomplete) | 0,159 | 0,676 | 0,393–1,165 |
| | Graduate school (complete or incomplete) | 0,551 | 0,777 | 0,339–1,780 |
| Smoking status | No | Ref | | |
| | Yes | 0,946 | 0,973 | 0,450–2,106 |
| Alcohol consumption | No | Ref | | |
| | Yes | 0,836 | 0,951 | 0,594–1,524 |
| Age at first period | | 0,533 | 1,046 | 0,908–1,205 |
| Age at first sexual intercourse | | 0,551 | 1,023 | 0,949–1,102 |
| Pregnancies | | 0,336 | 0,937 | 0,820–1,070 |
| Miscarriage | | 0,593 | 0,857 | 0,488–1,507 |
| Partner | | 0,130 | 1,026 | 0,992–1,061 |
| Oral contraceptive use | No | Ref | | |
| | Yes | 0,044 | 2,676 | 1,028–6,964 |
| Condom use | No | Ref | | |
| | Yes | 0,041 | 1,699 | 1,021–2,828 |

Ref: reference.

| | | P-value | Odds ratio | 95% Confidence interval |
|---|---|---|---|---|
| Anal sex | No | Ref | | |
| | Yes | 0,768 | 0,931 | 0,578–1,499 |
| Oral sex | No | Ref | | |
| | Yes | 0,229 | 2,178 | 0,612–7,748 |
| STI history | No | Ref | | |
| | Yes | 0,706 | 0,894 | 0,501–1,598 |
| Cytological abnormality | No | Ref | | |
| | Yes | 0,159 | 1,507 | 0,852–2,667 |
| Confirmed sexually transmitted infection | No | Ref | | |
| | Yes | 0,564 | 0,867 | 0,533–1,409 |
| *Trichomonas vaginalis* | No | Ref | | |
| | Yes | 0,001 | 2,243 | 1,386–3,630 |
| Ref: reference. | | | | |

# Discussion

HPV and TV infections are among the most common STIs worldwide, both associated with a variety of health consequences in men and women [12]. HPV is considered the etiological factor of cervical cancer; however, the fact that some women manage to eliminate HPV without the development of cervical lesions leads to the question that other cofactors can facilitate the persistence of this virus, thereby preventing its elimination and favoring cervical changes mediated by HPV [18].

Studies have shown that a previous history of infection with TV leads to an increased risk of HPV infection, mainly owing to the viral types of high oncogenic risk [23, 13, 18]. In this context, the present study reaffirmed the association of TV with HPV as well as with cytological changes identified in previous exams.

TV releases lytic enzymes that reduce the protective mucus layer of the vaginal wall, leading to a reduction in vaginal fluids [13]. This can lead to the development of micro lesions in the epithelium, thereby increasing virulence and allowing the integration of the viral DNA into the DNA of the host cell, which in turn leads to host cell DNA damage and the beginning of the carcinogenic process [12]. In addition, the inflammatory process can also rupture the basal layer of the cervical epithelium and thus facilitate its persistence in the cervical-vaginal epithelium tissue [19, 12].

Thus, the results in studies on the co-infection of HPV and TV in the genital tract of women without cervical cancer are justified. However, the differences in prevalence are observed globally, with 1.9% in Busan/South Korea [24], 3.1% in Shanghai/China [25], 5.6% in female sex workers in the Midwest region of Brazil [26], 5.7% in Bahia/Brazil [27], 31.4% in Kenya [28], 18.8% in Beijing/China [29], and 24% in the rural area around Ngaramtoni /Tanzania [13]. The results presented here demonstrate a 27% co-infection prevalence of HPV and TV, which is in line with the studies mentioned previously.

In addition to HPV infection, multivariate analysis showed that other cofactors were also associated with TV infection in the study population, such as the use of oral contraceptives and inconsistent condom use. Continued use of oral contraceptives can lead to changes in the surface of the endometrium, making it more susceptible to sexually transmitted infections. It is believed that estrogen and progesterone from oral contraceptives can interact with the hormone receptors present in the cervical tissue and influence the natural history of HPV infection [30, 31]. Previous studies have reported that oral contraceptives are strongly associated with HPV acquisition as well as with the significant increase in cervical intraepithelial neoplasia (CIN3) and invasive cancer [32, 30, 33].

Inconsistent condom use has also been associated with HPV and TV co-infections. Individuals who do not use condoms are at high risk of infection and reinfection by HPV and other STIs, which also contribute to the progression of cervical lesions [34], probably owing to the local inflammatory process and intensified immune system stimulation [35, 19, 12]. In contrast, the constant use of condoms is associated with a lower risk of HPV infection and regression of cervical injury rates, as it allows the immune system to act if repair of tissue injuries are taking place, preventing the progression of the wound [36].

This study, despite confirming the correlation between HPV and TV among women who sought to undergo cancer screening, had limitations regarding the identification of the causal relationship between these infectious agents, as the participants were evaluated in a cross-sectional study design. However, a prospective cohort study to assess the linkage between HPV and other genital infections and the development of cervical neoplasia will be both time consuming and resource intensive.

# Conclusion

TV infection was associated with HPV infection of the cervix as well as with cervical cytological abnormalities. The magnitude and prevalence of co-infections in our study population warrant attention by public health services and demonstrate the importance of condoms and the frequency with which among female sex workers undergo oncotic cytology examinations. Further studies could reveal the mechanisms by which these two organisms interact at the cellular level, with control for shared behavioral risk factors.

# Abbreviations

AIR - Australian Immunization Register

CEP-UFMA - Research Ethics Committee of the Federal University of Maranhão

DNA - Deoxyribonucleic Acid

ESF - Family Health Strategy

USA - United States of America

HIV - Human Immunodeficiency Virus

HPV - Human Papillomavirus

IARC - International Cancer Research Agency

STI - Sexually Transmitted Infection

MS - Ministry of Health

WHO - World Health Organization

PCR - Polymerase Chain Reaction

SUS - Unified Health System

FICF - Informed Consent Form

UBS - Basic Health Units

TV- Trichomonas vaginalis

WHO - World Health Organization

# Declarations

### Ethical Approval and Consent to participate

This research is in agreement with Resolution No. 466/2012 of the National Health Council and has the Research Ethics Committee of the Hospital Universitário Presidente Dutra, from Universidade Federal do Maranhão.

### Consent for publication

This research had the approval of the Ethics in Researches Committee at College Hospital, of the Federal University of Maranhão under the opinion number under opinion number 76328917.5.0000.5086. And followed the regiment of the Resolution 466/12 of the National Health Consul, in a way that the data were

collected only after the reading, comprehension and assignment of the Enlightened and Free Consent Term (EFCT).

## Availability of supporting data

No support data available

## Competing interests

There are no conflicts of interest


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## Authors' contributions

IKPB - research design, sampling collections, analyzed and interpreted the data and was a contributor in writing the manuscript.

APAC - sampling collections, analyzed and interpreted the data and was a contributor in writing the manuscript.

FPBM - sampling collections, analyzed and interpreted the data

LVM – Statistics, analyzed and interpreted the data

RGL - sampling collections and analyzed and interpreted the data

LHLC – sampling collections and analyzed the data

PM - sampling collections and analyzed data

MBF - sampling collections and analyzed data

GRBS - performed the citological examination of lamines

JLC - sampling collections, analyzed and interpreted the data

LGOB - reading and correcting the manuscript

LMOB - reading and correcting the manuscript

FCBV - contributor in writing the manuscript.

SCMM - research design, analyzed and interpreted patients' data and contributed in writing the manuscript

All authors read and approved the final manuscript.

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