

Prevalence of *Candida albicans* and *Trichomonas vaginalis* Infections in Women

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Abstract: Background and Objective: Fungi, parasites, bacteria and viruses are the causative agents of human genitourinary tract infections. One such infection, Vulvovaginal candidiasis, affects millions of individuals worldwide. *Candida albicans* (*C. albicans*) is responsible for 85%-95% of vaginal yeast infections. Trichomoniasis is the most widespread protozoal venereal disease after bacterial infections. The rate of *Trichomonas vaginalis* (*T. vaginalis*) contamination varies in different areas of the world. This study has been designed to determine the prevalence rates of *C. albicans* and *T. vaginalis* infections in woman who were referred to medical centers.

Materials and Methods: This is a descriptive study designed to determine the prevalence rates of *C. albicans* and *T. vaginalis* infections in women. Five-hundred vaginal discharge specimens were stained using Löffler (L) and diluted carbol-fuchsin (DC-F) stains, and cultures were done on Sabouraud dextrose agar and Dorset medium. The data were analyzed using chi-squared tests and Student's t-tests. **Results:** One-hundred and fifty participants (30%) were infected with the *Candida* species. *C. albicans* caused the infection in 67 of these participants (13.4%), with the remaining 83 participants (16.6%) infected with some other *Candida* species. *T. vaginalis* was detected in 7 out of 500 women (1.4%). **Conclusion:** Candidiasis and trichomoniasis are the two most common infections of the urogenital system in humans. In the present study, the incidence of candidiasis (30%) was higher than previous studies had documented, and the incidence of trichomoniasis (1.4%) was less than that reported in previous studies.

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Background:

Parasites, bacteria, viruses and fungi are the causative agents of human genitourinary tract infections. Candidiasis is one of the most important opportunistic fungal infections. Principally, *Candida* species present as normal flora on the skin and in the digestive and genital tracts, and they may metamorphose into pathogenic forms if the host's immune system weakens (1,2,3).

Vulvovaginal candidiasis (VVC) is one such infection affecting millions of individuals worldwide. In developing countries, the prevalence of this infection has increased in recent years (4,7).

The infection can be acute or chronic, superficial or deep, and its clinical *Candida*

spectrum is so wide that a more specific definition cannot be made(28). Increasing literature on infection shows no sign of narrowing the clinical and scientific interest in *Candida* infection, which is remains high(29).

Data of incidence of vaginal candidiasis suggest approximately two-thirds of women experience at least one episode during their lifetime and nearly 50% of women have multiple episodes. The majority of cases of vulvovaginal candidiasis are caused by *C. albicans*; however, incidences due to non-*albicans* species of *Candida* appear to be increasing(30).

C. albicans is responsible for 85%-95% of vaginal yeast infections (8,9,10). Currently, *Candida*

vaginitis is more common than trichomonal and bacterial vaginosis (7,11,12). Patients with Candida vaginitis may not show any particular symptoms, but symptoms such as itching, burning, caseous discharge, painful intercourse, and vulva edema and erythema may be reported (11,13). That have been linked to idiopathic vulvovaginal candidiasis (VVC) include changes or imbalance in reproductive hormones, as a result of oral contraception, pregnancy, or hormone replacement therapy (HRT), as well as antibiotic usage, and diabetes mellitus(31)

Trichomoniasis is the most widespread protozoal venereal disease after bacterial infections. This parasite is the second most common cause of sexual infections (14) and also is known to increase the risk of human immunodeficiency virus (HIV) infection and other nonulcerative sexually transmitted diseases (STDs) (15). The characteristic “strawberry cervix,” caused by intraepithelial hemorrhages, is seen in only 5% to 10% of patients(39,51). Virulence is highly variable among the different strains, and 25% to 50% of women who are culture positive for *Trichomonas vaginalis* are asymptomatic. *Trichomonas vaginalis* can be recovered from the urine in up to 30% of male sexual contacts of women with the infection; however, less than 20% of men with trichomonas in the urine are symptomatic(52). Annually, more than 170 million people are afflicted by this pathogen throughout the world (14,16,17,18). The rate of contamination by this parasite varies in different areas of the world. In Iran, various studies have determined the prevalence of trichomoniasis to be between 2% to 8%, but based on cultural and social status, this rate can exceed 30% (14). However, only 1 out of 7 infected people complain about its complications (19). *Trichomonas vaginalis* is a flagellate protozoan that colonizes in the human urogenital orifice. It lives only in the form of a trophozoite and proliferates with longitudinal binary divisions (20). The organism, slightly larger than a leukocyte, is a motile protozoan with four flagella. Its vigorous mechanical motion is felt to be cytotoxic, and causes a variable amount of vaginal mucosal erythema(50). Signs of contamination vary from a mild and chronic to an acute inflammatory reaction in the urogenital organs (21,22). The disease has a broad spectrum of other clinical signs and symptoms, as well. It may appear as an asymptomatic infection, intensive purulent vaginitis, ulcerative cervicitis and probably even cervical cancer (18,23). Pruritus is a more frequent symptom than in patients with bacterial vaginosis, but less common than in those with yeast vaginitis. Some patients report very little in the way of symptoms. The vulvar and intertriginous groin areas are spared, and the vaginal rugations are typically only mildly inflamed(43).

Such infections typically are asymptomatic in males but may lead to urethritis and prostatitis. This study has been designed to determine the prevalence rates of *C. albicans* and *T. vaginalis* infections in women who were referred to medical centers in Robat Karim, Iran.

Materials and Methods:

This descriptive study was conducted during the summer and autumn seasons. Participants included 500 housewives who were referred to hospitals and clinics, for different reasons, such as routine checkups, urogenital difficulties, family planning, etc. Women who were menstruating, pregnant, postmenopausal, or diabetic or who had an immune deficiency were excluded from the study. Before collection of the specimens, the participants were asked to complete a questionnaire and to undergo speculum examination during which samples were collected from each participant using 5 intravaginal cotton swabs. The first swab was used to determine the pH with a pH indicator, and the second swab was used for Giemsa and lacto phenol cotton blue staining. The third and fourth swabs were used for yeast cultures in Sabouraud dextrose agar and some other mycological media; in addition, a Germ tube test was conducted to detect *C. albicans*, and a culture was done on Dorset medium to detect *T. vaginalis*. The last swab was used for wet mount microscopy and staining by Löffler and carbol–fuchsin stains.

Results:

The results indicated that 150 of the participants (30%) were infected with a *Candida* species. Clamidoconidia production and the Germ tube test identified *C. albicans* in 67 of those 150 participants (13.4%). Also, the tests showed that 83 of the participants (16.6%) were infected with other *Candida* species.

T. vaginalis was detected in 7 out of the 500 participants (1.4%) whose vaginal discharge samples were examined. *T. vaginalis* was identified in 5 wet mount specimens and 7 cultured samples. No association between affliction with these two pathogens and variables such as age, education level, or parity was observed ($p > 0.05$). Most participants infected with these two organisms were between 20-30 years old, had not finished high school, and had less than 3 children. They had visited the clinics for routine checkups and family planning. No association was observed between infection with *T. vaginalis* and any methods of contraception ($p > 0.05$). The association between infection with *C. albicans* and certain contraception methods was significant. Only 1 out of 7 participants infected with *T. vaginalis* was asymptomatic; the other 6 participants showed

symptoms such as vaginal discharge, pruritus and burning sensations, and painful intercourse. 57.1% of participants had green or brown scanty, foamy discharge. A significant association was observed between pH and infection with these organisms. The pH of 62.1% of participants infected with *C. albicans* was 3-4, and the pH of the vaginal discharges of 71.4% of those infected with *T. vaginalis* was 6-7. Also, a significant association was observed between the white blood cell (WBC) counts in each microscopic field with $\times 40$ magnification and infection to these pathogens. Among participants infected with *C. albicans*, 40.3% had a WBC count of 2-10 in each wet mount microscopic field. In 57.1% of those infected with *T. vaginalis*, the WBC count was more than 20 in each wet mount microscopic field.

Discussion:

The initial evaluation of vaginal discharge requires an understanding of physiologic vaginal discharge and what differentiates it from abnormal, pathologic discharge. Substances from the vulvar, sebaceous, sweat, Bartholin's and Skene's glands, as well as exfoliated cells, cervical mucus, and secretions of the endometrial cavity and fallopian tubes constitute the normal physiologic secretions of the vagina. These secretions pool in the posterior fornix and do not adhere to the vaginal walls(41). The pH of normal vaginal secretions in women of childbearing age is between 3.8 and 4.5. The presence of sperm, blood, amniotic fluid, or cervical mucus raises the vaginal pH(41). The amount and fluidity of the discharge can vary over the menstrual cycle. Cervical mucus becomes more fluid around ovulation, and women frequently mistake this change in consistency for an abnormal discharge. Stress increases the rate of vaginal desquamation and thus the amount of discharge, which patients can also mistake for a pathologic discharge(53). Women generally do not have other symptoms if their discharge is physiologic. Candidiasis and trichomoniasis are the two most common infections of the urogenital system in humans. Although these infectious organisms are not fatal, they might lead to serious problems, including abortion, abrasive cervical ulcers, and inflammation of the urogenital system. Factors that contribute to the virulence of candida organisms are not well understood. *Candida albicans* has greater adherence to vaginal epithelial cells than *C. tropicalis*, and this may explain why more clinical infection is seen with this organism. Germinating cells that are more virulent also have greater adherence to epithelial cells(32). Although the symptoms of vaginal yeast infection are not strictly related to the number of organisms, the majority of

women with symptomatic yeast infection have large numbers of yeast present, as well as evidence of germination(33). Estrogens have been shown to enhance the ability of candida to adhere to vaginal epithelial cells, thus promoting infection(34). *Candida* strains have also been shown to have a receptor for female reproductive hormones, which may facilitate yeast mycelial formation and virulence(35,36,37). The world incidence of these diseases varies in different regions (18,19,23). It has been reported that in Iran, the incidence of *C. albicans* is 4-25.5% (24,25,26,27), and that of *T. vaginalis* is 2%-8% (12). In the present study, the incidence of candidiasis (30%) was higher than previously recorded. This may be explained by the severe economic poverty in the region, as well as by malnutrition, which negatively affects immune system functioning. On the other hand, blindly treating trichomoniasis with metronidazole without seeking a laboratory diagnosis might cause candidiasis to spread in the population under investigation (7,20). The incidence of trichomonas infection has been declining for the past 20 years, and currently this accounts for only 10% to 25% of vaginal infections, depending on the population studied(38,39,40). Sexually transmitted disease clinics report a higher incidence of this infection. Risk factors for trichomonas infection include lower socioeconomic status and having a greater number of sexual partners. Cigarette smoking as well as African-American race have also been linked to trichomonas infection, even after adjustment for income and level of education. In addition, women who stop smoking are no more likely to be infected than nonsmokers, suggesting that smoking increases trichomonas colonization through a physiologic effect on the vaginal environment(39). The organism has been found to coexist with a number of other infections, including gonococcal infection and bacterial vaginosis(41,42). The incidence of trichomoniasis was reported in this study as 1.4%, which is less than other reports. This lower rate of infection may be due to cultural propaganda, the promotion of STD awareness, decreased unrestrained sexual behavior, and the decrease in the age at which people get married in this area.

The most sensitive diagnostic modality is a trichomonas culture, which is relatively inexpensive(44,45). Despite the low cost, many laboratories do not culture trichomonas. This requires a special liquid medium (Dorset) that allows growth of the organism so that it can be subsequently visualized on wet mount. Rapid identification of these organisms by Löffler and diluted carbolfuchsin staining yielded variable results. Several other methods are also being evaluated for the

diagnosis of trichomonas infection, including a polymerase chain reaction–based test(46,47), a direct fluorescent antibody test, and an enzyme immunoassay(48). Sensitivities and specificities of these tests, however, are not clearly established(49). Fluorescent antibody tests are commercially available; however, the sensitivity of these tests is low, and use of a fluorescent microscope is needed. In regard to candida, the yeasts and mycelia were clearly stained and sharply identifiable. However, in regard to trichomonas, the results were not satisfactory, i.e., sometimes the flagella, karyosomes, and cytoplasmic organelles were not stained, and the

parasites could not be identified. The association between the infection and factors such as age, education, parity, use of contraception, clinical symptoms, pH, and the vaginal discharge count was studied. The results were comparable to those of previous national and international studies (13,18). Additionally, according to the results of this study, treating obstetric and gynecologic patients empirically, i.e. on the basis of clinical presentation alone, is not recommended. Laboratory confirmation should be sought before treating against these organisms.

Table 1. Frequency and distribution of infection with *Candida albicans* according to the symptoms

Symptoms	Positive cases		Negative cases	
	Number	Percent	Number	Percent
Discharge	55	%82.1	183	%47.3
Pruritus	42	%62.7	109	%29.8
Burning	33	%49.3	109	%28
Painful intercourse	39	%58.2	108	%29.2
Increase in menstrual blood flow	-	-	9	%1.8
Postmenstrual pain and pruritus	4	%6	14	%3.7

Table2. Frequency and distribution of infection with *Trichomonas vaginalis* according to the symptoms

Symptoms	Positive cases		Negative cases	
	Number	Percent	Number	Percent
Discharge	6	%85.7	233	%47.3
Pruritus	4	%57.1	147	%29.8
Burning	4	%57.1	138	%28
Painful intercourse	3	%42.9	144	%29.2
Increase in menstrual blood flow	-	-	9	%1.8
Postmenstrual pain and pruritus	-	-	18	%3.7

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References:

1. Steven L. Chang, MD, Linda D. Shortliffe, MD. Pediatric Urinary Tract Infections *Pediatr Clin N Am* 53 (2006) 379– 400
2. Etienne M, Caron F. Management of fungal urinary tract infections. *Presse Med* 2007 Dec;36(12 Pt 3):1899-906.
3. Svanborg-Edén C, de Man P, Jodal U, Linder H, Lomberg H. Host parasite interaction in urinary tract infection. *1987 Oct*;1(4):623-31.
4. ERIKA N. RINGDAHL, M.D. Treatment of Recurrent Vulvovaginal Candidiasis. *Am Fam Physician*. 2000 Jun 1;61(11):3306-3312
5. Wachtler B, Wilson D, Hube B. *Candida albicans* adhesion to and Invasion and damage of vaginal epithelial cells: Stage-specific inhibition by clotrimazole and bifonazole. *Antimicrobial Agent And Chemotherapy*, Sept. 2011, p. 4436-4439.
6. Mahmoudi Rad M, Zafarghandi ASh, Amel Zabihi M, et al. Identification of *Candida* species associated with vulvovaginal candidiasis by multiplex PCR. *Infectious Disease in Obstetrics and Gynecology*, Vol. 2012, Article ID 872169, 5 pages.
7. Riordan T, Macaulay ME, James JM, Leventhall PA, Morris EM, Neal BR, Rowland J, Evans BM. A prospective study of genital infections in a family- planning clinic: (1) microbiological findings and their association with vaginal symptoms. *Epidemiol Infect* 1990; 104(1):47-53.
8. Paul L, Fidel JR. Host defense against oropharyngeal and vaginal candidiasis: Site-

- specific differences. Rev Iberoam Micol 1999; 16: 8-15.
9. Harriott MM, Lilly EA, Rodriguez TE, et al. *Candida albicans* forms biofilms on vaginal mucosa. Microbiology (2010), 156, 3635-3644.
 10. Jun Z, Lili Z, Ting M, et al. Distribution of *Candida albicans* genotype and *Candida* species is associated with the severity of vulvovaginal candidiasis. J South Med Univ, 2011; 31(10).
 11. Mandell, Douglas, Bennet. Principle and Practice of Infectious Disease. New York: Churchill Livingstone, 1990; Chap. 247, Vol. 2, 2656-2674.
 12. Pellis V, Seta FD, Crovella S, et al. Mannose binding lectin and C3 act as recognition molecules for infectious agents in vagina. Clinical and Experimental Immunology, 2005, 139: 120-126.
 13. Odds FC. *Candida* and Candidosis. A Review and Bibliography. 2nd ed. London: Bailliere Tindall, 1988, Chap 2, p 270.
 14. Matini M, Rezaie S, Mohebbi M, et al. Prevalence of *Trichomonas vaginalis* infection in Hamadan city, western Iran. Iranian J Parasitol: Vol. 7, No. 2, 2012, pp.67-72.
 15. Patil MJ, Nagamoti JM, Metgud SC. Diagnosis of *Trichomonas vaginalis* from vaginal specimens by wet mount microscopy, in Pouch TV culture system and PCR. J Glob Infect Dis. 2012 Jan-Mar; 4(1): 22-25.
 16. Secor WE. *Trichomonas vaginalis*: treatment questions and challenges. Expert Rev. Anti Infect. Ther 10(2), 107-109(2012).
 17. Fiori PL, Rappelli P, Addis MF, Mannu F, Cappuccinelli P " Contact dependent disruption of host cell membrane skeleton induced by *Trichomonas vaginalis* " Infect Immun 1999; 1:149-156.
 18. Gilbert RO, Elia G, Beach DH, Klaessig S, Singh BN " Cytopathogenic effect of *Trichomonas vaginalis* on human vaginal epithelial cells culture in vitro" Infect Immun 1999; 68(7): 4200-4206 .
 19. Oormazdi H. Medical Parasitology. 1st ed. Tehran: Iran University of Medical Sciences Press, 1999, pp 138-148.
 20. Keneths W, Mahmood. Tropical and Geographical Medicine, 2nd ed. New York: McGraw Hill 1989; p 221-223.
 21. Topley WWC, Wilson GS. Microbiology and Microbial Infection. 2nd ed. London: Oxford University Press. 1998; Vol.5, Chap. 11; pp 203- 214.
 22. Cates W. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. STD 1999; 26: S 2-7.
 23. Dan WP, Lin RS. Epidemiologic differences between candidal and trichomonal infections as detected in cytologic smears in Taiwan. Public Health 1995; 109: 443-450.
 24. Abshar N. Comparison of trichomoniasis symptoms with microbial and fungal infections in vaginal environment. Thesis (M.S) No 1762, School of Public Health. Tehran University of Medical Sciences 1996, p105.
 25. Shahabee Gh. Survey in trichomoniasis and its relationship with bacterial and fungal agents in women referred to cytology and microbiology sections of Shahre Kord Health Center. Thesis (M.S) No.1568, School of Public Health. Tehran University of Medical Sciences. 1996, p 120.
 26. Manuchehry Rd MH. Evaluation of fungal and parasitical infections of vagina in South part of Tehran. Thesis (Laboratory Sciences) No.45, Collage of Applied Medical Sciences, Iran University of Medical Sciences 1995, p 101.
 27. Yasaei S. Investigation of *Trichomonas vaginalis* in women referred to Health Centers of Karaj. Thesis (M.S) No.43, Medical Collage, Iran University of Medical Sciences 1999, p 135.
 28. Kwon Chung, K.J. and Bennett, J.E.: Medical Mycology. Lea & Febiger, Philadelphia 1992.
 29. Odds, F.C.: *Candida* and Candidosis. Baillier Tindall, London. 1988.
 30. Richter, S. S., Galask, R. P., Messer, A. S., Hollis, R. J., Diekema D. J., Pfaller, M. A.: J. Clin. Microbiol. 2005, 43: 2155-2162.
 31. Sobel J.D, Faro s., ForceR., Foxman B., Summers P.R, vulvovaginal candidiasis: Epidemiologic, Diagnostic, and therapeutic considerations. (1998) AM Journal of Obstet Gynecol. 178;203-211.
 32. King RD, Lee JC, Morris AL. Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. Infect Immun. 1980;27:667-74.
 33. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol. 1985;152:924-34.
 34. Powell BL, Frey CL, Drutz L. Estrogen receptor in *Candida albicans*: a possible explanation for hormonal influences in vaginal candidiasis. 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy; October 24-26; Las Vegas, Nev. 1983. p. Abstract 751.
 35. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol. 1985;152:924-34.
 36. Kinsman OS. Effect of mammalian steroid hormones and luteinizing hormone on

- germination of *C. albicans*. American Society of Microbiology Conference on *Candida albicans*; May 16, 1987; Palm Springs, Calif. 1987. p. Abstract 27. Presented at the.
37. Br J Obstet Gynaecol. Vol. 92. 1985. The pill does not cause "thrush." pp. 1265–6.
 38. Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol. 1991;165:1168–76.
 39. Dunkelberg WE, Skaggs R, Kellogg DS, et al. Relative incidence of *Cornebacterium* vaginale, *Nesserria* gonorrhoea and *Trichomonas* among women attending a venereal disease clinic. Br J Vener Dis. 1970;46:187–90.
 40. James JA, Thomason JL, Gilbert SM, et al. Is trichomoniasis often associated with bacterial vaginosis in pregnant adolescents? Am J Obstet Gynecol. 1992;166:859–63.
 41. Hill LVH, Embil JA. Vaginitis: current microbiologic and clinical concepts. Can Med Assoc J. 1986;134:321–31.
 42. Weston TE, Nichol CS. Natural history of trichomonal infection in males. Br J Vener Dis. 1963;39:251–3.
 43. Alderete JF, Pearlman E. Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. Br J Vener Dis. 1984;60:99–105.
 44. Fouts A, Kraus S. *Trichomonas vaginalis*: re-evaluation of its clinical presentation and laboratory diagnosis. J Infect Dis. 1980;141:137–43.
 45. Mueller M, Meingassrer JG, Miller W, et al. The metronidazole resistant strains of *T. vaginalis*. Am Obstet Gynecol. 1980;138:808–12.
 46. Sobel JD. Recurrent vulvovaginal candidosis. What we know and what we don't. Ann Intern Med. 1984;101:390–2.
 47. Riley DE, Roberts MC, Takayama T, et al. Development of a polymerase chain reaction–based diagnosis of *Trichomonas vaginalis*. J Clin Microbiol. 1992;30:465–72.
 48. Spence M. The clinical and laboratory diagnosis of *T. vaginalis* infection. Sex Transm Dis. 1980;7:188–95.
 49. McLaren LC, Davis LE, Healy GR, et al. Isolation of *Trichomonas vaginalis* from the respiratory tract of infants with respiratory disease. Pediatrics. 1983;71:888–90.
 50. Underhill RA, Peck J. Causes of therapy failure after treatment of *T. vaginalis*. Br J Clin Pract. 1974;28:134.
 51. Eschenbach DA, Hillier SL. Advances in diagnostic testing for vaginitis and cervicitis. J Reprod Med. 1989;34:555–65.
 52. Hammill HA. *Trichomonas vaginalis*. Obstet Gynecol Clin North Am. 1989;16:531–40.
 53. Friedrich EG. Vaginitis. Am J Obstet Gynecol. 1985;152:247–51.
 54. Magurran AE. Ecological Diversity and Its Measurement. Princeton University Press, Princeton, New Jersey, 1988;179.

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