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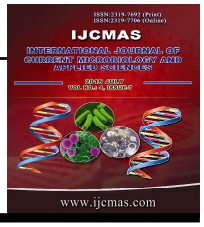

Original Research Article

Frequency and Etiology of Vaginitis in Women Refereed to Health Centers in Yazd city

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Trichomonas vaginalis, Bacterial vaginosis, *Candida albicans*, Yazd, Vaginitis

A B S T R A C T

The vaginal flora is a kinetic ecosystem, although four causes of vaginal discharges are which cover almost 95% of all them. Trichomoniasis with the cause of *Trichomonas vaginalis*, bacterial vaginosis and *Candida albicans* are well identified as sexual transmitted diseases. This study attempted to identify frequency distribution and etiology of vaginitis in women referred to health centers in Yazd, Iran. A cross-sectional study conducted from September 2012 to September 2013 in Yazd city. A total of 360 women were recruited. The collection of material for diagnosis is ideally performed during a comprehensive pelvic analysis using a speculum for diagnosis of bacterial vaginosis; *Candida albicans* and *Trichomonas vaginalis* with identical vaginal pH; smear preparation, staining and culturing. Finally all of 360 women that observed and tested, 120 (33.33%) of them have involved and 240(66.67%) have not involved. From 120 infected women, 55 paitents had bacterial vaginosis (incidence rate: 15.6%), 40 patients had *Candida albicans* (IR: 10.8%) and 25 patients had TV (*Trichomonas vaginalis*) (IR: 5.9%) there was not any coincidence of infection in one patient. Vaginitis symptoms declared by patients will be impressing to early detection and appropriate treatment of these infections, especially in low income community.

Introduction

In the women of reproductive age group, vaginal discharge is the most frequently explained complaint. Vaginal discharge formed a considerable problem for many women causing Discomfort, anxiety affecting women's quality of life and consuming considerable resources. Some vaginal discharges are normal and can vary with age, use of contraceptives, menstrual cycle and with the estrogen level. In our part

of world there is culture of silence, therefore in most of the patients there is delay in seeking help unfortunately. A pathological discharge may be relinquished by somewhere as normal physiological discharge was considered as abnormal by some fastidious women Vaginal discharge accounts for 1% of all consultations in UK, more over one woman in 10 can present with vaginal discharge in the course of a year. The vaginal flora is a kinetic ecosystem that can be easily altered, although there are four causes of vaginal discharges which cover almost 95% of cases. These are bacterial vaginosis, candidal vulvovaginitis, Trichomoniasis and normal physiological discharge. The management of vaginal evacuation is largely syndromic and empirical, it is usually based on nuded eye examination of vaginal discharge and that is unsatisfactory because the diagnostic accuracy is lost without microscopic examination.

Bacterial vaginosis (BV) is the most common type of vaginal infection among women of Reproductive age and accounts for at least one third of all Vulvovaginal infections. BV is not caused by a single pathogen but rather it is a polymicrobial clinical syndrome. Common agents of BV include *Gardnerella vaginalis*, *Mobiluncus*, Bacteroides saprophytes and *Mycobacterium hominis*. Candidiasis is mostly due to *Candida albicans* (CA) and may be with pregnancy, diabetes, and prolong use of antibiotics. Patient presents with vaginal discharge and pruritis. Discharge appears to be like curdled milk and deep erythema of vulva and vagina is often seen. Trichomoniasis is a sexually transmitted disease (STD) that results from infection with flagellated protozoa named as *Trichomonas vaginalis* (TV). The prevalence of Trichomoniasis in American women is 3–5 million. WHO estimates the world wide prevalence of Trichomoniasis to be 170 millions. The discharge is thin copious and pools in the vaginal vault. On vaginal and Vulvar erythema is noted. The strawberry cervix in trichomoniasis resulting from punctuate haemorrhage is usually observed with colposcopy.

Trichomoniasis caused by TV, Candidiasis caused by CA and Bacterial vaginitis caused by BV is the most common sexual transmitted disease (STD). They can conduct to preterm labour and lowbrith weight (LBW). Because of the incidence rate of AIDS epidemic currently is growing, STD needs to more attention for control and diagnosis.There has been growing concern about reproductive tract infections (RTIs) recent year, especially those that are sexually transmitted Vaginitis, whether infectious or not, constitutes one of the most common problems in clinical medicine ,and is one of the main motives for women seeking an obstetrician. Trichomoniasis, Candiasis and Bacterial vaginosis are responsible for 90% of cases of infectious origin (Akbarian *et al.*, 2005; Adad *et al.*, 2010). TV 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 labor has a worldwide distribution, and it has different prevalence in the different points of the world (Hay Wood and Browm, 2002; Shazia A Khan *et al.*, 2009). Trichomoniasis accounts for 15%–20% of all vaginitis, but is probably under diagnosed due to lack of confirmatory testing. Trichomonas is the

most prevalent in women aged 20–45 years. The prevalence ranges from less than 1% to more than 50% in different populations. Infection with the protozoan parasite TV is the most common nonverbal sexually transmitted infection STI, with prevalence estimates frequently surpassing those for gonorrhea and Chlamydia. Infection of the female genital tract can result in vaginitis, cervicitis, and urethritis, and trichomoniasis has been associated with adverse pregnancy outcomes. Though it was once virtually ignored, TV infection in men is now recognized as an important cause of nongonococcal urethritis and is associated with prostatitis and male factor infertility (Ali Fattahi Bafghi *et al.,* 2009; Marcia M. Hobbs *et al.,* 2006). TV is a flagellated protozoan considered to be sexually transmissible and related to low socioeconomic levels. 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 (Sardana *et al.*, 1994; Plourd, 1997).

Candida infections account for 33% of all vaginitis cases. It is the most common in pre- menopausal women. Candidiasis is an infection caused by a yeast-like fungus called CA. It can infect the mouth, vagina, skin, stomach, and urinary tract. About 75% of women will get a vaginal yeast infection during their lifetime, and 90% of all people with HIV/AIDS develop Candida infections.

Oral infections, called oral thrush, are most common in infants, elderly people, and those with a weakened immune system. Symptoms of candidiasis may include: Creamy white patches in the mouth or on the throat (oral thrush), painful cracks at the corners of the mouth, skin rashes, patches, and blisters found most commonly in the groin, between fingers and toes, and under the breasts, vaginal itching and irritation with a white discharge resembling cottage cheese (vaginal yeast infection). Symptomatic CA infection arises when there is an excessive proliferation of this microorganism in the vaginal flora, ceasing colonized starting to achieve outright adherence to the vaginal cells, consequently causing infection. Patient presents thick, fetid vaginal secretions with a granular appearance and an itchy vulva. The vagina becomes hyperemic and the vulva erythematous, and there may be excoriation and Dyspareunia (Taylor *et al.,* 2005).

BV is characterized by the substitution of the vaginal flora, normally dominated by lactobacilli, by a complex and abundant flora of strictly or optionally anaerobic bacteria that are normally found in the vagina (*Gardnerella vaginalis, Bacteroides* sp, *Peptostreptococcus, Mobiluncus* sp). Abundant foul-smelling vaginal secretions are the typical symptom of infection by *Gardnerella vaginalis*.

Recent research suggests that bacterial vaginosis, a common genital tract infection which has been linked in pregnant women to premature labor and low birth weight, may also be associated with HIV risk (Taylor *et al.,* 2005; Sewankambo *et al.,* 1997; Paxton *et al.,* 1998). The objective present cross-sectional study was undertaken to frequency distribution and etiology of vaginitis in women referred to health centers in Yazd, Iran.

Materials and Methods

After providing written informed consent, a cross- sectional comparative and prospective study of genital infections was conducted in health centers university-affiliated teaching in Yazd city, Iran. A total of 360 women were recruited.

Bacterial vaginosis

The collection of material for diagnosis is ideally performed during a comprehensive pelvic examination using a speculum. At the time of speculum examination, an evaluation of the nature of the discharge is made by the clinician, and a specimen from the lateral vaginal wall and posterior fornix can be taken with a sterile swab. The classical BV discharge is thin, homogeneous and grey/yellow in color. However, absence of the classic discharge does not rule out disturbed vaginal flora. For the purposes of laboratory based testing, the swab can be placed in a standard bacterial culture transport medium to maintain moistness or can be smeared onto a slide and air dried for later Gram stain. Transportation for either of these transport systems (acculturates or dried slide) can be at room temperature or 4°C.

This clinical diagnosis requires that three of the following four criteria be met: first, a vaginal pH of greater than pH 4.5; second, the presence of clue cells in the vaginal fluid; third, a milky, homogeneous vaginal discharge; and finally, the release of an amine (fishy) odor after addition of 10% potassium hydroxide to the vaginal fluid. The pH can be determined directly with the use of pH sticks placed on the vaginal wall or with the use of a swab which is touched on pH paper in the range covering pH 4.0 to pH 6.5. The swab is then extracted into 0.2 mL of physiological saline either on a glass slide or in a test tube; a drop of the extract is then placed on a glass slide. A drop of 10% potassium hydroxide is placed on another glass slide. The swab is then stirred in the 10% potassium hydroxide and immediately evaluated for the presence of a fishy odour. Both drops are then covered with a covers lip and examined at 400x magnification with a light microscope. Clue cells are identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured. If three of four criteria are met, then a clinical diagnosis of BV can be made (Krohn *et al.*, 1989).

Candida albicans

A wet-mount smear was examined within 15 minutes of collection for *Trichomonas vaginalis*. A KOH mount was examined for the presence of fungal hyphae. The slide was air dried, Gram stained, and microscopically examined. Diagnosis of bacterial vaginosis was determined according to a morphological scoring system based on the proportions of lactobacilli relative to gram-negative anaerobes. This is a descriptive study and was conducted in department of medical parasitology & mycology, Yazd Shahid Sadoughi University of medical sciences, Yazd, Iran. A total of 100 otherwise healthy women of reproductive age group with the complaint of excessive vaginal discharge were included in the study. We exclude the patients who are unmarried ,women with known skin disease and post menopausal. In addition to a detailed history every patient underwent complete clinical examination and relevant investigations, then the data recorded in proforma. While examining the nature, color and consistency of discharge the pH was also checked. The pH was measured with pH paper held with forceps and dipped into the vaginal discharge, care was taken to avoid contamination with cervical secretion as it falsely change pH. Additionally Whiffor Amine test was performed by mixing vaginal secretion with 10% KOH on the glass slide. The two plain cotton wool sterile vaginal swabs were used for High Vaginal Swab (HVS) for each patient. The swab was rubbed and rotated in post vaginal fornix. One swab was used immediately to prepare a wet mount with one to two drops of normal saline on a glass slide

and was examined by light microscopy for motility of Trichomonas vaginalis. The pus cells , budding yeast cells, pseudohyphal and clue cells were also looked for in the same wet mount. Other swab was immediately sent to the lab for gram's staining and reporting. The swab was inoculated on Sabouraud's agar and incubated at 35 ºC±2 ºC aerobically for 48 hours for the growth of Candida saprophytes. The growth was later examined for yeast cells. Infection with *Trichomonas vaginalis* was identified by characteristic morphology in a wet mount. *Gardnerrella vaginalis* and Group B *Streptococcus* were cultured (Kenji Uno *et al.*, 2007; Jones, 1991).

Trichomonas vaginalis

Diagnosis of TV made whit general appearance and PH monitoring of cervical culture. There are two methods: microscopic examination and culture on KUPPFER-BERG. Diagnosis of candidiasis is made with gram-stain and culture of cervical discharge on saboro it is confirmed with germ tube. Diagnosis of bacterial vaginosis is made with PH moitoring and whiff test at cervical discharge. We also are performed Gram stain and clue cell for diagnosis (Al-Saeed *et al.,* 2011).

Results and Discussion

Finally all of 360 women that observed and tested, 120 (33.33%) of them have involved and 240(66.67%) have not involved. from 120 infected women, 55 patients had BV (15.6%) 40 patients had CA (10.8%) and 25 patients had TV (5.9%) there was not any coincidence of infection in one patient (Table 1).

The subjects' age ranged from 18-45 years with a mean of 25±5.5. Most of the subjects (180 cases, 50%) belonged to the age group 18-24, followed by 101 cases (28.06%) to 25-34 year and 96 cases (21.94%) to 35–45 year. Regarding literacy, most of the subjects 112, (31.11%) had uneducated degrees, 104 (28.90%) had primary school certificate, 96 cases (26.70%) had diploma degrees, and the least of them, 48 cases (9.43%) were academic degree. The relationship between pathogens with different variables was examined. There was no statistically significant correlation between vaginitis caused by BV, CA, TV and factors such as gender and level of literacy (P>0.05) (Table 2).

Clinically, 240 cases out of 360 subjects (66.67%) lacked any type of clinical symptoms. The rest of the subjects showed clinical demonstration of which 120 cases (33.33%) had vaginal discharge. In 84 subjects (23.3%) had asymptomatic. 36 subjects (10%) symptomatic, 34 cases (9.4%) inflammation, 29 cases (8.06%) gray discharge, 36 cases (10%) irritation and itching, 24 cases (6.7%) unpleasant odor and 17 (4.7%) clue cell.

A positive test result for infection was added to clinical topical symptoms such as inflammation, discharge, itching, fetid smell, and strawberries vulvovaginitis. The relationship between pathogens with different variables was examined. There was no statistically significant correlation between vaginitis caused by BV, CA and TV, (P>0.05) (Table 3).

There was a statistically significant correlation between Bacterial vaginosis and its diagnostic method (P value <0.05) in that 28 cases with pH (50.91%), 17 cases with clue cell (30.91) and 8 cases (18.18) with whiff test the test result was positive (Table 4).

Also, there was a statistically significant correlation between Candida albicans and its diagnostic method (P value <0.05) in that 21 cases with pH (52.5%), 14 cases with clue cell (35%) and 5 cases (12.5) with whiff test the test result was positive (Table 5).

Finally, there was a statistically significant correlation between *Trichomonas vaginalis* and its diagnostic method (P value <0.05) in that 21 cases with pH (52.5%), 14 cases with clue cell (35%) and 5 cases (12.5) with whiff test the test result was positive (Table 6).

Today, Mere microscopic diagnosis should be avoided since inexperienced pathologists readily Mistake white or colorless vaginal discharge for semen and sure methods are more sensitive, Innovative and more specific methods should be used additionally, obstetricians and midwives should instruct their patients in this regard and notify the sexuality transmitted disease pathogens to medical laboratory personnel. Bacterial vaginosis, candidiasis and trichomoniasis are responsible for 90% of the cases of vaginal infections. Although culvovaginal candidiasis is the most common fungal disease in the world, little information is known about the distribution and etiology of candidiasis because microbiology tests are not routinely performed in laboratories (Seddigheh Esmaeilzadeh *et al.*, 2009).

New laboratory tests that that can diagnose trichomoniasis as well as other types of vaginitis with high sensibility and specificity, fast enough to give results immediately and acquirable for third world countries are a demand. In this study with relatively high number of cases with STIs risk factors, the LAT behave as expected diagnosing higher number of trichomoniasis cases than in previous study in a sample of pregnant women that attended obstetrics clinics where candidacies was the most prevalent cause of vaginitis (Octavio Fernandez Limia and Dra María Isela Lantero, 2004). From the declared risk factors and diagnosis was possible to observed a significant odds ratio >6 between trichomoniasis and promiscuous behavior confirming *Trichomonas vaginalis* STI condition. This was not the same for Gardnerella vaginalis where no relation was observed with STI risk factors.

Prevalence of Trichomoniasis was also high in this women's sample followed by Bacterial Vaginosis (BV) and very few cases of candidacies, which were also combined. Considering the presence of risk factors for STIs and no presence of pregnant women in this sample explains the predominance of trichomoniasis over BV and vulvovaginitis. Fast and reliable diagnoses of vaginitis lead to precise and fast treatment. A delay in diagnosing and treating STDs can lead to chronic complications and irreversible squeal. Women and children suffer the main consequences. In women, the most serious consequences are acute and chronic pelvic inflammatory diseases, infertility, ectopic pregnancy, and cervical cancer. Infection during pregnancy may cause abortion, stillbirth, prematurity, low birth weight, congenital syphilis, and *ophthalmic neonatorum* (Barbara *et al.*, 2007). The most frequent symptom was vaginal discharge in women who tested positive for bacterial vaginosis, candidacies and trichomoniasis; however, it was a symptom present in fewer than 50% of the women with positive samples. The other commonly but less detected symptoms in positive cases were colpitis, vulvar pruritus, abdominal pain, bleeding cervix and dyspaurenia, confirming that clinical criteria are not very useful for diagnosis. Vaginitis is one of the most common problems in clinical medicine, and it is the reason cited most often for visits to obstetricians and gynecologists.

Feature of vaginitis*

	Candida	Bacterial vaginosis	Trichomonas
Symptoms	Pruritus White discharge Dyspareunia	Gray discharge Odor	purulent discharge Odor Dyspureunia
Vaginal pH	4.0- 4.5	>4.5	4.0- 6.0
Sign	Vulvar erythema	Discharge	Vulvovaginal erythema
Wet mount	Psudohyphae WBCs	Clue cells	**WBSs Trichomonas
Amme test	Negative	Positive	Variable

* May have Mixed Infection **WBC=White Blood Cells

Table.1 Results of testing for vaginitis

Prevalence Status of Infection		N	%
Vaginitis	Bacterial vaginosis	55	**15.6**
	Candida albicans	40	**10.8**
	Trichomonas vaginalis	25	**5.9**
	Total	120	**33.33**
	Non- Vaginitis	240	**66.67**
	Total	360	**100**

Table.2 Characteristics study of women on Vaginitis

Characteristic	Number	percent
Age range (yr)		
18-24	180	**50**
25-34	101	**28.06**
35-45	69	**21.94**
Total		**100**
Literacy	**Number**	**percent**
Uneducated	112	**31.11**
Primary-High School	104	**28.90**
Diploma	96	**26.70**
Academic	48	**13.29**
Total		**100**

Table.3 Clinical features of bacterial vaginosis

Clinical observation		Number	Percent
1-Negative:	240		**66.67**
2-Positive:	120		33.33
Asymptomatic	084		23.30
Symptomatic	036		10.00
Inflammation	034		09.40
Gray discharge	029		08.06
Irritation & itching	036		10.00
Unpleasant odor	024		06.70
Clue cell	017		04.70

Table.4 Results of testing for bacterial vaginosis

Positive Specimens	Direct methods >4.5	Clue cell	Whiff test
28	+	+	+
17	-	+	+
8	-	-	+
Positive *			

* Of 55 positive tested specimens, 28 testes were positive by all methods.

Table.5 Results of testing for Candida albicans

Positive Specimens	Direct Methods Wet mount		Culture Method Giemsa staining
21	+	+	+
14	-	+	+
05	-	-	+
Positive*			

* Of 40 positive tested specimens, 21 testes were positive by all methods.

Table.6 Results of testing on *Trichomonas vaginalis*

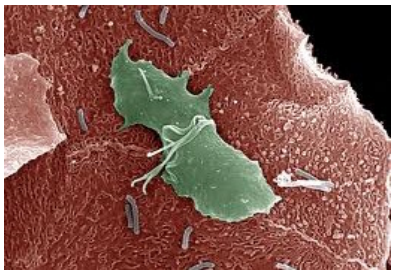
Positive Specimens	Direct Methods Wet mount		Culture Method Giemsa staining
15	+	+	+
07	-	+	+
03	-	-	-
Positive*			

* Of 25 positive tested specimens, 15 testes were positive by all methods.

a. Bacterial vaginosis

b. Candida albicans

c. *Trichomonas vaginalis*



Most women will have a vaginal infection, characterized by discharge, itching, or odor, during their lifetime. With the availability of complementary and alternative therapies and over-the-counter medications for Candidiasis, many simple microscopy of a wet mount of vaginal fluid allows differentiation between at least two types of abnormal bacterial vaginal flora: anaerobic bacteria vaginosis and aerobic vaginitis. There may be a degree of overlap, indicating that aerobic vaginitis and bacterial vaginosis can coexist, but whether one condition can evolve into the other has not yet been determined.

The clinical features associated with, and the host response to aerobic vaginitis, are so specific for the condition and differ so clearly from those for bacterial vaginosis, that we are convinced that aerobic vaginitis should be seen as a separate disease entity. Its pathogenesis, by the production of immense amounts of vaginal pro-inflammatory cytokines, makes it an ideal candidate for causing or promoting preterm labor, chorioamnionitis and preterm rupture of the membranes. Further studies to differentiate the effects of bacterial vaginosis and aerobic vaginitis on the outcome of pregnancy are therefore urgently needed, as they may hold part of the answer to the question why some studies have found no association between bacterial vaginosis or its treatment and pregnancy outcome, while others have found that restoring the flora to normal prevented preterm birth. Symptomatic women seek these products before or in addition to an evaluation by a medical provider.

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Conflicts of Interest

There are no Conflicts of interest.

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