

Liquid Based Pap Smears for Detection of Infective Organisms in a Tertiary Care Hospital

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Abstract

Background: Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonal vaginitis (TV) are the most common etiologies of infective vaginitis. The Papanicolaou (Pap) smear is a standard screening test for cervical precancerous lesions that can also be useful to identify infective organisms. This study aimed to identify the causative organisms for cervico-vaginal infection in liquid-based Pap smears.

Methods: This study was a cross sectional study and included 370 liquid based Pap smears with infective organisms.

Results: The most prevalent cervico-vaginal infection was BV, which was detected in 259 (70%) of the cases, followed by VVC in 64 (17.3%) and TV in 25 (6.8%) cases. Women aged 30-39 years were mostly affected.

Conclusion: The present study shows the usefulness of cervical Pap smear in diagnosis of infective vaginitis other than epithelial cell abnormalities.

Key Words: bacterial vaginosis; cervico-vaginal infection; infective organisms; liquid-based pap smears; trichomonal vaginitis; vulvovaginal candidiasis

Abbreviations

Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), trichomonal vaginitis (TV), Papanicolaou (Pap), BV-associated bacterium (BVAB), liquid-based cytology (LBC), the Bethesda System (TBS), Institutional Review Committee of Chitwan Medical College and Teaching Hospital (CMCIRC), statistical package for the social sciences (SPSS), Windows Microsoft Windows (MS-Windows)

Introduction

Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonal vaginitis (TV) are the most common etiologies of infective vaginitis, accounting for about 90% of cases [1]. A variety of possible microbial infections have been implicated in the disease process of BV, both individually and in combination. Gardnerella, Atopobium, Prevotella, Peptostreptococcus, Mobiluncus, Sneathia, Leptotrichia, Mycoplasma, and BV-associated bacterium 1 (BVAB1) to BVAB3 are among the probable agents [2].

TV is caused by the flagellate protozoan parasite *Trichomonas vaginalis*, which is sexually transmitted disease [3]. BV and TV frequently occur concurrently [4]. TV helps to create an anaerobic environment that favors the development of BV [5]. VVC is caused by the overgrowth of *Candida albicans* that is part of the vaginal flora [6]. VCC is thought to affect 75% of women at some point during their lives [7]. Other causes of infective vaginitis are *Actinomyces*, *Chlamydia trachomatis*, Herpes Simplex Virus, *Leptothrix vaginalis*, Cytomegalovirus, *Enterobius vermicularis*, and *Schistosoma* [8].

The Papanicolaou (Pap) smear is a standard screening test for cervical precancerous lesions. It is, nevertheless, useful for identifying infectious organisms [9]. Inflammatory Pap smear is the common diagnosis reported as negative for intraepithelial lesion and mostly under-investigated because of non-specific inflammation. Thus, the proper guidelines to work up and follow up of the patients with inflammatory Pap smears are lacking. Pathologists should intensively look for infectious organisms in inflammatory Pap smears.

The aim of the present study was to identify the causative organisms for cervico-vaginal infections in liquid-based Pap smears.

Methods

This study was a hospital-based cross-sectional study conducted in Department of Pathology, Chitwan Medical College and Teaching Hospital, Chitwan from September 2021 to February 2022 and included 370 symptomatic and asymptomatic women screened with Pap smears. The symptomatic patients had complained of discharge per vaginum, pain abdomen and abnormal bleeding. Pregnant women were excluded. Cervico-vaginal smears were collected for liquid-based cytology technique in Gynecology outpatient department by scraping cells from squamocolumnar junction using Rovex Cervex Cyto brush and the brush was dipped totally into a disposable liquid-based cytology (LBC) vial containing preservative fluid. The LBC vials were sent to Department of Pathology for cytological examination. The processing of samples and staining of smears was undertaken as per the prescribed protocol by BD SurePath™ method (LBC, BD Diagnostics, Becton, Dickinson and Company). The slides were analyzed by two consultant pathologists using an Olympus CX23 microscope and classified according to the Bethesda System (TBS) for Reporting Cervical Cytology 2014. An informed verbal consent was obtained from all the patients included in the study. Ethical approval was taken from the Institutional Review Committee of Chitwan Medical College and Teaching Hospital (CMC-IRC). Data analysis was done by using the statistical package for the social sciences (SPSS) version 20 for Windows Microsoft Windows (MS-Windows) (SPSS Inc., Chicago, Illinois, the United States).

- Diagnostic criteria for various infective organisms found in LBC Pap smear were as follows:
- Bacterial vaginosis- presence of clue cells (squamous cells covered by a layer of coccobacilli obscuring the cell membrane) and conspicuous absence of lactobacilli.
- Vulvovaginal candidiasis- presence of pseudohyphae and/or budding yeast forms.
- Trichomonal vaginitis- presence of pear-shaped, oval, or round cyanophilic organisms; few associated background changes like mature squamous cells with small perinuclear halo and 3-dimensional clusters of neutrophils (polyballs or cannon balls).
- Leptothrix- segmented, lengthy, and filamentous organisms; may be seen in association with *Trichomonas vaginalis*

Results

During the study period, 1117 LBC Pap smears were examined. Infective organisms were found in 370 (33.1%) cases of the 1117 Pap smears examined (Table 1 and 2). The most common infection encountered was BV (Figure 1) in 259 (70%) cases followed by VVC (Figure 2) in 64 (17.3%) cases and trichomonal vaginitis (Figure 3) in 25 (6.8%) cases (Table 1). Co-infection with mixed organisms (BV and VVC, BV and TV, VVC and TV) was seen in 20 (5.4%) cases (Table 1).

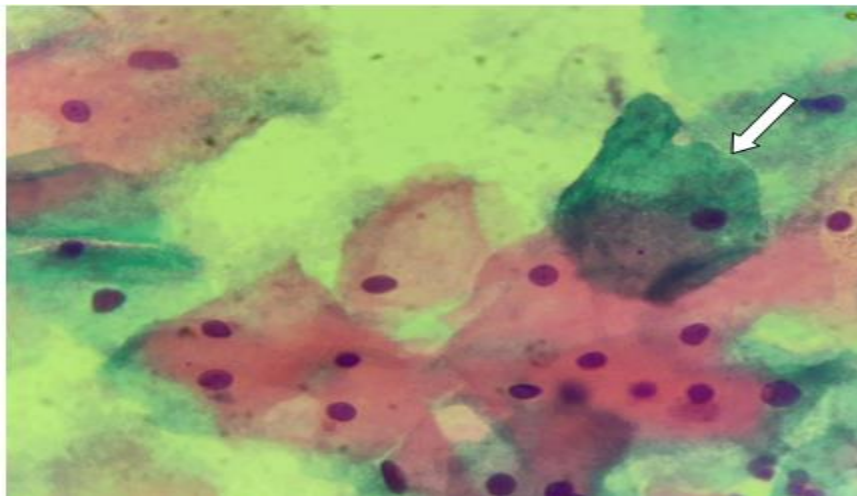


Figure 1: Bacterial Vaginosis with presence of clue cells (arrow) in Pap stain liquid-based cytology 400x..

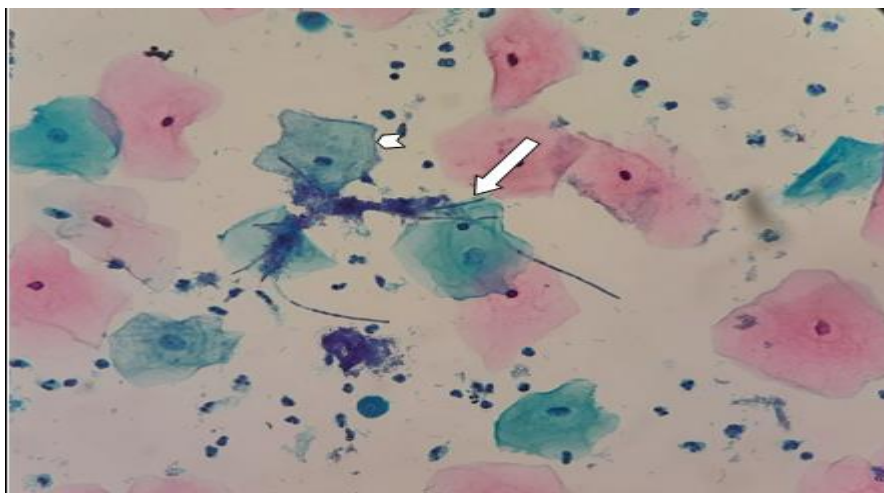


Figure 2: Candida (pseudohyphae- arrow) and Bacterial Vaginosis with presence of clue cells (arrowhead) in Pap stain liquid-based cytology 200x.



Figure 3: *Trichomonas vaginalis* (arrows) with polyballs (arrowhead) in Pap stain liquid-based cytology 200x.

Types of infection	n (%)
Overall prevalence (any type)	370 (33.1)
Single type of infection	
BV*	259 (70.0)
VVC†	64 (17.3)
TV‡	25 (6.8)
Leptothrix	2 (0.5)
Co-infection with mixed organisms	
BV and VVC	9 (2.4)
BV and TV	8 (2.2)
VVC and TV	3 (0.8)

* Bacterial vaginosis, † Vulvovaginal candidiasis, ‡ Trichomonal vaginitis

Table 1: Types of infection in LBC Pap smears

The age of women in the present study ranged from 20 to 67 years. Women aged 30-39 years were commonly affected; comprising 140 (37.8%) cases (Table 2). The lowest frequency was observed in the age group 60 years or above, constituting 13 (3.5%) cases (Table 2).

Age (Years)	BV* n (%)	VVC† n (%)	TV‡ n (%)	Leptothrix n (%)	BV+VVC n (%)	BV+TV n (%)	VVC+TV n (%)	Total n (%)
20-29	44 (11.9)	10 (2.7)	2 (0.5)	1 (0.3)	4 (1.1)	1 (0.3)	0 (0)	62 (16.8)
30-39	99 (26.8)	24 (6.5)	7 (1.9)	1 (0.3)	2 (0.5)	5 (1.4)	2 (0.5)	140 (37.8)
40-49	73 (19.7)	19 (5.1)	8 (2.2)	0 (0)	2 (0.5)	2 (0.5)	0 (0)	104 (28.1)
50-59	36 (9.7)	9 (2.4)	5 (1.4)	0 (0)	1 (0.3)	0 (0)	0 (0)	51 (13.8)
≥60	7 (1.9)	2 (0.5)	3 (0.8)	0 (0)	0 (0)	0 (0)	1 (0.3)	13 (3.5)

*Bacterial vaginosis, † Vulvovaginal candidiasis, ‡ Trichomonal vaginitis

Table 2: Age distribution of patients with infection in LBC Pap smears

Discussion

In sexually active women, vaginitis is one of the most frequent reproductive tract infections [10, 11]. Patients present clinically with abnormal vaginal discharge and irritation of vulva, vagina, or both [10, 12]. In Nepal, one in every three women requires gynecological evaluation due to abnormal vaginal discharge [11]. In the current study, 33.1% of women had infective vaginitis either due single or mixed organisms. This finding is comparable to the reports from Iran and Africa [13, 14]. However, the prevalence of infective vaginitis was extremely low among women in other study of Nepal [15]. Diverse study populations, varying climate, socioeconomic, and cultural situations, different sensitivity of diagnostic tests, and the use of different diagnostic methods all contribute to these variations.

Bacterial vaginosis was the most common type of infective vaginitis in the present study which is comparable to other reports [16-19]. When compared to prior other Nepalese research [16-19], we found a high prevalence of BV (70 %). Several microbiological techniques are compared with Pap smear for diagnosing BV. Gram stain is used for microbiological examination to compare diagnostic accuracy to Pap stain. According to Tokyol et al. [20], Pap smear is not sensitive enough for BV screening when compared to microbiological results, but due of its high specificity, it may be an adequate diagnostic criteria when it is positive.

VVC was the second most common infection, with 17.3% of women infected, which is lower than other Nepalese studies [17, 18]. Takei et al. [21] stated that the liquid-based Pap smear was more sensitive and specific for the identification of candida than other organisms (bacteria and *Trichomonas vaginalis*) due to the large size of candida in contrast to

other organisms. The larger organism may not be removed during the liquid preparation process, resulting in an increase in the number of organisms on the smear.

Trichomonas vaginitis was diagnosed in 6.8% of women in the present study. However, the other study from Nepal detected no cases of TV [22]. The most common clinical feature in infected women is symptomatic vaginitis [22]. De Glehn et al. [23] discovered that TV infection was linked to the number of lifetime sex partners. The risk of transmission of HIV increases by 2-fold in the presence of TV, which is assumed to be due to local inflammation and disruption of the cervical epithelium.³ In the study performed by Aslan et al. [24], liquid-based Pap smears were found to be considerably more sensitive and specific than conventional Pap smears in facilitating the identification of TV. The authors discovered that morphologic diagnosis of TV on LBC was extremely accurate, implying that patients may not need confirmation tests. Lara-Torre and Pinkerton observed that LBC smears have a sensitivity of 61.4 % and a specificity of 99.4 % for TV, and so the diagnosis of TV on the liquid-based preparations is more accurate and dependable, helping patients to be treated without further testing [25].

Leptothrix was discovered in 0.5 % of women in the present study. These segmented, lengthy, and filamentous organisms have a blue hue on Pap smears. The presence of Leptothrix is frequently related with T. vaginalis [26]. A very few articles about this organism have been published so far. Furthermore, none of the papers published have evaluated the Pap smear's sensitivity or specificity for this organism.

In the current study, 5.4% of women had co-infection with mixed microbes. The most common mixed infection in our study was BV and VVC co-infection, which was detected in 2.4 % of cases. Similar result was obtained in other research of Nepal conducted by Bhargava et al. [18] Since two or more of these infections may coexist, the signs and symptoms may not necessarily be typical. There are conflicting findings that Candida albicans and BV have an inverse connection. The environment of BV is assumed to be unsuitable for blastospore proliferation, hence fungal infection does not develop in the presence of BV [27]. According to Souza et al. [28], the most prevalent bacterial flora associated with Candida spp. was lactobacilli (73.63%), followed by mixed flora (25.40 %), and rarely BV (0.73 %). However, other researchers have shown that women with VVC have concurrent BV infection similar to our findings [29, 30], indicating the assumption that Candida spp. and BV do not coexist or that Candida spp. grows only in a lactobacillary dominated flora with normal pH is untrue. Furthermore, Wei et al. [30] discovered that BV can coexist with blastospores (14.2%), compared to pseudohyphae in 35.4%, suggesting that pseudohyphae could cause a considerable alteration in bacterial flora.

In a study by Pathak et al. [16], co-infection with BV and TV was discovered in only 2.8% of cases, which is similar to our findings (2.2%). According to a study published by Gatski et al. [31], BV and TV are the most prevalent infections diagnosed in HIV-positive women. Demirezen et al. and Heller et al. investigated the relationship of TV and BV on Pap smears [5, 32]. In their studies, 44.4% and 46.5% of TV-positive cases had BV, respectively. It has been proposed that TV elevates vaginal pH by lactobacilli phagocytosis [33], and this is the mechanism that helps BV to develop in the presence of TV. Similarly, Moodley et al. [27] have postulated that TV affects vaginal flora, rendering women at risk for BV. This suggests to an increased BV frequency in the presence of TV.

In our analysis, vaginitis was most common in women aged 30-39 years (37.8%), with the highest number of patients having either single infection with BV and VVC or mixed infections. However, we observed TV most commonly in the women aged 40 to 49 years. In contradiction to our findings, Kumar et al. [9] discovered that the highest number of co-infection was found in the age group 41-50 years, whereas the maximum

number of TV infection was identified in the age group 31-40 years. According to Bhargava et al. [18], women of reproductive age (20-29 years) had the highest number of vaginitis. The higher frequency of vaginitis in the 20-29 age range may be due to their high sexual exposure and, presumably, a higher tendency to change sexual partners in this age group.

Conclusions:

Bacterial vaginosis is the most common infective vaginitis, followed by vulvovaginal candidiasis and trichomonal vaginitis. The women aged 30-39 years are commonly affected. The present study shows the usefulness of cervical Pap smear in diagnosis of infective vaginitis other than epithelial cell abnormalities. The smears which are negative for intraepithelial lesion/malignancy with dense inflammation should be extensively evaluated to rule out infectious etiology as most of the time the infective organisms are obscured by inflammation. However, this problem has been lessened by liquid-based cytology, which removes background debris, hemorrhage, and significantly reduces inflammatory cells.

There are some limitations to this study. Since this was a hospital-based study, the sample size was relatively small and the participants did not represent a specific community. The time period of this study was also short. In addition, there are only a few past Nepalese research studies on the subject. To determine the true burden of infective vaginitis in Nepal, large-scale, multi-centric, and community-based investigations should be carried out.

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Conflict of Interest: None

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