The Pathogenic Microorganisms in Papanicolaou Vaginal Smears and Correlation with Inflammation

Esmat Barouti; M.D.[1], Farah Farzaneh; M.D.[1], Azadeh Akbari Sene; M.D.[2],
Zohreh Tajik; M.D.[1], Bahar Jafari; M.D.[3]

1 Infertility and Reproductive Health Research Center, Department of Obstetrics and Gynecology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2 Department of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Objective: Non-specific cervicitis or inflammatory changes in a smear report are common which are usually unclear for clinical approaches. To investigate the frequency of inflammation and pathogenic vaginal microorganisms in cervical smears among an Iranian population sample.

Materials and methods: This cross-sectional study was carried out on Pap smear samples of women referred to gynecological clinic of Taleghani Hospital in Tehran, Iran, between October 2008 and March 2009. This study was conducted on 528 conventional Papanicolaou cervical smears. The frequency and severity of inflammation and prevalence of bacterial vaginosis (BV), *Trichomonas vaginalis* (TV), and vaginal candidiasis (VC) was determined in the samples. Also co-infection of the microorganisms in Pap samples was evaluated. percentage, mean±standard deviation of the outcome parameters were calculated. The comparison between data was performed with the Pearson's chi square or Fisher's exact test.

Results: The prevalence of BV, VC, and TV in Pap samples was 17%, 11%, and 0.4% respectively. Overall, the prevalence of these microorganisms in women of reproductive age was higher than menopausal women. There was a significant association between VC and the presence of inflammation in our samples.

Conclusion: Based on our results, inflammation in the Pap smears can suggest an infection of VC and the patients should be considered for proper VC treatment.

Keywords: Pap smear, Trichomonas vaginalis, Bacterial vaginosis, Vaginal candidiasis, Inflammation

Introduction

The possible presence of infection in cervical smear tests is usually reported by the cytologists based on cytological criteria. Therefore, non-specific cervicitis or inflammatory changes in a smear report are common. These findings are usually unclear for clinical approaches and there is not an appropriate guideline for management of such patients. It is not known if these women should be recalled for cultures and treatment or inflammatory change should be considered as minor.

Vaginal discharge is a common problem among women. Changes in balance of normal vaginal flora

Correspondence:
Farah Farzaneh, Infertility and Reproductive Health Research Center, Imam Hossein Hospital, 1617763141, Tehran, Iran.
Email: medicalresco1@yahoo.com

can cause an overgrowth of pathogens that lead to discharge. Although it is a common complain among sexually active women, there are still gaps in our knowledge about it. Bacterial vaginosis (BV), vaginal candidiasis (VC) and trichomoniasis are the three infections most commonly associated with vaginal discharge (1). BV is the most common cause of vaginal discharge (2). A number of studies have indicated the association of BV with postoperative complications after gynecologic surgery as well as pregnancy complications (2- 5). *Trichomonas vaginalis* (TV) is also another common cause of vaginal discharge, but some studies have not found its association with pregnancy complications (3) or some other indicated that TV during pregnancy was a predisposing factor for preterm delivery and delivering low-birth weight infants (6).

The Pap smear test for detection of BV has showed a sensitivity of 88.2%, a specificity of 98.6%, and a positive predictive value (PPV) of 96.8% (7). The sensitivity, specificity, and PPV of Pap test for TV diagnosis has been reported 98%, 96%, and 88%, respectively (8). Also, some recent studies have demonstrated the co-infection of vaginal pathogenic organisms in Pap tests (2, 9, 10).

This study aimed to investigate the prevalence of pathogenic vaginal microorganisms and the presence of inflammation in Pap smear among an Iranian women sample and to evaluate the possible co-infection of these organisms.

Materials and methods

This cross-sectional study was carried out on Pap smear samples of women referred to gynecological clinic of Taleghani Hospital in Tehran, Iran, between October 2008 and March 2009. Exclusion criteria were pregnancy, smoking, use of oral contraception and/or corticosteroids, regular use of vaginal douche, and women with chronic systemic diseases or systemic immunosuppression. Ethical clearance was obtained from the Ethical Committee of Shaheed Beheshti Medical University. Prior to data collection, the objectives of the study were explained in community meetings. Informed written consent was obtained from all study participants. All women received their laboratory results in written form, and results were explained during a consultation. Women were recalled to the clinic for treatment if an infection was confirmed. The study protocol was also approved by the Institutional Review Boards of department of Gynecology and Obstetrics.

Data were obtained in privacy using an information form applied by one investigator, followed by gynecological examination and specimen collection all done by one gynecologist. Cervical smear samples, based on the conventional method, were prepared with a spatula and fixed on glass slides and were stained with the Papanicolaou technique. Cytology smears were reviewed twice by two independent cytologists. BV was diagnosed based on identifying *coccobacilli* or clue cells. TV was defined as the presence of trophozoites in Pap smears; VC was diagnosed if fungal hyphae or budding yeasts were present in Pap smears.

The presence of inflammation in the smears was divided into mild, moderate, and severe categories. Mild inflammation was defined as less than 30 inflammatory cells/high-power field; moderate inflammation was defined as 30 to 100 inflammatory cells/high-power field; and severe inflammation was defined as more than 100 inflammatory cells/high-power field. The frequency of pathogenic microorganism included BV, TV, and VC were determined in specimens. The frequency of inflammation and infection compared between pre- and post-menopausal women.

Statistical significance was analyzed using Statistical Package for Social Sciences (SPSS software version 17). Percentage, mean±standard deviation of the outcome parameters were calculated. The comparison between data was performed with the Pearson's chi square or Fisher's exact test. The results were considered significant when probability was less than 0.05.

Results

Totally, 528 Pap smear samples were evaluated. The mean age of patients was 45 (ranged 20-77) years. Inflammation was reported in 408 (77.2%) samples. Mild inflammation was noted in 136 (26.1%), moderate in 155 (29.4%) and severe in 117 (22.2%) of them (Table 1).

Among all samples, BV (the presence of clue cells) was the most common pathogenic microorganism which was seen in 91 samples (17.2%), followed by VC (n=56; 10.6%). TV (n=2; 0.4%) was shown to have a low prevalence among our patients (Table 1).

The overall prevalence of pathogenic microorganisms was greater among reproductive age participants, meanwhile the prevalence of BV as well as VC was significantly different (p=0.042 and

p=0.006, respectively) between non- and post-menopausal women.

Table 1: The frequency of pathogenic vaginal microorganisms and inflammation among Pap smear samples

	n (%)
Microorganism	
Bacterial vaginosis	91 (17.2%)
Candida	56 (10.6%)
Trichomonas vaginalis	2 (0.4%)
Inflammation	
Yes	408 (77.2%)
No	126 (23.9%)
Severity of inflammation	
Mild	136 (26.1%)
Moderate	155 (29.4%)
Severe	117 (22.2%)

Inflammation was detected in 76% (n=402) of Pap smears, in which 80.1% (n=322) were reported among reproductive age women. However, the severity of inflammation was not significantly different between non- and post- menopausal women.

An association between microorganisms and inflammation reported in Pap smears was also investigated. Overall, the presence of inflammation was significantly associated with VC (p=0.002), but its severity was not correlated to the infection. Although, there was no significant relation between the presence of inflammation and BV, the severity of inflammation in specimen was significantly increased in association with BV (p<0.001).

The concurrent existence of pathogenic organisms in Pap samples was also assessed. There was no significant association between BV and TV or VC. Likewise, there was no association between TV and VC.

Discussion

In our study, the prevalence of BV among Pap smear specimens of women living in Tehran was 17% which is similar to Azargoon's study (16%) on a population in Semnan (another city in center of Iran) (3), but it had a higher rate in Hamadan (a western city of Iran) and has been reported 28.5% (11). In comparison with other Asian countries, in India, the prevalence of BV among sexually active women was 19% (12). It is estimated that about 10 to 20% of U.S. white women have BV (2). But it was 38% in an African study in Botswana on 703 pregnant women (13). Some predisposing factors for BV include having

sexual relationship with more than one partner, early onset of sexual activity, orogenital sexual contacts, coitus during menstruation, use of IUD, and smoking (2, 14). Differences in sexual behavior and risk factors in different societies can justify the differences in BV prevalence of mentioned studies. An Indian study by Madhivanan et al (12). on reproductive age women indicated that the prevalence of BV in Muslim women was lower than non-Muslims.

Although the prevalence of TV in Pap samples of our study was much less than several studies, similar reports demonstrated by Depuydt et al in Flanders detected by real-time PCR (0.37%) (15). However, this rate was 18% in the study in Hamedan (11), 8.5% among Indian women (16), and 19% in the African study (13). A probable reason for these variations could be differences in Pap sampling quality and cytologists' skill.

Several studies show an increased vaginal pH (≥5) in both BV and TV infection (3, 5, 17). Demirezen et al in Turkey on 600 Pap tests detected TV in 6% of women and BV in 44% of TV cases. They also found a significant relationship between TV and BV infection, which might be due to this hypothesis that TV by phagocytosis the vaginal lactobacilli increases the vaginal pH and generates an anaerobic environment, providing an appropriate condition for the growth of anaerobic microorganisms (18, 19). Also, it was confirmed by Heller's study on 400 Pap samples which concluded that BV diagnosis should be considered at the presence of TV in Pap smears (2). In our study, we found no significant association between BV and TV in conventional Pap smear sampling, which might be because of low prevalence of TV in our samples. The effect of habits and behaviors on vaginal flora among different population can be evaluated in further assessments.

In our study, only the presence of *Candida* in Pap smear samples was associated with the report of inflammation. Therefore, reporting inflammation in Pap smear test might be considered for candidiasis evaluation and treatment. Several studies investigated the association between infection and existence of inflammation in cervical smears (20- 23). Some of them found that inflammation on Pap smear had a relatively low predictive value for the presence of infection (21). They stated that the inflammation was not exactly a consequence of infection and other causes might be responsible for this appearance (20). In contrast, others indicated a significant association between inflammatory smear and reproductive tract

infections (22, 24). As Burke et al (22) in their study on 256 women found an evidence of inflammation in 9.7% of smears and genital tract infection in 29.2% of their participants overall. They found infection in 48% of women having inflammatory changes on smear test vs. 27.3% of women whose smear tests showed no evidence of inflammation. They discovered that the prevalence of infection with *Chlamydia trachomatis*, *Candida*, *Bacteroides* and *Gardnerella vaginalis* was higher in the inflammatory smear group. They concluded that women with an inflammatory smear were more likely to mask the infection than women whose smear shows no evidence of inflammation (22).

Conclusion

Prevalence of BV infection (17%) in this study was similar to most other studies, which was not true for TV (<1%). The use of other diagnostic methods may be probably more valuable than conventional Pap smear for evaluation of TV among Iranian women and it needs further investigations. As the report of inflammation in our samples was very high, use the newer Pap smear techniques such as ThinPrep® (liquid base preparation) in the further studies is recommended. Based on our results, inflammation reported in the Pap smears demands the evaluation for VC and its proper treatment.

Acknowledgement

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