

Clinical importance of detection of bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces in Papanicolaou smears

N. Güdücü¹, G. Gönenç¹, H. İşçi¹, A. Başgül Yiğiter¹, N. Başsüllü², İ. Dünder¹

¹Department of Obstetrics and Gynecology, ²Department of Pathology, Istanbul Bilim University, Avrupa Hospital, Istanbul (Turkey)

Summary

Objective: The aim of this study was to determine the role of Papanicolaou (pap) smears in the diagnosis of lower genital tract infections. **Materials and Methods:** A retrospective study was planned by reviewing charts of patients for trichomonas vaginalis, bacterial vaginosis, actinomyces, candida and nonspecific vaginitis. **Results:** Charts of 9,080 patients were reviewed and 1,733 women had a diagnosis of lower genital tract infection in the pap smear or had had a clinically treated lower genital tract infection. Only 33.5%, 30.4%, 43.3%, and 0% of patients with bacterial vaginosis, trichomonas vaginalis, candida and actinomyces, respectively on pap smear were diagnosed and treated clinically. Postmenopausal patients had a higher rate of trichomonas vaginalis infection and a lower rate of candida infection when compared to women of the reproductive age group. Patients using an intrauterine device for contraception had a statistically significantly increased rate of trichomonas vaginalis and candida infection when compared to women using other contraceptive methods or those who were not using any contraception. **Conclusions:** Finding trichomonas vaginalis, bacterial vaginosis and actinomyces infections in pap smears might be considered an indication for treatment without performing other diagnostic tests. Treatment of asymptomatic infections can prevent complications in selected patients. Candida can be a commensal bacteria in the vagina, therefore asymptomatic patients may not require treatment. Detection of a higher rate of trichomonas vaginalis and candida infection in IUD users shows that IUDs can increase the risk of vaginal infections and associated complications.

Key words: Bacterial vaginosis; Trichomonas vaginalis; Candida albicans; Actinomyces; Pap smear.

Introduction

The Papanicolaou (pap) smear is a simple screening test used routinely for detection of cervical cancer and precancerous lesions of the cervix. Although the main aim of the pathologist is to focus on the detection of cervical intraepithelial neoplasia, it is common practice to report genital tract infections as trichomonas vaginalis, bacterial vaginosis, candida albicans, actinomyces and nonspecific infections within the pap smear results whenever detected. The Bethesda System also supports making a diagnosis of microorganisms. It seems logical to use a test performed periodically for cervical cancer screening to detect asymptomatic genital infections or to confirm a clinically diagnosed infection. Trichomonas vaginalis is a sexually transmitted infection [1] and bacterial vaginosis increases the risk of obstetric and gynecological morbidity including pelvic inflammatory disease, postoperative infections, cervicitis, endometritis, chorioamnionitis, preterm birth [2], HIV acquisition [3] and cervical intraepithelial neoplasia [4].

There is no national cancer screening program in Turkey, but in our gynecology clinic smear tests are performed periodically. The objective of this study was to determine the role of pap smears in the diagnosis of lower genital tract infections.

Materials and Methods

This is a retrospective study carried out by searching charts of patients who attended the gynecology clinic at Avrupa Hospital of Bilim University between 2002 and 2010. Every woman attending our clinic receives a yearly cervical smear. Samples were collected by a cytobrush by rotating the brush 360°. The collected material was spread on a glass slide and fixed with spray. The samples were stained by the conventional Papanicolaou technique. All smears were evaluated by pathologists (not by cytotechnicians) according to Bethesda system criteria. Exclusion criteria consisted of the following: pregnancy, presence of vaginal bleeding, use of vaginally applied medications three days prior to the sample collection and sexual intercourse or vaginal douching within 24 hours of the sample collection. Cases with nonspecific vaginal infections, bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces on pap smear reports were identified. Also patients that had been treated for vaginitis but showed no signs of vaginal infection on the pap smear report were recorded. The study protocol was within the ethical guidelines of the Declaration of Helsinki.

Results

Results of 9,080 women were suitable for analysis. Of these, 1,733 women had a clinically diagnosed lower genital tract infection or had a normal clinical examination but had had a microorganism reported within the pap smear results. Mean age at the time of smear test was 37.9 ± 11.4 (range 17-79). Mean gravida was 2.17 ± 2.32 (range 0-16), mean abortus was 0.11 ± 0.41 (range 0-4), and mean termination of pregnancy on demand was 1 ± 1.6 (range 0-14) (Table 1). Of the patients included in this

Revised manuscript accepted for publication August 22, 2011

study (1,566/1,733), 90.4% were in the reproductive age group. A lower genital tract infection was diagnosed in 13.6% of the women in pap smears. In this study 11.4% (197/1733), 3.3% (56/1733), 8.1% (141/1733), 0.1% (2/1733) and 48.5% (840/1733) of pap smears were positive for bacterial vaginosis, trichomonas vaginalis, candidiasis, actinomycosis and nonspecific vaginitis, respectively. Infections detected in pap smears were clinically diagnosed and treated in only 33.5%, 30.4% and 43.3% of bacterial vaginosis, trichomonas vaginalis and candida infections, respectively. When patients were stratified by age, detection rate of trichomonas in pap smears after 40 years of age increased statistically significantly (Table 2). Stratification of patients into age groups statistically significantly increased the detection rate of trichomonas infection in pap smears in patients older than 40 years, but this did not increase the clinical diagnosis and appropriate treatment rates. In contrast patients younger than 40 years received appropriate treatment for bacterial vaginosis and candida infection before the detection of infection in pap smears when compared to patients older than 40 years, $p = 0.001$ and $p = 0.019$, respectively (Table 2). Patients older than 50 years had an increased rate of diagnosis of nonspecific infection in pap smear (Table 2).

When the menopausal status of patients were taken into consideration the rate of trichomonas vaginalis infection in pap smears was significantly less common in the reproductive age group when compared to the postmenopausal group, 45/1,566 (2.9%) and 11/167 (6.6%), respectively, $p < 0.01$ (Table 3). In contrast the rate of candida infection in pap smears was more common in the reproductive age group when compared to the postmenopausal group, 135/1,566 (8.6%) and 6/167 (3.6%) respectively, $p = 0.02$. Similarly nonspecific infection was more common in the reproductive age group when compared to the postmenopausal group, 465/1,566 (29.7%) and 29/167 (17.4%), respectively.

As a contraceptive method 222 patients were using an intrauterine device (IUD) and 12.4%, 5.5%, 11.9%, 0.009% had bacterial vaginosis, trichomonas vaginalis, candida and actinomyces infection detected with a pap smear, respectively (Table 4). In women who were not using a contraceptive method or using contraceptive methods other than an IUD, the infection rates detected with pap smears were lower, and 11.6%, 2.7%, 7.8%, 0% had bacterial vaginosis, trichomonas vaginalis, candida and actinomyces infection, respectively. Patients using an intrauterine device for contraception had statistically significantly more trichomonas and candida infection detected in pap smears when compared to patients using other contraceptive methods or no contraception, 12/222 (5.4%) and 44/1511 (2.9%), $p = 0.05$ for trichomonas and 27/222 (12.2%) and 114/1511 (7.5%), $p = 0.019$ for candidiasis.

Smears showing ASCUS (atypical squamous cells of undetermined significance) and LGSIL (low grade squamous intraepithelial lesion) constituted 1.7% (30/1733) of smears. Similarly 1.7% (29/1733) of this population had genital warts. Patients presenting with high-grade lesions were excluded.

Table 1. — Demographic characteristics of the study population.

Characteristics	Mean \pm SD
Age	37.9 \pm 2.32
No. of pregnancies	2.17 \pm 2.32
No. of deliveries	1.1 \pm 1.13
No. of abortus	0.11 \pm 0.41

Table 2. — Results of pap smears after stratification of patients according to age groups.

Age	Trichomonas vaginalis	p	Bacterial vaginosis	p	Candida	p	Nonspecific infection	p
20-29	1.8%	0.01	44.2%	0.01	8.4%	0.359	46.8%	0.004
30-39	2.6%		50%		7.9%		48.2%	
40-49	4.9%		23.6%		9.9%		41.6%	
50-59	3.8%		14.3%		5%		63.6%	
> 60	5.6%		33.3%		5.6%		61.1%	

Maentel Haenszel Test.

Table 3. — Comparison of patients in the postmenopausal group and reproductive age group.

Pap smear result	Reproductive age group	Postmenopausal group	p value
Bacterial vaginosis (+)	11.6% (181/1566)	9.6% (16/167)	0.44
Trichomonas vaginalis (+)	2.9% (45/1566)	6.6% (11/167)	0.01
Candida (+)	8.6% (135/1566)	3.6% (6/167)	0.02
Non-infectious	29.7 (465/1566)	17.4% (29/167)	0.001

Chi-square test.

Table 4. — Comparison of patients using an intrauterine device (IUD) and other methods.

Pap smear result	IUD (+)	IUD (-)	p value
Bacterial vaginosis (+)	13.1% (29/222)	11.1% (168/1511)	0.394
Trichomonas vaginalis(+)	5.4% (12/222)	2.9% (44/1511)	0.05
Candida (+)	12.2% (27/222)	7.5% (114/1511)	0.019
Non-infectious	23.4% (52/222)	29.3% (442/1511)	0.072

Chi-square test.

Discussion

Bacterial vaginosis is characterized by decreased concentration of lactobacilli and an overgrowth of mixed anaerobic flora [5]. In gynecology clinics the prevalence of bacterial vaginosis ranges from 9-38% [6-8]. Clinically bacterial vaginosis is diagnosed by using Amsel's criteria [9] or by gram-stained vaginal smears [10]. Platz-Christensen reported the sensitivity and specificity of pap smears as 90% and 97%, respectively and showed that pap smears could be used for clinical diagnosis of bacterial vaginosis as accurate as Amsel's criteria [4] and as gram-stain [11]. Later other studies supported the high sensitivity and specificity of pap smears in the detection of bacterial vaginosis [12-15] and other studies showed contradictory results [8]. Tokyol *et al.* reported that the sensitivity of pap smears is not enough to screen for bacterial vaginosis but that it can be used as a diagnostic test when positive because of the high specificity [16]. Considering the results we obtained from this study we suggest that pap smears can be used to detect asymptomatic and clinically missed cases of bacterial vaginosis.

Lamont *et al.* reported that sensitivity and specificity of the smear for the diagnosis of bacterial vaginosis increased from 81% to 87% and 91% to 97%, respectively when smears were evaluated by cytotechnicians and cytopathologists [17]. In Turkey we have no cytotechnicians, so all smears are evaluated by pathologists. This may be the reason for the increased detection of clinically undiagnosed infections in pap smears.

A survey conducted in the United Kingdom revealed that all four of Amsel's criteria are used only by 30% of clinics [18]. This may decrease the detection rate of bacterial vaginosis. On the other hand there are reports that using only two of Amsel's criteria has enough sensitivity and specificity for the detection of bacterial vaginosis [19, 20]. For some clinics it can be time consuming or expensive to use all of Amsel's criteria and gram-stain, or they may not have adequate equipment. In this case it is a money saving procedure to use the routine pap smear for the diagnosis of lower genital tract infections. Half of the cases [21], can be detected and further diagnostic work-up can be planned. Some propose that it can be time consuming to look for signs of vaginal infection in the smear test and that it can direct the cytologist away from the main purpose, diagnosing cervical intraepithelial neoplasia. If the hypothesis proposing cervical intraepithelial neoplasia as a complication of bacterial vaginosis is established then diagnosis and treatment of bacterial vaginosis will gain more importance.

Trichomonas vaginalis is a sexually transmitted protozoal infection [1]. Fifty percent of trichomonas infections are asymptomatic [22]. In this case asymptomatic patients may act as a reservoir and transmit the protozoa to their partners. This may lead to pelvic inflammatory disease, premature rupture of the membranes, preterm delivery and abortion [23]. Wet mount was reported to be less sensitive than culture and pap smear [24]. Burja *et al.* reported that 68% of asymptomatic cases presenting for a gynecological examination were left untreated, in 61% of cases infection was detected only in pap smears and in 28% clinically detected infections were confirmed by pap smears [25]; our results present similar findings. Loo *et al.* reported sensitivity and specificity of pap smears in detection of trichomonas vaginalis as 96% and 98%, respectively [26]. Nearly 70% of cases detected by pap smears are clinically missed, therefore we agree with previous reports that the pap smear is a valuable test in detecting and treating trichomonas vaginalis infections [27, 28].

In previous studies an increased rate of trichomonas vaginalis infection was diagnosed in women using an IUD when compared to women using other contraceptive methods [29, 30]. In this study also an increased rate of trichomonas vaginalis infection was detected in pap smears of IUD users ($p < 0.05$). When the asymptomatic nature of the infection is considered, pap smears can be used to treat trichomoniasis and to decrease complications such as pelvic inflammatory disease, which increases both in IUD users and trichomoniasis.

Spinillo *et al.* performed a study in symptomatic vaginitis patients and found an increased prevalence of

trichomonas vaginitis in postmenopausal women when compared to women of childbearing age, 10.8% and 1.92%, respectively [31]. In our study 6.6% of postmenopausal women and 2.9% of the reproductive age group had trichomoniasis. This may be the result of changes in vaginal cytology in the postmenopausal period that makes the diagnosis of trichomonas vaginalis easier or may be the result of increased parabasal cells in estrogen deficient women that could be diagnosed as trichomonas trophozoites by mistake. Also in postmenopausal patients there is an increased rate of nonspecific infection, which can be.

Seventy-five percent of sexually active women will experience at least one episode of vulvovaginal candidiasis in their lifetime [32]. Sixty-six percent of women with candida detected in pap smears were reported to be asymptomatic [33]. As confirmed with this study, in postmenopausal women candida rates decrease when compared to women of childbearing age. Our results are very similar to the report of Shurbaji *et al.*, where the candida detection rate with pap smears in clinically asymptomatic women was 66% [34]. Audisio *et al.* found the specificity of pap smear for candida infection to be 98.5% [27]. Candida can be a commensal bacteria in the vagina and is not associated with any serious gynecologic or obstetric complications, so detection of candida in pap smears may not require any treatment at all.

Gupta *et al.* reported an increased presence of actinomyces infection in women with IUDs [35]. Although for actinomyces infection presence of an IUD is not a must, in our study population no actinomyces was detected in women without an IUD. This may be the result of increased attention of the cytologist when the presence of an IUD has been reported by the clinician. Actinomyces was positive in 11% of women with IUDs in a previous study [36], but in our study group this was only 0.9%. Although this presents a small group of patients, clinically none of them were recognized.

In conclusion finding trichomonas vaginalis, bacterial vaginosis and actinomyces infections in pap smears might be considered an indication for treatment without performing other diagnostic tests. Treatment of asymptomatic infections can prevent complications associated with these infections. Candida can be a commensal bacteria in the vagina, therefore asymptomatic patients may not require treatment. Detection of a higher rate of trichomonas vaginalis and candida infection in IUD users shows that IUDs can increase the risk of vaginal infections and associated complications.

References

- [1] World Health Organization: "Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates". In: WHO Geneva, 2001, 27.
- [2] O'Brien R.F.: "Bacterial vaginosis: many questions-any answers?". *Curr. Opin. Pediatr.*, 2005, 17, 473.
- [3] Larsson P.G., Bergstrom M., Forsum U., Jacobsson B., Strand A., Wolner-Hanssen P.: "Bacterial vaginosis. Transmission, role in genital tract infection and pregnancy outcome: an enigma". *APMIS*, 2005, 113, 233.

- [4] Platz-Christensen J.J., Larsson P.G., Sundstrom E., Bondeson L.: "Detection of bacterial vaginosis in Papanicolaou smears". *Am. J. Obstet. Gynecol.*, 1989, 160, 132.
- [5] Forsum U., Holst E., Larsson P.G., Vasquez A., Jakobsson T., Mattsby-Baltzer I.: "Bacterial vaginosis-a microbial and immunological enigma". *APMIS*, 2005, 1113, 81.
- [6] Lamont R.F., Morgan D.J., Wilden S.D., Taylor-Robinson D.: "Prevalence of bacterial vaginosis in women attending one of three general practices for routine cervical cytology". *Int. J. STD. AIDS*, 2000, 11, 495.
- [7] Yen S., Shafer M.A., Moncada J., Campbell C.J., Flinn S.D., Boyer C.B.: "Bacterial vaginosis in sexually experienced and non-experienced young women entering the military". *Obstet. Gynecol.*, 2003, 102, 927.
- [8] Davis J.D., Connor E.E., Clark P., Wilkonson E.J., Duff P.: "Correlation between cervical cytologic results and gram stain as diagnostic tests for bacterial vaginosis". *Am. J. Obstet. Gynecol.*, 1997, 177, 532.
- [9] Amsel R., Totten P.A., Spiegel C.A., Chen K., Eschenbach D.A., Holmes K.K.: "Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations". *Am. J. Med.*, 1983, 74, 14.
- [10] Spiegel C.A., Amsel R., Holmes K.K.: "Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid". *J. Clin. Microbiol.*, 1983, 18, 170.
- [11] Platz-Christensen J.J., Larsson P.G., Sundstrom E., Wiqvist N.: "Detection of bacterial vaginosis in wet-mount, Papanicolaou stained vaginal smears and gram stained smears". *Acta Obstet. Gynecol. Scand.*, 1995, 74, 67.
- [12] Giacomini G., Calcinaï A., Moretti D., Cristofani R.: "Accuracy of cervical/vaginal cytology in the diagnosis of bacterial vaginosis". *Sex Transm. Dis.*, 1998, 25, 24.
- [13] Eriksson K., Forsum U., Bjornerem A., Platz-Christensen J.J., Larsson P.G.: "Validation of the use of Pap-stained vaginal smears for diagnosis of bacterial vaginosis". *APMIS*, 2007, 115, 809.
- [14] Discacciati M.G., Simoes J.A., Amaral R.G., Brolazo E., Rabelo-Santos S.H., Westin M.C.A., Morternor E.B.L.: "Presence of 20% or more clue cells: An accurate criterion for the diagnosis of bacterial vaginosis in Papanicolaou cervical smears". *Diagn. Cytopathol.*, 2005, 34, 272.
- [15] Vardar E., Maral I., Inal M., Özgüder Ö., Tasli F., Postaci H.: "Comparison of gram stain and Pap smear procedures in the diagnosis of bacterial vaginosis". *Infect. Dis. Obstet. Gynecol.*, 2002, 10, 203.
- [16] Tokyol Ç., Aktepe O.C., Cevrioglu A.S., Altundi M., Dilek F.H.: "Bacterial vaginosis: comparison of Pap smear and microbiological test results". *Mod. Pathol.*, 2004, 17, 857.
- [17] Lamont R.F., Hudson E.A., Hay P.E., Morgan D.J., Modi V., Ison C.A. et al.: "A comparison of the use of Papanicolaou-stained cervical cytological smears with gram-stained vaginal smears for the diagnosis of bacterial vaginosis in early pregnancy". *Int. J. STD AIDS*, 1999, 10, 93.
- [18] Keane F.E., Maw R., Pritchard C., Ison C.A.: "Methods employed by genitourinary medicine clinics in the United Kingdom to diagnose bacterial vaginosis". *Sex Transm. Infect.*, 2005, 81, 155.
- [19] Gutman R.E., Peipert J.F., Weitzen S., Blume J.: "Evaluation of clinical methods for diagnosing bacterial vaginosis". *Obstet. Gynecol.*, 2005, 105, 551.
- [20] Simoes J.A., Discacciati M.G., Brolazo E.M., Portugal P.M., Dini D.V., Dantas M.C.: "Clinical diagnosis of bacterial vaginosis". *Int. J. Gynecol. Obstet.*, 2006, 94, 28.
- [21] Georgijevic A., Cjukic-Ivancevic S., Bujko M.: "Bacterial vaginosis. Epidemiology and risk factors". *Srp Arh Celok Lek*, 2000, 128, 29.
- [22] Spence M.R., Hollander D.H., Smith J., McCaig L., Sewell D., Brockman M.: "The clinical and laboratory diagnosis of Trichomonas vaginalis infection". *Sex Transm. Dis.*, 1980, 7, 168.
- [23] Moodley P., Wilkinson D., Connolly C., Moodley J., Sturm A.W.: "Trichomonas vaginalis is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus". *Clin. Infect. Dis.*, 2002, 34, 519.
- [24] Petersen C.S., Carl L., Alnor D., Thomsen U., Thomsen H.K.: "Ignored trichomonal infestation diagnosed by Papanicolaou smear". *Genitourin Med.*, 1995, 71, 257.
- [25] Burja I.T., Shurbaji M.S.: "Clinical impact of identifying trichomonas vaginalis on cervicovaginal (papanicolaou) smears". *Diagn. Cytopathol.*, 2001, 24, 195.
- [26] Loo S.K.F., Tang W.Y.M., Lo K.K.: "Clinical significance of trichomonas vaginalis detected in Papanicolaou smear: a survey in female Social Hygiene Clinic". *Hong Kong Med. J.*, 2009, 15, 90.
- [27] Audisio T., Pignini T., de Riutort S.V., Schindler L., Ozan M., Tocalli C., Bertolotto P.: "Validity of the Papanicolaou smear in the diagnosis of candida spp. trichomonas vaginalis and bacterial vaginosis". *J. Lower Gen. Tract Dis.*, 2001, 5, 223.
- [28] Lara-Torre E., Pinkerton J.S.: "Accuracy of detection of trichomonas vaginalis organisms on a liquid-based papanicolaou smear". *Am. J. Obstet. Gynecol.*, 2003, 188, 354.
- [29] Sönmez T.G., Keçeli Ö.S., Yücesoy G., Gacar G.: "The relation between trichomoniasis and contraceptive methods". *Türkiye Paraziol Derg.*, 2009, 33, 266.
- [30] Ceruti M., Canestrelli M., Condemi V.: "Methods of contraception and rates of genital infections". *Clin. Epid. Obstet. Gynecol.*, 1994, 119.
- [31] Spinillo A., Bernuzzi A.M., Cevini C., Gulminetti R., Luzi S., De Santolo A.: "The relationship of bacterial vaginosis, candida and trichomonas infection to symptomatic vaginitis in postmenopausal women attending a vaginitis clinic". *Maturitas*, 1997, 27, 253.
- [32] Eckert L.O., Hawes S.E., Stevens C.E., Koutsky L.A., Eschenbach D.A., Holmes K.K.: "Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm". *Obstet. Gynecol.*, 1998, 92, 757.
- [33] Sobel J.D.: "Candidal vulvovaginitis". *Clin. Obstet. Gynecol.*, 1993, 36, 153.
- [34] Shurbaji M.S., Burja I.T., Sawyer W.L.: "Clinical significance of identifying candida on cervicovaginal smears". *Diagn. Cytopathol.*, 1999, 21, 14.
- [35] Gupta P.K., Hollander D.H., Frost J.K.: "Actinomyces in cervicovaginal smears: an association with IUD usage". *Acta Cytol.*, 1976, 20, 295.
- [36] Chatwani A., Amin-Hanjani S.: "Incidence of actinomycosis associated with intrauterine devices". *J. Reprod. Med.*, 1994, 39, 585.

Address reprint requests to:
N. GÜDÜCÜ, M.D.
Istanbul Bilim University
Department of Obstetrics and Gynecology
Kısıklı cad. No:106 Altunizade
34692 Istanbul (Turkey)
e-mail: nilgun.kutay@gmail.com