CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

Candida albicans infection of cervix and comparison of Pap smear and culture in diagnosis

T. Cengiz[1], T. Toka Özer[2], F. Kılınç[3], R. Selimoğlu[4], H. Yılmaz[5]

[1]*Department of Obstetrics and Gynecology, Ortadoğu Hospital, Adana (Turkey)*
[2]*Department of Medical Microbiology, Konya Hospital, Konya (Turkey)*
[3]*Department of Pathology, Medical Faculty, Necmettin Erbakan University, Konya (Turkey)*
[4]*Department of Medical Microbiology, Anıt Hospital, Konya (Turkey)*
[5]*Department of Obstetrics and Gynecology, Şemdinli State Hospital, Hakkari (Turkey)*

Summary

Purpose: The Pap smear is a routine screening test for the detection of cervical abnormalities viral, bacterial, and fungal infections of the uterine cervix, The aim of this study is to investigate if Pap smear is an alternative to cervicovaginal culture in the diagnosis of asymptomatic *Candida. Materials and Methods:* A retrospective analysis of 133 non-pregnant asymptomatic cases were included. *Candida spp.* positive cases in Pap smear and/or culture were compared. *Results: Candida spp.* was found in 45 cases in culture and 40 cases of *Candida* in Pap smear examination. The sensitivity of Pap smear was 88%, specificity was 100%, the positive predictive value was 100%, the negative predictive value was 94%, and the overall power of the test (test validity) was 96%. It was detected that 33.83% of the asymptomatic cases had *Candida* infection. *Conclusion:* Pap smear can be used as a first-line examination method.

Key words: Pap smear; Candida infections; Cervicovaginal culture; Sabourad dextrose agar; Screening test.

Introduction

Papanicolaou (Pap) smear is a screening test for detecting cervical epithelial abnormalities, and there are a number of factors that limit its adequacy. One of the most important of these factors is cervicovaginal infections. Indeed, studies have reported that by examining the relationship between the inflammatory cervical smear and the diagnosis of malignancies, even invasive cervical squamous cell carcinomas cannot be detected due to cancer or pre-cancer changes are masked by infection and underlying infections are treated even certain invasive cervical squamous cell carcinomas can not be detected. [1] One of the frequently encountered cases of these infections is vulvovaginal candidiasis (VVC). As stated in the literature, the main complaint of the majority of women with *C. albicans* infection is pruritus. Burning and irritation may occur less frequently. Usually, a whitish, cheesy vaginal discharge, and the erythema at the vaginal entrance are shown, more rarely generalized vulvar erythema, erosion, and satellite pustules. On the other hand, *non-albicans Candida* vaginitis, burning, and irritation is usually shown although there is no discharge or itching. On physical examination, there is no feature. [2-5]

While 40-75% of sexually active women experience symptomatic vaginal candidiasis [6], 20% of asymptomatic non-pregnant women have them in their vaginal flora and they are harmless. However, in immunosuppressed patients as pregnancy or HIV infection, they can spread to life-threatening cases by developing as opportunistic pathogens [7].

Although the most common cause of fungal infections in immunocompromised patients is *C.albicans, Candida (Torulopsis) glabrata, Candida parapsilosis, Candida krusei, Candida tropicalis, Candida kefyr*, and *Saccharomyces cerevisiae* can also be causative agents [3, 8, 9]. The cause of the more frequent occurrence of *Candida albicans* is that they had a family of genes with genetic codes that secrete aspartic proteinases that are associated with virulence [10-12]. Because of this feature, it is more pathogenic and more successful colonizing than other *Candida* species. During infections, the proteinases digest the host proteins for nutrient supply and evade the host defenses by degrading immunoglobulins and complement proteins. [13] Individual members of the gene family may have their own special role in infection, and this may be reflected in a differential pattern at various stages of the infection process. [1] However, the pathogenicity of *C. albicans* is not solely dependent on dominant virulence factors. There is also a number of immunosuppressive factors such as radioimmunoassay (RIA), anemia, DM, the use of corticosteroids, antibiotics, and chemotherapeutic agents [7, 14].

Published: 15 April 2020

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVII, n. 2, 2020
doi: 10.31083/j.ceog.2020.02.5193

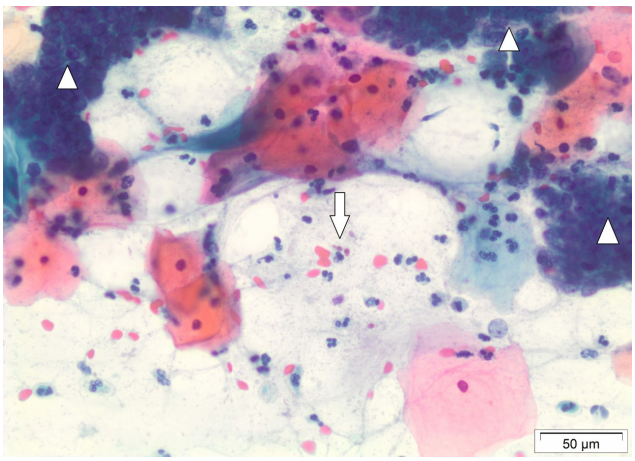

Figure 1. — *Candida* in Pap smear.

Clinically, other factors are found in diagnosis of *Candida* in 50% of cases [15]. Because of this reason, various laboratory methods besides clinical diagnosis are used in the diagnosis of Candidiasis. Indeed, infectious agents have also been reported in Pap smears for the purpose of screening for precancerous and invasive cancers. One of the most common of these factors is *Candida albicans*. The aim of this study was to determine the efficacy of the Pap stain for the detection of *C. albicans* in non-pregnant asymptomatic women.

Materials and Methods

Of the 3,303 cases with Pap smear test in pathology laboratories and 448 cases with cervicovaginal discharge culture in microbiology laboratory were screened retrospectively. The incidence of *Candida* diagnosed cases by both methods was calculated. Of these cases, 133 cases of asymptomatic and non-pregnant who had both Pap smears and culture samples were included in our study. The files of the patients were investigated and their age, method of contraception they used, and marital status were noted. Pap smear preparations previously examined were re-evaluated. The culture results of 133 patients were evaluated and *Candida* diagnosed cases were determined. Both methods were compared.

In the present clinic, Pap smear and after that culture samples were taken, prepared, and evaluated as described below. Individuals who presented with heavy vaginal discharge were excluded from the study because this leads to false positive Pap smear results. The specimens were prepared using the Pap smear method examined by microscopy and subsequently cultured in Sabouraud dextrose agar.

The specimens were collected to obtain cellular samples with swab sticks for culture and an Ayre's spatula for cytology with the support of a speculum. Samples on the Ayre's spatula were transferred immediately to clean dry glass slides and spread on the same slides for conventional cytology. The smears were fixed immediately with 95% alcohol for 15-30 minutes. They were then stained by the Pap method as follows: 50% alcohol for ten minutes, rinsed in distilled water, Harris's Haematoxylin without acetic acid for two minutes, rinsed under weak stream of tap water for one minute and differentiated in 1% acidalcohol for two seconds, and washed in tap water for one minute. Smears were taken to lithium carbonate for two minutes, washed in tap water for 30 seconds, then taken to 95% alcohol two times and stained in OG6 for two minutes, rinsed in 95% alcohol two times, and stained in EA 50 for four minutes. Smears were then taken to two changes of absolute alcohol, xylene until clear at least 15 minutes, and mounted in DPX. The stained smear slides were examined under the light microscopy at low and high magnification for the presence of *Candida albicans*.

Table 1. — *Distribution of Candida detected cases by both methods according to age groups.*

	(Group 1)	(Group 2)	(Group 3)	Total
Age groups (years)	18-39 n (%)	40-59 n (%)	≥60 n (%)	n (%)
Number of cases in groups	67 (50.38)	53 (39.85)	13 (9.77)	133 (100.00)
Pap smear-*Candida* (+)	28 (70)	11 (27.50)	1 (2.5)	40 (30.08)
Culture-*Candida* (+)	31 (68.89)	12 (26.67)	2 (4.44)	45 (33.83)

The vaginal discharge was examined by adding saline or potassium hydroxide for demonstration of budding yeast cells and pseudohyphae under the microscope, diagnosed with pseudohy-phae (mycelium), specimens from the swab sticks and Sabouraud dextrose agar (SDA), and blood agar and incubated at 37 °C for 48 hours and examined for the growth of yeasts. Pasty, creamy, and smooth colonies were considered as yeasts and then further identified by germ tube test and by chlamydospore production on corn meal agar. Gram's staining was used to determine the presence of yeast cells, leucocytes, and bacterial morphotypes.

Descriptive statistics were expressed as a mean ± standard deviation, nominal values as a number (n), and percentage (%). Sensitivity, specificity, positive predictive value, negative predictive value, and overall power of the test (test validity) of Pap smear for *Candida* were calculated as culture gold standard.

Results

Of the 3,303 Pap smears, 455 (13.78%) were *Candida* and 448 (38.5%) *Candida* were detected in the cervicovaginal discharge culture. However, the study group consisted of 133 asymptomatic women simultaneously receiving both Pap smear and current culture. All of the patients were married and between 18 and 76 years of age. 67 (50.38%) of the patients were in the 18-39 age group, 53 (39.85%) were between the ages of 40-59, and ≥ 60 ages were 13 (9.77%). *Candida* was detected in 45/133 (33.83%) cases in the cervicovaginal discharge culture and 40/133 (30.07%) in the Pap smears. *Candida* in Pap smear was shown on the Figure 1. The results of Pap smear and current culture and the distribution were compared according to age groups are shown in Table 1. Thirty-one out of 67 (46.26%) of the patients who were diagnosed as Candidiasis in the cervicovaginal discharge culture were in the 18-39 age group, 12/53 (22.64%) in the 40-59 age group, and 2/13 (15.38%) ≥ 60 age group. Sixteen of the *Candida* (35.6%) was *C. albicans* and 29 of the *Candida* (64.4%)

was *Candida spp*. A small amount of *Candida* in 20 patients, much of *Candida* in 14 patients, and a very excessive amount of *Candida* in five patients were produced in the culture medium. In six of the 45 cases, one to two colonies were reported to be *Candida*, additionally in three of them to be *E. coli*, in one to be *Enterococci spp.*, in one to be *Klebsiella spp*., and in one of them to be *Candida* as the one to two colony in normal vaginal flora.

In the Pap smear, 28/67 (41.79%) were seen in the 18-39 age group, 11/53 (20.75%) in the age group of 40-50, and 1/13 (7.69%) in the ≥ 60 age group. In culture, five of six cases (one to two colony *Candida* with *E. Coli* or *Enterococcus spp.* or *Klebsiella spp.*). *Candida* was not seen but reported as inflammation and as infection-related competence has been limited in Pap smear.

In the reproductive age group of 18-39 years of age, 17 of them were using RIA, five of them were using the oral contraceptive pill (OK), and five of them were using a condom as a contraceptive method. In the 40-50 age group, seven of them were using RIA. Thirty-four (75.55%) patients were using contraceptive method in total: 24/45 (53.33%) RIA and ten (22.22%) OK.

The sensitivity of Pap smear in *Candida* diagnosis was 88%, specificity was 100%, the positive predictive value was 100%, the negative predictive value was 94%, and the overall power of the test (test validity) was 96% as the culture considered as gold standard.

Discussion

The Pap smear is a routine screening test for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix. It also detects certain viral, bacterial, and fungal infections of the cervix and vagina [16]. Cancers of the cervix constitute an important cause of vaginal discharge among non-infectious causes (3.3%) [17]. *C. albicans* is the second most common after bacterial vaginosis in the US and the most common cause of vaginitis in Europe. During life, 75% of women become infected with *C. albicans* and 5% have recurrent episodes. It is difficult to identify uncomplicated, asymptomatic candidiasis, and risk factors [18].

In this study, despite the cases were asymptomatic, *C. albicans* was produced in 35.6% of cases and *Candida spp.* was produced in 64.4% of them. It is known that non-albicans *Candida* vaginitis generally has no discharge but has burning and irritation and there is no finding on physical examination [2-5], 64.4% of the cases have *Candida spp*. seems to be consistent with the literature. According to some studies, using oral contraceptive pills, using diaphragm spermicide or Intrauterine device (IUD), performing oral sex, frequent sexual intercourse or sexual intercourse at a young age are among the risk factors [8, 19-21]. Chassot *et al.* [21] found yeast cells tightly bound on the RIA and showed that copper was holding the yeast cells in place. The reason for this high concentration is that the rope of the RIA can facilitate the uptake of yeast cells in the vagina by playing a bridge between the vagina and the upper genital system. In these cases, recurrences are solved only by removal of RIA. The fact that 34 of 45 patients have *C. albicans* were using RIA, OK supports the view that these methods are risk factors in the literature.

Many different methods are used in the diagnosis of *C. albicans*. Culture, latex agglutination test, wet microscopy and Pap smear are some of the methods. Malkawi *et al.* [22], in Pap smear as 1.2%, Lessa *et al.* [23] as 5.8%, Adad *et al.* [24] as % 22.5 in 20.356 cases, Roeters *et al*. [25] as 9.8%, Kalantari *et al.* [26] (as 6.7 % (in 2248 cases from 33600 cases) detected Candidiasis. However, cervical drainage culture has not been confirmed. Pap smear was diagnosed with candidiasis at a rate of 13.78% (455/3303) but candidiasis diagnosed as 31.25% (140/448) in culture. In these cases, both Pap smear and culture were performed simultaneously in 133 cases. Candidiasis was detected in 33.83% (45/133) in culture and 30.08% (40/133) in the smear. In both methods, the most common group was the 18-39 age group and at least common group was ≥60 age group, which is similar to the literature. [16, 27]

Avwioro *et al.* [16] reported that the rate of the *C. albicans* infections were 7.6% with Pap smear and 30.1% with culture in 1000 women. Pap smears can not identify moderate to mild-severe infections that can be identified in culture. Sabourad dextrose agar culture compared to Pap smear, that number of diagnosed candidiasis is less than cultures. The authors concluded that positive cultures were strongly related to a number of clinical signs and symptoms, but Pap smears were not sensitive for diagnosing symptomatic fungal infection. Thus, the culture is the gold standard, Pap smears should not be used as an alternative for the diagnosis of *C. albicans*. In our results, the rate of *C. albicans* diagnosis is similar in both methods and does not overlap with the view of Avwioro *et al.* [16]. We think that our results are not similar because the number of our patient in the study is smaller than Avwioro *et al.* [16] and our cases are asymptomatic. On the other hand, we agree that the severity of the disease can not be determined and that it is difficult to detect cases with low intensity in the Pap smear. Zdolsek *et al.* [27] surveyed 983 women using RIA. They found *Candida* in wet smear as 13.9%, and in culture as 13.2%. Both methods have equal sensitivity in symptomatic and asymptomatic VVC, so they suggested that both methods can be used in VVC diagnosis, even if wet smear microscopy is the first-line method, and samples should be cultured although clinical complaints and symptoms indicate that VVC if microscopy is negative. Unlike Zdolsek e*t al.* [27], the present results were a more similar wet microscopic examination of the vulvovaginal discharge than the smears prepared for cytology purposes.

Siapco *et al.* compared culture results and cervical smear in 31 cases with suspicious of *Candida* infection [28]. *Can-*

dida albicans in culture in 20 (64.5%) of the 31 cases, *C. paratropicalis* in one (3.2%), *C. albicans*, and *C. glabrata* in two (6.4%). In only two (6.4%) specimens, *C. glabrata* was produced. Twenty (80%) of 25 fungal-positive specimens were fungus-positive cervical smears and five (20%) specimens were fungus-negative cervical smears. They reported that there were no cases of cervical smear-positive but culture negative. They reported that sensitivity to fungi detection in culture-positive patients is 80% and cervical smear may be for the rapid detection of *C.albicans* when blastospores and pseudomycelium are present [28].

In the present study, while candida was positive in cervical smear, there was no case that was candida negative in culture. However, in spite of one to two colonies in culture, no candida was observed in the smear of five cases. Pap smear sensitivity was 88%, specificity was 100%, the positive predictive value was 100%, the negative predictive value was 94%, and the overall power of the test was 96% (test validity) in the statistical analysis. The present results were compatible with the work of Siapco *et al.* [28]

Pap smear *Candida* can be used as a first step examination method which is cheaper and shorter in the diagnosis of *Candida*. However, if symptomatic cases are present, there is no agent on microscopic examination, there is resistance to treatment and/or recurrences, there is *nonalbicans-Candida,* the authors believe that culture should be performed.

References

[1] Kelly B.A., Black S.A.: ''The inflammatory cervical smear: a study in general practice''. *Br. J. Gen. Pract.,* 1990, *40*, 238.

[2] Sobel J.D.: ''Vaginal infections in adult women''. *Sex. Transm. Dis.,* 1990, *74*, 1573.

[3] Edwards L.: ''The diagnosis and treatment of infectious vaginitis''. *Dermatol. Ther.,* 2004, *17*, 10210.

[4] Erdem H., Cetin M., Timuroglu T., Cetin A., Yanar O., Pahsa A.: ''Identification of yeasts in public hospital primary care patients with or without clinical vaginitis''. *Aust. N. Z. J. Obstet. Gynaecol.,* 2003, *43*, 3126.

[5] Dan M., Poch F., Levin D.: ''High rate of vaginal infections caused by *non C. albicans Candida* species among asymptomatic women''. *Med. Mycol.,* 2002, *40*, 3836.

[6] Eckert L.O., Hawes S.E., Stevens C.E., Koutsy L.A., Eschenbach D.A., Holmes K.K.: ''Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm''. *Obstet. Gynecol.*,1998, *92*, 757.

[7] Odds F.C.: ''Candida and Candidosis''. London: Baillière Tindall (W. B. Saunders), 1988.

[8] Sobel J.D., Faro S., Force R.W., Foxman B., Ledger W.J., Nyirjesy P.R. *et al.*: ''Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations''. *Am. J. Obstet. Gynecol.,* 1998, *178*, 203.

[9] Spinillo A., Capuzzo E., Egbe T.O., Baltaro F., Nicola S., Piazzi G.: ''*Torulopsis glabrata* vaginitis''. *Obstet. Gynecol.,* 1995, *85*, 993.

[10] Monod M., Hube B., Hess D., Sanglard D.: ''Differential regulation of SAP8 and SAP9, which encode two new members of the secreted aspartic proteinase family in *Candida albicans*''. *Microbiology.* 1998, *144*, 2731.

[11] Cassone A., De Bernardis F., Mondello F., Ceddia T., Agatensi L.: ''Evidence for a correlation between proteinase secretion and vulvovaginal candidosis''. *J. Infect. Dis.,* 1987, *156*, 777.

[12] Staib P., Kretschmar M, Nichterlein T, Hof H., Morschha J.: ''Differential activation of a *Candida albicans* virulence gene family during infection''. *PNAS,* 2000, *97*, 6102.

[13] Hube B.: ''Possible role of secreted proteinases in *Candida albicans* infection''. *Rev. Iberoam. Micol.,* 1998, *15*, 65.

[14] Calderone R.A.: ''Recognition between *Candida albicans* and host cells''. *Trends. Microbiol*., 1993, *1*, 55.

[15] Papanicolaou G.N.: ''A new procedure for staining vaginal smears''. *Science,* 1942, *95*, 438.

[16] Avwioro O.G., Olabiyi Oe., Avwioro To.: ''Sensitivity of a Papanicolaou smear in the diagnosis of *Candida albicans* infection of the cervix''. *N. Am. J. Med. Sci.,* 2010, *2*, 97.

[17] Sivaranjini R., Jaisankar T., Thappa D.M., Kumari R., Chandrasekhar L., Malathi M., *et al.*: ''Spectrum of vaginal discharge in a tertiary care setting''. *Trop. Parasitol.,* 2013, *3*, 135.

[18] Foxman B.: ''The epidemiology of vulvovaginal candidiasis: risk factors''. *Am. J. Public. Health*, 1990, *80*, 329.

[19] Spinillo A., Capuzzo E., Nicola S., Baltaro F., Ferrari A., Monaco A.: ''The impact of oral contraception on vulvovaginal candidiasis''. *Contraception,* 1995, *51*, 293.

[20] Hooton T.M., Roberts P.L., Stamm W.E.: ''Effects of recent sexual activity and use of a diaphragm on the vaginal microflora''. *Clin. Infect. Dis.,* 1994, *19*, 274.

[21] Chassot F., Negri M.F.N., Svidzinski A.E., Donatti L., Peralta R.M., Svidzinski T.I., Consolaro M.E.: ''Can intrauterine contraceptive devices be a *Candida albicans* reservoir?'' *Contraception,* 2008, *77*, 355.

[22] Malkawi S.R., Abu Hazeem R.M., Hajjat B.M., Hajjiri F.K.: ''Evaluation of cervical smears at King Hussein Medical Centre, Jordan, over three and a half years. *East. Mediterr. Health. J.,* 2004, *10*, 676.

[23] Lessa P.R., Ribeiro S.G., Lima D.J., Nicolau A.I., Damasceno A.K., Pinheiro AK.: '' Presence of high-grade intraepithelial lesions among women deprived of their liberty: a documental study''. *Rev. Lat. Am. Enfermagem.,* 2012, *20*, 354.

[24] Adad S.J., de Lima R.V., Sawan Z.T., Silva M.L., de Souza M.A., Saldanha J.C., *et al.*: ''Frequency Of *Trichomonas Vaginalis*, *Candida spp.* and *Gardnerella vaginalis* in cervical-vaginal smears in four dif-ferent decades''. *São. Paulo. Med. J.,* 2001, *119*, 200.

[25] Roeters A.M., Boon M.E., van Haaften M., Vernooij F., Bonteoe T.R., Heintz A.P.: ''Inflammatory events as detected in cervical smears and squamous intraepithelial lesions''. *Diagn. Cytopathol.*, 2010, *38*, 85.

[26] Kalantari N., Ghaffari S., Bayani M.: ''*Trichomonas, Candida*, and *Gardnerella* in cervical smears of Iranian women for cancer screening''. *N. Am. J. Med. Sci.,* 2014, *6*, 25.

[27] Zdolsek B., Hellberg D., Fróman G., Nilsson S., Mårdh P.A.: ''Culture and wet smear microscopy in the diagnosis of low-symptomatic vulvovaginal candidosis''. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 1995, *58*, 47.

[28] Siapco B.J., Kaplan B.J., Bernstein G.S., Moyer D.L.: ''Cytodiagnosis of *Candida* organisms in cervical smears''. *Acta. Cytol.,* 1986, *30*, 477.

Corresponding Author:
T. TOKA ÖZER, M.D.
Department of Medical Microbiology, Konya Hospital
Şemsi Tebrizi mah. Şerafettin cad. No. 95/A
42030 Karatay, Konya (Turkey)
e-mail: tozer73@hotmail.com