

Sensitivity of a papanicolaou smear in the diagnosis of candida albicans infection of the cervix

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Abstract

Background: Candida albicans infections of the cervix are not adequately diagnosed in Papanicolaou smears when compared with culture in Sabouraud dextrose agar. **Methods:** Cervical smears were collected from 1000, non-pregnant, asymptomatic women. The specimens were prepared using the Papanicolaou (Pap) smear method examined by microscopy and subsequently cultured in Sabouraud dextrose agar. **Results:** The overall incidences of C. albicans in cervical smears cultured in Sabouraud dextrose agar was 30.10%, while the incidence in the Pap smears was 7.60%, making the sensitivity of Pap smears for the diagnosis of C. albicans to be 25.25%. Mild, and some moderate, infections detectable in Sabouraud dextrose agar could not be detected in the Pap smear specimens. Vulnerability to C. albicans infection decreased with age, the highest infection rate being between 20 and 39 years of age while the least was between 60 and 69 years of age. Papanicolaou's method stained the nuclei, chromatin and nucleoli very well and enhanced differentiation between malignant and non-malignant cells. It also stained the cytoplasm and its contents such as keratin, vacuoles and granules and differentiated between acidophilic and basophilic materials. Non-cellular substances such as fibrin, crystals and pigments were also stained using this method. In addition, Papanicolaou's method also stained some fungal and bacterial species, but did not provide differential staining characteristics seen in Gram staining technique. **Conclusions:** Papanicolaou stain should not be used as an alternative to culture in the diagnosis of Candida albicans.

Keywords: Pap smear, culture, Candida albicans infections, cervix, Sabouraud dextrose agar.

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Introduction

In most healthy people, Candida albicans is a harmless microflora but, in immune compromised patients, C. albicans develops into an opportunistic pathogen that can cause life-threatening disseminated infections [1]. Although the immune status of the host is the major factor that determines whether C. albicans can become a pathogen and cause infection, the fact that C. albicans is by far the most frequent cause of fungal infections in such debilitated patients, indicates that it must possess traits that make it a more successful colonizer and pathogen than other medically important Candida species [2].

The relationship between inflammatory cervical smears and the diagnosis of malignancies has been examined and it was reported that underlying infections could mask cancerous or precancerous changes and that certain invasive cervical squamous cell carcinomas could not be detected until the infections were treated [3]. C. albicans is a major cause of the disease called vulvovaginal candidiasis and it possesses a gene family which encodes secreted aspartic proteinases [4.] which have been linked with the virulence of the fungus [5]. During infections, the proteinases digest the host's proteins for nutrient supply and evade the host defenses by degrading

immunoglobulins and complement proteins [6]. Individual members of the gene family might have their own special role in infection, and this might be reflected by a differential expression pattern at various stages of the infection process [3].

It is a general view that the pathogenicity of *C. albicans* is not caused by single dominant virulence factors [7], rather, it seems to be the high adaptability of *C. albicans* to many different host niches, as illustrated by the possession of many different adhesins that mediate binding to a variety of tissues, which allows the fungus to colonize and infect virtually all body locations [8]. A prerequisite for this adaptability is the capacity to respond to complex environmental signals, representing the different host niches, by the expression of an appropriate set of virulence-related genes. Intra-amniotic and placental infections with *Candida albicans* are rare during pregnancy, but these have been described [9] and have also been found in association with the intrauterine device (IUD)[10,11]. The IUD, being the facilitator of the infection.

The Pap smear is a routine screening test used for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix [12]. It also detects certain viral, bacterial, and fungal infections of the cervix and vagina [13]. There is also epidemiological and experimental evidence that Pap smears are beneficial in detecting infections that are risk factors associated with cervical cancer, such as human papilloma virus [14]. The aim of this study was to determine the suitability of Papanicolaou stain for the detection of *C. albicans* in cervical specimens.

Patients and Methods

From 2005 to 2007, twin cervical specimens were collected from 1000, asymptomatic, non-pregnant women between the ages of 20 and 70 years in South Western Nigeria. Individuals who presented with heavy vaginal discharge were excluded from the study because this often leads to false positive Pap smear results. The specimens were collected with swab sticks and an Ayre's spatula with the support of a speculum. Smears from the Ayre's spatula were made on clean dry slides. The smears were fixed immediately in equal volumes alcohol and ether while they were still wet for 1 hour.

They were then stained by the Papanicolaou method as follows: Harris's haematoxylin without acetic acid for 5 minutes, rinsed in tap water and differentiated in 1% acid-alcohol for 30 seconds and blued in Scott's water for 2 minutes. Smears were taken to 95% alcohol and stained in OG6 for 2 minutes, rinsed in 95% alcohol and stained in EA 35 for 2 minutes. Smears were then taken to two changes of absolute alcohol, xylene and mounted in DPX. The stained smears were examined under the light microscope at low and high power objectives for the presence of *Candida albicans*.

Specimens from the swab sticks were cultured on Sabouraud dextrose agar (SDA) with 0.05 mg/mL chloramphenicol 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 chlamydo-spore production on corn meal agar. Colonies were smeared on slides for Gram's standard staining technique. Wet preparations of the swabs in sterile normal saline were also examined with low and high power objectives.

Results

A total of 1000 cervical specimens were examined. These were arranged into 5 age groups of 200 each. Incidence of *Candida albicans* in Papanicolaou smears were compared with incidence of *Candida albicans* in microscopy and culture. The over all incidences of *C. albicans* in cervical smears cultured in SDA was 30.10%, while the incidence in the Pap smears was 7.60%, making the sensitivity of Pap smear for the diagnosis of *C. albicans* to be 25.25%. Culture growths on Sabouraud dextrose agar plates ranged from mild, moderate to profuse growths. Mild, and some moderate, infections detectable in culture could not be detected in the Pap smears. Vulnerability to *C. albicans* infection decreased with age, the highest infection rate being between 20 and 39 years while the lowest was between 60 and 69 years (Table 1).

Table 1 Comparative study of *C. albicans* in Papanicolaou smears with microscopy and culture

Age group	specimen	+Ve by Papanicolaou	+Ve by microscopy & culture	Sensitivity of Pap smear compared with culture (%)
20-29	200	19	68 (34%)	27.94
30-39	200	18	67 (33.5%)	26.87
40-49	200	14	62 (31%)	22.58
50-59	200	13	54 (27%)	24.07
60-69	200	12	50 (25%)	24.00
Total	1000	76 (7.6%)	301(30.1%)	25.25

Papanicolaou's method stained the nucleus, chromatin and nucleolus very well and enhanced differentiation between malignant and non-malignant cells. It also stained the cytoplasm and its contents such as keratin, vacuoles and granules and differentiated between acidophilic and basophilic materials. Non cellular substances such as fibrin, crystals and pigments were also stained using this method. In addition, Papanicolaou's method also stained some fungal and bacterial species but it did not provide the unique differential staining characteristics seen in Gram staining technique.

Discussion

Papanicolaou's is the most important staining method in cytology, because it enhances differentiation between malignant and non-malignant cells and also stains the cytoplasm and its contents. Being able to differentiate

between acidophilic and basophilic materials as well as its ability to stain non-cellular substances such as fibrin, crystals and pigments, make it an essential stain in cytology. Papanicolaou's method also stains fungi and bacteria but it does not provide differential staining characteristics seen in Gram staining technique [13].

This study confirms that *C. albicans* is detectable by the Pap technique, but the major disadvantage is in its inability to detect mild infections as it is only 25.25% sensitive when compared with microscopy and culture results in Sabouraud dextrose agar. Women below the age of 20 years were excluded from the study, because they are often excluded from routine Pap smear tests and it was difficult to get volunteers who were below 20 years of age. Of the 1000 cases studied, 301(30.10%), had *C. albicans* in their cervix by the culture method while only 76 (7.60%) were positive by the Pap smear technique. This result is slightly lower than a previous study [15] where, in samples taken from pregnant women, 40% were positive in culture versus 20% in the Pap smears. 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 infections.

In a separate study, *C. albicans* was detected in 42.3% of samples when an immunologic latex agglutination test method was used for the detection of *C. albicans* in the vagina [16] but this was not confirmed by culture. A low value of 1.2% *C. albicans* was detected in Pap smears in a study conducted in Jordan [17] although they also did not compare their results with culture. Yet another study reported a striking low value of 14% observed in the cultures of vaginal specimens of pregnant women [18].

In some countries, people have unlimited access to drugs including antifungal drugs and use them at will. This may account for the wide variation in the presence of the organisms in the cervix from one country to another. The immune status, hygiene and level of infection of patients may also have a profound influence on the ability of *C. albicans* to cause an infection in the cervix as a 50% incidence of *C. albicans* in the vagina of patients that presented with vaginal itching and discharge were observed [19].

Conclusion

Culture is therefore, the gold standard for the diagnosis of *Candida albicans* and Pap smears should not be used as an alternative for the diagnosis of *C. albicans* because it cannot detect mild and some moderate infections.

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