Content available at: https://www.ipinnovative.com/open-access-journals

IP Archives of Cytology and Histopathology Research

Journal homepage: https://www.achr.co.in/

Case Report

Aspergillus as a contaminant on conventional cervicovaginal papanicolaou smear

Kuladeepa Ananda Vaidya K[1],*, Aashish Sharma K[1]

[1] *Dept, of Pathology, Srinivas Institute of Medical Sciences & Research Centre, Mangalore, Karnataka, India*

ARTICLE INFO

Article history:
Received 27-08-2022
Accepted 21-10-2022
Available online 30-11-2022

Keywords:
Aspergillus
fungi
Papanicolaou test
contaminant

ABSTRACT

Majority of the cases of fungal infections to be detected in the cervicovaginal smears, are caused by Candida species. Aspergillus infections are not just rare, but should also raise alarm regarding the immune status of the patient. Here we report the case of an adult female with cervicovaginal smears showing non-Candida fungal elements, going on to prove contamination of the wooden spatula by Aspergillus fumigatus.

1. Introduction

Candida is the most common fungal species seen in cervicovaginal Pap smear, whereas infection with other fungal species such as Aspergillus is a rare occurrence.[1] Aspergillus is a ubiquitous fungus known to cause aspergillosis in immunocompromised patients.[2] Identification of a fungus like Aspergillus which is known to cause systemic infection should alert one to look for risk factors, that lead to immunocompromised state.[3] Detection of organisms does not always indicate infection but a high index of suspicion is necessary to detect potential contamination.[4] The collection of cervicovaginal smears and their processing till the step of interpretation involve various steps which may potentially produce a contamination in ill-controlled environment.[2] Contaminants from various sources are curious findings in cervicovaginal smears and may pose diagnostic challenges.[2,4]

Here we report a rare presentation of spores and hyphae of Aspergillus species in a routine Papanicolaou (Pap) smear which we later on confirmed to be contamination rather than a primary infection with thorough investigations.

2. Case Report

A 36-year-old lady came to gynaecology department for routine examination and underwent a health screening. Her general physical examination, biochemical, and hematologic investigations were within normal limits. Routine and microscopic examination of urine was normal. A screening Pap smear was sent for cytopathological assessment. The cytologic examination of the cervicovaginal Pap smear showed superficial and intermediate squamous epithelial cells interspersed with a few clusters of endocervical cells and sparse acute inflammatory cells. Low magnification revealed a collection of intermeshed fungal hyphae and spores [Figure 1]. At higher magnification, the hyphae were uniform, 4 to 6 μm in diameter, hyaline and septate, with parallel walls and acute-angle branching. These spores and hyphae were lying at the same plane of the cervical cells. [Figure 2]

As the patient was immunocompetent, not having any clinical symptoms such as vaginal discharge, considering the disproportionately minimal inflammation in Pap smear, we signed out the report as contaminant.

Detection of fungal hyphae and spores in conventional Pap smears led to the screening of all smears received in our lab during that month to evaluate the source of

* *Corresponding author.*
E-mail address: ks.aashish@gmail.com
(Kuladeepa Ananda Vaidya K).

https://doi.org/10.18231/j.achr.2022.061

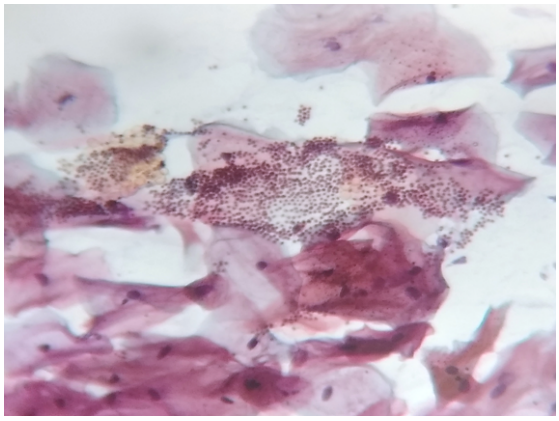

Fig. 1: Conventional Pap smear showing numerous fungalspores. No significant inflammatory cells. (Pap stain, x400)

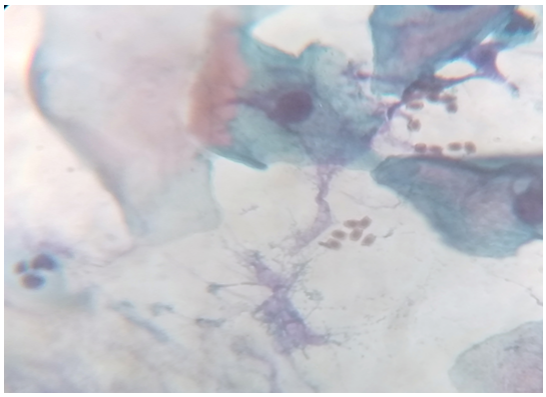

Fig. 2: Smear showing fungalspores and occasional hyphae. Pap stain x1000.

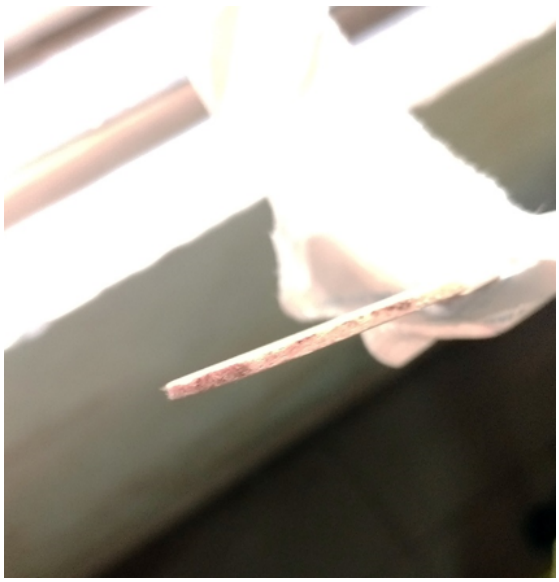

Fig. 3: Edge of Ayre's wooden spatula showing whitish-brown powdery growth

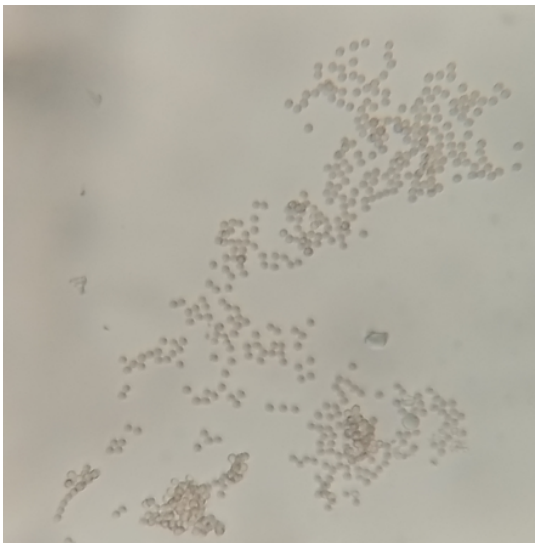

Fig. 4: Spores of a fungus seen as seen in Wet mount (scrape from spatula)

Fig. 5: SDA slant showing characteristic growth

Fig. 6: Aspergillus fumigatus fruiting body. Lactophenol cotton blue stain x400

contamination. Fifty-two cervical smears were received from the gynaecology department on that month. The smears were prepared on clean slides, fixed in alcohol, and stained by the rapid Pap technique. On review, two of the 52 cases showed occasional spores of similar morphology. All smears were negative for malignancy.

Possibility of contamination from lab side was ruled out by staining few random blank slides/ slides without any material, using the same reagents/ fixatives and stains. These slides failed to show any contaminants. Random examination of the stored wooden spatulas procured from the gynaecology department showed whitish to brownish powdery substance on the surface [Figure 3], these were scraped and part of it was visualized under microscope as wet mount [Figure 4], rest of it was handed over for culture. Culture with Sabouraud's Dextrose Agar slant showed characteristic growth [Figure 5]. Lactophenol Cotton Blue stain showed fruiting body of Aspergillus fumigatus, shown in Figure 6.

A repeat Pap smear with due precaution using sterilized new set of spatulas and a glass slide under similar environmental conditions did not show any evidence of Aspergillus species, clearly indicating that the initial presentation of the fungus was due to contamination. As clinical follow-up was unremarkable, further microbiologic evaluation or antifungal treatment was not recommended.

3. Discussion

Contamination by microorganisms are more likely in conventional Pap smears compared to liquid based smears. Fungi and bacteria are the usual culprits.

Fungal infections usually detected in cevicovaginal Pap smears are mostly attributed to Candida spp. Infection by other fungi such as Aspergillus, Paracoccidioides are rare, but have been reported.[4] The identification of Aspergillus species in cervicovaginal smears should prompt the clinician for further investigation to look for any coexisting systemic disease.[1]

Aspergillus species in a cervicovaginal smear is rare. It may represent a contamination or a true infection following prolonged antibiotic therapy or immunosuppression.[1,5] The most relevant of the Aspergillus species are fumigatus, flavus, and niger. These grow in the natural environment, and in tissues and cultures in the form of hyphae that produce conidia upon exposure to air.[3]

Numerous contaminants can be visualised in routine Pap smears such as glove powder, pollen, plant cells, etc. The presence of microorganisms should be of concern, their nature has to be assessed for the provision of proper therapy.[4] Airborne contamination of the Pap smears acquired at the time of staining procedure should be considered while screening to avoid unwarranted treatment.[6]

If fungal elements such as fruiting bodies and hyphae of Aspergillus species are found in Pap smears, distinguishing between infection and contamination is very important.[7] The isolated presence of fungal spores, without hyphae or fruiting bodies need to be distinguished from cocci and erythrocytes. Morphometry may be useful in this regard, as fungal spores are generally larger than bacteria but smaller than RBCs. Elements of Candida species most of the times are oval in shape, show yeast forms displaying budding with or without pseudo hyphae.[4]

Most authors emphasize that it is essential to rule out contamination with the Aspergillus species due to use of improperly sterilized spatulas in the colposcopic clinic before diagnosing it as a true infection.[1] There could be multiple sources of contamination, the possibility of contamination at each level has to be given due diligence in each case of cervicovaginal smear wherein fungal elements are detected.[2] The source of the contamination could be either during the smear preparation or during processing.[4] The speculums, spatula, endocervical brushes, are all prone for contamination if not stored or strilised in a proper fashion. Even the lubricant used to facilitate specimen draw may also be contaminated. The glass slides on which smear are made may also get contaminated.[2] In hot, humid tropical and sub-tropical climates, fungi grow easily on surfaces of glass slides, spatulas, and containers, specially those that are stored for long intervals before use. Similar contaminants are sometimes detected within the laboratory, in the stains and solutions used. Such accidental contamination of the samples could either be before or during the staining process of the smears.[4] Culture for characterization of fungus before initiating

therapy is important to avoid unnecessary treatment due to contaminants. However, growth in culture also needs to be interpreted with caution because of ubiquitous nature of conidial spores.[2] The powdery substance that was seen on the stored wooden spatulas in our case could most probably be the spores of Aspergillus found as a contaminant in the atmosphere. When it was scraped and sent for culture, the material grew Aspergillus fumigatus.

Compared to liquid-based preparations, chance of contamination is more likely in conventional smears. Spatulas can even get contaminated even by bacteria that exist in the environment, including Bacillus spp.[4]

The fruiting body provides clue about the particular fungus and its species. Determination of the length and width of conidiophores, shape of vesicles, phialides, and chains of conidia are some of the useful characteristic features to correctly identify the species.[1] In our case the hyphae lying at the same plane of the cervical cells suggest that the fungi were deposited at the same time as the cervical cells thereby favouring contamination of the spatula rather than the contamination of slide or staining reagents.

4. Conclusion

Aspergillus species in a cervicovaginal smear is rare. It may represent a contamination or a true infection in certain scenarios as prolonged use of antibiotics or immunosuppression. Contamination of Pap smears by fungus must be distinguished from true infection. Procedures in the lab may reduce such occurrences, but eliminating similar factors in the clinician's setup may be tough. The interpreting pathologists need to be aware lest they mischaracterise it as an infection leading to unnecessary therapy.

5. Source of Funding

None.

6. Conflicts of Interest

There is no conflict of interest.

References

1. Gupta P, Goyal S, Kaushal M. Concomitant aspergillus species infection and squamous cell carcinoma diagnosed on Pap smear. *Turk Patoloji Derg*. 2016;32(1):54–6.
2. Verma AK, Kaur O. Aspergillus and cervicovaginal papanicolaou smear: a review Int. *Clin Pathol J*. 2017;4(1):33–6.
3. Garza-Guajardo R, Canales-Martínez LC, Rodríguez-Sánchez IP, Sánchez-Chaparro M, Gómez-Macías GS, Vilches-Cisneros N, et al. Aspergillus in liquid-based cervicovaginal cytology in a postmenopausal patient: A case report. *Biomed Rep*. 2017;7(4):306–8.
4. Kini H, Kini JR, Suman E, Rai S. Fungal spores and fruiting bodies in cervicovaginal smears: Contaminant or infection. *Diagn Cytopathol*. 2017;45(3):191–4. doi:10.1002/dc.23658.
5. Hoda RS, Colello C, Roddy M, Houser PM. Fruiting body" of aspergillus species in a routine cervico-vaginal smear (Pap test). *Diag Cytopathol*. 2005;33(4):244–5. doi:10.1002/dc.20267.
6. Martínez-Girón R, Fernández-García C. Aspergillus/Penicillium sp. spores as a contaminant on conventional Pap smear. *Diagnostic Cytopathology*. 2009;37(12):899–900. doi:10.1002/dc.21017.
7. Chandra S, Gaur D, Harsh M, Chaturvedi J, Kishore S. An unusual presentation of Aspergillus species in a routine cervicovaginal pap smear. *Acta cytologica*. 2009;53(2):229–31.

Author biography

Kuladeepa Ananda Vaidya K, Professor

Aashish Sharma K, Assistant Professor

Cite this article: Kuladeepa Ananda Vaidya K, Aashish Sharma K. Aspergillus as a contaminant on conventional cervicovaginal papanicolaou smear. *IP Arch Cytol Histopathology Res* 2022;7(4):266-269.