Fluorescence of \textit{Candida} in diagnosis of oral candidiasis

Kumaraswamy Naik LR, Pushparaja Shetty\textsuperscript{1}, Krishna Prasad MS\textsuperscript{2}, Vimal Kumar Karnaker\textsuperscript{3}, Sarosh E Shroff\textsuperscript{1}, Lal P Madathil\textsuperscript{1}

\textbf{ABSTRACT}

\textbf{Background:} Many pathogenic fungi fluoresce in hematoxylin and eosin stained sections, and Papanicolaou (PAP)-stained smears under ultraviolet illumination. In theory, this phenomenon could aid in the diagnosis of common fungal infections without the delay which is usually associated with special stains.

\textbf{Objective:} To evaluate the role of fluorescence as a rapid screening technique for oral infections caused by \textit{Candida} organisms in exfoliative smears of oral candidiasis.

\textbf{Materials and Methods:} Two smears and one swab were collected from each of 62 clinically diagnosed cases of oral candidiasis. Smears were stained with (PAP) and periodic acid–Schiff stain (PAS). Both smears were evaluated under light microscopy (LM). Later, PAP smears were observed under fluorescent microscopy (PAP-FM). The swab was inoculated on Sabouraud’s agar plate. Each technique was evaluated for sensitivity and specificity.

\textbf{Results:} It was found that the PAS-stained smears were more reliable for detection of \textit{Candida} species than other methods (sensitivity = 100%; specificity = 66.7%). The PAP-LM and PAP-FM showed less sensitivity (67.9% and 85.7%) and specificity (66.7% and 33.3%), respectively. Combined results of both light and fluorescent microscopy of PAP (LM + FM) showed increased sensitivity (89.3%) but reduced specificity (16.7%).

\textbf{Conclusion:} PAP autofluorescence is less sensitive than PAS, still it accentuates the distinct morphological features of \textit{Candida}.

\textbf{Key words:} Autofluorescence, \textit{Candida}, cytology, Papanicolaou, periodic acid Schiff

Since the time oral candidiasis has been recognized as a clinical entity, methods for identification of \textit{Candida} species are available and are best accompanied by a combination of morphologic features, culture, and biochemical characteristics.\cite{1,2} Although time-consuming procedures, established methods of identifying \textit{Candida} organisms on a morphological basis by a periodic acid–Schiff stain (PAS) or Gridley’s or Gomori’s methenamine silver staining and mycological culture are accepted and routinely being followed.\cite{2,3} Rapid cytological detection of infective agents by direct fluorescent microscopic evaluation is a well-established procedure. Autofluorescence (AF) of infective agents in cytological smears is useful as it allows their rapid identification, much before the results of culture and special stains are available.\cite{4,6} AF of fungi has been demonstrated in tissue sections stained with routine hematoxylin and eosin (H and E).\cite{7,8} Moreover, there have been few studies applying the property of AF for rapid detection of fungal organisms in the Papanicolaou (PAP) stained, nonoral cytological smears (PAP-FM).\cite{9,10} However, to the best of our knowledge, there are no documented studies with regard to its use on PAP-stained oral smears. The aim of the present study was to assess the diagnostic reliability of autofluorescence of \textit{Candida} species in PAP-stained oral smears in comparison with PAS stain and fungal culture.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Quick Response Code: & Website: www.iijdr.in \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
DO: & 10.4103/0970-9290.199592 \\
\hline
\end{tabular}
\end{table}

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Kumaraswamy Naik LR, Shetty P, Krishna Prasad MS, Karnaker VK, Shroff SE, Madathil LP. Fluorescence of \textit{Candida} in diagnosis of oral candidiasis. Indian J Dent Res 2016;27:618-22.
MATERIALS AND METHODS

The study was undertaken after getting approval from the Independent Ethics Committee of the institution. The study group comprised 62 patients, who were clinically diagnosed as suffering from oral candidiasis. The clinical diagnosis of oral candidiasis was made according to Lehner (1966).[11] From each patient, a swab for microbial culture was obtained from lesional tissue, which was directly inoculated onto the Sabouraud’s agar plate. The plates were incubated at 37°C for 48 h. Two smears were prepared from the clinically suspected area by scraping the lesional tissue and/or from the fitting surface of the denture (in the case of chronic atrophic candidiasis) with a sterile metal spatula. The collected sample was spread over the glass slide. All the slides were fixed for half an hour in a Coplin jar containing ether-alcohol (1:5). One slide was then stained with PAP and the other with PAS. Three observers examined all the slides. All the slides were observed under ×40 for presence (+) or absence (−) of organisms on the morphological (for consistent usage) basis.[12] Grades for visibility in each technique were recorded as +, ++, ++++, and ++++ depending on the number, the delineation, and the fluorescence of Candida in contrast with the other materials such as epithelium, mucus, debris, and inflammatory cells as depicted in Table 1. Final grades of visibility were calculated for least value of number of organisms, delineation, and fluorescence of Candida. The graded data were then tabulated for statistical analysis by evaluating each technique for sensitivity, specificity, positive predictive value, and negative predictive value. Fluorescent microscopic evaluation was done with the help of incident light fluorescent microscope POLYVAR 2 Reichert-Jung. A 100 Watt HBO high-pressure mercury lamp with 220-volt AC mains was used as the source. The excitation filter used was of band pass 450–495 in blue excitation range (VB1). The barrier filter was of long pass 520 with a dichroic illuminator mirror with a specification of spectral reflection and edge of transmission range in (DS) 510. PAS-stained smears were examined under a light microscope, and appropriate grades were recorded [Table 1].

The PAP smear grading of Candida species on light microscopy (PAP-LM) was appropriately recorded using ×40 objective [Table 1]. Subsequently, AF evaluation of PAP-stained smears (PAP-FM) was done under ×40 objective of a fluorescent microscope. The area of light microscopically examined site was focused first to look for the presence (positive) or absence (negative) of AF. Candida species was identified by its characteristic morphology. Later, other areas of the same slide were screened for any improvement in visibility of organisms. The improvement in visibility was recorded accordingly [Table 1]. After incubation of the organisms in Sabouraud’s agar plates, they were examined for the presence (+) or absence (−) of yeast growth. Plates without growth at the end of 48 h were incubated for up to 2 weeks before being discarded as negative.[13] A small suspension of the culture was then taken and stained with Gram’s standard staining technique. The readings were documented as positive (+) or negative (−) depending on the presence or absence of yeast.

RESULTS

Of the 62 clinically diagnosed cases of oral candidiasis, 56 were positive on culture (90.30%). All positive growths (100%) showed round, oval, Gram-positive budding yeast cells. In PAS-stained smears, Candida species were identified as tangled, magenta masses of elongated hyphae, and pseudohyphae with numerous budding yeast cells [Figure 1]. Fifty-eight (93.5%) of 62 lesions demonstrated the presence of organisms [Table 2]. In PAP-stained smears (PAP-LM), Candida species were either not seen or seen as lightly stained, pale pink or light blue colored masses of hyphae with budding yeast cells [Figure 2 and Table 3]. On fluorescent microscopy of the PAP-stained smears (PAP-FM), Candida species were fluorescing with varying intensities of yellow/green colors, and they were identified as thread-like elongated masses of hyphae/pseudohyphae with budding yeast cells [Figure 3a–c and Table 4]. Other elements such as epithelial cells, inflammatory cells, bacteria, mucin, and cellular debris were also fluorescing in a similar manner interfering with the identification of Candida species [Figure 4]. The combined PAP-LM and PAP-FM results were compared with the culture results [Table 5].

DISCUSSION

In 62 clinically diagnosed cases of oral candidiasis, 6 were negative in culture. This included 4 cases of median rhomboid glossitis and a case of acute and chronic atrophic candidiasis. We had deviated from original classification of Lehner (1966) by including 13 cases of median rhomboid glossitis in our study as literature reports a significant number of cases that are associated with Candida species.[14]

Demonstration of Candida species, especially as hyphae, by PAS stain is considered as one of the diagnostic methods.[15]
In the present study, two false positive results were seen accounting for 100% sensitivity and 66.7% specificity. Mucus which also stains magenta with PAS stain interfered with the identification of organisms although *Candida* could be well delineated by morphology.

In the present study, the organisms were poorly stained with PAP. Moreover, when the organisms were less in number, it was difficult to demonstrate them on LM (PAP). When the organisms are good in number in well-stained PAP smear, identification of *Candida* species is not that difficult, and a need for PAS also does not arise. In addition, when organisms are less in number there will be a need for other techniques for identification. Although forty smears (64.5%) showed the presence of organisms, only 2 (3.6%) showed the visibility criteria of ++++, and none of the slides showed ++++

Organisms such as *Mycobacteria* and *Pneumocystis carinii* have been shown to exhibit the property autofluorescence on PAP-stained smears (PAP-FM); hence, the PAP-FM technique serves as a fast and inexpensive screening technique.
Fluorescence of Candida in oral cytosmears Naik, et al.

Table 4: Comparison of Papanicolaou-stained smears under fluorescent microscopy and culture

| Culture | PAP-FM Negative | Positive | Total |
|---------|-----------------|----------|-------|
| Negative | 2 (33.3) | 8 (14.3) | 10 (16.1) |
| +       | 2 (33.3) | 18 (32.1) | 20 (32.3) |
| ++      | 2 (33.3) | 21 (37.5) | 23 (37.1) |
| +++     | 9 (16.1) | 9 (14.5)  |        |
| Total   | 6 (100)   | 56 (100)  | 62 (100) |

Sensitivity=85.7%, Specificity=33.3%, Positive predictive value=92%, Negative predictive value=20%. PAP=Papanicolaou, FM=Fluorescent microscopy

Table 5: Comparison of culture and combination of light and fluorescent results of Papanicolaou-stained smears

| Culture | Combination of PAP-LM and PAP-FM | Total (%) |
|---------|----------------------------------|-----------|
| Negative| - + ++ +++ ++++                   | 6 (100)   |
| Positive| 1 3 27 10                        | 56 (100)  |
| Total   | 7 16 29 10                       | 62 (100)  |

Sensitivity=89.3%, Specificity=16.7%, Positive predictive value=91%, Negative predictive value=14%. PAP=Papanicolaou, FM=Fluorescent microscopy, LM=Light microscopy

method for the identification of these organisms. Similarly, autofluorescence of fungi in tissue sections has been advocated for rapid diagnosis of fungal infection.[7,8] A few reports on applications of autofluorescence property of fungi for locating them in cytological smears are also on record.[9,10,16] Our literature search reveals that none of the previous studies utilized the property of autofluorescence in the smear diagnosis of oral candidiasis. Fluorescent dyes such as calcofluor white and Fungiqual were described to demonstrate Candida species. However, these techniques are known to have nonspecific fluorescence and other artifacts due to keratin.[3,21] Moreover, these methods are time-consuming. The advantage of PAP staining is that it is routinely done in most cytology laboratories for all types of cytological samples including the oral smears. Similar to PAP-stained smears, H and E stained frozen/paraffin embedded sections also exhibit autofluorescence of microorganisms.[3,18] As per the literature, there has been less stress on the fluorescent property of the Candida species in PAS smears and in unstained fungal culture.[18] The fluorescence of fungi depends on the type of fungus. The Candida exhibits an excellent delineation under fluorescence,[10] though the mechanism of its autofluorescence is yet to be understood.

PAP-LM showed a low sensitivity and specificity of 67.9% and 66.9%, respectively, while PAP-FM revealed an improved sensitivity of 85.7% with a lower specificity of 33.3%. Although PAP-LM revealed the Candida species, most of them were poorly delineated and faintly visualized. The same organisms could be well demonstrated under fluorescent microscopy of the same smear and field with more visibility grading.

Comparison of the sensitivity and specificity of PAP under light and fluorescence microscopy (PAP [LM] + PAP [FM]) with special stain (PAS) was done. Of all these methods, PAS was proved to be the most reliable with 100% sensitivity and 66.7% specificity. As for the other two methods, when the organisms were not seen with PAP (LM), the same organisms could be demonstrated with PAP (FM) and the combined (PAP [LM] + PAP [FM]) increased the sensitivity to 89.3% (which is still less than the PAS) but reducing the specificity to 16.7%. Increased visibility of PAS is related to the specific site in the Candida cell wall, which takes up brilliant magenta color due to rich glycogen content.[21] This makes observer to identify Candida easily in the background of lightly stained epithelial cells.

CONCLUSION

PAS unquestionably remains the mainstay of identification of fungi in routine cytological preparations. Although PAP autofluorescence is less sensitive, it accentuates the distinct morphological features of Candida, thereby facilitating its differentiation from other components of oral smears. In addition, PAP fluorescence is more reliable for diagnosis when the light microscopic findings (PAP [LM]) are dubious due to poorly stained hyphae. As most of the cytopathology laboratories utilize PAP for routine staining and if the fluorescent microscope is available, the demonstration of Candida species in the same smear under ultraviolet light could be useful as a screening test, which when compared to other tests is less economic and is time saving.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jegannathan S, Chan YC. Immunodiagnosis in oral candidiasis. A review. Oral Surg Oral Med Oral Pathol 1992;74:451-4.
2. Scully C, el-Kabir M, Samaranayake LP. Candida and oral candidosis: A review. Crit Rev Oral Biol Med 1994;5:125-57.
3. Monheit JE, Cowan DF, Moore DG. Rapid detection of fungi in tissues using calcofluor white and fluorescence microscopy. Arch Pathol Lab Med 1984;108:616-8.
4. Ghali VS, Garcia RL, Skolom J. Fluorescence of Pneumocystis carinii in Papanicolaou smears. Hum Pathol 1984;15:907-9.
5. Pfitzer P, Wehle K, Blanke M, Bürrig KF. Fluorescence microscopy of Papanicolaou-stained bronchoalveolar lavage specimens in the diagnosis of Pneumocystis carinii. Acta Cytol 1989;33:557-9.
6. Wright CA, van Zyl Y, Burgess SM, Blumberg L, Leiman G. Mucocutaneous autofluorescence in Papanicolaou-stained lymph node aspirates: A glimmer in the dark? Diagn Cytopathol 2004;30:257-60.
7. Graham AR. Fungal autofluorescence with ultraviolet illumination. Am J Clin Pathol 1983;79:231-4.
8. Mann JL. Autofluorescence of fungi: An aid to detection in tissue sections. Am J Clin Pathol 1983;79:587-90.
9. Gonzales MF, Brown RW, Bhathal PS. Fluorescence of fungi – Not autofluorescence. Am J Clin Pathol 1984;81:142-3.
10. Shet T, Naik L, Rege J. Autofluorescence of fungi in Papanicolaou
stained smears, an aid to rapid diagnosis of soft tissue mycosis. J Cytol 2002;19:87-91.

11. Bastiaan RJ, Reade PC. The prevalence of Candida albicans in the mouths of tobacco smokers with and without oral mucous membrane keratoses. Oral Surg Oral Med Oral Pathol 1982;53:148-51.

12. Samaranayake LP, MacFarlane TW. Oral Candidosis. London: Wright-Butterworth; 1990.

13. Epstein JB, Pearsall NN, Truelove EL. Quantitative relationships between Candida albicans in saliva and the clinical status of human subjects. J Clin Microbiol 1980;12:475-6.

14. Samaranayake LP, Nair RG. Oral Candida infections - A review. Indian J Dent Res 1995;6:60-82.

15. Williams DW, Lewis MA. Review of oral microbiology. Isolation and identification of Candida from the oral cavity. Oral Dis 2000;6:3-11.

16. Eduard W, Blomquist G, Herbert Nielsen B, Kulvik Heldal K. Recognition errors in the quantification of micro-organisms by fluorescence microscopy, Ann Occup Hyg 2001;45:493-8.

17. Fradkin A, Patrick ZA. Fluorescence microscopy to study colonization of conidia and hyphae of Cochliobolus sativus by soil microorganism. Soil Biol Biochem 1982;14:543-8.

18. Hettlich C, Küpper TH, Wehle K, Pfitzer P. Aspergillus in the Papanicolaou stain: Morphology, fluorescence and diagnostic feasibility. Cytopathology 1998;9:381-8.

19. Og A, Oe O, To A. Sensitivity of a Papanicolaou smear in the diagnosis of Candida albicans infection of the cervix. N Am J Med Sci 2010;2:97-9.

20. Siapco BJ, Kaplan BJ, Bernstein GS, Moyer DL. Cytodiagnosis of Candida organisms in cervical smears. Acta Cytol 1986;30:477-80.

21. Cannon RD, Holmes AR, Mason AB, Monk BC. Oral Candida: Clearance, colonisation, or candidiasis? J Dent Res 1995;74:1152-61.