Diagnostic yield of calcofluor white in the identification of Candida albicans in oral squamous cell carcinoma

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Abstract

Background: Although oral cancer is multifactorial in origin only few had evaluated the diagnostic parameters for candidial infection in oral squamous cell carcinoma (OSCC).

Aims: To compare and assess the accuracy of Calcofluor White (CFW) to that of conventional staining methods to identify the presence of candidal infection in OSCC.

Methods: Archival collections of 43 OSCC were retrieved for this investigation. Standard staining protocol was followed for the index test (CFW) and reference standard (Periodic Acid Schiff). Two examiners were blinded for either one of the staining techniques. Diagnostic test evaluation and Kappa statistic was calculated using MedCalc software.

Results: The study comprised 37 males, six females, and their mean age at the time of presentation was 51 (range 23 to 75 years). The sensitivity and specificity of CFW was 75.8% (CI 57.7 to 88.9%) and 10.0% (CI 0.2 to 44.5%). While the positive predictive and negative predictive values were 63.1% (CI - 50.6-74.0%) and 67.0% (CI - 49.2-81.0%).

Conclusion: Our results show minimal agreement with PAS with a Kappa score of 0.148 (95% CI = 0.39 to 0.09). Overall detection rate was similar for both the index test and reference standard although there was considerable number of intermediate test results. Sensitivity of CFW was comparable to that of earlier studies but specificity was low and the degree of concordance was 60.4%. Although CFW staining can come with high false positive they are a useful test for ruling out candida infection when the test result is negative.

Keywords: Calcofluor white, candida albicans, periodic acid Schiff, leukoplakia

INTRODUCTION

Candida is a commensal that has the potential for opportunistic infection. Candidal invasion in leukoplakia was first reported in 1960s.[1] Since then there has been several reports of their association to malignant and potentially malignant lesions.[3] Some consider Candida...
as the forerunner for the dysplastic changes where the degree of dysplasia positively correlated to the level of invasion. Proponents of this theory attribute increased mitotic activity and or the production of carcinogenic nitrosamine by certain strains to be directly responsible for oral neoplasms. Systematic review of 16 reports identified candidal association in 6.8% to 100% of oral leukoplaikas. Of them, three identified malignant transformation in 2.5%, 6.5% and 28.7% of the cases. This led to renewed interest in candida as a probable causative organism for oral squamous cell carcinoma (OSCC). On the contrary, a three-year prospective study identified 10 of the 28 patients with oral leukoplaikas had superimposed candidal infection at the time of presentation. Yet only 1 of the 28 patients with no previous history of candidal infection developed OSCC. Findings where C. albicans infection is not present in the lesional tissue pose considerable challenges in ascertaining the precise role of C. albicans in the initiation of OSCC. Hence, epithelial dysplasia did not lead to carcinoma always, so there is also less direct evidence of a causal role, but a mere coincidence. While more than 90% of OSCC are of the squamous type, that is multifactorial in origin, the role of candidal infection in carcinogenesis is less clear.

Studies on diagnostic accuracy essentially involve comparison of one or more test results to that of the reference standard. Histopathological investigation helps in discriminating infection from colonisation and is the preferred method for identifying chronic hyperplastic candidiasis (CHC). Periodic Acid Schiff (PAS) is often used as the preferred reference standard for detecting candidal species in tissue biopsy. The thin hyphae of C. albicans are easily visible when stained with PAS. Yet, the conventional methods can be time consuming and prone for false negative in 5-15% of the cases. Studies that compared Calcofluor White (CFW) to that of traditional methods like PAS demonstrated superior diagnostic ability of CFW in detecting Candidal infection in histological sections of OSCC. Despite oral cancer being highly prevalent and multifactorial in origin very few studies have reported the diagnostic potential of CFW in the identification of C. albicans in OSCC. Hence diagnostic accuracy tests are imperative we decided to assess C. albicans presence and compare the diagnostic potential of fluorescent stain to that of PAS to help determine the most reliable test for diagnosis of candidal infection in archival records of OSCC.

METHODS

We adhered to STARD 2015 updated list of essential items for reporting diagnostic accuracy studies. Archival records of formalin fixed biopsy tissues of OSCC (n = 43) were retrieved for examination in the department of oral pathology and microbiology, Surendera Dental College and Research Institute. An Institutional Ethical Committee approval (SDCRI/IEC/2017/012) was obtained for this research work. Paraffin wax blocks with sufficient tissue material where the surface epithelium and lesional tissue was intact were included. Sections were made of 4 µm thickness, Hematoxin and Eosin (H&E) staining was used to reconfirm the diagnosis in the archival collection. After which, two serial sections were made from each block. Standard staining protocol was followed for CFW and PAS. For CFW, Nikon Eclipse 80i (Japan) microscope with fluorescent attachment was used and LX400-Labomed Inc (USA) microscope was used for PAS.

Earlier reports of Candidal infection in OSCC identified a prevalence between 3 and 66%. Since the ratio of cases where C. albicans infection is present or absent may not reflect the true prevalence in our archival records we included prevalence data from previous studies along with the reference standard (PAS) prevalence value in the present study (76.7%) in calculating the overall mean prevalence of 51.6%. Statistical analyses for the index test evaluation were performed using MedCalc for Windows, version 19.2.6 (MedCalc Software, Ostend, Belgium). The mutually exclusive paired observations of the index test (CFW) and the reference standard (PAS) was recorded independently, following standard criteria, by two trained oral pathologists. The observers were blinded to avoid intra and inter examiner variability where the degree agreement will be determined using Cohen’s K statistic.

RESULTS

The archival data include 37 males, six females and their mean age at the time of presentation was 51 (range 23 to 75 years). The histopathological sections were categorised accordingly as well differentiatated (n = 22), moderately differentiatated (n = 17) and poorly differentiatated (n = 4) OSCC. Records of the past medical history identified 27 and 10 of the men used smokeless tobacco (oral tobacco = 23) and alcohol. Table 1 shows the distribution of different grades of OSCC where 58% (n = 25) tested positive for C. albicans in both CFW and PAS. Table 2 shows results of the index test and reference standard in 2 × 2 table. The diagnostic accuracy parameters for CFW are presented in Table 3. Cohen’s K determined slight agreement between the two observers with a Kappa score of 0.148 (SE 0.1, 95% CI = 0.39 to 0.09).
Our results identified a sensitivity of 75.8% and specificity of 57.74% to 89.0% as either false positive or false negative. Our study is free of verification bias, the sensitivity and specificity values is in line with previous diagnostic accuracy study where CFW was compared to PAS archival records of OSCC. In general, tests that have high sensitivity are often accompanied with low values for specificity. The high sensitivity is also accompanied with high rate of false positive that leads to disease-free individuals being subjected to more invasive procedures. Mammogram is an excellent example of this.

There are many reasons why intermediate test results can arise, imperfect reference standard, technical reasons in classification due to retrospective analysis of archival data can pose a challenge while assessing the performance of diagnostic tests. Frequencies of up to 40% had been reported in literature for intermediate test outcomes that varies from one test to the other. Such findings can lead to erroneous outcome. Disease prevalence and sample size also play crucial role in diagnostic accuracy studies. Although, our study includes a small number of archival records yet it is not uncommon in diagnostic accuracy studies to have small sample numbers. As such, any generalisation or blanked extrapolation of our findings will need to be interpreted with caution.

**DISCUSSION**

*C. albicans* is the most prevalent of the Candidal species where >80% of isolates are of this type. They can present in health and disease as yeast, pseudohyphae or hyphae. Under favourable condition, the hyphae withstand adverse oral environment with the propensity for host tissue invasion by circumventing macrophageal phagocytosis. Both PAS and CFW can detect fungal elements under direct microscopic examination.

To the best of our knowledge, only three studies had reported the diagnostic parameters comparing CFW and PAS in the identification of *C. albican* in OSCC. Of them, only two had used PAS as reference standard where it is reported that CFW had a sensitivity and specificity of 4.9% and 4.3% more to that of PAS. Despite this, limited information is available as to how the intermediate test results (both false positive and false negative) were assessed in the final analysis particularly when the reference standards in itself are not an error-free gold standard.

Accurate diagnosis of candida infection is important as some of the untreated CHC may endow dysplastic characteristics and a potential risk for the development of OSCC. But the critical question, was there candidal infection prior to the initiation of OSCC or the association was more of casual rather causal is yet to be explored and longitudinal studies are needed to address this.

**Ethics**

An Institutional Ethical Committee approval (SDCRI/IEC/2017/012) was obtained.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Jepsen A, Winther JE. Mycotic infection in oral leukoplakia. Acta Odontol Scand 1965;23:239-56.
2. Firth NA, O’Grady JF, Reade PC. Oral squamous cell carcinoma in a young person with candidiasis endemicophaty syndrome: A case report. Int J Oral Maxillofac Surg 1997;26:42-4.
3. McGurk M, Holmes M. Chronic muco-cutaneous candidiasis and oral
neoplasia. J Laryngol Otol 1988;102:643-5.
4. Böckle B, Wilhelm M, Müller H, Gösch C, Sepp N. Oral mucous squamous cell carcinoma—an anticipated consequence of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). J Am Acad Dermatol 2010;62:864-8.
5. Barrett A, Kingsmill V, Speight P. The frequency of fungal infection in biopsies of oral mucosal lesions. Oral Dis 1998;4:26-31.
6. Shakir B, Smith C, Martin M. Epithelial mitotic activity during the induction of palatal candidosis in the Wistar rat. J Oral Pathol Med 1986;15:375-80.
7. Krogh P. The role of yeasts in oral cancer by means of endogenous nitrosation. Acta Odontol Scand 1990;48:85-8.
8. Sankari SL, Gayathri K, Balachander N, Malathi L. Candida in potentially malignant oral disorders. J Pharm Bioallied Sci 2015;7(Suppl 1):S162-4.
9. Shukla K, Vun I, Lov I, Laparidis G, McCamley C, Ariyawardana A. Role of Candida infection in the malignant transformation of oral leukoplaikia: A systematic review of observational studies. Transl Res Oral Oncol 2019;4:2057178X19828229. doi: 10.1177/2057178X19828229.
10. Bakri MM, Cannon RD, Holmes AR, Rich AM. Detection of Candida albicans ADH1 and ADH2 mRNAs in human archival oral biopsy samples. J Oral Pathol Med 2014;43:704-10.
11. Russell C, Jones J. The histology of prolonged candidal infection of the rat's tongue. J Oral Pathol Med 1975;4:330-9.
12. Sitheeque MAM, Samaranayake LP. Chronic hyperplastic candidosis/candidiasis (candidal leukoplaikia). Crit Rev Oral Biol Med 2003;14:253-67.
13. Bao F, Fan Y, Sun L, Yu Y, Wang Z, Pan Q, et al. Comparison of fungal fluorescent staining and ITS rDNA PCR-based sequencing with conventional methods for the diagnosis of onychomycosis. J Eur Acad Dermatol Venereol 2018;32:1017-21.
14. Yao Y, Shi I, Zhang C, Sun H, Wu L. Application of fungal fluorescent staining in oral candidiasis: Diagnostic analysis of 228 specimens. BMC Microbiol 2019;19:96.
15. Jahanshahi G, Shirani S. Detection of Candida albicans in oral squamous cell carcinoma by fluorescence staining technique. Dent Res J 2015;12:115-20.
16. Padilha CML, Picciani BLS, Santos BMd, Silva Júnior A, Dias EP. Comparative analysis of Gram’s method and PAS for the identification of Candida spp. samples from the oral mucosa. J Bras Patol Med Lab 2014;50:325-8.
17. Kumar RS, Ganvir S, Hazarey V. Candida and calcofluor white: Study in precancer and cancer. J Oral Maxillofac Pathol 2009;13:2-8.
18. Gall F, Coilella G, Di Onofrio V, Rosselli R, Angelillo IF, Liguori G. Candida spp. in oral cancer and precancerous lesions. New Microbiol 2013;36:283-8.
19. Sanketh DS, Patil S, Rao RS. Estimating the frequency of Candida in oral squamous cell carcinoma using Calcofluor White fluorescent stain. J Investig Clin Dent 2016;7:304-7.
20. McCullough MJ, Clemens KV, Stevens DA. Molecular epidemiology of the global and temporal diversity of Candida albicans. Clin Infect Dis 1999;29:1220-5.
21. Saghrouni F, Ben Abdeljelil J, Boukadida J, Ben Said M. Molecular methods for strain typing of Candida albicans: A review. J Appl Microbiol 2013;114:1559-74.
22. Barrett AW, Kingsmill VJ, Speight PM. The frequency of fungal infection in biopsies of oral mucosal lesions. Oral Dis 1998;4:26-31.
23. Gow NA, Van De Veerdonk FL, Brown AJ, Netea MG. Candida albicans morphogenesis and host defence: Discriminating invasion from colonization. Nat Rev Microbiol 2012;10:112-22.
24. Gow NA, Brown AJ, Odds FC. Fungal morphogenesis and host invasion. Curr Opin Microbiol 2002;5:366-71.
25. Uwamahoro N, Verma-Gaur J, Shen H, Qu Y, Lewis R, Lu J, et al. The pathogen Candida albicans hijacks pyroptosis for escape from macrophages. MBio 2014;5:e00003-14.
26. Slutsky B, Buffo J, Soll DR. High-frequency switching of colony morphology in Candida albicans. Science 1985;230:666-9.
27. Soll D. Candida commensalism and virulence: The evolution of phenotypic plasticity. Acta Trop 2002;81:101-10.
28. Bhavasar RS, Goje SK, Takalkar AA, Ganvir SM, Hazarey VK, Gosavi SR. Detection of Candida by calcofluor white. Acta Cytol 2010;54:679-84.
29. Begg CB, Greenes RA, Iglewicz B. The influence of uninterpretability on the assessment of diagnostic tests. J Chronic Dis 1986;39:575-84.