

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 candidal 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

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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.^[2] Some consider *Candida*

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as the forerunner for the dysplastic changes where the degree of dysplasia positively correlated to the level of invasion.^[2-5] Proponents of this theory attribute increased mitotic activity^[6] and or the production of carcinogenic nitrosamine by certain strains to be directly responsible for oral neoplasm.^[7,8] Systematic review of 16 reports identified candidal association in 6.8% to 100% of oral leukoplakias. Of them, three identified malignant transformation in 2.5%, 6.5% and 28.7% of the cases.^[9] 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^[10] identified 10 of the 28 patients with oral leukoplakia 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.^[11,12] 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.^[13] 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.^[14-17] 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 μ m thickness, Hematoxylin 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%.^[5,18,19] 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 differentiated ($n = 22$), moderately differentiated ($n = 17$) and poorly differentiated ($n = 4$) 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 the 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).

Table 1: Histological differentiation

OSCC	No of cases	PAS positive+	CFW positive	Both positive
Well differentiated	22	16	19	15
Moderately differentiated	17	13	12	8
Poorly differentiated	4	4	3	3
Total	52	33 (77%)	34 (79%)	25 (58%)

Table 2: CFW compared to reference standard

Technique	PAS		Total
	Positive	Negative	
CFW			
Positive	25	9	34
Negative	8	1	9
Total	33	19	43

Table 3: CFW diagnostic parameters

	% value	95% CI
Sensitivity	75.8%	57.74% to 89.0%
Specificity	10.0%	0.25% to 44.5%
Positive likelihood ratio	0.8	0.63 to 1.1
Negative likelihood ratio	2.4	0.34 to 17.1
Disease prevalence (*)	51.7%	
Positive predictive value (*)	47.3%	40.42% to 54.42%
Negative predictive value (*)	27.8%	5.18% to 73.15%
Accuracy (*)	44.0%	28.89% to 59.92%

Decimals rounded off. * These values are dependent on disease prevalence.

DISCUSSION

C. albicans is the most prevalent of the Candidal species where >80% of isolates are of this type.^[20] They can present in health and disease as yeast, pseudohyphae or hyphae.^[21,22] The progression from yeast to hyphae by a phenomenon called “switching” is considered critical in the expression of their virulence, adhesion, tissue penetration and secretion of proteases.^[12] Under favourable condition, the hyphae withstand adverse oral environment with the propensity for host tissue invasion by circumventing macrophageal phagocytosis.^[12,23-27] 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.^[15,28] 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.

Our results identified a sensitivity of 75.8% and specificity of 10.0% with a degree of concordance of 60.4% for

C. albicans. Seventeen of the 43 tests results between the observers showed disagreement and had to be misclassified 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 in archival records of OSCC.^[15] 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.^[29] 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.

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.

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