



Candidal Association With Oral Precancer And Cancer – A Candid View

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ABSTRACT

BACKGROUND : *Candida albicans*, a diploid asexual fungus, is an opportunistic pathogen which colonizes human mucosal epithelial cell surfaces. *C.albicans* are innocuous oral commensals and when optimal conditions supervene, they cause diseases. *C.albicans* is much more frequently detected in OSCC than in controls. Candidal infection along with other co-factors may also induce epithelial atypia and dysplasia leading to malignant transformation. The present study evaluates the association of *C.albicans* with potentially malignant and malignant lesions of oral cavity and compare with normal control group.

AIM : Quantitative analysis of candida albicans in formalin fixed paraffin embedded tissue blocks of oral leukoplakia, oral squamous cell carcinoma, oral submucous fibrosis, normal mucosa using Periodic Acid Schiff stain.

MATERIALS AND METHODS : 45 paraffin embedded tissue blocks which included 20 potentially malignant (10 - oral leukoplakia and 10 - OSMF), 15 OSCC and 10 normal mucosa which served as a control were stained with PAS stain and viewed under the microscope. All the observations were tabulated and the results obtained were subjected to statistical analysis. The Candidal load comparison between premalignant and malignant were tested with Mann Whitneys test. The observations from the four groups were subjected to Kruskal Wallis test.

RESULTS: Out of 45 biopsy tissues stained for presence of candida using PAS stain, 17 tissues showed Fungal forms. In OSCC (the *Candida albicans* count was more accounting for 53% whereas it was 40% of OSMF, 30% in oral leukoplakia and 20% in normal controls.

CONCLUSION : A significant association of candidal load were seen more often in malignant lesions than in premalignant lesions. However statistically no significant difference was seen (p - value = 0.085).

KEYWORDS : *Candida albicans* , oral dysplasia , pas stain , oral leukoplakia

INTRODUCTION:

Candida albicans, a diploid asexual fungus, is an opportunistic pathogen which colonizes human mucosal epithelial cell surfaces. *Candida albicans* (*C.albicans*) are innocuous oral commensals and when optimal conditions supervene, they cause diseases.¹

Previous studies have shown the association of *Candida* with various precancer and cancer lesions as a causative agent.^{2,3,4,5} In 1960s, it was reported that there is a possible association between *Candida* spp. and oral neoplasia,^{6,7} with later reports which suggested an association between the presence of *C.albicans* in the oral cavity and the development of oral squamous cell carcinoma.^{8,9,10} *C.albicans* is much more frequently detected in oral squamous cell carcinoma than in controls.¹¹

Studies have shown that there are some non- candidal oral mucosal lesions which are associated with candida including oral leukoplakia, oral submucous fibrosis.¹² Candidal infection along with other co-factors may also induce epithelial atypia and dysplasia leading to malignant transformation.¹

AIMS AND OBJECTIVES:

Quantitative analysis of *C.albicans* in oral leukoplakia (OL), oral squamous cell carcinoma (OSCC), oral submucous fibrosis (OSMF), normal mucosa.

- To Compare Candidal count in each lesion.
- To discuss the probable role of candida in the pathogenesis of these lesions.

MATERIALS AND METHODS:

The study is to quantitatively assess the presence of candidal species in Periodic Acid Schiff stained histopathologic sections of intraoral lesions. The present study comprised of total 45 formalin fixed paraffin embedded tissue blocks which included 20 potentially malignant (10- oral leukoplakia and 10- oral submucous fibrosis), 15- oral squamous cell carcinoma and 10 – normal mucosa which served as a control were stained with Periodic Acid Schiff stain and viewed under the microscope. PAS stain which is suitable to demonstrate the presence of fungal forms was used. The candidal hyphae in PAS stained sections were observed by counting the candidal species in each field at 40x magnification. All the observations were tabulated and the results were subjected to statistical analysis. The Candidal load comparison between premalignant and malignant were tested with Mann Whitneys test. The observations from the four groups were subjected to Kruskal Wallis test.

RESULTS:

Out of 45 biopsy tissues stained for presence of candida using PAS stain, 17 tissues showed Fungal forms. In oral squamous cell carcinoma, *C.albicans* count was more accounting for 53% (8 out of 15 tissues) whereas it was 40% (4 out of 10) in oral submucous fibrosis, 30% (3 out of 10) in oral leukoplakia and 20% (2 out of 10) in normal controls. Malignant lesions showed more candidal load compared to premalignant although statistically no significant differences (p - value = 0.085) were observed .

(table 1, Graph 1)

Table 1: Comparison of Mean candidal count among potentially - malignant and malignant cases

Comparison of Mean candidal load among potentially-malignant and malignant lesions using Mann Whitney U test at P<0.05										
Groups	N	Mean	SD	SE	Mean Diff	95% CI of the Diff		z	df	P-Value
						Lower	Upper			
Pre-Malignant Lesions	20	2.2	3.19	0.71	-2.067	-4.593	0.46	-1.724	33	0.085
Malignant Lesions	15	4.27	4.17	1.08						

Graph 1: Graph showing Mean candidal load more in malignant than in pre-malignant lesions

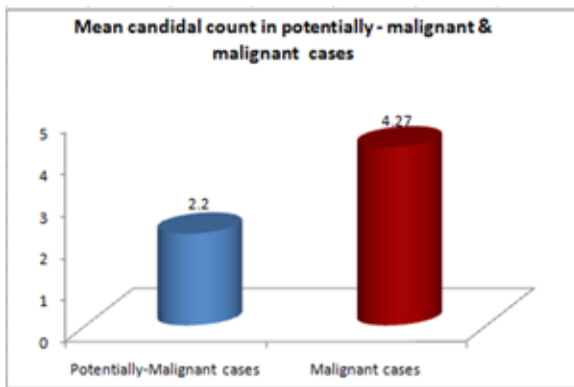


Table 2: Comparison of Mean Candidal Load among various study groups

Comparison of Mean Candidal load among various study groups using Kruskal Wallis Test at P<0.05										
Groups	N	Mean	SD	SE	95% CI for Mean		Min	Max	P-Value	
					Lower	Upper				
Oral Leukoplakia	10	1.5	2.46	0.78	-0.26	3.26	0	6	0.097	
OSMF	10	2.9	3.78	1.20	0.19	5.61	0	8		
OSCC	15	4.27	4.17	1.08	1.96	6.57	0	9		
Control	10	0.9	1.91	0.61	-0.47	2.27	0	5		

Graph 2: Showing Mean Candidal load in different study groups

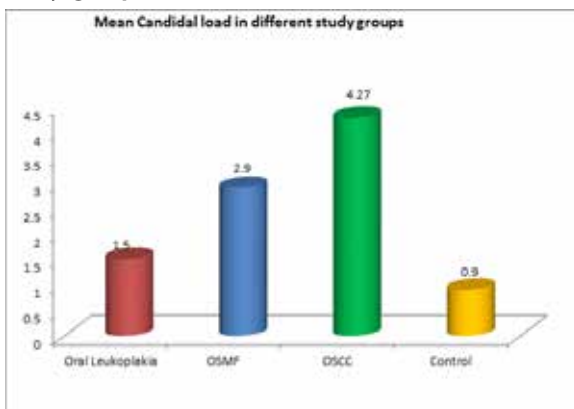


Figure 1: Candida albicans seen in superficial layers of epithelium in Oral leukoplakia

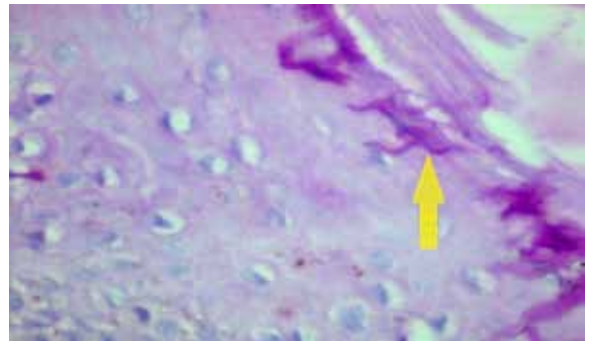


Figure 2: Candida albicans crossing the basement membrane zone in Oral submucous fibrosis

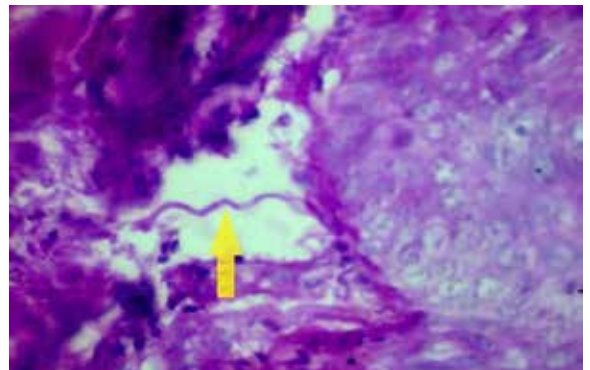
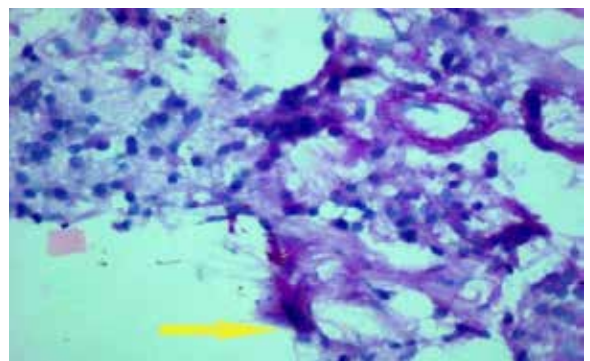


Figure 3: Candidal cluster seen in Oral squamous cell carcinoma in deeper connective tissue



DISCUSSION:

Candida is an opportunistic dimorphic yeast like fungus that are detectable in 40- 50% of individuals, the predominant species being C.albicans which can occur in both yeast and hyphae form.^{13,14} It has a potential to infect any tissue within the body, however its predominantly found in oral and vaginal mucosa.^{15,11} It is a normal commensal of oral mucosa and it can colonise the buccal mucosa, dorsum of tongue, in denture prosthesis. It can as well adhere to oral bacteria and pellicle of dental plaque.

The degree of colonization may vary from person to person which may be due to physiological changes related to age, body fluids, natural barriers against yeast colonization, habits of the individuals and changes to the ecological environment of the oral cavity.¹⁶ Oral cancer is one of the most common malignancy accounting for about 2-4% of all malignancies in developed countries. The causative role of the species in the development of oral cancer is explained by means of endogenous nitrosamine,^{17,18} oligosaccharide and lectin like component production.¹⁹

The present study was undertaken to detect the presence of *C. albicans* in potentially malignant & malignant oral mucosal lesions and to evaluate its role in oral carcinogenesis. To evaluate the presence of fungal organisms in deeper layers of the epithelium, histopathologic specimen were prepared. Many studies, such as those by Seto, Fotos *et al.*, and Barrett *et al.* recommended the use of PAS stain for the detection of fungi.^{20,21,22} Cawson and Cawson and Lehner demonstrated the presence of *Candida* in oral Candidal leukoplakia by using PAS was investigated.^{23,1}

In the present study, *Candida* was seen in 17 out of 45 biopsy tissues (37.7%). In oral squamous cell carcinoma (fig 3) the *C. albicans* count was more accounting for 53% (8 out of 15 tissues) whereas it was 40% (4 out of 10) in oral submucous fibrosis (fig 2), 30% (3 out of 10) in oral leukoplakia (fig 1) and 20% (2 out of 10) in normal controls. In malignant lesions, immunity of the patient is compromised, hence candidal load is increased in malignant compared to potentially malignant disorders. However no significant difference were observed between the above groups(p-value- 0.085). Among potentially malignant lesion & condition, oral submucous fibrosis had maximum candidal load followed by oral leukoplakia. It may also be possible that their increased colonization and prevalence in these lesions is entirely coincidental and merely reflects a changed local environment that allows for the proliferation of these common oral commensals.^{24,25} Thus the presence of candida itself may not be sensitive enough to draw any definite conclusion regarding malignant potential.¹²

In this study we have also considered the depth of candidal invasion

where in, candidal organisms are seen in deeper epithelium and crossing the basement membrane in oral squamous cell carcinoma, oral submucous fibrosis and oral leukoplakia. In addition, lot of inflammatory cells are seen in these lesions.

In oral leukoplakia, candidal hyphae were seen in superficial epithelium, basal cell layer and near basement membrane zone..

In oral submucous fibrosis, candida were seen deep into the connective tissue supporting its malignant potential.

In oral squamous cell carcinoma, more number of candida were seen in deeper epithelium and in connective tissue proving its malignant potential.

CONCLUSION :

A significant association of candidal load were seen more often in malignant lesions than in potentially malignant lesions. However no significant differences were observed between the discussed lesions to come to a definite conclusion. Histologically, candidal hyphae were observed which may indicate that the yeast have invaded the tissue, although they are not as sensitive as cultural methods. That would certainly influence the results, because of the uneven distribution of *C. albicans* throughout the oral cavity. To conclude, *C. albicans* seems to play a role in malignant transformation of oral leukoplakia, oral submucous fibrosis. This commensal appears as a reservoir of infection and when optimal conditions supervene they may cause disease. In potentially malignant lesions as the oral mucosa is compromised, it can be supported with a fact that the presence of candidal species does play a role in carcinogenesis by elaborating the nitrosamine compounds which may act directly on oral mucosa or interact with other chemical carcinogens to activate specific proto-oncogenes and there by initiate oral neoplasia. Thus the relationship of *C. albicans* with precancer and its malignant transformation is still debatable.

However, further studies are definitely required to correlate the association of candida and epithelial dysplasia in precancer and cancer cases, as to determine the nature of candida; whether it is one of the causal factors or is it a sole etiological factor.

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