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Isolation and Identification Of Candida Albicans In Smokers With Epithelial Dysplasia And Squamous Cell Carcinoma: A Histopathological Correlation.

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Abstract
Background:*Greater adaptability of Candida to the host niche makes it the most common opportunistic pathogen. Its role as a commensal or pathogen in development of premalignancies and its progression to malignancies is of considerable debate. Present study was undertaken to determine the role of Candida albicans(CA)in progressionof oral cancer by isolation and identification by mycological methods in smokers without oral lesions and histopathologically diagnosed cases of epithelial dysplasia (ED) and oral squamous cell carcinoma (OSCC).*
Methodology:*The study comprised of 120 cases that included study group of 40 smokers with oral lesions, 40 smokers without oral lesions and 40 non-smokers as control group. Clinical examination was followed by oral rinse sample collection and biopsy for histopathological confirmation. Sedimented oral rinse was inoculated on Sabouraud's dextrose agar (SDA). Isolated Candida colony after identification with Gram stain was subjected to germ tube and chlamydospore formation test for confirmation of CA. The data was statistically analysed using chi square test.*
Results:*Colonisation of CA was 52.5% in the study group and 5% in the control group, was found to be statistically significant (p<0.001).Association of CA in smokers with varying histopathologic grades of oral lesions showed no evidence of a correlation. However, positive correlation was found in OSCC (n=19, 95%) and showed statistical significance (p<0.001).*
Conclusion:*The present study showed an increase in colonization of CA in malignant lesions suggesting that CA in conjunction with tobacco usage may play a role in oral carcinogenesis.*
Keywords:*Candida albicans; saliva; smoking; squamous cell carcinoma; tobacco.*

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I. Introduction:

Incidence of oral cancer is particularly high among smokers and its etiology remains multifactorial. Oral microbes act as opportunistic pathogens, most notably *Candida* species.[1] In recent years, role of *Candida* infection is recognized as a significant factor in the development of potentially malignant disorders (PMD).[2]There is an enduring discussion whether *Candida* infection can be a cause of PMD or a superimposed infection in a pre-existing lesion.

A major virulent attribute of *Candida* is its ability to invade superficial layers of the epithelium, aided in particular by their hyphal appendages.[3] In addition alterations in diet, medications, habits and host immune status lead to overgrowth of minor components of oral microflora predisposing the site to disease. Among more than 150species of *Candida* isolated, approximately 10 colonize the oral cavity and*Candida albicans* (*C.albicans*) is the most prevalent speciesrecognized both in healthy and diseased (70 to 75% of isolates), followed by *Candidaglabrata* and *Candidatropicalis*(7% of isolates).[4]Although rarely fatal in the absence of other serious underlying disease, oral candidiasis serves as a useful clinical marker for the presence of significant predisposing conditions.[5]

The hyperplastic response of the epithelium when invaded by *Candida*has been confirmed.[6]*Candida albicans* is capable of promoting cancer by several mechanisms, such as production of carcinogenic byproducts, triggering of inflammation and molecular mimicry.[3]Leukoplakia with candidal infection or candidal leukoplakia has been shown to have a higher rate of malignant transformation than those not infected with *Candida.*[7]

Tobacco smoke exposure has been shown to promote microbial biofilm formation.[8]Specifically, data demonstrates that cigarette smoke favours *C. albicans* adhesion and growthand also promoted transition from blastospore to hyphal form promoting pathogenicity.[9,10]Thus smoking is a relative risk factor for the presence of *Candida* in the oral cavity. There is a strong dose-response relationship between the use of tobacco and the development of oral cancer.[11]It has been proposed that tobacco carcinogens act as initiators and *Candida* components as promoters according to classical theories of carcinogenesis.[12]

In individuals with leukoplakia and oral squamous cell carcinoma (OSCC), *C. albicans* produced higher levels of aspartyl proteinases which further accelerates colonization.[13]Nitrosamines, a chemical carcinogen produced by certain strains of *Candida,*which either act directly on oral mucosa or interact with other chemical carcinogens to activate specific proto-oncogenes and thereby initiating oral neoplasia.[14]Thus, a synergistic effect with candidiasis and life-style factors may exist in oral carcinogenesis.

The present study was conducted to identify the association of *C. albicans* speciesin the study groups comprising of OED & OSCC and normal control group using microbiological methods to understand the correlation between *Candida*, smoking habit and histopathological grading of oral lesions.

II. Materials And Methods

The study was conducted at K.V.G. Dental College & Hospital, Sullia and approved by the Institutional Research Ethics committee[IEC/KVGMC/10/2016]. A total of 120 outpatient subjects were included in the study after obtaining an informed consent from the patients. The patients were grouped as:

Group A (n=40): Study group of smokers with oral lesions(OED & OSCC) (Fig I).

Group B (n=40): Study group of smokers without oral lesions.

Group C (n=40): Healthy control group without smoking habits and without any oral lesion

The participants of the study group were only males due to social stigma about smoking in females and the regional culture. Therefore, the control group was also restricted to male participants.

Fig I: *Study group of smokers with oral lesions (OSCC)*

Oral sample collection:

A provisional diagnosis of the oral lesions was made clinically. Oral rinse sample was collected from study and control groups. Oral rinse samples were obtained by asking patients to rinse their mouths with 10 ml of phosphate-buffered saline (PBS; pH 7.2, 0.1 M) for 60 seconds and to expectorate the rinse into a sterile container.

Isolation and identification of Candida species:

The mouth rinse sample was centrifuged at 1700 g for 10 min, the sediment was inoculated on Sabouraud's Dextrose agar (SDA) and incubated for 48 h at 37ºC. The isolates were identified as *C. albicans* by colony morphology, Gram's stain, germ tube formation and chlamydospore demonstration. [15]

Very small inoculum from an isolated *Candida* colony was suspended in a test tube containing pooled human serum (0.5 mL). The mixture was incubated at 37°C for 2 h and examined for germ tube formation.

Confirmatory test for the identification of *C. albicans* was done by subjectingGerm tube-positive samples for chlamydospore production by inoculation and incubation in cornmeal agar. [16] (Fig II)

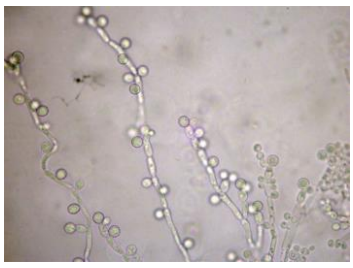

Fig II:Chlamydospore formation on corn meal agar(× 400X)

Histopathological evaluation:

Incisional biopsy or punch biopsy was obtained from the representative sites after applying toludine blue and under all aseptic precautions. Samples were processed according to standard procedures for histopathological evaluation.[17]

Statistical analysis was carried out using SPSS 20 software version. Chi square test was used to find the significance between the colonization of *C. albicans* among smokers in various study groups. Kruskal Wallis tests were used to analyse the comparison of colonisation among healthy control, smokers without lesion and smokers with lesion.

III. Results

Candida species was isolated from both study groups, whereas the healthy control group showed no growth. Isolation rate of *Candida* from group A and group B was 52.5%and 5% respectively. There was a highly significant (p<0.001) difference in the isolation rate of *Candida* among the groups (Table I).

Table I: Isolation rate of Candida albicans in various groups

Groups	**Colonization**		***p*-value**
	Positive No (%)	**Negative No (%)**	
Group A (n=40)	21 (52.5)	19 (47.5)	< **.001**
Group B (n=40)	38 (95)	02 (5)	
Group C (n=40)	0	40(100)	

In Group A, higher percentage of *Candida* isolated from the age group of 46-55 years9(42.9%) followed by 56-65 years 6(28.6%), 36-45 years 4(19%) and least from the age group of 65-75 years 2(9.5%).Culture positive samples when further subjected to germ tube and chlamydospore formation, only study group A showed positivity with statisticalsignificance(p< 0.001).

The rate of colonization of *Candida* was compared among the groups. The range of colony-forming units among cases of Group A varied from $15x10^3$ to $30x10^3$CFU/mL. Majority of cases in Group A (57.1%) showed a colonization in the range of $10x10^3$ to 20 $x10^3$ CFU/mL. Whereas in Group B, colony forming units were in the range of $3x10^3$ to $12x10^3$(Table II).

Table II: Distribution of colony-forming units in study groups.

Range of CFU	**Group A**	**Group B**
	No of cases (%)	**No of cases (%)**
$10x10^3$-$20x10^3$	12 (57.1)	02 (5)
$20x10^3$-$30x10^3$	08 (38.1)	-
$30x10^3$-$40x10^3$	01 (4.8)	-
Total	21 (52.5)	2 (5)

When isolation of *C. albicans* was compared among the grades of epithelial dysplasia, it showed positivity with one case each in moderate and severe grades of dysplasia. (Table III) When isolation of *C.albicans* was compared among the grades of squamous cell carcinoma, 19 (90.47%) cases showed positivity, among that 11 were well differentiated squamous cell carcinoma (WDSCC), 7 were moderate differentiated squamous cell carcinoma (MDSCC) and 1 poorlydifferentiated squamous cell carcinoma (PDSCC). Colonisation of *C. albicans* among smokers was compared with the histopathological grading of epithelial dysplasia and OSCC was not statistically significant (Table IV).

Table III: Colonization of C. albicans among histopathological grades of epithelial dysplasia

Histopathological grades of epithelial dysplasia	**Colonisation of *C.albicans***	**Total (N)**	***p*-value**	
Present **Absent**				
Mild	0	9	9	
Moderate	1	7	8	
Severe	1	1	2	
Total	2	17	19	**0.111**

Table IV: Colonization of C. albicans among histopathological grades of OSCC

Histopathological grades of OSCC	Colonisation of *C. albicans*		Total (N)	*p*-value
	Present	Absent		
WDSCC	11	0	11	
MDSCC	7	2	9	
PDSCC	11	0	11	
Total	**19**	**2**	**21**	**.229**

IV. Discussion:

Candida albicans is both a commensal and pathogen that exhibits yeast, hyphal, or pseudohyphal morphology.[18]Depending on the host defence mechanisms or local oral microenvironment, *Candida* can transform from a harmless commensal to the pathogenic organism causing oral mucosal infection.[19]Hence, identification of *Candida* species is crucial for successful clinical management.

In our study *Candida* isolation rate was found to be 52.5% in OED and OSCC and 12.5% in smokers without oral lesions, which was similar with previous studies.[8, 14, 17]This revealed a significant correlation between *Candida* colonization and the severity of oral lesions.

Age wise analysis showed a high positivity for *Candida* culture in the age group of 46-55 years among smokers with lesions. This was in accordance with studies conducted for isolation, identification, and carriage of *Candida* species.[8, 11]The result suggested that aging process does not act as a promoter, but act indirectly as predisposing factor. This could be due to reduced oxygen supply or reduced resistance of oral tissues. The participants of the study group were restricted to males. Thus, the influence of gender on yeast carriage could not be estimated in this study. Several other studies have shown absence of statistical significance of *Candida* growth in relation to gender.[20, 21]

We observed highly significant association of *Candida*in oral cancer than in precancer. Of the 21 cases of OSCC, 19 cases showed colonization. Culture positivity revealed a significant correlation between *Candida* colonization and the severity of oral lesions as observed by the histopathological grades of epithelial dysplasia and squamous cell carcinoma.

Studies based on histopathological staining and fluorescent staining techniques for *Candida* evaluation have shown the similar results.[21,22]In another comparative study, the association of *C. albicans* with malignant patients was highly significant ($p<0.001$) compared to other study groups. [23, 24]In agreement with other studies our results further fortifying the association of yeast and its role in malignant transformation.

Comparison of colony forming units among the groups showed the mean colony count to be equally distributed in different grades with minimum variation. It was observed that cases with a higher degree of malignancy had a higher frequency of isolation of *Candida* colony. Thus the persistent presence of *C.albicans*in smokers and increase with severity of oral lesions is proved in the present study. The precise mechanism through which oral *Candida* carriage is affected by tobacco still remains unclear. Earlier studies have tried to explain the possible mechanism.[1, 9, 10]Though the direct role of *C. albicans* in oral lesions is still debatable, our hypothesis suggests that *C. albicans* in conjunction with tobacco usage enhances the process of carcinogenesis.

V. Conclusion:

Candida albicans was significantly isolated in the group of smokers with malignant lesions in comparison to potentially malignant lesions. The results indicated that *C. albicans* plays a vital role in the advancement and deterioration of the condition as was seen associated with different grades of cancer. Thus we propose that *C. albicans* in association with smoking will enhance the process of carcinogenesis.

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References:

[1] Sanjaya Pr, Gokul S, Gururaj Patil B, Raju R.Candida In Oral Pre- Cancer And Oral Cancer. Med Hypotheses 2011;77(6):1125-8.

[2] Sankari Sl, Gayathri K, Balachander N, Malathi L. Candida In Potentially Malignant Oral Disorders. J Pharm Bioallied Sci 2015;7(1):162-4.

[3] Mohd Bakri M, Mohd Hussaini H, Rachel Holmes A, David Cannon R, Mary Rich A. Revisiting The Association Between Candidal Infection And Carcinoma, Particularly Oral Squamous Cell Carcinoma. J Oral Microbiol2010;21(2):1-6.

[4] MeurmanJh, Siikala E, Richardson M, Rautemaa R. Non-Candida Albicans Candida Yeasts In The Oral Cavity. Méndez-Vilas A. Ed. Communicating Current Research And Educational Topics And Trends In Applied Microbiology. Formatex Microbiology. Badajoz 2007;1(2):719-31.

[5] Lynch Dp.Oral Candidiasis. History, Classification, And Clinical Presentation. Oral Surg Oral Med Oral Pathol 1994;78(2):189-93.

[6] Brock M. Fungal Metabolism In Host Niches. Curr OpinMicrobiol2009;12:371-6.

[7] Mccullough M, Jaber M, Barrett Aw. Oral Yeast Carriage Correlates With Presence Of Oral Epithelial Dysplasia. Oral Oncol 2002;38(4):391-3.

[8] Muzurović S, Hukić M, Babajić E, Smajić R. The Relationship Between Cigarette Smoking And Oral Colonization With Candida Species In Healthy Adult Subjects. Med Glas (Zenica) 2013;10(2):397-9.

[9] Semlali A, Killer K, Alanazi H, Chmielewski W, Rouabhia M. Cigarette Smoke Condensate Increases C. Albicans Adhesion, Growth, Biofilm Formation, And Eap1, Hwp1 And Sap2 Gene Expression. BmcMicrobiol 2014;14(61):1-9.

[10] Alanazi H, Semlali A, Perraud L, Chmielewski W, Zakrzewski A, Rouabhia M. Cigarette Smoke-Exposed Candida Albicans Increased Chitin Production And Modulated Human Fibroblast Cell Responses. Biomed Research International. Volume 2014, Article Id 963156. 11 Pages. Doi.Org/10.1155/2014/963156.

[11] Pelucchi C, Gallus S, Garavello W, Bosetti C, Vecchia Cl. Cancer Risk Associated With Alcohol And Tobacco Use: Focus On Upper Aero Digestive Tract And Liver. Alcohol Res 2006;29(3):193-198.

[12] Arendorf Tm, Walker Dm, Kingdom Rj, Roll Jr, Newcombe Rg.Tobacco Smoking And Denture Wearing In Oral Candidial Leukoplakia. Br Dent J 1983;155:340-3.

[13] Krogh P. The Role Of Yeasts In Oral Cancer By Means Of Endogenous Nitrosation. Acta Odontol Scand 1990; 48:85-8.

[14] Saigal S, Bhargava A, Mehra Sk, Dakwala F. Identification Of Candida Albicans By Using Different Culture Medias And Its Association In Potentially Malignant And Malignant Lesions. Contemp Clin Dent 2011; 2(3):188-93

[15] White Pl, Williams Dw, Kuriyama T, Samad Sa, Lewis Ma, Barnes Ra.Detection Of Candida In Concentrated Oral Rinse Cultures By Real-Time Pcr.J Clin Microbiol 2004;42(5):2101-7.

[16] Fisher F, Look Mb. Fundamentals Of Diagnostic Mycology. 2nded. Wb Saunders Co., Philadelphia;1998. P.200-202.

[17] Rajendran R. Shafer's Textbook Of Oral Pathology. 6th Ed. Elsevier India, 2009. P 91-92.

[18] Pei-Wen Tsai, Yu-Ting Chen, Po-Chen Hsu, Chung-Yu Lan.Study Of Candida Albicans And Its Interactions With The Host: A Mini Review. Biomedicine 2013; 3(1):51–64.

[19] Bouza E, Muñoz P. Epidemiology Of Candidemia In Intensive Care Units. Int J Antimicrob Agents 2008; 32 (Suppl 2):87-91.

[20] Dany A, Kurian K, Shanmugam S. Association Of Candida In Different Stages Of Oral Leukoplakia. Jiaomr 2011;23(1):14-6.

[21] Hebbar Pb, Pai A, Sujatha D. Mycological And Histological Associations Of Candida In Oral Mucosal Lesions. J Oral Sci 2013; 55(2):157-60.

[22] Odedra S, Chawda J, Rupapara R, Rupakar P. Presence Of Candida In Oral Dysplastic Lesions - A Casual Involvement Or A Causal Role?. Indian Journal Of Public Health Research& Development 2013; 4(3):267-71.

[23] Srinivasprasad V, Dineshshankar J, Sathiyajeeva J, Karthikeyan M, Sunitha J, Ragunathan R. Liaison Between Micro-Organisms And Oral Cancer. Journal Of Pharmacy &Bioallied Sciences. 2015;7(Suppl 2):S354-S360. Doi:10.4103/0975-7406.163451.

[24] Gall F, Colella G, Di Onofrio V, Rossiello R, Angelillo If, Liguori G. Candida Spp. In Oral Cancer And Oral Precancerous Lesions. New Microbiol 2013;36(3):283-8.