

# Isolation of Candida by using Sabouraud's Dextrose Agar Media and its Association with Oral Mucosal Lesions

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**Abstract:** Background: Candida is the most common fungal pathogen in the human oral cavity. So they have the ability to cause opportunistic infection called as oral candidiasis. This study was designed to isolate Candida by using Sabouraud's Dextrose agar media and its association with Oral mucosal lesions. Methods: 45 patients of clinically diagnosed oral mucosal lesions, 5 each was of Leukoplakia, Lichen Planus, Lichenoid Reaction, Chewer's Mucosa, Squamous cell Carcinoma, Angular Cheilitis, Denture Stomatitis, Aphthous ulcers were selected. Samples were collected from oral mucosal lesions for the candidal growth. The data obtained was statistically analyzed by using Chi Square test using SPSS version 16. Results: Out of 45 patients, 14 showed positive Candidal growth in which 5 Cases were of Leukoplakia, 3 of Lichen Planus, 2 each of Fibroma, Squamous cell carcinoma, Denture stomatitis. Conclusion: 14 Patients with Oral Mucosal Lesions showed positive fungal growth, so these patients are more susceptible for developing Oral Candidiasis as compared to the lesions with no Candidal growth.

**Keywords:** Candida, Sabouraud's Dextrose agar media, Oral mucosal lesions, Candidiasis

## 1. Introduction

Candida is harmless commensal of the healthy human oral cavity [1] and the most common fungal pathogen is Candida Albicans. [2] Any alteration in the balance of flora or in debilitation of the host or environment can lead to overgrowth of fungus and infection to the host. [3], [4] So, Oral candidiasis is the most common opportunistic fungal infection affecting humans. [5] About 40-60% of healthy adults harbor commensals Candida in the oral cavity with or without any signs or symptoms of Candidiasis. [6], [7]

Overgrowth of Candida is facilitated by local and systemic predisposing factors including immunologic imbalances, genetics and malignant diseases, ill fitting dentures, tobacco smoking. [7] Candida associated denture stomatitis is a common infection in most of the denture wearers. [8], [9], [10]

Studies have shown that Candida infection associated with Oral Mucosal Lesions, for example Candidal Leukoplakia carries a significant risk of malignant malformation [11], [12], [13], [14] and also Candida plays a causative role in carcinogenesis as they are capable of producing endogenous nitrosamines, oligosaccharide and lectin – like component production. [11], [15], [16], [17] Many non Candida oral mucosal lesions are reported to be associated with Candida including Leukoplakia, Oral Lichen Planus, Fibroma, [19], [20] Actinic Cheilitis, Lichenoid Reaction, Chewers Mucosa, Denture Stomatitis and Aphthous Ulcers.

There is an increased colonization and prevalence of Candida in these oral mucosal lesions because of an altered

local environment that favors proliferation of the oral fungal commensals. [5], [20] Association of Candida with oral mucosal lesions will help to initiate anti-mycotic therapy as first line treatment prior to any other treatment. [5], [20] The present study was carried out to isolate Candida by using Sabouraud's Dextrose agar media and its association with Oral mucosal lesions.

## 2. Material and Methods

45 subjects of either sex with clinically diagnosed cases of Oral mucosal lesions were selected from the patients reporting to the outpatient department of Oral Medicine and Radiology, KLE V.K. Institute of Dental Sciences and Research centre, Belagavi. Subjects willing to participate in the study and signed an informed consent and were included in the study. Patients with systemic diseases, receiving radiotherapy, steroid therapy and under long term local and systemic drug therapy, also immunocompromised patients were excluded.

Among 45 patients, 5 each were of Leukoplakia, Lichen Planus, Lichenoid reaction, Chewer's mucosa, Squamous cell carcinoma, Angular Cheilitis, Denture Stomatitis, Aphthous ulcers. Patients were examined on dental chair with artificial light using mouth mirror and probe. Routine oral examination was carried out and the site of lesion was evaluated by the examiner. The entire procedure was explained to the patients in their local language.

The samples were collected from oral mucosal lesions using a sterile cotton swab. The swabs were inserted in the tube which were kept at room temperature and was carried to

Basic Science Research Laboratory at KLE Dental College, Belagavi for analysis of Candidal growth. Each sample was inoculated onto Sabouraud Dextrose Agar media for 48-72 hours at 37°C and the culture was inspected on a daily basis for yeast growth on inoculated plates. Candida appeared as white colored colonies. [Figure 1]

The candidal growth was recorded and the data obtained was tabulated. The data obtained was statistically analyzed by using Chi Square test using SPSS version 16.

### 3. Results

Total of 45 patients participated in the study and 5 each were of Leukoplakia, lichen Planus, lichenoid reaction, chewer's mucosa, Squamous cell carcinoma, Angular Cheilitis, Denture stomatitis, aphthous ulcers were selected. Out of 45 patients, 14 showed positive Candidal growth in which 5 Cases were of Leukoplakia, 3 of Lichen Planus, 2 each of Fibroma, Squamous cell carcinoma, Denture stomatitis [Table 1]. Positive Candidal growth was seen in 11 Males and 3 Female patients [Table 2]. 11 patients showed the presence of Candidal growth in buccal mucosa and 3 patients were found positive for Candidal growth in Palate [Table 3].

Statistically significant difference was found in patients with Oral mucosal lesions showing the presence or absence of Candidal growth with  $p$  value  $< 0.005$ . No significant results were found with respect to gender and site in patients with Candida positive oral mucosal lesions.

### 4. Discussion

Candida is normal commensals fungi that are found colonizing the oral mucosa and is also "opportunistic pathogens". [3], [20] Depending on the host defense mechanisms or local oral microenvironment, Candida can transform from a harmless commensals to the pathogenic organism causing oral mucosal infection. [6], [9], [21]

Candida albicans has the ability for the development of pathological conditions and premalignant changes. [11], [12], [21] Candida Albicans was considered an etiologic factor for potentially malignant disorders as it can produce carcinogenic compounds, like nitrosamines, N-nitrosobenzylmethylamine. [3], [21] Studies have shown link between the presence of Candida albicans in the oral cavity and the development of oral Squamous cell carcinoma. [15], [18], [21] Also, the pathogenic mechanism of Candida involved in carcinogenesis is because of the ability to convert nitrite and nitrate in nitrosamines and other substances to produce acetaldehyde. [13], [17], [19], [22] Candida albicans has also been implicated in oral and esophageal cancer development. [4], [22] Candida also acts as a cofactor in Denture Stomatitis. Leukoplakia with candidal infection has been shown to have a higher rate of malignant transformation than in those not infected with Candida. [23] Hence in the current study Candida isolation and its association with different mucosal lesions was evaluated.

In the present study, a total of 45 subjects of with clinically diagnosed cases of Oral mucosal lesions were included. The samples were collected and inoculated onto Sabouraud Dextrose Agar media and the culture was inspected for yeast growth which revealed that there was statistically significant difference in patients with Oral mucosal lesions with Candidal growth. So, patients with positive growth with or without clinical signs and symptoms initiate the use of antifungal therapy. [1], [23]

In the present study different Candida species were not considered. The Identification of Candida species which are responsible for of human infections is important in successful clinical management and for determining appropriate control measures to prevent transmission of resistant candidal pathogens. [24] Also the Culture method is reliable to assess the presence of Candida species in advance and possibly establish a treatment for oral mucosal lesions on the basis of the antifungal susceptibility patterns shown by isolated strains' [2], [24] The present data should be considered preliminary, further studies with larger samples should be carried out to substantiate the results of the present study.

### 5. Conclusion

Patients with Candida positive growth in patients with Leukoplakia, Lichen Planus, Denture Stomatitis, Squamous Cell Carcinoma and Fibroma were more susceptible for developing Oral Candidiasis as compared to the lesions with no Candidal growth.

**Table 1**

Lesion	Positive Growth of Candida	No growth of Candida	p value
Leukoplakia	5 100.0%	0 0.0%	0.004
Lichen Planus	3 60.0%	2 40.0%	
Lichenoid Reaction	0 0.0%	5 100.0%	
Chewers Mucosa	0 0.0%	5 100.0%	
Squamous Cell Carcinoma	2 40%	3 60%	
Fibroma	2 40%	3 60%	
Aphthous Ulcer	0 0.0%	5 100.0%	
Denture Stomatitis	2 40%	3 60%	
Angular Cheilitis	0 0.0%	5 100.0%	

Table 1 showing the presence or absence of candidal growth in patients with Oral mucosal lesions

**Table 2**

Gender	Positive Growth of Candida	No Growth of Candida	p Value
Male	11 40.7%	16 59.3%	0.087
Female	3 16.7%	15 83.3%	

Table 2 showing gender distribution among the patients with presence or absence of Candidal growth in Oral mucosal lesions

**Table 3**

Localization	Positive Growth of Candida	No Growth of Candida	p Value
Buccal Mucosa	11 39.3%	17 60.7%	0.144
Tongue	0 0.0%	2 100%	
Palate	3 60.0%	2 40.0%	
Buccogingival Complex	0 0.0%	2 100%	
Alveolar Ridge of Mandible	0 0.0%	3 100%	
Corner of Mouth	0 0.0%	5 100%	

Table 3 showing the site of obtaining swab among the patients with presence or absence of candidal growth in Oral mucosal lesions

**Figure 1**



**Figure 1:** Showing white coloured colonies of Candida

## References

- [1] Neppelenbroek KH, Seo RS, Urban VM, Silva S, Dovigo LN, Jorge JH, et al. Identification of Candida species in the clinical laboratory: a review of conventional, commercial, and molecular Techniques. Oral Diseases 2013; 20: 329–344.
- [2] Galle F, Colella G, Onofrio VD, Rossiello R, Angelillo IF, Liguori G. Candida spp. in oral cancer and oral precancerous lesions. New Microbiologica, 2013; 36:283-288.
- [3] Sankari SL, Gayathri K, Balachander N, Malathi L. Candida in potentially malignant oral disorders. J Pharm Bioallied Sci. 2015 ; 7: 162 –164.
- [4] Bakri MM, Hussaini HM, Holmes AR, Cannon RD, Rich AM. Revisiting the association between candidal infection and carcinoma, particularly oral Squamous cell carcinoma. J Oral Microbiol. 2010; 2, 3030–44.
- [5] Hebbar PB, Pai A, Sujatha D. Mycological and histological associations of Candida in oral mucosal lesions. Journal of Oral Science 2013; 55: 157-160.
- [6] Bouza E, Munoz P. Epidemiology of candidemia in intensive care units. Int J Antimicrob Agents.2008 ;32:87–91
- [7] Shenoy MP, Puranik RS, Vanaki SS, Puranik SR, Shetty P, Shenoy R. A comparative study of oral Candidal species carriage in patients with type1 and type2 diabetes mellitus. J Oral and Maxillofac Pathol 2014; 11: 60–65
- [8] Thein ZM., Seneviratne CJ, Samaranayake YH, , Samaranayake LP. Community lifestyle of *Candida* in mixed biofilms: a mini review. Mycoses.2009; 52:467-75
- [9] Marol S, Yuesoy M. Molecular epidemiology of Candida species isolated from clinical specimens of intensive care unit patients. Mycoses. 2008 Jan;51:40–9
- [10] Dahlen G, Linde A, Moller AJ, Ohman A. A retrospective study of microbiologic samples from oral mucosal lesions. Oral Surg Oral Med Oral Pathol.1982; 53:250-5
- [11] McCullough M, Jaber M., Barrett AW, Bain L, Speight PM, Porter SR. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncology 2002; 38: 391–393
- [12] Rindum JL, Stenderup A, Holmstrup P. Identification of Candida albicans types related to healthy and pathological oral mucosa. J Oral Pathol Med.1994; 23:406-12.
- [13] Sitheeque M, Samaranayake LP, Chronic Hyperplasic Candidosis/Candidiasis (Candidal Leukoplakia). CROBM.2003;14:253-67
- [14] Cawson R.A. Leukoplakia and oral cancer. Proc roy Soc Med.1969;62:610-614
- [15] Daftary DK, Mehta FS, Gupta PC, Pindborg JJ. The Presence of Candida in 723 Oral Leukoplakias among Indian Villagers. Scand J dent. Res. 1972; 80:75–79
- [16] Farah CS, Lynch N, and McCullough MJ. Oral fungal infections: an update for the general practitioner. Aust Dent J.2010; 55:48-54
- [17] Rossano F, Gombos F, Liguori G, Gaeta GM, Esposito C, Serpico R, et al. Malignant transformation of oral Lichen Planus by Candida albicans. Int J Immunopathol Pharmacol. 1993 ;6 :125-134
- [18] 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: 148-151
- [19] Scardina GA, Ruggieri A., Messina P. Chronic hyperplasic Candidosis: a pilot study of the efficacy of 0.18% isotretinoin. J Oral Sci.2009; 51: 407- 410.
- [20] Singh Siddharth Kumar, Gupta Anjali, Rajan SY, Padmavathi BN, Mamatha GP, Mathur Hemant, et al. Correlation of Presence of Candida and Epithelial Dysplasia in Oral Mucosal Lesions. J Clin Diagn Res. 2014;8: 31-35
- [21] Rodriguez MJ, Schneider J, Moraques MD, Martinez-Conde R, Ponton J, Aquirre JM. Cross-reactivity between Candida albicans and oral Squamous cell carcinoma revealed by monoclonal antibody C7. Anticancer Res.2007 ;27: 3639-3643
- [22] Samaranayake LP, Keung Leung W, Jin L. Oral mucosal fungal infections. Periodontol. 2009; 49:39-59

- [23] Sarkar R., Rathod GP, Clinicopathologic assessment of Candida colonization of oral Leukoplakia. Indian J of Dermatol Venereol Leprol. 2014 ;80:413-8
- [24] Mehdipour Masoumeh, Zenouz Ali Taghavi, Hekmatfar Somayeh, Adibpour Mohammad, Bahramian Aila, Khorshidi Reza . Prevalence of Candida Species in Erosive Oral Lichen Planus. J Dent Res Dent Clin Dent Prospect. 2010 ; 4: 14–16

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