

## ORIGINAL ARTICLE

# Comparison of salivary flow and candidal carriage in patients with oral submucous fibrosis

Bhavana Gupta, Shaleen Chandra<sup>1</sup>, Vineet Raj, Vivek Gupta<sup>2</sup>

Department of Oral Pathology, Saraswati Dental College and Hospital, <sup>1</sup>Department of Oral Pathology, King George's Medical University, Lucknow, <sup>2</sup>Department of Periodontology, Rama Dental College and Hospital, Kanpur, Uttar Pradesh, India

## Address for correspondence:

Dr. Bhavana Gupta,  
C-2/600 Sector C, Jankipuram, Lucknow,  
Uttar Pradesh, India.  
E-mail: drbhavanagupta02@gmail.com

Received: 04-02-2015

Accepted: 12-08-2015

## ABSTRACT

**Background:** Oral submucous fibrosis (OSMF) is a high-risk precancerous condition that predominantly affects Indians due to prevalent gutka chewing. Changes in the salivary flow rate and its effect on candidal carriage in patients suffering from OSMF have not been extensively explored. **Aim:** The aim of this study is to compare the salivary flow rate and salivary candidal carriage in OSMF patients and healthy individuals. **Materials and Methods:** This pilot study included a total of 30 OSMF patients and 30 healthy individuals. Salivary flow was estimated using preweighed cotton rolls placed at the openings of major salivary duct for 5 min. The cotton rolls were then removed from the oral cavity and weighed again. The difference in weight was recorded. Salivary samples were collected by the oral rinse technique and cultured on Sabouraud agar medium. The cultured yeast colonies were identified based on Gram's staining, periodic acid-Schiff (PAS) and germ tube formation. **Result:** There was statistically significant ( $P < 0.001$ ) decreased salivary flow rate in OSMF individuals as compared to the control. Salivary flow rates constantly reduced with different grades of OSMF patients, although candidal carriage was seen in grade II and grade III OSMF patients. **Conclusion:** A higher incidence of *Candida* was observed in OSMF patients when compared to the healthy individuals. The results of our study suggest that a higher candidal carriage in grade II and grade III OSMF patients could be related to decreased salivary flow rate.

**Key words:** *Candida albicans*, oral submucous fibrosis, salivary flow rate, Sabouraud dextrose agar

## INTRODUCTION

Oral submucous fibrosis (OSMF) is a well-known precancerous condition, which clinically presents with reduced mouth opening, decreased salivary flow rate, blanching of oral mucosa and burning sensations.<sup>[1]</sup> Submucous fibrosis is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx, occasionally, it is preceded by and/or associated with vesicle formation and is always associated with a juxta-epithelial inflammatory reaction followed by progressive hyalinization of the lamina propria. The later subepithelial and submucosal fibrosis leads to stiffness of the oral mucosa and deeper tissues with progressive limitation in

opening of the mouth and protrusion of the tongue, thus causing difficulty in eating, swallowing and phonation. Epithelial atrophy is marked in advanced stages of the disease.<sup>[2-4]</sup>

The name *Candida* is derived from a custom in ancient Rome for a *candidatus*, a candidate for public office, to dress in white. *Albico* means "to be white," so the name *Candida albicans* is redundant. Among more important pathogenic species, *C. albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida lusitanae* and *Candida glabrata*,<sup>[5]</sup> *C. Albicans* is the primary cause of oral candidiasis. *Candida*

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

**How to cite this article:** Gupta B, Chandra S, Raj V, Gupta V. Comparison of salivary flow and candidal carriage in patients with oral submucous fibrosis. J Oral Maxillofac Pathol 2015;19:158-63.

## Access this article online

### Quick Response Code:



### Website:

www.jomfp.in

### DOI:

10.4103/0973-029X.164526

species are opportunistic organism which shows lesions in patients with reduced salivary flow.<sup>[6]</sup> The presence of *Candida* in the mouth together with epithelial changes may predispose individuals to candidal infection. Epithelial changes of the oral mucosa, such as atrophy, hyperplasia and dysplasia, may compromise the mucosal barrier and facilitate candidal invasion, especially in the event of epithelial atrophy.<sup>[7]</sup> These opportunistic fungal pathogens may colonize, invade and induce lesions in any part of the oral cavity in both immunocompetent and immunocompromised individuals. However, those who are immunocompromised are affected at a significantly higher frequency than healthy individuals.<sup>[8]</sup> According to Shinozaki *et al.*, the salivary flow rate is negatively correlated with candidal carriage.<sup>[9]</sup>

Hence, the present study was undertaken to compare and assess the salivary flow rate and salivary candidal carriage in the OSMF patients and healthy individuals.

## MATERIALS AND METHODS

The present study comprised sixty patients, 30 OSMF patients of different grades (nine patients of grade I OSMF, 14 patients each of grade II and III, respectively and two patients of grade IV OSMF) and control group comprised 30 healthy individuals who were not having any relevant medical, dental and habit history. Candidal infection was not suspected in these patients. The systemic illnesses have been ruled out in all these OSMF patients. There was no history of any treatment such as antibiotic or steroid usage in any of these patients. All the OSMF patients were divided into four groups according to severity, following the criteria from a study done by Ranganathan *et al.*<sup>[10]</sup> The criteria considered was mouth opening that is graded as follows:

Grade I: Only symptoms, with no demonstrable restriction in mouth opening

Grade II: Limited mouth opening, twenty millimeters and above

Grade III: Mouth opening <20 mm

Grade IV: OSMF advanced with limited mouth opening. Precancerous or cancerous changes were seen throughout the mucosa.

Salivary flow was estimated using preweighed cotton rolls placed at the openings of major salivary gland duct for 5 min. The cotton rolls were then removed from the oral cavity and weighed again. The difference in weight was recorded. Salivary samples were collected by the oral rinse technique<sup>[10]</sup> and cultured on Sabouraud dextrose agar (SDA) medium.

The tests performed for the identification of *Candida* species were Gram stain, periodic acid-Schiff (PAS) technique and germ tube test.

## Preparation of culture media

### For fungus growth

One hundred gram of SDA was mixed in distil water to obtain a uniform suspension. It was heated and boiled and then sterilized in an autoclave at a temperature of about 121–123°C for 15 min. A drop of streptomycin was added before pouring the solution in a test tube. A slant is prepared in the test tube for the growth.

### For germ tube test

According to Chin and Saat<sup>[11]</sup> ever since *C. albicans* was reported to form germ tubes on incubation in serum for 3 h at 37°C, the germ tube test has been widely used to identify and differentiate *C. albicans* from other species of *Candida*. According to Saigal *et al.*,<sup>[8]</sup> human serum from patient's venous blood was collected and kept vertically to form clot; then it was centrifuged in centrifuge machine for about 3000 rpm for 10 min. About 0.3–0.5 mL of human serum obtained from this method was used for the detection of germ tube. A small inoculum from an isolated candidal colony was picked up by sterile inoculating loop and mixed it with the human serum in a disposable sterile container. The mixture was incubated at 42°C for 2–3 h. A drop of obtained mixture is kept on a clean glass slide and covered with a coverslip. This was then examined under a microscope.

### Saliva culture technique for candida

The subjects in both groups were asked to rinse their mouth with 10 mL of phosphate buffered saline for 2 min and then expectorate in a sterile container. The samples were then centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the remaining material was inoculated in SDA media. The samples were incubated at 37°C for 48 h. The growth appeared in 48 h as cream/white colored, smooth and pasty colonies [Figure 1].



Figure 1: Candidal colony in Sabouraud dextrose agar media

### Statistical analysis

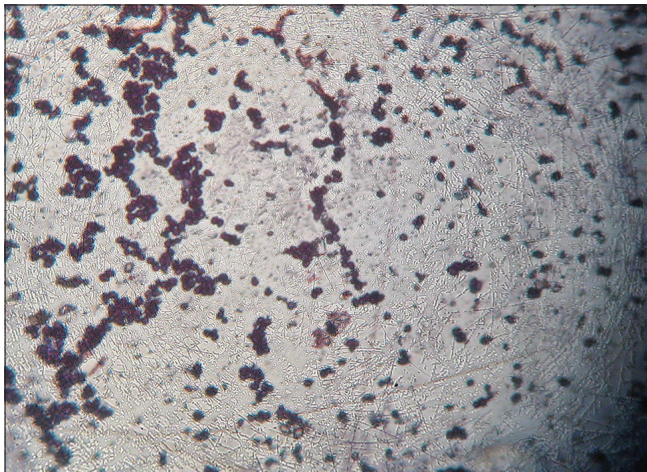
Student *t*-test and Chi-square test were done to compare between two groups.

### RESULTS

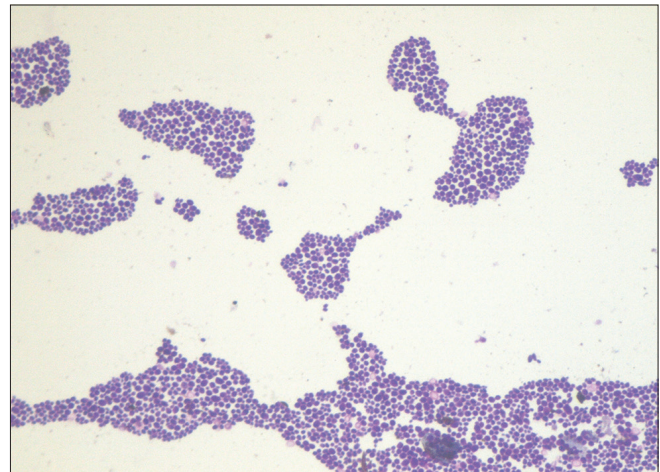
Three tests were performed for the identification of *Candida* species. They were Gram stain, PAS technique and germ tube test.

- Gram stain- Gram staining, showing candidal spores [Figure 2]
- PAS technique - showing candidal yeast cells was recorded as a positive finding [Figure 3]
- Germ tube test- A germ tube was seen in the wet smear preparation, obtained from human serum [Figure 4] which confirmed the presence of fungal hyphae in the wet smear [Figure 5] (according to Saigal *et al.*<sup>[8]</sup> and Chin and Saat<sup>[11]</sup>).

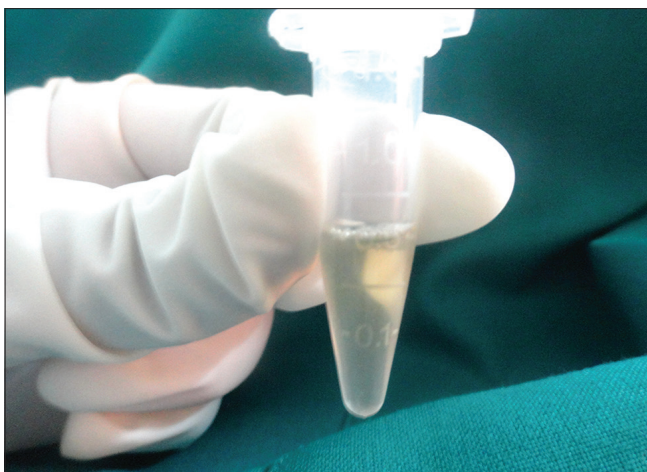
When comparing saliva flow rate between OSMF patients and control group, there was a statistically significant ( $P < 0.001$ ) decrease in the salivary flow rate in OSMF individuals as compared to the control [Graph 1]. The results also showed that there was a constant decrease in the salivary flow rate among different grades of OSMF patients [Graph 2] which was statistically significant ( $P < 0.001$ ). There were absence of candidal carriage in healthy individuals (control) as well as in grade I and grade IV OSMF patients as compared to grade II and grade III OSMF patients (grade III, IV) which were also statistically significant ( $P < 0.001$ ) [Graph 3 and 4]. There was a statistically significant difference in the salivary flow rate and candidal carriage in the OSMF patients as compared to the healthy individual/control group ( $P < 0.001$ ) [Graph 5]. Salivary flow rate was constantly reduced with different grades of OSMF patients, although candidal carriage was seen in grade II and grade III OSMF patients.



**Figure 2:** Gram staining, showing candidal spores. (Gram stain, x100)



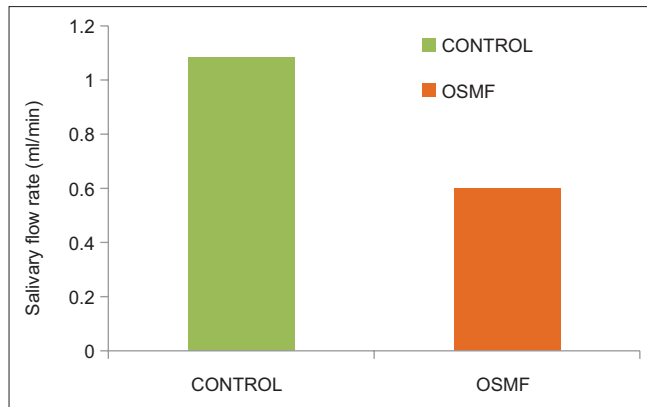
**Figure 3:** Periodic acid-Schiff technique showing candidal yeast cells recorded as a positive finding. (PAS stain, x100)



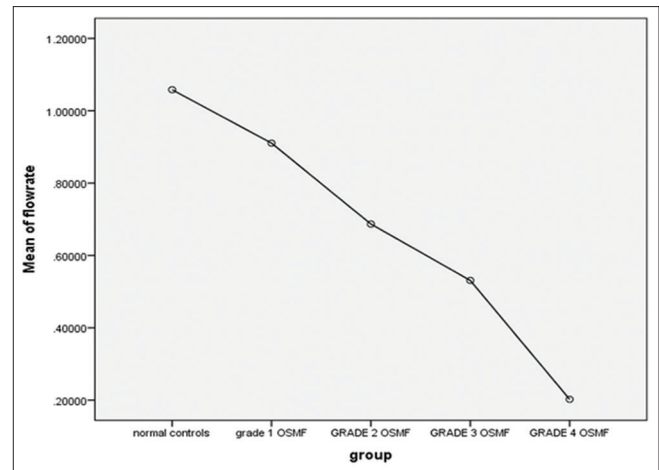
**Figure 4:** Human serum containing small inoculum from an isolated candidal colony



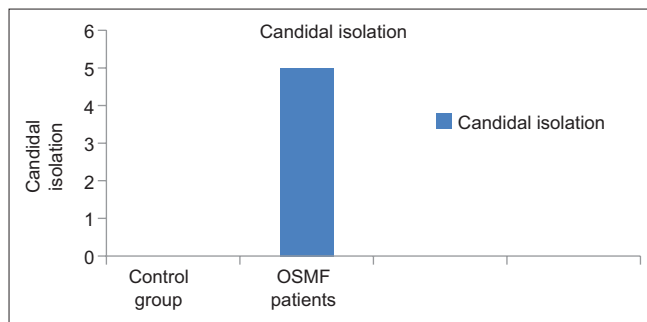
**Figure 5:** Germ tube formation in human serum.(Wet smear, x400)



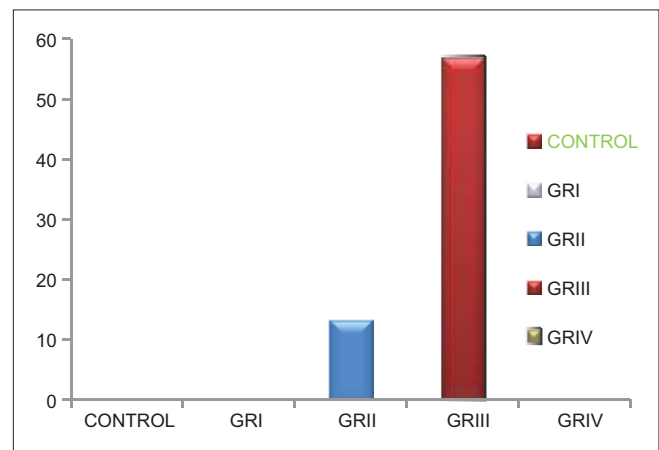
**Graph 1:** There was statistically significant ( $P < 0.001$ ) decreased salivary flow rate in the oral submucous fibrosis individuals as compared to the control./changes in the salivary flow rate between control and oral submucous fibrosis as a single group



**Graph 2:** Changes in the salivary flow rate between the groups of oral submucous fibrosis



**Graph 3:** Comparison of candidal isolation between control and oral submucous fibrosis as a single group



**Graph 4:** Comparison of candidal isolation between control and different grades of oral submucous fibrosis patients

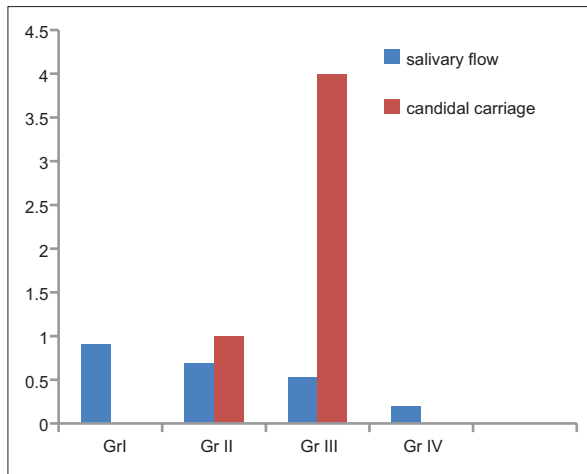
## DISCUSSION

Healthy individuals carry 3–47% of *Candida* species as a component of normal oral flora.<sup>[12]</sup> The predominant species is *C. albicans*, which has the potential to infect any tissue within the body under immunocompromised conditions. An association between *Candida* and various precancerous and cancerous lesions has been reported in the literature.<sup>[13]</sup> Candidal carriage varies in individuals from 1.0% to 80.6% depending upon the population surveyed. A delicate balance clearly exists between the potentially damaging effects of candida virulence factors and the nature of the immune response elicited by the host. Frequently, it is changes in the host factors that lead to candida seemingly changing from a commensal to pathogenic existence. However, given the often reported heterogeneity in morphological and biochemical factors that exist between *Candida* species and indeed strains of *C. albicans*, it may also be the fact that colonizing strains differ in the way they exploit the resources, to allow persistence at mucosal surfaces and as a consequence this too may affect the way *Candida* interacts with epithelial cells.<sup>[14]</sup>

Results of our study suggest an inverse relationship between salivary flow and candidal colony, which was in accordance

to the study of Torres *et al.*<sup>[15]</sup> Factors such as poor oral hygiene, denture wearing, oral epithelial dysplasia, smoking habits, orthodontic appliances, mouth breathing and chronic irritants have been found to be the local factors that predispose to candidiasis.<sup>[16]</sup> Shinozaki *et al.* compared OSMF and healthy individual with xerostomia and found significantly decreased salivary flow rate, increased rate of oral mucosal symptoms and higher numbers of *Candida* in OSMF patients than a healthy individual. Salivary flow rate was negatively correlated with the number of *Candida*, which is in agreement with the findings of our study.<sup>[9]</sup> Our result was in accordance with the result of Saigal *et al.*, which found no fungal growth in the normal control group and 3 out of 15 cases of OSMF showed fungal growth.<sup>[8]</sup> The results of our study were similar to that of Kamat *et al.*, who suggested that OSMF favors the colonization of *Candida*.<sup>[17]</sup>

Field *et al.* suggested that *C. albicans* plays an important role in the development of oral cancer by means of endogenous



**Graph 5:** Association/relation or comparison between salivary flow rate and *Candida* carriage

nitrosamine, oligosaccharides and lectin-like component production.<sup>[18]</sup> Our results confirmed the findings of Ariyawardana *et al.*, who showed higher candidal carriage in OSMF patients, using the oral rinse technique. As oral mucosa is compromised in OSMF patients, it can be argued that *Candida* may predispose the individual to candidal infection and invasion.<sup>[19]</sup> A study done by Anila *et al.* suggested a higher incidence of candidal carriage in OSMF patients and increased salivary flow in OSMF patients, which contradicts our study.<sup>[7]</sup> On the contrary, Reichart *et al.* reported that there was no significant difference in the candidal carriage rate between the OSMF patients and control group.<sup>[20]</sup> At present, the results of our study suggest that a higher candidal carriage in grade II and grade III OSMF patients could be related to decreased salivary flow rate. Candidal carriage was not seen in grade IV OSMF patients; the reason might be because of small sample size of this group, as this study was done on random number of patients.

## CONCLUSION

A higher incidence of *Candida* was observed in the OSMF patients when compared to the healthy individuals. Also, the study showed that there is a constant decrease in the salivary flow rate among the different grades of OSMF patients from grade I to grade IV. Absence of candidal carriage was seen in the control group as compared to the OSMF patients. Prospective studies with larger sample size of OSMF patients and different culture media to identify various species of *Candida* along with long-term follow-up will help in assessing the role of increased candidal carriage in the coarse and malignant transformation of OSMF.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Rajendran R, Sivapathasundharam B. Shafer's Textbook of Oral Pathology. 6<sup>th</sup> ed. New Delhi: Elsevier; 2009.p. 364-6.
2. Rajendran R. Oral submucous fibrosis: Etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-96.
3. Pindborg JJ, Singh B. Formation of vesicles in oral submucous fibrosis. Acta Pathol Microbiol Scand 1964;62:562-6.
4. Sirsat SM, Pindborg JJ. Subepithelial changes in oral submucous fibrosis. Acta Pathol Microbiol Scand 1967;70:161-73.
5. Vincent S. Origin of the Names of Species of *Candida*. Available from: <http://www.antimicrobe.org>. [last accessed on 2015 Aug 21].
6. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and Maxillofacial Pathology. 3<sup>rd</sup> ed. Philadelphia: Saunders; 2009.p. 213-21.
7. Anila K, Hallikeri K, Shubhada C, Naikmasur VG, Kulkarni RD. Comparative study of *Candida* in oral submucous fibrosis and healthy individuals. Rev Odontol Cien 2011;26:71-6.
8. 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;2:188-93.
9. Shinozaki S, Moriyama M, Hayashida JN, Tanaka A, Maehara T, Ieda S, *et al.* Close association between oral *Candida* species and oral mucosal disorders in patients with xerostomia. Oral Dis 2012;18:667-72.
10. Ranganathan K, Devi U, Elizabeth J, Arun B, Rooban T, Viswanathan R. Mouth opening, cheek flexibility and tongue protrusion parameters of 800 normal patients in Chennai, South India. A base line study to enable assessment of alterations in oral submucous fibrosis. JIDA 2001;72:78-80.
11. Chin CS, Saat I. Do germ-tube positive *Candida tropicalis* occur? Malays J Pathol 1983;6:51-3.
12. Samaranyake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. J Oral Pathol 1986;15:386-8.
13. Kumar RS, Ganvir S, Hazarey V. *Candida* and calcofluor white: Study in precancer and cancer. J Oral Maxillofac Pathol 2009;13:2-8.
14. Niimi M, Cannon RD, Monk BC. *Candida albicans* pathogenicity: A proteomic perspective. Electrophoresis 1999;20:2299-308.
15. Torres SR, Peixoto CB, Caldas DM, Silva EB, Akiti T, Nucci M, *et al.* Relationship between salivary flow rates and *Candida* counts in subjects with xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:149-54.
16. Jorge AO, Koga CY, Gonçalves CR, Fantinato V, Unterkircher CS. Presence of *Candida* genus yeasts in the saliva of patients with different predisposing factors and of control individuals. Rev Odontol Univ Sao Paulo 1997;11:279-85.
17. Kamat MS, Vanaki SS, Puranik RS, Puranik SR, Kaur R. Oral *Candida* carriage, quantification, and species characterization in oral submucous fibrosis patients and healthy individuals. J Investig Clin Dent 2011;2:275-9.
18. Field EA, Field JK, Martin MV. Does *Candida* have a role in

- oral epithelial neoplasia? J Med Vet Mycol 1989;27:277-94.
19. Ariyawardana A, Panagoda GJ, Fernando HN, Ellepola AN, Tilakaratne WM, Samaranayake LP. Oral submucous fibrosis and oral yeast carriage – A case control study in Sri Lankan patients. Mycoses 2007;50:116-20.
  20. Reichart PA, Schmidtberg W, Samaranayake LP, Scheifele C. Betel quid-associated oral lesions and oral *Candida* species in a female Cambodian cohort. J Oral Pathol Med 2002;31:468-72.