Comparison of Salivary Flow and Candida Carriage in Patients with Oral Sub Mucous Fibrosis: An *In Vivo* Study

Ashish Bhaskar Zope¹, Lata Madhukar Kale², Jyoti Dasharath Magare³,
Aishwarya Madhukar Kale⁴

¹MDS, Lecturer, Department of Oral Medicine & Radiology, C.S.M.S.S. Dental College & Hospital, Kanchanwadi, Paithan Road, Aurangabad, Maharashtra, India; ²MDS, Dean, Professor & H.O.D., Department of Oral Medicine & Radiology, C.S.M.S.S. Dental College & Hospital, Kanchanwadi, Paithan Road, Aurangabad, Maharashtra, India; ³PhD, Reader, Department of Pathology & Microbiology, C.S.M.S.S. Dental College & Hospital, Kanchanwadi, Paithan Road, Aurangabad, Maharashtra, India; ⁴M.D.S., Periodontist, Aurangabad, Maharashtra, India.

ABSTRACT

Objective: To compare salivary flow & candida carriage in patients with oral submucous fibrosis (OSMF) as OSMF causes a reduction in salivary flow making odontogenic structures caries prone & increasing in candida causing oral thrush, angular cheilitis & in some cases malignancies.

Material & Methods: A total of 40 subjects were included in the study & divided into 2 groups. The 1st group included patients without OSMF & the 2nd group included patients with OSMF. The duration of the study was 1 year. For every subject, inter incisal distance was measured using vernier callipers. The saliva sample was collected in pre-weighed cotton rolls & the difference was noted. A separate saliva sample was collected & inoculated on chrome agar for candida growth. Descriptive statistics like mean, median & standard deviation were used to summarize the variable. Differences in the mean across the groups were tested by Student's t-test.

Results: The difference in salivary flow was statistically significant at a 5% level of significance (P-value < 0.000). The control group did not show candida growth. In contrast, the OSMF group showed the presence of candida in 16 out of 20 samples.

Conclusion: Salivary flow was decreased significantly & candida carriage was increased significantly in patients with OSMF as compared with healthy control group subjects. As the grades of OSMF increased, the salivary flow was decreased & candida carriage was increased.

Key Words: Oral Submucous Fibrosis (OSMF), Inter-incisal distance, Visual blanching of oral mucous membranes, Stiffness of buccal mucosae on palpation, Salivary flow rate, Candida carriage

INTRODUCTION

Oral submucous fibrosis (OSMF) is a potentially malignant disorder and presents clinically with reduced mouth opening, decreased salivary flow rate, blanching of oral mucosa and burning sensations.¹ It is insidious in onset, chronic & any part of the oral cavity can be affected by it. Sometimes it follows vesicle formation, or vesiculation may occur simultaneously. Juxta epithelial inflammatory reaction always occurs simultaneously, which is followed by progressive hyalinization of lamina propria. It is followed by subepithelial and submucosal fibrosis, which leads to stiffness of the oral mucosa and deeper tissues, with progressive limitation in mouth opening and tongue protrusion. It causes difficulty in eating,

swallowing and phonation. There is epithelial atrophy in advanced stages.^{1,2}

It is a chronic, progressive and irreversible disease. It is a potentially malignant disease, caused by areca nut chewing and is associated with significant morbidity and an increased risk for malignancy. The habit of areca nut chewing has a major social and cultural role in communities throughout the Indian subcontinent and hence there is a high prevalence of OSMF in people of the Indian subcontinent and South Asian ethnicity.³

Oral candidiasis is also known as oral candidosis, oral thrush, oropharyngeal candidiasis, moniliasis, candidal stomatitis. These are a mycosis type of fungal infection of Candida species

Corresponding Author:

Dr. Ashish Bhaskar Zope, 35, Zambad estate, New Shreyanagar, Aurangabad-431005, Maharashtra, India.

Email: drabyz@rediffmail.com

ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 03.05.2021

Revised: 06.07.2021

Accepted: 02.09.2021

Published: 13.12.2021

on the mucous membrane of the mouth.⁴ Atrophic, hyperplastic and dysplastic epithelial changes in oral mucosa may cause compromise of the mucosal barrier and facilitate the invasion of candida.¹⁰ Hence, the present study was conducted, to check the salivary flow and candida carriage in OSMF patients and healthy individuals.

MATERIAL AND METHODS

Study design & data collection

The study was started after obtaining ethical clearance from the Institutional Ethical Committee of the college (Vide letter no. MUHS/PG-T/E-1/2593/2017, dated 07.12.2017) & obtaining written informed consent from all the participants before their inclusion in the study. The study was carried out following the Helsinki Declaration.⁵

The sample size was calculated using the following formula

$$N = \frac{2[Z\alpha + Z\beta]^2}{\left(\frac{\mu_1 - \mu_2}{\sigma}\right)^2}$$

& a sample size of 20 per group was considered.

It was a descriptive cross-sectional study, in which a total of 40 subjects reporting to the Department of Oral Medicine & Radiology, were included & divided into 2 groups. The 1st group included patients without OSMF & the 2nd group included patients with OSMF. The OSMF group patients had a history of tobacco use of an average of 7 to 10 years. There were no gender restrictions, for participation. The duration of the study was 1 year. The age range for participation in the study was between 18 years to 55 years. The OSMF patients were graded from grades I to IV, as given by Ranganathan et al.⁶ Those with immunocompromise, chronic systemic illnesses of cardiovascular, renal, endocrinal and/or other systems, evidence of medications, age below 18 years & above 55 years & those with salivary gland disease were excluded.

A thorough clinical examination was carried out & clinical findings were recorded. Vernier calliper was used to measure maximum mouth opening, which was the distance between the centres of incisal edges of maxillary central incisors and mandibular central incisors. (Fig 2). Mouth opening measurement, visual blanching of oral mucous membranes (Fig 1), stiffness of buccal mucosae on palpation were the criteria used for concluding diagnosis of OSMF.

Culture Technique

A saliva sample was collected in pre-weighed cotton rolls, by placing the latter sublingually for 5 minutes & the difference between the latter was noted. A separate saliva sample was

collected for inoculation of candida on chrome agar, by the rinse & spit method. The saliva samples were inoculated on chrome agar & incubated at 37°C for 24 to 48 hours. After growth, Gram's staining was performed. Candida albicans on chrome agar gives green coloured colonies (as per HI media instructions) (Fig 3). Microscopically, Gram-positive yeast-like cells were seen with pseudohyphae (Fig 4).

Descriptive statistics like mean, median & standard deviation were used to summarize the variable. Differences in the mean across the groups were tested by Student's t-test. The data was tabulated in excel spreadsheets and analyzed with IBMSPSS 20 statistical software. The whole sample was divided in two groups- control and OSMF groups and the values were assigned randomly. The difference between the salivary flows of the groups was calculated and then compared statistically. The number of candida carrier patients were tabulated.

RESULTS

In the present study titled "Comparison of salivary flow and candida carriage in patients with oral submucous fibrosis – an *in vivo* study" a total of 40 subjects were included. 20 patients were of the OSMF group and 20 patients were of the healthy control group. Salivary flow and candida carriage among the control group & OSMF group was compared.

Graph 1 shows salivary flow and candida carriage in the control group and OSMF group. Presenting with both the parameters in each of the groups with significant difference of candidal carriage in OSMF group.

DISCUSSION

The oral, oropharyngeal and sometimes the oesophageal mucosa is affected by OSMF. It is a chronic, progressive and irreversible disease.⁷ There is a significant association between the stage/type of lesion and candidal growth; and type of habit with the colonization of the Candida species.⁸

The main culprit is pan use. Pan is areca nut, tobacco and crude lime wrapped in betel leaf. Arecoline, an alkaloid component of the areca nut, can induce fibroblast proliferation and collagen synthesis and may penetrate the oral mucosa causing cross-linking of collagen fibres, resulting in OSMF, experimentally.⁷

Reportedly, 7.6% of OSMF cases progress to malignancies. Many factors are responsible for causing OSMF, but there is no agreement upon single pathophysiology. Hence, no effective treatment exists. Thus, the management of OSMF poses a great challenge.⁹

The study showed, decreased salivary flow rate in OSMF in-

dividuals as compared to the controls. The incidence of candida was higher in OSMF patients, as compared with healthy individuals. It suggested an inverse relationship between salivary flow and candida colony, all the above observations matched with the findings of a study conducted by Gupta et al.¹⁰

The mucosal changes in OSMF that rendered the patients to increased susceptibility to candida infection matched with the conclusion of the study conducted by Panditray et al.¹¹ & it also showed the increased incidence of candida found in OSMF patients when compared with healthy individuals. This matched with the conclusion of a study carried out by Anila et al.¹²

The patients with OSMF are prone to develop *Candida albicans* infection as compared to the control group was observed in the study. This matched the observation of the study by Beena George.⁴

A study by Chaudhary et al.¹³ revealed that the rate of oral yeast carriage was relatively higher in OSMF patients than in healthy individuals. OSMF patients harboured yeast species more as compared with controls.¹² This was in agreement with the findings of the present study.

As concluded by Somashekhar et al.¹⁴ *C. tropicalis*, a non *C. albicans* species, was the major isolate in OSMF. There are immunological changes in the oral environment caused due to betel quid chewing and OSMF, as is evident by the finding of their study. They also found the presence of candida in OSMF patients, which was similar to the findings of this study.

The study by Ariyawardana et al.¹⁵ was the first study to investigate oral candidal carriage in patients with OSMF. Further, the study was the first to isolate *C. dubliniensis* from Sri Lankan individuals. Although not statistically significant, this study demonstrated a higher candida carriage in patients with OSMF. But it didn't show the presence of candida in a few OSMF samples, as was the observation in this study also. This study also showed more vulnerability of OSMF patients for candida infections.

This study is also consistent with Kamat et al.¹⁶ who affirmed that oral submucous fibrosis favours the colonization of *Candida*. The alteration in mucosa caused due to underlying disease or betel quid chewing may lead to candidal colonization.

This study is also supported by Nayak et al.¹⁷ who concluded, that there was a significant increase in the candida carriage rate in the OSMF group than in normal controls which could be attributed to the altered mucosa in these patients. The study findings suggest that *Candida* might act as a co-factor in the development of pre-malignancies. The present study also showed increased candida carriage in OSMF patients.

The marked decrease in salivary flow rate (SFR) and taste perception to salty and sour among Stage II OSMF when compared to Stage I OSMF subjects was found by Dyasanoor et al.¹⁸ Their findings related to SFR were similar to the present study. Sangeeth et al.¹⁹ concluded that there is an increase in salivary flow rate, salivary copper and salivary iron in gutkha chewers with oral submucous fibrosis. The observation of increased salivary flow rate was in contradiction with the findings of the present study.

The findings related to reduced salivary flow and increased candida carriage in OSMF patients compared to normal control, in majority studies, was similar to the findings of this study. Thus, this study confirms the findings that there is a reduction in salivary flow and an increase in candida carriage amongst patients with OSMF.

This study shows that the salivary flow is reduced and candida carriage is increased in OSMF. It included a moderate sample size of both the control and OSMF groups. A study with a larger sample size would shed more light on the role of candida in OSMF.

However, it signifies that patients with OSMF are more prone to the ill effects of candida related illnesses such as oral thrush, angular cheilitis & in some cases malignant transformation, because of the increased candida count. These patients are also more prone to xerostomia related sequelae such as dental caries and other opportunistic oral infections. Hence patient counselling along with specific treatment such as antioxidants, multivitamins, in initial stages & surgical intervention in advanced stages, is of utmost importance in OSMF patients as this illness affects not only the soft tissues of the oral cavity but also causes xerostomia that affects odontogenic structures as well.

CONCLUSION

The present *in vivo* study was conducted to study the salivary flow rate and candida carriage in OSMF patients and to compare the same with healthy controls.

In the study, it was observed that the salivary flow rate in OSMF patients was significantly decreased as compared to healthy controls. Secondly, the candida carriage in OSMF patients was increased as compared to healthy controls.

Limitations & future prospects:

In the future, a study with a larger sample size would give more precise comparisons between salivary flow and candida carriage in OSMF patients and healthy controls. More technologies could be used, such as polymerase chain reaction & 16S ribosomal studies for the identification of specific microbial species. This is advisable as OSMF makes the

patients prone to candida infections as well as makes the odontogenic tissues caries prone due to associated xerostomia.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Source of Funding: Nil.

Conflicts of interest: Nil.

REFERENCES

- Pindborg JJ, Singh B. Formation of vesicles in oral submucous fibrosis. *Acta Pathol Microbiol Scand*. 1964; 62:562-6.
- Sirsat SM, Pindborg JJ. Subepithelial changes in oral submucous fibrosis. *Acta Pathol Microbiol Scand*. 1967; 70:161-73.
- Kalvizhi E B, Charanya D, Sasirekha B, Megalaa N, Kanmani R. A review on oral submucous fibrosis. *IJCR*. 2017 July; Vol. 9, Issue, 07: pp.55116-55120.
- Beena G. Evaluation of the prevalence of candida Albicans infection in patients with oral submucous fibrosis in comparison with healthy individuals. *International Journal of Bioassays* 4.10 (2015): 4411-4413.
- World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964; amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. *Bulletin of the World Health Organization* 2001; 79 (4): 373-374.
- 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 baseline study to enable assessment of alterations in oral submucous fibrosis. *J Int Bioassay*. 2001;72:78-80.
- Afroz N, Hasan SA, Naseem S. Oral Submucous Fibrosis A Distressing Disease with Malignant Potential. *Indian Journal of Community Medicine*. 2006 October-December; Vol. 31, No. 4: 270-271.
- More C, Peter R, Nishma G, Chen Y, Rao N. Association of Candida species with Oral Submucous Fibrosis and Oral Leukoplakia: A Case-Control Study. *Ann Clin Lab Res*. 2018; Vol.6 No.3, 248: 1-5.
- Agarwal N, Singh D, Sinha A, Srivastava S, Prasad RK, Singh G. Evaluation of the efficacy of turmeric in management of oral submucous fibrosis. *Journal of Indian Academy of Oral Medicine & Radiology*. 2014;26(3): 260-263.
- Gupta B, Chandra S, Raj V, Gupta V. Comparison of salivary flow and candidal carriage in patients with oral submucous fibrosis. *Journal of Oral and Maxillofacial Pathology*. 2015 May-Aug; Vol. 19 Issue 2:158-163.
- Panditray S, Bepari K, Sahu SK, Palai S, Deepakraj V. Prevalence of candida and salivary flow rates in oral submucous fibrosis patients. *J Evolution Med. Dent. Sci*. 2018; 7(04): 424-428.
- Anila K, Hallikeri K, Shubhada C, Naikmasur V G, Kulkarni R D. Comparative study of candida in oral submucous fibrosis and healthy individuals. *Rev OdontoCienc*. 2011; 26(1):71-76.
- Chaudhary M, Chandak T, Gawande M, Holani A, Gadail A. Candidal Carriage in Oral Submucous Fibrosis Patients a case-control study. *J D M I M S U*. 2012; Vol.7 No.1:5-9.
- Somashekhar P, Kamath V.V, Ramanna C. Oral candidal carriage and species identification among betel quid chewers and oral submucous fibrosis patients. *J Adv Res Clin Ins*. 2016; 3: 133-138.
- Ariyawardana A, Panagoda GJ, Fernando HN, Ellepola ANB, Tilakaratne WM, Samaranyake LP. Oral submucous fibrosis and oral yeast carriage – a case-control study in Sri Lankan patients. *Mycoses*. 2007; 50:116–120.
- 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. *Journal of Investigative and Clinical Dentistry*. 2011; 2: 275–279.
- Nayak SR, Madhavan Nirmal R, Gokul S, Adarsh H, Manjunath N. Analysis of Candidal carriage in oral submucous fibrosis – a case-control study. *International Journal of Dental Update*. 2012;2(1):10-15.
- Dyasanoor S, Abdul Khader NF. Alteration in salivary properties and taste perception in OSMF. *Contemp Clin Dent*. 2016;7:146-52.
- Sangeeth S, Ashok L, Sujatha GP. Estimation of Unstimulated Salivary Flow Rate, pH, Copper and Iron in Gutkha Chewers with and without Oral Submucous Fibrosis –A Preliminary Study. *Res J Pharm, Bio Chem Sci*. 2014 May June; 5(3): 300-306.

Figure 1: OSMF patient buccal view.

Figure 2: Interincisal distance measurement.

Figure 3: Positive candida growth on inoculation.

Graph 1

Figure 4: Candida growth as seen under the compound microscope.