PREVALENCE OF CANDIDA AND SALIVARY FLOW RATES IN ORAL SUBMUCOSAL FIBROSIS PATIENTS

Siddharth Panditray¹, Kamalini Bepari², Sunil Kumar Sahu³, Shrabani Palait, Deepakraj V5

1Postgraduate Resident, Department of ENT and Head Neck Surgery, VIMSAR, Burla.
2Assistant Professor, Department of ENT and Head Neck Surgery, VIMSAR, Burla.
3Postgraduate Resident, Department of Microbiology, VIMSAR, Burla.
4Postgraduate Resident, Department of SPM, MKCG, MCH, Berhampur.
5Postgraduate Resident, Department of ENT and Head Neck Surgery, VIMSAR, Burla.

BACKGROUND
Candida species belong to the normal microbiota of an individual’s mucosal oral cavity, gastrointestinal tract and vagina. The alteration in the homeostasis between Candida, host immune system and normal oral bacterial flora causes damage to tissue by resisting host defense and production of hydrolytic enzymes. Salivary gland hypofunction may alter the oral microbiota and increase the risk of oral candidiasis. Oral submucosal fibrosis patients are prone to the above pathologies.

The objective of this study is to study the prevalence of candida species and to determine the salivary flow rates of patients with oral submucosal fibrosis.

MATERIALS AND METHODS
42 patients presenting to ENT OPD of VIMSAR, Burla, with clinically diagnosed oral submucosal fibrosis (OSMF) between September 2015 and August 2017 were chosen for the study. The patients were compared with age and gender matched controls (n = 42). Samples for candida colony count were collected by oral rinse technique and salivary flow rates in mL per minute were calculated by saliva collection techniques. Patients were staged from stage 1 to 4 OSMF clinically. Candida was quantified as colony forming units (CFU) and species identification was done by standardised methods. Data was tabulated in Excel Sheets and statistical analysis was done by Mann-Whitney U Test in SPSS software version 16.0. Statistical significance was set at p < 0.05.

RESULTS
There was significant difference between cases and controls with regards to prevalence of candida and salivary flow rates (p<0.000). Candida albicans was the most common species identified in both cases and controls. Salivary flow rates progressively decreased from stage 1 to 4, while CFUs were highest in stage 3 and lowest in stage 1 OSMF.

CONCLUSION
The mucosal changes in OSMF render the patients to increased susceptibility to Candida infection. With clinical progression of OSMF, salivary flow rates decrease. This study may be helpful for deciding prophylactic management of fungal infection as well as xerostomia in OSMF patients.

KEYWORDS
Oral Submucosal Fibrosis, Candida, Salivary Flow Rates, Xerostomia in OSMF, Oral Fungal Infections in OSMF.


BACKGROUND
Candida species belong to the normal microbiota of an individual’s mucosal oral cavity, gastrointestinal tract and vagina,[3] and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections.[2]

In the oral cavity, the Candida niches include the tongue followed by palate and buccal mucosa.[3] The alteration in the homeostasis between Candida, host immune system and normal oral bacterial flora causes damage to tissue by resisting host defense and production of hydrolytic enzymes such as proteases, phospholipases and haemolysin.[4]

Epithelial changes of the oral mucosa such as atrophy, hyperplasia and dysplasia breach the mucosal barrier and facilitate candidal invasion.[5] Candida causes infections in both immunocompetent and immunocompromised host.[6][7]

The increase in the incidence of infections in immunocompromised individuals is due to their greater adaptability to divergent host niches.[8] Candida albicans is the primary cause of oral candidiasis. Candida colonisation of oral surfaces serve as a reservoir for disseminated infections, such as asperate pneumonia and gastrointestinal infections.[9] Candidal infection leads to malignant transformation through the release of carcinogenic nitrosamine compounds.[10] Oral submucous fibrosis (OSMF), a chronic potentially malignant disorder, is of multifactorial origin with tobacco chewing as a predominant causative agent.[11] The tobacco contents (nicotine, polycyclic aromatic hydrocarbons, polonium and nitrosoproline), which provide nutrition for Candida and promote their proliferation.[12] They increase the colonisation of Candida by causing an increase in epithelial keratinisation, decrease in salivary immunoglobulin A and leucocyte function, and oral epithelial changes such as atrophy, hyperplasia and dysplasia which disrupts the epithelial...
Saliva is important for oral health as it buffers acids, helps in preventing erosion of gingival mucosa and also contains antibodies and immunoglobulins. Risk of developing candidiasis increases when salivary flow rate is diminished.\[^{[14]}\]

Saliva contains antimicrobial proteins such as lysozyme, lactoperoxidase, immunoglobulins, histatins and lactoferrin. Histatins have potent antifungal activity. IgA present in saliva inhibits adhesion of Candida alibicans in the oral cavity. The myelomonocytic L1 protein or calciprotein, which bind to calcium in saliva acts as defense against oral candidiasis in HIV-infected patients.\[^{[15,16]}\] Salivary gland hypofunction may alter the oral microbiota and increase the risk of oral candidiasis, which is the most prevalent opportunistic infection affecting the oral mucosa caused by Candida species.\[^{[14]}\]

**MATERIALS AND METHODS**
A cross-sectional study with total of 42 patients were selected from those visiting the outpatient department of ENT and HN Surgery at VIMSAR, Burla and Odisha between September 2015 and August 2017. Age and gender matched normal individuals are taken as controls for comparison. Informed consent was obtained from all participants prior to inclusion in the study.

**Inclusion Criteria**
The study participants were in the age group of 20 - 45 years with equal males and females in the groups. They were categorised into two groups including OSMF patients in one and healthy individuals in the other group.

A detailed clinical history was obtained from each participant and clinical staging was noted using the criteria established by Chandra am More et al (2011)\[^{[17]}\] as follows-

- **Stage 1 (S1):** Stomatitis and/or blanching of oral mucosa.
- **Stage 2 (S2):** Presence of palpable fibrous bands in buccal mucosa and/or oropharynx with/without stomatitis.
- **Stage 3 (S3):** Presence of palpable fibrous bands in buccal, mucosa and/or oropharynx, and in any other parts of oral cavity with/without stomatitis.
- **Stage 4 (S4) as follows:** A. Any one of the above stages along with other potentially malignant disorders, e.g. Oral leukoplakia, oral erythroplakia, etc. B. Any one of the above stage along with oral candida.

Patients were divided into Groups Based on Addiction Habits, as follows-
- **Group A:** Gutkha (tobacco + supari);
- **Group B:** Supari only;
- **Group C:** Smoking;
- **Group D:** Smoking + Gutkha.

**Exclusion Criteria**
The study excluded individuals-

- Using antifungal agents, antibiotics, non-steroidal anti-inflammatory drugs/steroids within the past 12 weeks.
- With systemic disorders such as diabetes mellitus, hepatitis B, hepatitis C, HIV and acquired immunodeficiency syndrome.
- Denture wearers.

**Sample Collection and Processing**
The oral rinse technique described by Samaranayake et al was used to collect samples. Subjects were asked to rinse their mouth with 10 mL of phosphate buffered saline (PBS) for 2 mins and expectorate into a sterile container. The sample was immediately transported to the laboratory where it was centrifuged at 2500 g for 10 mins. The pellet was suspended in PBS; 100 μL of this solution were plated onto Sabouraud’s dextrose agar and incubated for 48 h at 37°C. Candida species recognition was done based on the morphology of the colonies (cream coloured, smooth and pesty), gram staining, germ tube test, chlamydospore formation and sugar assimilation tests. The number of yeast colonies was counted and expressed as colony forming units per millilitre (CFU/mL) of the collected sample.

For measurement of salivary flow rates, a method suggested by Navazesh Mahvash et al was used.\[^{[18]}\]

The patient is advised to refrain from intake of any food or beverage one hour before the test session. Smoking, chewing gum and intake of coffee also are prohibited during this hour. The subject is advised to rinse his or her mouth several times with deionized (distilled) water and then to relax for five minutes.

The patient is then told the following: “I will first obtain measures of saliva. Flow while you are at rest. This means that before and during the collection you should make every effort to minimise movement, particularly movements of your mouth. To begin a collection trial, I will ask you to swallow to void the mouth of saliva. Then you should lean your head forward over the test tube and funnel” (Demonstrate). “Keep your mouth slightly open and allow saliva to drain into the tube. Keep your eyes open. At the end of the collection period, I will ask you to collect any remaining saliva in your mouth and spit it into the test tube. This movement should be done very quickly and should be done in the same manner from trial to trial. This is very important. Do you understand the procedures?”

**RESULTS**

<table>
<thead>
<tr>
<th></th>
<th>Cases and Controls</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salivary Flow Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Cases</td>
<td>42</td>
<td>.6776</td>
<td>.40099</td>
<td>.06187</td>
</tr>
<tr>
<td><strong>Colony Forming Unit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Cases</td>
<td>42</td>
<td>3.402</td>
<td>80.510</td>
<td>12.423</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Table 1. Group Statistics</td>
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</tbody>
</table>
Mean age of the study was (37.76 ± 8.58) years for cases and (35.28 ± 6.80) years for controls.

The mean CFU/mL in cases was (435.17 ± 38.163) more than controls (34.02 ± 80.510). This difference was highly statistically significant (p= 0.000).

The mean SFR/ min in cases was (.2455 ± .22283) less than controls (.6776 ± .40099). This difference was highly statistically significant (p= 0.000).

The mean CFU for cases and controls in males= 239 ± 337.8 and mean SFR= 0.51 ± 0.42

The mean CFU in females= 220 ± 357.2 and mean SFR= 0.30 ± 0.15

There was no statistically significant difference between genders in both parameters of the study (CFU- p= 0.757; SFR- p= 0.106).

Comparing the median value for CFU (Cases= 537.5 vs. Controls= 0) and SFR (Cases= 0.17 vs. Controls= 0.53) and IQR for CFU (Cases= 0 - 798 vs. Controls= 0 - 0) and SFR (Cases= 0.10 - 0.31 vs. Controls= 0.40 - 0.87).

Comparing the median value of CFU (Male= 0 vs. Female= 0) and SFR (Male= 0.42 vs. Female= 0.29) and the IQR for CFU (Male= 0 - 550.25 vs. Female= 0 - 517.0) and SFR (Male= 0.17 - 0.73 vs. Female= 0.17 - 0.37).

25 out of 42 cases and 8 out of 42 controls had candida isolated from their oral cavities.

Comparing cases and controls, total no. of patients with Candida albicans were (n= 13/25) vs. (n= 4/8), with Candida tropicalis were (n= 4/25) vs. (n= 1/8), Candida krusei (n=4/25) vs. (n= 1/8) respectively.

Multiple species were found in both cases and controls as C. albicans + C. tropicalis (n= 3/25 vs. n= 0/8), C. albicans + C. krusei (n= 1/25 vs. n= 1/8), C. krusei + C. tropicalis (n= 0/25 vs. n= 1/8).

Hence, Candida albicans was the dominant species found in both cases and controls followed by C. tropicalis.

### Table 2

<table>
<thead>
<tr>
<th>Stage</th>
<th>CFU</th>
<th>SFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>149 ± 255.5</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>499 ± 383.8</td>
<td>0.31</td>
</tr>
<tr>
<td>3</td>
<td>536 ± 391.2</td>
<td>0.73</td>
</tr>
<tr>
<td>4</td>
<td>396 ± 389.03</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The mean CFUs in each stage of OSMF are as follows-

1. Stage 1 (149 ± 255.5),
2. Stage 2 (499 ± 383.8),
3. Stage 3 (536 ± 391.2),
4. Stage 4 (396 ± 389.03).

Candida count is lowest in stage 1 followed by stage 4 and 2. Highest prevalence of candida is seen in stage 3.

Comparing stage 3 and 1 values, we found statistically significant difference in values (p= 0.030).

### Table 3

The Mean SFUs in each stage of OSMF are as follows-

1. Stage 1 (0.517±0.168),
2. Stage 2 (0.295±0.314),
3. Stage 3 (0.218±0.170),
4. Stage 4 (0.122±0.05).

The salivary flow rates are lowest in stage 4, progressively increasing to stage 1.

Comparing stages 4 and 1, we found extreme statistical significance in difference of value (p= 0.0001). This indicates that better the stage of OSMF, less the chance of xerostomia.

Combining results from Table 2 and 3, stage 3 patients were worst hit on both parameters.

### Table 4

<table>
<thead>
<tr>
<th>Addiction</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gutkha (Tob + Sup)</td>
<td>18</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Supari only (Areca Nut)</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Smoking</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Smoking + Gutkha</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>32</td>
<td>10</td>
<td>42</td>
</tr>
</tbody>
</table>

Majority of patients were in Group A.

Mean CFU in Group A were (339.21 ± 392.4), Group B were (526 ± 387.8), Group C were (567.4 ± 277.4) and Group D were (620 ± 422.3). As evident, patients who had both smoking and gutkha habits were at higher risk of Candidal carriage as other groups.

Mean SFR in Group A was (0.28 ± 0.27), Group B was (0.177 ± 0.09), Group C was (0.197 ± 0.12) and Group D was (0.215 ± 0.09).

Interestingly, patients who consumed supari alone were at the highest risk of xerostomia with the lowest salivary flow rates.

### Table 5

<table>
<thead>
<tr>
<th>Stage</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Majority of patients in all groups were in stages 2 (15) OSMF followed by stage 3 (13). Patients in Group D presented with advanced stages of disease.
DISCUSSION
The equilibrium between Candida, host immune system and normal oral bacterial flora determines the role of Candida species as either saprophytes or opportunistic pathogens in the oral cavity.\textsuperscript{[15,20,21,22]} About 3%-47% of Candida species are inhabitants of normal oral flora in healthy individuals.\textsuperscript{[23]}

The prevalence of Candida in oral cavity is regulated by endogenous factors such as: (a) Oral epithelial cell antimicrobial peptides such as defensins, cathelicidins and histidine and epithelial integrity, (b) Salivary constituents such as salivary immunoglobulin A, lysozyme, histidine-rich polypeptides, lactoferrin, and lactoperoxidase and (c) Oral cavity temperature and exogenous factors such as high carbohydrate diet.\textsuperscript{[24,25,26,27]} The epithelial cells promote Candida adhesion.\textsuperscript{[28]} The high carbohydrate diet also facilitate Candida adherence to epithelial cells by reduction in pH due to degradation of carbohydrate in saliva.\textsuperscript{[29]} The earlier studies have reported C. albicans to have greater adhesion to oral epithelial cells followed by non-albicans candida group.\textsuperscript{[30,31]} The presence of more α-L-fucose remnants promote greater adhesion of C. albicans.\textsuperscript{[30]}

Candida is associated with various precancerous and cancerous lesions.\textsuperscript{[32,33,34]} Reichart et al has studied oral candidal species in betel quid chewers. C. albicans was the most commonly isolated species in Cambodian people, but non-albicans group of candida predominated in the Padaung population.\textsuperscript{[35,36]} Ariyawardana et al has studied the prevalence of candida species in OSMF patients and healthy individuals. Candida was isolated from 63.6% of the test group and 50% of the control group.\textsuperscript{[37]} Our study revealed a higher candidal prevalence in OSMF patients (50%) when compared to control group (10%), and mean scores of candidal growth were also higher in OSMF patients than controls. The results of the present study were similar to those presented by Anila et al.\textsuperscript{[38]} C. albicans was the predominant species isolated (87.5%) in OSMF patients in this study.

A study done by Gupta et al showed a higher incidence of Candida in OSMF patients when compared to 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,\textsuperscript{[39]} corroborating with our findings.

Nadig et al showed that there was a significant negative correlation between SFRs and Candida counts in patients with xerostomia.\textsuperscript{[40]} In a study done by Torres et al, there was a significant inverse relationship between salivary flow and Candida CFU counts.\textsuperscript{[15]} In a study by Shinozaki et al compared with controls, patients with xerostomia exhibited significantly decreased whole salivary flow rate, increased rate of oral mucosal symptoms and higher numbers of Candida.\textsuperscript{[41]}

Our study complied with previous studies in terms of salivary flow rates, but no negative correlation could be demonstrated with candidal prevalence. Stage 3 OSMF in our study demonstrated highest candidal counts.

CONCLUSION
• OSMF is a precancerous condition resulting from chronic insult to the mucosa.
• Candidal prevalence significantly increases in patients with OSMF than controls.

• Xerostomia increases in severity with increasing grade of OSMF.
• This study could be applied in prophylactic management of xerostomia and candidal infections in OSMF patients.

REFERENCES


