A Study to determine the Association between Tobacco Smoking Habit and Oral Candidal Infection in Median Rhomboid Glossitis by Cytologic and Histopathologic Methods

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ABSTRACT

Objectives: To determine the association, if any, between tobacco smoking and candidal infection in median rhomboid glossitis (MRG) and to histopathologically evaluate the presence of dysplastic changes in it.

Study design: Hospital-based study consisting of 100 subjects who satisfied the clinical criteria of MRG. Smears and swabs were collected to ascertain the presence of Candida and sub-typing was done utilizing the CHROMagar technique. Biopsy and histopathological examination was done to determine the presence or absence of dysplasia of the lesional tissue.

Results: All the subjects were male and had the habit of smoking. Smears and swabs taken from glossal lesion of 60 subjects' showed 100% candidal positivity. Tissue section from 31 subjects showed histological picture consistent with MRG and 23 (74%) showed dysplastic changes.

Conclusion: MRG is a Candida-associated lesion and the tobacco-smoking habit in our cohort may play an important role in increasing the candidal colonization. As some of our cases exhibited mild epithelial dysplasia, both candidal colonization and smoking habits may have contributed to the dysplastic changes.

Keywords: Atrophic tongue, Candidal infection, Glossitis.


Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

‘Glossite lasangigue mediane de la face dorsale de la langue’ was the name given initially by Brocq and Pautrier1 to a benign lesion which occurs in the posterior midline of the dorsum of the tongue at about the middle third or in front of the circumvallate papillae. Median rhomboid glossitis (MRG) is described as rhomboid reddish smooth and shiny lesion in the midline of the base of the tongue that is devoid of papillae, or elevated and nodular with some opalescent spots.2 The prevalence of median rhomboid glossitis in general population is less than 1% and in the Indian population varies between 0.04 and 0.01%3 with adult males being more commonly affected than females.4,4

Researchers have suggested both developmental and infectious causes specifically Candida for its occurrences.7 Candida is a commensal in the oral cavity with carrier rates in the general population ranging from 2 to 71%.8 Despite the high carrier state among the general population only a small percentage of the population develop oral candidiasis. Smoking and denture wearing appear to be the major predisposing local factors in candidal colonization and the infection in mouth.9-12

Smoking tobacco is considered as an important local factor influencing candidal growth on the dorsal surface of the tongue causing MRG. Studies conducted have shown that candidal species replicate using polycyclic aromatic hydrocarbon as their source of carbon and energy which are present in smoking tobacco.9,10

Though the diagnosis of median rhomboid glossitis is essentially a clinical, the role of Candida in MRG can be proved by the isolation of pathogenic Candida species from the lesion by laboratory techniques, such as smear, culture of Candida on Sabouraud’s dextrose agar, colony forming units and CHROMagar test.

The characteristic histopathological features have been studied by Sammet8 which include loss of papillae with varying degrees of parakeratosis, downward proliferation of the spinous layer forming elongation of the rete ridges which branch and anastomose, lymphocytic proliferation and presence of fungal hyphae readily seen by periodic acid-schiff (PAS) stain.

Studies conducted on animal models by Jones and Russel have shown evidence of epithelial dysplasia in MRG.13 But no reported studies in the literature shows histopathological examination on human subjects with MRG.

Therefore, the primary aim of our study was to determine the association between tobacco smoking habit and candidal colonization in MRG through clinical, cytological, microbiological methods and the secondary aim was to histopathologically evaluate the presence of dysplastic changes, if any associated with MRG.
METHODS

Study Design and Population

The present study is a cross-sectional hospital-based study. Hundred subjects reporting to outpatient clinic of Ragas Dental College and Hospital, Uthandi, Chennai, for routine dental treatment with morphologically depapillated area on the mid dorsum of the tongue resembling MRG were included in the study irrespective of age, sex and socioeconomic background.

OBTAINING APPROVAL FROM THE AUTHORITIES

Permission from the ethical committee of the dental hospital was obtained before starting the study for interpretation and examining patients and also for performing biopsy as and when needed. An informed consent was also obtained from the patients forming the study sample, to participate in the study and to undergo any investigatory procedure, if any, in course of the study.

Selection Criteria

Inclusion Criteria

Patients with oval to rhomboid-shaped depapillated area, reddish, smooth and shiny seen in the mid dorsum of the tongue anterior to the circumvallate papillae which is consistent with the diagnosis of MRG were included in the study.14

Exclusion Criteria

Patients with a history of systemic diseases namely diabetes mellitus, anemia and other forms of immunosuppression were excluded. Patients with history of corticosteroid medications and broad-spectrum antibiotic therapy over the past 3 months were excluded. Patients with removable partial dentures were excluded.

Clinical Examination and Sample Collection

A detailed history and thorough clinical examinations were done and the findings recorded which included habits, such as smoking, chewing tobacco and alcohol consumption. Type of substance used, duration in years and frequency per day were recorded.

Smears to identify Candida

Tongue scrapings were taken with the help of the wooden spatula from the lesion present on the dorsum of the tongue and smeared onto the glass slide and fixed immediately with 70% isopropyl alcohol and the slides are stained with PAS staining to identify the presence of Candida.15

Candidal Culture

In order to assess the candidal carriage from specific site (tongue), swab technique was done. A sterile swab was rubbed on the erythematous area of the tongue and inoculated into the petri dishes containing Sabouraud’s dextrose agar at room temperature. The petri dishes were incubated at 37°C for 48 hours. Pastey, creamy white spherical colonies of varying sizes were identified as candidal colonies.15

Colonies forming units per milliliter of saliva (CFU/ml) were counted. One per 1,000 dilution can be obtained by adding 0.1 ml of sample to 99.9 ml of buffer. Once the dilution is made, an aliquot can be plated on an agar plate using the spread plate technique or in an agar medium using the pour plate technique.15

\[
\frac{0.1 \text{ ml of sample}}{0.1 \text{ ml of sample} + 99.9 \text{ ml of buffer}} = \frac{0.1 \text{ ml}}{0.1 \text{ ml} + 99.9 \text{ ml}} = \frac{0.1 \text{ ml}}{100 \text{ ml}} = 1 \times 0.001 = 10^{-3}
\]

Germ Tube Test

It was done to identify of *Candida albicans* obtained from clinical specimen, as the other subtypes do not form germ tube. A loopful of the colonies from Sabouraud’s dextrose agar is suspended in 1 ml of human serum (sterile), which is incubated at 37°C for 2 hours. After 2 hours, a drop of the mixture is placed on a clean glass slide and examined under light microscope for the growth of germ tube which is characterized by the growth of long tube like projections from the round yeast cell with no constriction at the junction of the yeast cell and the tube, which is a characteristic feature of *Candida albicans*.15

CHROMagar was used to identify the candidal subtypes based on morphology and color. A loopful of candidal colonies was smeared onto the petri dishes containing CHROMagar. After 48 hours of incubation at 37°C, the presumptive identification of yeast isolates was based on the color of the colonies.16

Biopsy and histopathological analysis was done. Incisional biopsy was taken from the lesional tissue under local anesthesia and sent for histopathologic examination to identify the presence of *Candida* and to identify epithelial dysplasia, if any.

Statistical methods used in the study was mean and significance of difference was tested using Chi-square test.
RESULTS

Subject population and clinical manifestation: The study population comprised of 100 subjects of which all 100 (100%) subjects were males and our subjects ranged in age between 35 and 70 years of age (Table 1).

All 100 subjects had the habit of smoking tobacco of which 77 (77%) subjects smoked cigarettes and 23 (23%) subjects smoked beedi (a thin, South Asian cigarette filled with tobacco flake and wrapped in a tendu leaf tied with a string at one end).

A significant relation between duration and frequency of smoking habit and increased occurrence of MRG was not seen, as MRG was found equally distributed in recent smokers (<10 years of duration) as well as long-term smokers (>20 years of duration; Graphs 1 and 2; Table 2).

Oral Candida Identification

Of the 100 subjects with MRG, 60 subjects consented for cytological and microbiological analysis (Fig. 1). The samples from all the 60 (60%) subjects showed the presence of candidal hyphae on PAS staining and exhibited positive candidal growth on Sabouraud’s dextrose agar (Figs 2 and 3). The mean colony forming units (CFU/ml) among smokers with MRG was $1350 \times 10^3$ CFU/ml.

Germ Tube Test

Germ tube test was done in positive candidal cultures of which 20 (33%) samples showed germ tube formation confirming the speciation of albicans subtype.

Oral Candidal Species Isolation

Speciation of Candida was done in the positive candidal cultures using CHROMagar, of which 19 (31.6%) samples showed green color growth indicating the presence of albicans subtype, and 41 (68.3%) samples showed blue
color indicating positivity for tropicalis (Graph 3, Fig. 4, Table 3).

**Candida and Epithelial Dysplasia**

Thirty-one tongue biopsies were taken from MRG subjects; all of them showed candidal hyphae in the superficial epithelial layer and histopathological features were consistent with that of MRG (Fig. 5).

Twenty-three (74%) of tongue biopsies showed varying levels of dysplasia. According to the severity, seven (30%) subjects showed mild dysplasia, 13 (57%) subjects showed moderate dysplasia and three (13%) subjects showed severe dysplasia (Graph 4, Figs 6 and 8, Table 4).

**Correlations among Clinical and Histopathological Parameters**

A significant positive correlation was found between distribution of epithelial dysplasia and habit of smoking, according to age group and material used for smoking, age group and duration of smoking habit, age group and number of cigarettes or beedis smoked per day [(p-value: 0.00369, 0.01492, 0.00665), p < 0.05] (Graphs 5 and 6 and Tables 5 to 7).

**DISCUSSION**

The present data have confirmed the association between smoking habit and candidal infection in MRG. All the subjects were males and had smoking habit. Cigarette smoking might lead to localized epithelial alteration which allows colonization by *Candida* and smoking is known to increase the threshold level of candidal colonization in the oral cavity which shifts a normal candidal commensal becomes symptomatic.3,17-19

In our study, the highest prevalence (38%) of MRG was seen in 41 to 50 years group, which is consistent with studies done by Bruce14 and Van der Wall.12

Since *Candida* is a normal commensal in the oral cavity, about 2 to 70% of healthy population harbors *Candida* species intraorally without signs and symptoms of clinical candidiasis. Measurement of CFU/ml of saliva
is a definitive quantitative method to classify individuals into carriers and those suffering from candidal infection. Epstein JB et al.\textsuperscript{20} classified individuals with less than 400 CFU/ml as carriers and with more than 400 CFU/ml as having candidal infection. In our study, the mean CFU was 1,350, which is higher than 400 CFU seen in carriers which indicates that MRG could be classified under chronic candidal infection.

Though there are numerous studies reporting speciation of *Candida* in other forms of clinical candidiasis, to our knowledge, this is the first study quantifying *Candida* species in MRG cases. In the present study, 41 samples (68.3\%) were *Candida tropicalis* and 19 (31.6\%) *Candida albicans*.

Histopathologically, presence of candidal hyphae penetrating into the upper half of the epithelium has been observed by various authors,\textsuperscript{12,18-20} thus proving the association of *Candida* with MRG.

Although few studies have reported the association between fungal infection and epithelial dysplasia, Joues and Rousell\textsuperscript{13} have studied the histopathology of chronic candidal infection in rat tongue and observed histopathological features consistent with that of MRG with mild dysplasia in the epithelium. Considering MRG as a chronic candidal infection, histopathological examination of the lesional tissue in 31 subjects showed candidal hyphae in superficial epithelium and histological picture was consistent with MRG with loss of papillae and presence of parakeratotic layer. Acanthosis branching and anastomosing of rete ridges lymphocyte and plasma cell infiltrate present in connective tissue. Presence of neutrophils within parakeratotic layer,\textsuperscript{4} PAS staining showed presence of candidal hyphae and 23 (74\%) sections showed the presence of candidal hyphae and varying degrees of epithelial dysplasia. In consideration of the above-mentioned features, it could be stated that smoking alters the mid dorsal lingual mucosa which promotes candidal colonization and thereby increase the overall candidal carriage of the patient.

**Table 3:** Distribution of candidal subspecies in culture-positive median rhomboid glossitis subjects

<table>
<thead>
<tr>
<th>Subspecies of <em>Candida</em></th>
<th>Subjects</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>19</td>
<td>31.6</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>41</td>
<td>68.3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 4:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to its severity

<table>
<thead>
<tr>
<th>Grading of dysplasia</th>
<th>Subjects</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>Moderate</td>
<td>13</td>
<td>57</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

**Fig. 6:** Photomicrograph showing mild epithelial dysplasia

**Fig. 7:** Photomicrograph showing moderate epithelial dysplasia

**Fig. 8:** Photomicrograph showing severe epithelial dysplasia
A Study to determine the Association between Tobacco Smoking Habit and Oral Candidal Infection

**Graph 3:** Distribution of *Candida* subspecies in culture-positive median rhomboid glossitis subjects

**Graph 4:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to its severity

**Graph 5:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to age group and duration of smoking

**Graph 6:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to age group and number of cigarettes or beedis smoked per day

**Table 5:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to age group and materials used for smoking

| Age  | Cigarette | | | Beedi | | | Total | |
|------|-----------|---|---|---|---|---|---|---|---|
|      | No | % | No | % | No | % | No | % |   |
| <40  | 3  | 100.0 | —  | —  | —  | —  | 3  | 13.0 |
| 41-50| 8  | 80.0 | 2  | 20.6 | 10 | 43.5 |
| 51-60| 4  | 50.0 | 4  | 50.0 | 8  | 34.8 |
| >60  | 1  | 50.0 | 1  | 50.0 | 2  | 8.7 |
| Total| 16 | 69.9 | 7  | 30.4 | 23 | 100.0 |

p-value: 0.00369

**Table 6:** Distribution of *Candida*-positive biopsy subjects with epithelial dysplasia according to age group and duration of smoking

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>&lt;10 (yrs)</th>
<th>11-20 (yrs)</th>
<th>21-30 (yrs)</th>
<th>&gt;30 (yrs)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt;40</td>
<td>3</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>20.2</td>
<td>5</td>
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</tr>
<tr>
<td>51-60</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>50.0</td>
<td>1</td>
</tr>
<tr>
<td>&gt;60</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>21.7</td>
<td>9</td>
<td>39.1</td>
<td>3</td>
</tr>
</tbody>
</table>

p-value: 0.01492
SUMMARY

Through this study, we were able to prove that MRG is a chronic candidal infection, and tobacco smoking plays a significant role in increasing the candidal colonization above the threshold level from normal commensal to clinical candidiasis. Though higher CFUs emphasize positive correlation between Candida albicans and MRG, further study showing the resolution of MRG with antifungal therapy will prove a definite cause and effect relationship. In addition, various degrees of epithelial dysplasia seen in our study underscores the role of Candida in oral epithelial dysplasia, however, the exact mechanism of candidal infection in development and progression of epithelial dysplasia remains unclear. Since presence of epithelial dysplasia implies a higher risk of malignant transformation, we as dental professionals should identify and treat MRG and take adequate measures to counsel and educate the patient about the ill effects of smoking.

REFERENCES


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Table 7: Distribution of Candida—positive biopsy subjects with epithelial dysplasia according to age group and number of cigarettes or beedis smoked per day

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>&lt;10 (yrs)</th>
<th>11-20 (yrs)</th>
<th>21-30 (yrs)</th>
<th>&gt;30 (yrs)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>&lt;40</td>
<td>3</td>
<td>100.0</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>41-50</td>
<td>1</td>
<td>10.0</td>
<td>8</td>
<td>80.0</td>
<td>1</td>
</tr>
<tr>
<td>51-60</td>
<td>1</td>
<td>12.5</td>
<td>3</td>
<td>37.5</td>
<td>3</td>
</tr>
<tr>
<td>&gt;60</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
</tbody>
</table>

Total | 5 | 21.7 | 11 | 47.8 | 6 | 26.1 | 1 | 4.3 | 23 | 100.0 |
p-value: 0.00665