

# Epithelial Thickness and Mouth Opening Negatively Correlates with the Burning Sensation of the Oral Cavity: A Pilot Clinicopathological Study

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## ABSTRACT

**Aim and objectives:** The present study aimed to quantify the burning sensation in oral submucous fibrosis (OSMF) patients and correlated then with epithelial atrophy, degree of fibrosis, and mouth opening.

**Materials and methods:** Total 53 OSMF cases without ulcerations and vesicle formation were recruited in the present study. The severity of burning sensation of the oral cavity was determined by a 10-mm visual analog scale (VAS, numeric scale type), in which 0 demonstrates no pain and 10 shows severe intolerable pain. The inter-incisal distance was calculated using Vernier caliper while the epithelial thickness and fibrosis grades were evaluated histopathologically under the light microscope.

**Results:** The mean VAS score in OSMF patients observed was  $4.43 \pm 1.53$ . A weak negative correlation was observed between VAS score and epithelial thickness ( $r = -0.245$ ) as well as VAS score and mouth opening ( $r = -0.431$ ). The epithelial thickness values were correlated with the mouth opening from OSMF patients. With regard to the grade of fibrosis, the VAS score was maximum in advanced stages ( $6.81 \pm 0.6$ ) and showed decreasing trend in intermediate ( $4.44 \pm 0.5$ ) and early stages ( $2.66 \pm 0.48$ ). There were statistically significant differences in the VAS score in all the grades of fibrosis ( $p < 0.00001$ ).

**Conclusion:** The burning sensation increases with a decrease in the epithelial thickness, mouth opening, and fibrosis in the connective tissue stroma. Since intra-epithelial nerve endings are the main carriers of burning sensation, maintaining the disturbed homeostasis of oral epithelium with the restoration of epithelial thickness could mitigate the burning sensation in OSMF patients.

**Clinical significance:** The results of the present study showed that the burning sensation in OSMF patients negatively correlates with the epithelial thickness. Moreover, increased fibrosis was associated with increasing the burning sensation. Maintaining epithelial homeostasis with medicinal intervention could probably help in reducing the burning sensation of the oral cavity in OSMF patients.

**Keywords:** Burning sensation, Epithelial thickness, Fibrosis, Mouth opening, Oral submucous fibrosis.

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## INTRODUCTION

There are myriad oral potentially malignant disorders in the oral cavity with varying degrees of malignant transformation rates.<sup>1,2</sup> Oral submucous fibrosis is a potentially malignant disorder with characteristic manifestations such as fibrous bands, blanching of the oral mucosa, limited mouth opening, burning sensation of the oral cavity, etc.<sup>3</sup> Majority of these features are part of outcome measures not only in the assessment of the quality of life but also in various clinical trials reported in the literature.<sup>4</sup> Among these, mouth opening and burning sensations have been consistently used in various studies to test the efficacy of various treatment modalities.<sup>5</sup> Despite extensive use of these vital outcome measures, very little attention has been given to the in-depth analysis of burning sensation.<sup>6,7</sup> Hence, it becomes paramount importance to investigate burning sensation in detail with related clinicopathological correlations.

Burning sensation in OSMF patients has been very scarcely investigated in terms of identifying the exact pathogenesis.<sup>6,7</sup> Various hypothetical speculations state that burning in OSMF occurs due to vesicles and ulcerations manifested in the oral cavity of the patient.<sup>8</sup> However, with regards to the commonness of burning sensation manifestation in OSMF patients, ulcerations and vesicle formation has been rarely observed manifestation in day-to-day practice. Hence, it is quite conceivable to rule out the vesicle formation and ulceration as the major determinant for

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burning sensation in OSMF patients leaving the scope for further investigations in this area. Degranulated mast cells were also a

positive predictor for burning sensation in the mild to moderate category of burning sensation.<sup>9</sup>

It is a known fact that oral epithelium harbors peripheral nerve endings which are responsible for various kinds of sensation.<sup>10</sup> These nerve endings are limited to the basal and para-basal layer of the epithelium and are quite away from the surface layer.<sup>11</sup> It has been proposed in the literature that atrophy of the oral epithelium in OSMF patients might cause shortening of the distance of epithelial nerve endings to the surface of the epithelium leading to increased responsiveness to external stimuli.<sup>6</sup> Hence, the atrophic epithelium has been proposed as a cause for the burning sensation of the oral cavity. If this proposition holds, then there has to be a correlation between the thickness of the epithelium and the degree of burning sensation. Since, epithelial atrophy is linked with the degree of fibrosis in the submucosa, which ultimately depends on the clinical stage of the OSMF, the correlation between these parameters and burning sensation becomes an imperative research question.

With this view in mind, the present study is designed to quantify the burning sensation in OSMF patients and correlated then with epithelial atrophy, degree of fibrosis, and mouth opening.

## MATERIALS AND METHODS

The study was proved by the Institutional Research and Recognition Committee, and Ethics Committee of the University (DYPC/EC/99/18). Total 53 OSMF cases were recruited in the present study based on the clinical criteria given by Pindborg.<sup>12</sup> To avoid the confounding effect on burning sensation, we made sure to exclude patients with ulcerations and vesicle formation to avoid any bias in determining burning sensation. Before the incisional biopsy, all the demographic data and required clinical data were collected from the patient. The inter-incisal distance was measured using Vernier caliper for obtaining the mouth opening measurement in millimeters (Fig. 1). After obtaining all the demographic and clinical data, an incisional biopsy was performed on buccal mucosa using a 6-mm diameter punch under local anesthesia.

### Burning Sensation Analysis

The severity of burning sensation of the oral cavity was determined by a 10-mm visual analog scale (VAS, numeric scale type), in which 0 demonstrates no pain and 10 shows severe intolerable pain. The



**Fig. 1:** Mouth opening measurement in oral submucous fibrosis patient using Vernier caliper

site of the burning sensation was noted and cases showing burning sensation on buccal mucosa were enrolled in the present study.

### Measurement of Epithelial Thickness

After obtained punch biopsy from buccal mucosa, the specimens were routinely processed using the paraffin-embedded technique. The hematoxylin and eosin-stained sections of each patient were examined under 400× magnification using a compound microscope. The cell layers were counted from basal cell layer to superficial layer at five places and averages were calculated. Due to the loss of epithelial ridges, the complexities in counting due to rete ridges were not present (Fig. 2).

### Fibrosis in the Stroma

The degree of fibrosis in the stroma was graded according to methodology given by Rangnathan and Mishra.<sup>13</sup> The hematoxylin and eosin-stained slides were examined under the compound microscope under 400× total magnification and fibrosis was graded into early, intermediate, and advanced stages (Fig. 2).

### Statistical Analysis

Mean and standard deviation were used to summarize the VAS score, mouth opening, epithelial thickness, and other demographic data. The mean VAS score was correlated with mouth opening and epithelial thickness using the Pearson correlation coefficient. The mean VAS score was compared in different fibrosis grades using one-way ANOVA and *post hoc* Tukey HSD. All of the analyzes were carried out with the use of SPSS version 17.0 and the significance of all the tests were set at a *p* value < 0.05.

## RESULTS AND OBSERVATIONS

### Demographic Data

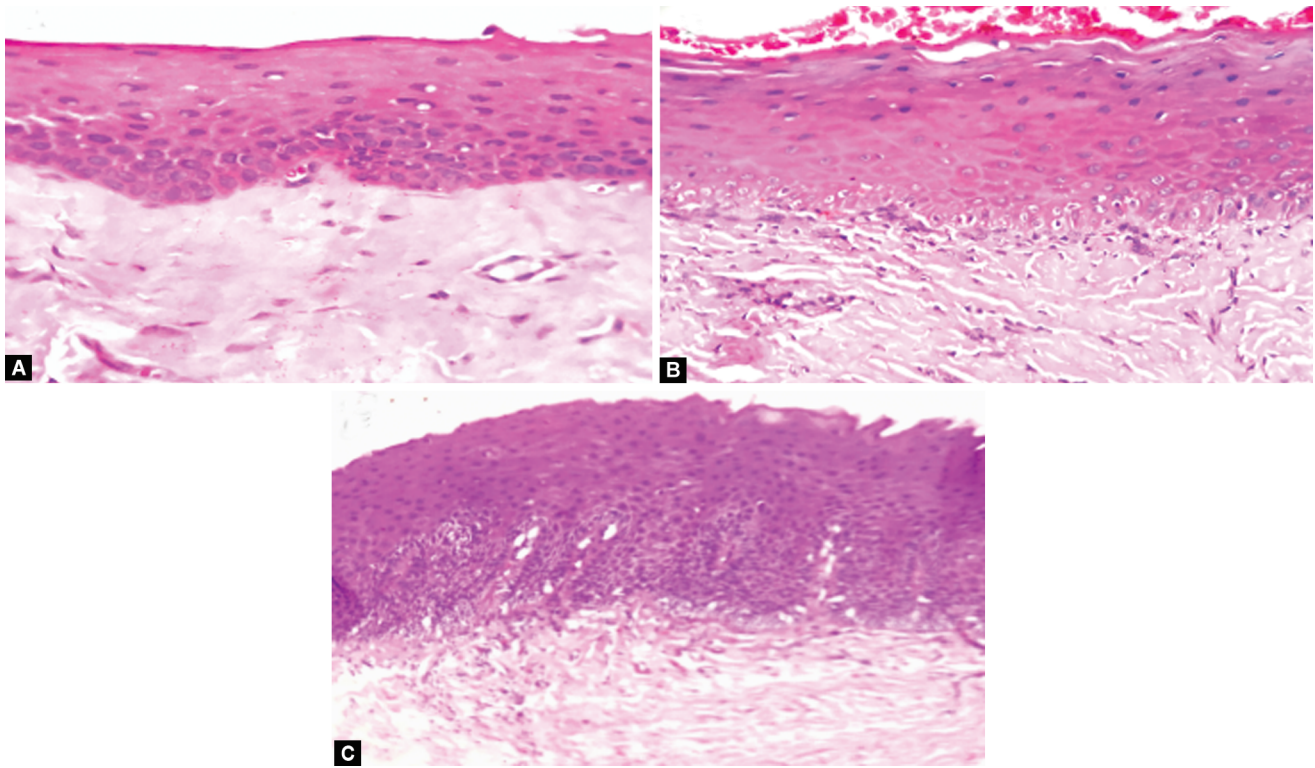
The present study comprised 53 subjects, which were clinically and histopathologically diagnosed with OSMF. The mean age of the OSMF patients was  $31.62 \pm 11.01$  (age range = 16–70 years). OSMF group comprised 46 males and 7 females with a male:female ratio of 6.5:1. This corresponds with the higher prevalence of betel quid chewing habits in a male in the Indian population. Mouth opening for OSF patients ranged from 0 to 39 mm with a mean of  $21.06 \pm 7.9$ . The most common habit was *Gutkha* chewing (44), followed by *Gutkha* + tobacco (6), *Gutkha* + Mawa (1), Mawa alone (1), and areca nut alone (1).

### Burning Sensation Analysis

The burning sensation values obtained from the VAS were correlated with other quantitative parameters such as epithelial thickness and mouth opening. The mean VAS score in OSMF patients observed was  $4.43 \pm 1.53$ .

A weak negative correlation was observed between the VAS score and epithelial thickness ( $r = -0.245$ ). A similar weak negative correlation was observed between the VAS score and mouth opening of OSMF patients ( $r = -0.431$ ). The epithelial thickness values were correlated with the mouth opening from OSMF patients. A moderate positive correlation was reported ( $r = 0.5529$ ), which means that there is a tendency for higher epithelial thickness scores to go with the high mouth opening (and *vice versa*) (Table 1).

The fibrosis in the stroma was graded into early-stage (15), intermediate stage (27), and advanced stages (11). The VAS score was maximum in advanced stages ( $6.81 \pm 0.6$ ) and showed a decreasing trend in intermediate ( $4.44 \pm 0.5$ ) and early stages ( $2.66 \pm 0.48$ ).



**Figs 2A to C:** Photomicrograph shows various grades of fibrosis in the stroma and its correlation with the epithelial thickness. As the grade of fibrosis increased, the epithelium shows an increasing reduction in the total thickness. (A) Advanced-grade fibrosis; (B) Intermediate grade fibrosis; (C) Early grade fibrosis (hematoxylin and eosin stain; total magnification 400x)

**Table 1:** Correlation analysis of visual analog scale with epithelial thickness and mouth opening in oral submucous fibrosis patients

Parameter	n	Mean $\pm$ SD	Significance
Burning sensation (A)	53	4.43 $\pm$ 1.53	A and B: (-0.245): WNC
Epithelial thickness (B)	53	7.20 $\pm$ 1.45	A and C: (-0.431): WNC
Mouth opening (C)	53	21.06 $\pm$ 7.9	B and C: (0.5529) MPC

**Table 2:** Comparison of VAS score in different fibrosis grades in oral submucous fibrosis patients

Fibrosis grade	n	Mean $\pm$ SD	f value	p value	Significance
Early (A)	15	2.66 $\pm$ 0.48	200.5	<0.00001	A and B: ( $p < 0.0001$ )
Intermediate (B)	27	4.44 $\pm$ 0.5			A and C: ( $p < 0.0001$ )
Advanced (C)	11	6.81 $\pm$ 0.6			B and C: ( $p < 0.0001$ )

One-way ANOVA with *post hoc* Tukey HSD (beta)

There were statistically significant differences in the VAS score in all the grades of fibrosis ( $p < 0.00001$ ) (Table 2).

## DISCUSSION

In the medical research field, VAS is the most commonly used instrument for the measurement of pain sensation.<sup>14</sup> Visual analog scale is particularly suited for use in everyday practice (by both patients and healthcare providers) since it is simple and intuitive to use (requiring no training), reproducible, sensitive, and suitable for everyday use. It has also been widely used in the dental field to elicit the pain response in patients with odontogenic

and non-odontogenic pathologies.<sup>15</sup> Among non-odontogenic pathologies, VAS was used predominantly in mucocutaneous disorders including aphthous ulcers, lichen planus, pemphigus, OSMF, etc.<sup>16–18</sup> In the present study, VAS was used to determine the burning sensation score in OSMF patients. Although there is a perception difference in the pain and burning sensation, VAS has been consistently used in various clinical trials on OSMF patients as an outcome measure.<sup>19–21</sup> The VAS score obtained in the present study was comparable to the others studies on OSMF in the literature.<sup>19–21</sup> The burning sensation values obtained from the VAS were then compared with other quantitative parameters such as epithelial thickness and mouth opening.



In the present study, the burning sensation increased with a decrease in epithelial thickness. This finding indirectly supports the proposition of the epithelial nerve ending hypothesis for the causation of burning sensation in OSMF patients.<sup>6</sup> According to this proposition, due to epithelial atrophy the intra-epithelial nerve endings located at the basal and suprabasal layer come closure to the surface layer. Hence, any external stimulus can easily able to reach the nerve ending causing a burning sensation in the oral cavity. We have taken utmost care to avoid any confounding effect due to the presence of vesicles and ulcerations in the oral cavity by excluding such cases on clinical examination. Hence, the possibility of burning sensation due to epithelial atrophy becomes high. However, for further authentication of this proposition, it is mandatory to study nerve endings and their locations in biopsy specimens of the OSMF tissues.

Mouth opening is an important clinical parameter that reflects the severity of disease in OSMF patients.<sup>22</sup> In the majority of the studies, mouth opening was used as an outcome measure in determining treatment efficacies.<sup>19–21</sup> However, the correlation of mouth opening with burning sensation was never reported in the literature. In the present study, the burning sensation was increased with decreasing mouth opening of the patient. It is quite possible that decreasing mouth opening could also decrease the epithelial thickness in OSMF patients, thus leading to an increase in the burning sensation.

In this regard, we observed a moderate positive correlation between mouth opening and epithelial thickness, which means with decreasing mouth opening there was a decrease in the epithelial thickness.

OSMF is primarily a connective tissue disorder with a defect in the fibroblast functioning.<sup>23</sup> Hence, fibrosis is considered as one of the primary histopathological manifestations in OSMF patients, with ramifications in the form of epithelial atrophy.<sup>24</sup> In the present study, the fibrosis in the stroma was graded into early-stage (15), intermediate stage (27), and advanced stages (11), and then compared with the burning sensation. The burning sensation was maximum in advanced stages and showed a decreasing trend in intermediate and early stages. It is a very well fact that fibrosis corresponds with the clinical grade of OSMF<sup>23</sup> and hence, the atrophy of oral epithelium. Thus, all the parameters including fibrosis, mouth opening, and epithelial atrophy are in sink with each other and with the burning sensation of the oral cavity.

The present study has some limitations, which need to be addressed in future research. The VAS is a highly subjective methodology as perception and tolerance levels can vary from person to person. However, this is an inherent limitation and currently, there are no measures that can address this issue more objectively and accurately. The burning sensation is a symptom that can be a manifestation of other well-known systemic pathologies such as anemia, nutritional deficiency, burning mouth syndrome, etc.<sup>25</sup> These pathologies can act as confounders and should be ruled out with appropriate experimentations in the future. Future studies are recommended on analyzing the intra-epithelial neural network in OSMF patients and its closeness to surface epithelium to further authenticate the findings of the present study. Non-inclusion of the control group of determining the normal epithelial thickness of buccal mucosa was one minor limitation of the present study. However, due to ethical reasons, a biopsy of normal buccal mucosa was avoided.

In corroboration with the aim, the inference drawn from the present study is that the burning sensation increases with a decrease in the epithelial thickness, mouth opening, and fibrosis in the connective tissue stroma.

## CONCLUSION

This is the first kind of study that correlates burning sensation in OSMF patients with various clinicopathological parameters. The burning sensation increases with a decrease in the epithelial thickness, mouth opening, and fibrosis in the connective tissue stroma. Since intra-epithelial nerve endings are the main carriers of burning sensation, maintaining the disturbed homeostasis of oral epithelium with the restoration of epithelial thickness could mitigate the burning sensation in OSMF patients. However, future studies are recommended on the status of intra-epithelial nerve endings and drugs targeting epithelial differentiation and maturation.

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