**Salivary pooling: an association with region specificity of oral submucous fibrosis?**

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**ABSTRACT**

Despite extensive research, the pathophysiology of oral submucous fibrosis (OSMF), a premalignant condition affecting primarily the mucosa is still unclear, though areca nut chewing is known to be the primary cause. While a clear association exists between areca nut and OSMF, very little has been published on the reason for its sporadic incidence in the mouth. Many studies suggest the habitual site of quid placement but this hypothesis fails to explain multiple sites of OSMF in unilateral chewers.

We hypothesised that the salivary pooling pattern might play an important role in affecting the distribution of OSMF by carrying the chemicals responsible for mucosal damage. In this study of 174 patients, we evaluated the sites of habitual quid placement, and the areas of saliva pooling and whether these were associated with the incidence of OSMF.

The majority of chewers (136/174, 78%) placed the quid in the buccal vestibule though other sites were also found including lip vestibule, tongue and floor of mouth. The results were analysed using Chi-squared tests with Yates’s continuity correction. The standardised residuals suggested statistically significant associations (P<0.001) between salivary pooling and OSMF indicating the effect of saliva pooling on oral mucosal surfaces in which it comes in contact.

Our results demonstrated that quid was not the only source for OSMF causation but that salivary pooling also plays an important role. These results provide a possible mechanism for the sporadic incidence of OSMF. To our knowledge this is the first study to evaluate saliva pooling as a contributory factor in OSMF and it may help to explain the pattern of distribution. Further work is needed in this area to understand the relationship more fully.

**Keywords: oral submucous fibrosis. Saliva pool, gutkha, areca nut, pathogenesis**

**INTRODUCTION**

Oral submucous fibrosis (OSMF) is a complex, debilitating, and precancerous conditionassociated with abnormal collagen metabolism. 1 Despite research spanning more than three decades, its pathogenesis is still not fully understood,1,2 though it is thought to be multifactorial with many influencing factors. Areca nut is thought to be the primary aetiological factor.3-8

Areca-nut contains alkaloids, flavenoids, and copper, which interfere with homeostasis of the extracellular matrix.1 Many studies provide evidence of a casual relationship between OSMF and copper in areca nut and drinking water.3, 8-12 It has been suggested that chewing areca nut significantly raises the concentration of soluble copper in saliva and as a consequence upregulates local lysyl oxidase activity in the oral mucosa, promoting fibrosis by the crosslinking of collagen fibers.8

In south Asia, betel quid and gutkha are the most commonly used commercially freeze-dried areca-nut products, the latter having replaced most areca-nut preparations. Gutkha contains high concentrations of areca nut along with tobacco.1 When chewed, gutkha dissolves quickly in saliva and provides central stimulation, which is reported as being more intense than tobacco alone.1

Gutkha is usually placed into the buccal or labial vestibule, and sometimes sublingually. It is chewed for up to an hour until the nut softens and dissolves in saliva. The excess is then either spat out or swallowed. Some patients have been known to place it in the buccal vestibule while they sleep. As increased copper concentrations have been found in fibrotic tissue in unilateral OSMF patients, and it has been postulated that the site where the patient habitually chews the preparation raises the local copper levels sufficiently to cause fibrosis.13, 14

While a clear association exists between gutkha / areca nut chewing and the incidence of OSMF, 10,11 very little has been published on the cause of its sporadic pattern of distribution in the oral cavity. Persistently chewing gutkha quid on a specific site has been suggested as the reason for the diverse distribution of OSMF, so unilateral chewers will have unilateral OSMF. However, this finding is based on experimental data rather than clinical observation.9-11

It has been proposed that the proximity of the gutkha / areca quid to oral mucosa is responsible for local OSMF development. Furthermore, it is also demonstrated experimentally that exposure of the oral mucosa to saliva containing dissolved products of the quid results in OSMF.10, 11 Based on these findings it was postulated that the gutkha quid and the saliva containing its chemicals are the primary cause of OSMF due to absorption of chemical gutkha contents in to the oral mucosa through these two sources.10.11 This hypothesis fails to explain incidence of OSMF in multiple sites among unilateral chewers sparing some surfaces unaffected.

However, it may be due to prolonged exposure of the oral mucosa to saliva containing dissolved products of the quid which occurs when the saliva pools in a specific area of the oral cavity. Therefore it could be speculated that the pooling pattern might play an important role in affecting the distribution of OSMF in the oral cavity.

We investigated whether the sites of OSMF are related to where the quid was chewed and/or where the pooling of saliva occurred. We documented the site of gutkha chewing and the sites of saliva pooling to determine whether they were associated with the location of OSMF.

**MATERIALS AND METHODS**

Local ethical committee approval was granted for this study which was conducted in the Yadgir district of the Hyderabad–Karnataka region in India. We randomly recruited 174 gutkha / areca chewers who had OSMF confirmed by clinical examination by one of the author of the study (GA). We avoided the histopathological examination because the pain resulting from the biopsy procedure may influence on the salivary pooling pattern leading to change of gutkha chewing pattern and hence the pooling of saliva. Hence we followed a standard clinical diagnosis protocol laid by Khanna and Andrade.15

Those who previously had surgery for OSMF were excluded as were those who had OSMF but were not currently chewing gutkha.

Patients were informed about the examination procedure only during documentation to avoid any influence on saliva pooling site. A detailed explanation was given to all patients about the gutkha quid placement and saliva pooling sites in their native language.

Saliva pooling was described to them as the collection of pooled saliva under pressure in one part of oral cavity during the process of chewing and the saliva pooling surface that mainly contained the gutkha ingredients in high concentration. For the sake of convenience we divided the oral mucosal surfaces in to six following categories: right buccal mucosa, left buccal mucosa, tongue, lip, floor of the mouth and posterior oral orifice.

Each patient (holding the gutkha quid for 5 minutes) was shown the oral cavity of a healthy volunteer for the six oral mucosal surfaces and questioned about quid placement and saliva pooling surfaces.

Quid placement and saliva pooling surfaces were documented by one of the authors (GA). A panel of three examiners (medical physiology staff) examined patients repeatedly to assess the site of OSMF surfaces. Histopathological examination of all the surfaces involved in this large cohort was beyond the scope and aims of this study.

The patient’s age and occupation was also questioned and documented. Isolated cases of OSMF were tabulated separately with quid placement and saliva pool surfaces. Any secondary changes in mucosal surfaces were confirmed for malignancy transformation by histopathological examination and recorded separately for the sites involved.

**Statistical methods:**

The nominal (categorical) data were analysed and assessed for the association between habitual quid placement site, saliva pool, and clinically affected sites. The analysis of association between groups was carried out by chi-squared tests with Yates continuity correction for 2x2 contingency tables. The chi-squared test is an approximate test, and Yates continuity correction should make it more exact, specifically in small samples. The Yates continuity correction makes the chi-squared result more conservative and this is thought to be beneficial because of the interdependence of the cells in a 2x2 table. The correction subtracts 0.5 from the difference between each observed and expected value and so reduces the size of test statistic, thus increasing the probability value. While the use of Yates continuity correction for large samples makes little difference to the outcome of chi-squared test, the correction has been applied here to show that significant associations were identified with the most conservative application of the chi-squared test. A significance level of P<0.05 was adopted for all tests. Data were analysed using SPSS v20.0 software.

**RESULTS**

174 male subjects with a mean (SD) age of 26.0 (7.9) years were included. Quid was most commonly placed in the left buccal mucosa (45.4%, 79/174) followed by the right buccal mucosa (32.8%, 57/174), the lower labial vestibule (13.2%, 23/174), ventral surface of tongue / lingual sulcus, (4.6%, 8/174) and anterior two thirds of the tongue (4.0%, 7/174) (Table 1). While the majority of salivary pooling occurred at each quid placement site, there were other sites of pooling found (Table 2).

The clinical diagnosis of OSMF followed the pattern of saliva pooling in all patients (Table 1). Chi-square analysis with Yate’s continuity correction showed a significant association (p<0.001) and the standardised residuals suggested the most significant associations were between where there was saliva pooling and OSMF (Table 1).

We found 22/174 cases of isolated OSMF (13%) all of which were found in association with the quid placement and saliva pool sites indicating the chewing site as a primary site of OSMF involvement (Table 3).

21/174 (12%) patients who were very conscious about their appearance and needed communication skills as part of their occupation (including doctors and software engineers) often had saliva pooling and OSMF in the posterior surface of the oral cavity.

A total of five patients presented with histologically confirmed malignant transformation.

**DISCUSSION**

Our results show that the sites of areca nut chewing and saliva pool are important factors in the oral distribution of OSMF.

When chewed, particles of gutkha are reduced in size by mastication and softened by salivation. The effects of saliva pooling depend on the permeability and absorptive capacity of the oral mucosa. It is generally accepted that oral mucosa is not an effective barrier to the penetration of substances. 16 Oral mucosal permeability is related to the thickness and degree of keratinisation and becomes less permeable moving from the sublingual to buccal and palatal mucosa. 16, 17

As buccal mucosa is less permeable than sublingual mucosa, it is a favourable route of sustained delivery of gutkha ingredients as well as being the most convenient anatomical site for placement of gutkha. 16 Therefore the buccal mucosa can be considered the most vulnerable surface for OSMF. Additionally, the rapid absorption of gutkha in the lingual sulcus results in an early loss of its taste, and therefore, this site might not be preferred by the patient subconsciously. 16 Placing betel quid other than the buccal sites may hinder speech and interestingly, patients who preferred these uncommon sites (Lip, tongue & floor of the mouth) were in jobs that required few skills in communication (garage workers, drivers, night duty watchman, and carpenters). Interestingly, patients with occupation requiring a good appearance and verbal commination skills had saliva pooling in the posterior surface to mask the gutkha chewing appearance. OSMF of soft palate, facial and retromolar tissue was detected in these cases.

In our study, the site of quid placement was the commonest region to be affected by fibrosis. We also observed increased severity of fibrosis (in terms of number of fibrotic bands, burning sensation, erosion and fragility) in the site of quid placement compared with other areas of the oral cavity. It is likely that the saliva pool containing concentrated gutkha grains initiates an early fibrotic reaction.

However, there were also sites of OSMF found where saliva pooling was absent. These patients gave an earlier history of saliva pooling prior to fibrosis in these areas. It could be speculated that the change in the saliva pooling pattern occurred when the mucosal surface became fibrosed, thereby reducing absorption of the quid contents and the change continued until all of the oral surfaces became affected by OSMF.

There was no isolated fibrosis in upper lip mucosa but fibrosis was always preceded by lower lip OSMF. This may be due to gravity which makes it a favourable site for gutkha placement and that upper lip sparing may be because it is protected by the upper incisors when present.

**CONCLUSION**

Our preliminary study has confirmed the role of salivary pooling in the pathogenesis of OSMF and could account for the sporadic distribution of this condition. Additional studies are needed to investigate this further.

**Conflict of Interest**

None

**Ethics statement/confirmation of patient permission**

Institutional research ethical committee (Human) of the medical college and Hospital

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