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Estimation of Unstimulated Salivary Flow Rate, pH, Copper and Iron in Gutkha Chewers with and without Oral Submucous Fibrosis – A Preliminary Study.

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ABSTRACT

Oral submucous fibrosis (OSMF) is well recognized as one of the most potentially premalignant condition of the oral mucosa in India. Based on clinical, epidemiological and animal experimental studies Gutkha is considered as the main predisposing factor for Oral submucous fibrosis. The present case control study was carried out to estimate and correlate salivary flow rate, pH, iron and copper in gutkha chewers with and without oral submucous fibrosis. Study included 20 gutkha chewers without oral submucous fibrosis, 20 gutkha chewers with oral submucous fibrosis and 20 age and sex matched controls. The saliva was collected. After collection salivary flow rate was measured & expressed in ml/min. The pH determination of saliva was done by pH analyzer Elico-L1-612. The estimation of copper and iron in saliva was done by atomic absorption spectrophotometry, AAS-203-CHEMITO. The salivary flow rate in gutkha chewers with and without oral submucous fibrosis was raised and was significant when compared with subjects without any habits (P was < .001). The salivary pH was not altered. The salivary copper and iron were raised in patients of gutkha chewers with oral submucous fibrosis when compared with patient without oral submucous fibrosis (P was < .01). The increased salivary flow rate in gutkha chewers with and without oral submucous fibrosis could be due to parasympathomimetic activity of arecoline in the arecanut and the increase in copper and iron could be due to release of these elements present in arecanut while chewing gutkha. The present preliminary study was able to estimate copper and iron in saliva of gutkha chewers for a small group of population and showed increased levels.

Keywords: Oral submucous fibrosis, Gutkha, Saliva, Copper.

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic inflammatory disease, affecting the oral mucosa exact etiology is unknown, but found to be strongly associated with the chewing of areca-nut preparations such as Gutkha. Gutkha is a preparation of crushed areca nut, tobacco, catechu, lime and sweet or savory flavorings and is a powdery, granular light brownish to white substance. Areca nuts contain magnesium, phosphorous, iron, sodium also several trace elements such as copper, manganese, zinc, nickel and lead. Within moments, the gutkha begins to dissolve and turn deep red in color. It imparts upon its user a "buzz" somewhat more intense than that of tobacco. Copper content in Areca catechu (betel nut) products is associated with oral submucous fibrosis. Areca nut has a copper content (302 nmol/g), a substantial amount of which is released into saliva while chewing. It is known that the copper dependent enzyme, lysyl oxidase, secreted by the fibroblasts, facilitates the cross-linking of collagen, thereby inhibiting its degradation. Arecanut contains four major alkaloids: arecoline, arecaidine, guvacoline and guvacine. Due to the parasympathomimetic activity of arecoline, Arecanut chewers have high Salivary Flow Rate which also influences the pH of saliva. In recent years saliva has attracted much attention, as saliva is being used for the diagnosis of a wide range of diseases and is proven to be an easily available, reliable and noninvasive diagnostic medium. It is a real alternative for determining plasma levels because saliva lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears". Few studies have been done on the influence of gutkha chewing on the salivary parameters. Given the paucity of literature on the influence of gutkha chewing on salivary flow rate and pH, the present study was undertaken to estimate the alteration in Salivary flow rate, pH, copper and iron in gutkha chewers with and without oral submucous fibrosis.

MATERIALS AND MEHODS

Study was conducted in the outpatient Department of Oral Medicine and Radiology, Bapuji Dental College and Hospital. 20 patients of Gutkha Chewers without oral submucous fibrosis, 20 patients of Gutkha Chewers with Oral Submucous Fibrosis and 20 controls were included in the study. The study was done after obtaining approval from the institutional ethical committee.

Patients were divided into three groups A, B, C. Group A & Group B patients had Gutkha chewing habits for more than six months. Patients with clinically and histologically proven Oral Submucous Fibrosis will be included in group A. Clinical Staging was done according to Khanna JN, Andrade NN (1995). Patients without Oral Submucous Fibrosis will be included in group B. Group C includes age & sex matched controls. Patients not having any known systemic disorder & not on any drugs were included. Before the beginning of the study, information was given to all the participants regarding the need and design of the study and the need for collection of saliva. Only those subjects, who gave a signed informed consent on an institutionally approved consent form, were participated in the study. Detailed history of gutkha chewing habit was recorded. Frequency of the habit, duration of the habit and habit of keeping the quid was noted. The site of chewing or keeping the quid, duration of chewing or keeping the quid and whether

he swallows the juice of gutkha or spits it out were recorded. Symptoms like burning sensation to normal food or spicy food, restricted mouth opening, difficulty in swallowing and speech and increased or decreased salivation and their duration was noted. The clinical examination was carried out and the relevant data were entered into the proforma [1]. Each patient was made to sit on a dental chair and was asked to spit in a graduated test tube every 1min for 10 mins. After collection salivary flow rate is measured & expressed in ml/min [1,2]. pH determination of saliva was done by pH analyzer Elico-L1-612.

The estimation of copper, iron in saliva was done by atomic absorption spectrophotometry, AAS-203-CHEMITO. First stock solutions were prepared & analysis was done by Atomic Absorption spectrophotometry. Later saliva sample analysis was done.

For copper, dissolve 1gm of copper metal in 1:2 nitric acid. For iron, dissolve 1 gm iron powder in 1:2 hydrochloric acid.

These are standard solutions which were used first before testing saliva sample & the absorbance of these solutions were recorded for reference. For copper and iron, saliva sample was aspirated directly into Atomic absorption spectrophotometer absorbance of saliva were recorded. These readings were transmitted to the detector then given by computer using the software AAS 201/203.

Statistical Analysis

ANOVA was used for multiple group comparisons & students't' test for two group comparisons. Results were expressed as Mean \pm SD and range values. Unpaired t-test was used for comparing means of two groups. Pearson's correlation coefficient was used to assess the relationship between salivary parameters. A P-value of 0.05 was set for statistical analysis.

RESULTS

All the 40 (100%) Gutkha Chewers in study group and 20 (100%) in control group were males in the age range of 18 – 30 years. All 40(100%) subjects in the study group had the habit of gutkha chewing. The mean salivary flow rate in controls was 0.39 ± 0.08 ml/min, in gutkha chewers with oral submucous fibrosis was 0.60 ± 0.09 ml/min, in gutkha chewers without oral submucous fibrosis was 0.61 ± 0.07 ml/min. On comparison of controls with gutkha chewers with oral submucous fibrosis, and without oral submucous fibrosis the salivary flow rate was increased and P was $< .001$ which was Highly Significant (Table 1, Graph 1). The mean salivary pH in controls was 6.83 ± 0.01 , in gutkha chewers with oral submucous fibrosis was 6.93 ± 0.23 , in gutkha chewers without oral submucous fibrosis was 6.88 ± 0.22 . Salivary pH was not significant (Table 1, Graph 2). The mean salivary copper in gutkha chewers with oral submucous fibrosis was 0.49 ± 0.10 ppm, in gutkha chewers without oral submucous fibrosis was 0.29 ± 0.06 ppm. The mean difference for salivary copper was 0.20, 't' value was >0.47 and P was $< .01$ which was Significant (Table 1, Graph 3). The mean salivary iron in gutkha chewers with oral submucous fibrosis was 0.27 ± 0.09 ppm, in gutkha chewers without oral submucous fibrosis

was 0.20 ± 0.07 ppm. The mean difference for salivary iron was 0.07, 't' value was 2.76 and P was $< .01$ which was Significant (Table 1, Graph 4).

Table 1: Comparison of Salivary Flow Rate, pH, Copper and Iron in Gutkha Chewers with Oral Submucous Fibrosis and without Oral Submucous Fibrosis

Groups	Particulars	Salivary Flow Rate (ml/min)	Salivary pH	Salivary Copper (ppm)	Salivary Iron (ppm)
With OSMF 20 Cases Group A	Mean \pm SD Range	0.60 ± 0.09 (0.5 – 0.8)	6.93 ± 0.23 (6.51 – 7.48)	0.49 ± 0.10 (0.36 – 0.78)	0.27 ± 0.09 (0.17 – 0.48)
Without OSMF 20 Cases Group B	Mean \pm SD Range	0.61 ± 0.07 (0.5 – 0.8)	6.88 ± 0.22 (6.63 – 7.28)	0.29 ± 0.06 (0.19 – 0.61)	0.20 ± 0.07 (0.10 – 0.39)
Controls 20 Cases Group C	Mean \pm SD Range	0.39 ± 0.08 (0.3-0.5)	6.83 ± 0.010 (6.80-6.84)		
Group A vs Group B	Mean Diff	0.01	0.05	0.20	0.07
	t	0.40	0.67	$>.47$	2.76
	P	0.69 NS	0.51 NS	$< .01$ S	$< .01$ S
Group A vs Group C	Mean Diff	0.21	0.10		
	t	$>.80$	1.94		
	P	$< .001$ HS	0.06 NS		
Group B vs Group C	Mean Diff	0.22	0.05		
	t	9.26	1.02		
	P	$< .001$ HS	0.31 NS		

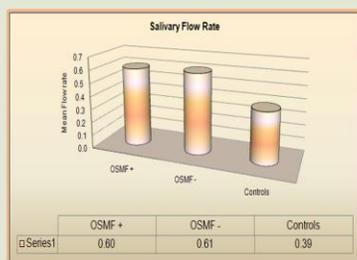
DISCUSSION

Gutkha, the word itself would invoke terror, given its destructive capability and yet, this substance is so popular amongst people, young and old, men and even women. The rapidly increasing adoption of this habit can be judged from the reports that the Indian market for pan masala and gutkha is worth the economy of 25 billion [3]. Habitual chewing of pan masala/gutkha is associated with earlier presentation of oral submucous fibrosis than betelquid use.

Oral Submucous Fibrosis is one of the most prevalent premalignant conditions that occur predominantly among Indians and in South East Asia. In the present study, saliva collection and salivary flow rate estimation was done, similar to a study to know the effect of chewing arecanut on resting whole mouth Saliva [1]. The measurements of pH of saliva were made using pH meter [4].

Present Study is a preliminary study to estimate salivary flow rate, Salivary pH, salivary copper and Salivary iron in gutkha chewers with and without oral submucous fibrosis. Among the 20 Gutkha chewers without Oral Submucous Fibrosis, there were maximum number of cases in the age group of 21-25yrs that made up of 50% of cases and was consistent with that reported in epidemiological study [5] of Mohammed sami et al and Sandeep K [6] et al.

GRAPH 1-COMPARISON OF SALIVARY FLOW RATE IN GUTKHA CHEWERS WITH ORAL SUBMUCOUS FIBROSIS AND WITHOUT ORAL SUBMUCOUS FIBROSIS

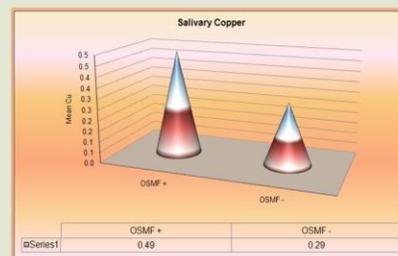


GRAPH 2-COMPARISON OF SALIVARY pH IN GUTKHA CHEWERS WITH ORAL SUBMUCOUS FIBROSIS AND WITHOUT ORAL SUBMUCOUS FIBROSIS

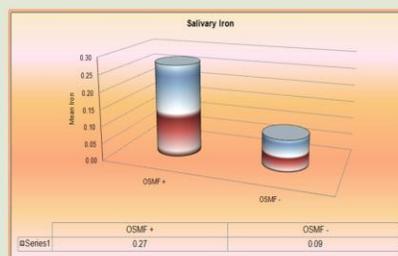


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GRAPH 3-COMPARISON OF SALIVARY COPPER IN GUTKHA CHEWERS WITH ORAL SUBMUCOUS FIBROSIS AND WITHOUT ORAL SUBMUCOUS FIBROSIS



GRAPH 4-COMPARISON OF SALIVARY IRON IN GUTKHA CHEWERS WITH ORAL SUBMUCOUS FIBROSIS AND WITHOUT ORAL SUBMUCOUS FIBROSIS



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The study group mean age was 25.0 ± 2.7 yrs can be comparable to study where the mean age was 29.1 yrs [7]. The higher incidence of Oral Submucous Fibrosis in younger age is attributed to the chewing habit which they were addicted to, because of the easy accessibility [5], low cost, popularity of gutkha and the youngsters are easily habituated to gutkha [5] which makes them susceptible for Oral Submucous Fibrosis. Among the 20 Oral Submucous Fibrosis subjects, all were male patients. The male predominance is seen in many studies with male to female ratio of, 4:1 [8], 9:5 [9]. All 40 (100%) patients had the habit of gutkha chewing which is considered as main etiologic factor based on epidemiological studies [7,10], case reports [11], case control studies [12,13], tissue culture study [14]. Thus, the present study is consistent with all the above studies. Arecoline and Arecaidine are the main alkaloids and present in arecanut which play a major role in the pathogenesis of OSMF by stimulating the human fibroblasts and collagen synthesis [15]. The copper present in the arecanut is released during chewing and is deposited in oral tissues, and the lysyl oxidase activity is upregulated, which leads to cross linking of collagen and elastin, making them less degradable by collagenase [16]. Copper of areca nut get deposited in the tissues and stimulate the lysyl oxidase enzyme, which in turn stabilizes the collagen fibers and makes them nearly ten

times resistant to the action of collagenase[16]. We found that the salivary flow rate was higher in both the study groups with significant difference ($P < .001$) when compared with control group.

The study found no significant difference (P was 0.51) of salivary pH in both the study groups when compared with control group.

Though, we are not able to compare our measurements with any studies done in the past, since ours is a preliminary study and there is scarcity of literature regarding the measurements of salivary flow rate and pH in gutkha chewers with and without oral submucous fibrosis in literature. In one study they have been done on arecanut chewers[1] in various forms where they found that the salivary flow rate was increased in raw arecanut chewers. This they have attributed probably due to the parasympathomimetic activity of arecoline. This study is consistent with the present study.

In the present study, the salivary copper was not detected in control group, this could be because of presence of copper in very minute quantity (i.e, in part per billion) in non gutkha chewers, when compared to study group (in part per million) as the sensitivity of atomic absorption spectrophotometer used in present study is only to detect part per million. In one study[17], soluble concentrations of copper in saliva were significantly ($p < 0.001$) higher than stimulated salivary samples collected without areca-nut chewing. They substantiated it to amounts of copper released from areca products induces lysyl oxidase activity upregulating collagen synthesis by fibroblasts, facilitating its cross linking and, thereby, inhibiting its degradation. This study supports present study, as one of the main constituent of gutkha is arecanut which contains copper; this copper is incorporated into lysyl oxidase during its biosynthesis[18]. Areca nuts have been shown to have a high copper content, and chewing areca nuts for 5–30 min significantly increases soluble copper levels in oral fluids. This increased level of soluble copper could act as an important factor in Oral Submucous Fibrosis by stimulating fibrogenesis through up-regulation of Lysyloxidase activity[16]. In another study[19], the mean salivary copper was higher than in control group.

The mean salivary iron in gutkha chewers with oral submucous fibrosis was 0.27 ± 0.09 ppm, in gutkha chewers without oral submucous fibrosis was 0.20 ± 0.07 ppm. We are not able to compare our measurements with any studies done in the past, since there is scarcity of literature regarding the measurements of salivary iron in gutkha chewers.

CONCLUSION

Oral submucous fibrosis has always been a challenging disease with high prevalence in India. Iron and copper are the transition metal ions that are involved in the catalytic process of Reactive oxygen species generation. To conclude it is clear that there is increase in salivary flow rate, salivary copper and salivary iron in gutkha chewers with Oral Submucous fibrosis. The presence of salivary copper and iron increase the prevalence of malignant transformation as

these elements promote formation of high levels of reactive oxygen species close to the buccal mucosa, overwhelming the protective enzymes and thus causing direct damage to the tissue.

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