ORIGINAL RESEARCH

Evaluation of Effect of Duration of Gutkha Chewing Habit on Different Salivary Parameters- A Cross Sectional Study.

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Abstract

Background and Objective: Reputation of smokeless tobacco (ST) is growing rapidly and its prevalence of use is rising globally including India. The study was done to evaluate the possible alterations in salivary parameters such as salivary flow rate, salivary pH, salivary buffer capacity and salivary alkaline phosphatase level in gutkha chewing and effect of duration of gutkha chewing habit on these parameters.

Methodology: Unstimulated saliva sample from healthy individuals and gutkha chewers (n=29 each) was collected and estimated various parameters such as salivary flow rate, pH buffer capacity and alkaline phosphatase level. The results were analysed statistically using independent sample t test and one way Anova and p≤0.005 is considered statistically significant.

Result: In the present study, we observed the mean salivary flow rate of 0.37 ± 0.03 mL/min, salivary pH of 5.92 ± 0.29, salivary buffer capacity of 4.64 ± 0.37 and salivary alkaline phosphatase level of 6.89±1.23. All parameters were significantly lower when compared to the normal healthy subjects (p value of ≤ 0.001.). No significant difference was notes in any of the evaluated parameters between Gutkha chewers of different duration.

Conclusion : A significant negative association was found between Salivary flow rate, salivary pH, salivary buffer capacity and salivary alkaline phosphatase level with gutkha chewing suggesting that gutkha use has deleterious effect on salivary gland function. Thus in addition to direct effect on oral mucosa, through altering quality and quantity of saliva, gutkha chewing habits poses a potential threat to oral health.

KEY WORDS: Smokeless tobacco, Saliva, Salivary flow rate, Salivary pH, Salivary buffer capacity, salivary alkaline phosphatase, Gutkha.

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Introduction

Worldwide, tobacco consumption either smoke or smokeless form is considered as one of the most important public health problems and is the most important etiological factor in the development of oral cancer. The reputation of smokeless tobacco (ST) is growing rapidly and its prevalence of use is rising globally. Utilization of Gutkha, an addictive form of ST, is mostly common among many Indian communities. Use of smokeless tobacco (ST) is increasing in popularity due to unsupported perception of safety, indoor smoking bans, ability to conceal use, increased social acceptance, and reported "positive" physiological effects, such as relaxation, increased concentration, heightened alertness, and diminished hunger.[1] Gutkha, which is placed between the gum and cheek, is usually sucked or chewed and it contains a combination and mixture of areca nut, slaked lime, catechu, and a number of spices.[2-4] Saliva plays an important role in the maintenance of oral health, by means of antibacterial activity, in the lubrication and repair of the oral mucosa and in the taste and digestion. Several systemic and local factors can influence salivary secretion and composition and serve as an efficient medium for monitoring health[3-7]. Average rate of secretion saliva is 0.4 ml/min unless otherwise stimulated. Salivary pH levels of healthy individuals range between 6.5 and 7.5 and is generally affected by the physiological and pathological factors. Optimal function of saliva in keeping health of oral cavity, is maintained as long as its pH, buffer capacity (BC), and salivary flow rate (SFR) remain normal.[5,6] Alkaline phosphatase (ALP) is an intracellular enzyme present in the saliva and most tissues, organs and bones, including the epithelial, inflammatory cells, bacterial organisms and mineralising tissue cells. The enzyme is related to cell injury and

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death. ALP most commonly correlates with bone metabolism and is present in the osteoblast cell membrane and polymorphonuclear leukocyte granules. Destructive processes in the alveolar bone can lead to increased ALP activity. This enzyme is a nonspecific phosphomonoesterase that functions through a phophosery l intermediate to produce free inorganic phosphate.[7] Estimation of salivary alkaline phosphatase was done by some investigators in connection with periodontal disease and dental caries.[8,9] Salivary alkaline phosphatase is also identified as one of the sensitive markers for the detection of oral carcinoma.[10] There are also studies on salivary alkaline phosphatase in smokers which identified a significant decrease in the same[11]. Similarly there are reports on decrease in salivary flow rate, pH and buffering capacity among users of different types of ST[12]. These findings clearly point to the fact that tobacco both, smoked or smokeless form alter salivary parameters including pH and buffering capacity which in turn might adversely affect the functions of salivary alkaline phosphatase, which probably affects the oral mucosal permeability to harmful substances from ST. In terms of the current research, no study evaluated the salivary parameter such as SFR, pH and buffering capacity in gutkha chewers and possible relationship between these salivary parameters and salivary ALP activity. So the need for the study is identified and the present study is designed to analyze salivary flow rate (SFR), salivary pH, Buffer capacity (BC) and salivary alkaline phosphatase (S-ALP) level in gutkha chewers and compare the obtained findings with that of normal healthy individual and also to compare the above salivary parameters in subjects having habits of different duration.

Aims and Objectives

To determine the possible alterations in salivary parameters such as salivary flow rate, salivary pH, salivary buffering capacity and salivary alkaline phosphatase level induced by gutkha chewing and effect of duration of gutkha chewing habit on these parameters.

Objective of the study is to analyze the salivary flow rate, salivary pH, salivary buffering capacity and salivary alkaline phosphatase level in gutkha users of duration 1-5 years and normal healthy individuals without any habits, and to Comparison of above salivary parameters in subjects having habits of different durations.

Methods:

Source of data: Fifty eight (29 subjects in each group) participants were randomly chosen from the patients reporting to the Department of Oral Medicine and Radiology in Yenepoya Dental Hospital who having habits of gutkha chewing. Age matched healthy individuals was chosen from Yenepoya University campus who don't have any habits as control group.

Study Group:

Participants chosen were divided into two different groups according to habit history: Group A: Gutkha users of duration 1-5 years as study subjects, Group B: Normal healthy individuals without any habits as controls. Subjects with systemic diseases, Individuals with habits less than 1 year, Participants with periodontal disease and oral mucosal lesions. After explaining the purpose of the study and procedure, a written consent was taken from every participant. A case history form was used, to record the demographic data and general information about the tobacco habit includes the type of ST use, frequency, duration etc. following which an oral examination was done to check for any existing mucosal changes. After recording the above information, unstimulated whole saliva samples were collected by spitting method. Saliva sample was collected between 9:00 am and 12:00 noon to avoid diurnal variation. The patients were advised not to eat, drink, and smoke or to chew 1 hour before and during the entire procedure. Subjects were comfortably seated in the dental chair and allowed to relax for few minutes before collecting saliva sample. Subjects were asked to rinse their mouth with water and 10 min later, they were advised to sit upright with head slightly tilted forward to collect saliva in the floor of the mouth and then spit into a graduated container, through a glass funnel every 1 min for 5 min. During saliva collection, subjects were instructed not to speak or swallow. After the collection, the following parameters were analyzed.

Salivary Flow Rate is evaluated by collecting saliva c within 5 minutes in the container with graduated marks was noted down. Flow rate (ml/min) of saliva was determined by dividing the volume of saliva collected by 5 and expressed as mL/min. The pH values for all salivary characteristics were assessed with the help of ECO TESTER pH meter (OAKTON PH1 TESTER). The pH meter was standardized using a standard protocol, using pH calibration solutions ranging from pH 4, 7 and 10. Buffer capacity is determined by quantitative test using a hand-held pH meter method. This method involves the addition of 0.5 ml of saliva to 1.5 ml of 5 mmol/L Hcl. Salivary alkaline phosphatase levels was done in Spectrophotometry (fig 8) Mispa CXL Pro is proven HCFG (Holographic Concave Flat Field Grating) rear spectrophotometry which reduces ambient light interferences, and photo-spot technology to reach super micro analysis. A volume of 3 ml saliva was centrifuged at 3000 rpm for 15 min, and the supernatant saliva was obtained. 10 µl of the supernatant was mixed with 500 µl of ALP reagent (Alkaline Phosphatase (ALP)-AGAPPE kit (Fig 9), Biosystems S.A. The vial with sample and ALP solution is aspirated of around 300µl, and analysed in kinetics for each 1 min (Delta 1, Delta 2, Delta 3) then the mean value generated by the instrument was taken as the final value which is expressed in IU/L. The data obtained was subjected to statistical analysis using Independent sample t test for comparing within the group and ANOVA for comparing between the groups. Statistical software SPSS17 and MS Excel was used to analyze the data. $p \leq 0.05$ was considered to be significant and $p \leq 0.01$ was considered to be highly significant.

Results:

In the present study we have analyzed salivary flow rate, pH, buffer capacity and salivary alkaline phosphatase level of 58 subjects. Study subjects were in 2 groups (n= 29 in each group) namely healthy individuals and gutkha chewers. Out of 58 subjects participated the mean age group of healthy individuals were 30.7±5.7 and 35.1±5.08 in gutkha chewers. (Table 1) The salivary pH in healthy individuals ranged from 6.7 to 7.9 with a mean value of 7.26 ± 0.32, while in gutkha chewers ranged from 5.5 to 6.4 with a mean value of 5.92 ± 0.29. The salivary buffer capacity was recorded in healthy individuals, the values ranged from 4.0 to 5.4 with a mean of 4.64 ± 0.37 and in gutkha chewers 2.7 to 3.7 with a mean value of 3.15±0.46. The salivary alkaline phosphatase level in healthy indi-

viduals ranged from 24.0 to 39.3 with a mean value of 29.70 ± 4.03. The salivary alkaline phosphatase level in gutkha chewers ranged from 5.2 to 8.6 with a mean value of 6.89±1.23. In gutkha chewers the salivary flow rate, salivary pH, salivary buffer capacity and salivary alkaline phosphatase level is significantly low when compared to healthy controls (Table 2).

In order to identify the possible effect of duration of gutkha use, various salivary parameters were compared in gutkha chewers of different duration of 2 years, 2-4 years and more than 4 years. The mean salivary flow rate in gutkha chewers of 2 years was 0.37±0.014 and 2-4 years was 0.38±0.032 and more than 4 years was 0.36±0.037. The salivary pH in gutkha chewers of different duration of 2 years the mean is 5.6± 0.14 and 2-4 years is 5.9 ± 0.30 and more than 4 years is 6.1 ± 0.29. The salivary buffer capacity in gutkha chewers of different duration of 2 years the mean is 2.6± 0.14 and 2-4 years is 3.1 ± 0.47 and more than 4 years is 4.4 ± 0.57. The salivary alkaline phosphatase in gutkha chewers of different duration of 2 years the mean is 6.8± 0.422-4 years is 6.8 ± 1.31 and more than 4 years is 7.4 ± 0.95. The p value in different durations of all salivary parameters are not statistically significant. (Table 3)

Discussion

Saliva is a complex body fluid that is of high importance, which influences oral health through specific and nonspecific physical and chemical properties. Though the importance of saliva in our everyday activities and its protective functions are often taken for granted, any alteration in the quality or quantity of saliva is reflected as detrimental effects on oral and systemic health. The quantity and quality of saliva may be affected by multitude of factors one of which can be use of tobacco[13].

In the present study salivary flow rate, salivary pH, salivary buffer capacity and salivary alkaline phosphatase levels were low when compared to healthy individuals.

A low unstimulated flow rate not only makes a person susceptible to xerostomia but greatly delays clearance of food from the mouth. People with a low salivary flow rate are particularly susceptible to dental caries because of the loss of many protective effects of saliva, including sugar clearance, acid clearance and remineralization property. All these properties are directly related to salivary secretion. The mucous glycoproteins of saliva, such as MUC5B, MUC7 and proline-rich glycoproteins, play a major role in lubricating oral tissues. This lubrication reduce trauma to the soft tissues during mastication, swallowing and speaking. These glycoproteins also help maintain an intact layer of saliva in contact with the oral mucosa, which prevents it from drying out. When salivary flow is low, areas of the mucosa become dried out and are much more susceptible to abrasion. Furthermore, in patients with a low unstimulated salivary flow rate, clearance of bacteria and desquamated epithelial cells is reduced greatly, increasing the tendency for halitosis to develop. An important feature of having a continuous flow of unstimulated saliva into the mouth is that it reduces the probability that oral bacteria will be able to ascend the salivary ducts and infect the glands. Salivary pH can be largely influenced by extrinsic factors including dietary intake, but also by intrinsic factors and the flow rate and buffering capacity. The low salivary pH provides an acidogenic environment for the growth of aciduric bacteria leading to dental caries which again further lowers the salivary pH leading to a vicious cycle. By causing decreased salivary pH, Gutkha chewing results in compromised oral health[6,4]. The protective functions of the saliva are not limited to the flow rate. Saliva also has some important biological properties such as its capacity to act as a buffer against the acids produced by micro-organisms or ingested through the diet, allow to keep a relatively constant oral pH[20] Buffering capacity of saliva is a significant property of saliva which ensure the pH of the oral cavity is maintained. The buffer capacity depends on the acids and bases contained in the secreted saliva[21]. Moreover, salivary parameters such as salivary flow and pH are related to one another. Bicarbonate is the main buffer that opposes acids, but is completely effective only at high salivary flow rates, because its concentration increases markedly with salivary flow rate rise[22]. The salivary buffering capacity is an important factor for dental caries resistance. Thus gutkha chewing can poten-

Table 1: Mean ages (in years) in the study group.

Study group	Total number of subjects (n)	Age range	Mean age ± SD
Healthy individuals	29	23-45	30.7±5.7
Gutkha chewers	29	25-46	35.1±5.08

SD= Standard deviation

Table 2 Comparison of salivary parameters between healthy individuals and gutkha chewers

Variable	Mean ±SD		F value (p value)
	Healthy indiduals (n= 29)	Chewers (n= 29)	
SFR	0.59 ± 0.05 (0.53- 0.68)	0.37 ± 0.03 (0.35- 0.44)	0.00 (0.00)
pH	7.26 ± 0.32 (6.7- 7.9)	5.92 ± 0.29 (5.5- 6.4)	0.00 (0.00)
BC	4.64 ± 0.37 (4.0- 5.4)	3.15 ± 0.46 (2.7- 3.7)	0.00 (0.00)
S-ALP	29.70 ± 4.03 (24.0- 39.3)	6.89 ± 1.23 (5.2- 8.6)	0.00 (0.00)

SD= Standard deviation; SFR= salivary flow rate; BC=buffer capacity; S-ALP= salivary alkaline phosphatase.

Table 3 Comparison of salivary parameters between gutkha chewers of different duration.

Duration	Mean Difference ±SD			
	SFR	pH	BC	S-ALP
2 yrs	0.37± 0.014	5.6± 0.14	2.6± 0.14	6.8± 0.42
2-4 yrs	0.38±0.032	5.9 ± 0.30	3.1 ± 0.47	6.8 ± 1.31
>4 yrs	0.36± 0.037	6.1 ± 0.29	4.4 ± 0.57	7.4 ± 0.95
P value	0.689	0.180	0.139	0.767

tially hamper the oral environment through its effect of salivary buffering capacity[23]. Alkaline phosphatase is a membrane-bound glycoprotein found on most cell membranes in the body and physiologically occurs during bone formation in developmental stages. It is a hydrolase intracellular enzyme that takes part in the metabolic processes of the cells. It is produced by many cells within the periodontal environment, the principal source being PMNs leukocytes, bacterial fibroblast and osteoblast activity which is disturbed due to diabetes, smoking, etc., pathologically.[16] ALP is one of the potentially powerful markers of periodontal disease activity and ALP levels increases in periodontal diseases.[17,18] ALP is recognized as an important marker of induction of tumor cell differentiation, ALP belongs to hydrolase group of enzymes which are biocatalysts synthesized in living cells.[14] Alkaline phosphatase role is to catalyze the hydrolysis of monoesters of phosphoric acid and also transphosphorylation reaction in the existence of large concentrations of phosphate acceptors.[15]

From the observations of this study, it can be established that use gutkha, has adverse effects on saliva which affects the physical and chemical properties of saliva. Salivary parameters can be affected by many external and internal factors; therefore it may be difficult to relate the findings exclusively to the effect of Gutkha. In order to confirm the findings, further studies with more controlled selection of subjects is recommended. Thus in addition to the direct adverse of chemical constituents on oral mucosa, gutkha hampers the oral health through effect on salivary parameters. To conclude, it is very clear that gutkha chewing poses a potential treat to oral health and predisposes oral tissue to various diseases.

Conclusion

The role of salivary parameters is to maintain oral health. From the present study conducted with objectives if determining the adverse effects of gutkha chewing in the salivary parameters, we came to following conclusion; the salivary flow rate of the gutkha chewers was low when compared to the healthy individuals. The salivary pH and salivary buffer capacity of the gutkha chewers was also found to be low when compared to the healthy individuals. The salivary alkaline phosphatase level in the gutkha chewers was drastically very low when compared to the healthy individuals. Though the duration of ghutka chewing did not show significant influence on salivary parameters studied the progressive reduction on the duration is increasing. So the gutkha chewing adversely affect the salivary gland function and resulting in the reduced salivary parameters, which in turn damages the mucosa contributing to pathogenesis of oral lesions.

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