Original article

Salivary proteins and amylase activity in smokers with Leukoplakia

Suma GN, Goel S, Dayashankar Rao JK, Nagarajappa R, Ramesh G

Abstract: Salivary diagnostics offer an easy and cost-effective method for assessing various biological molecules. Salivary amylase and protein levels in oral squamous cell carcinoma (OSCC) are altered. The establishment of these changes in leukoplakia the most common potentially malignant disorder in the oral cavity may help in preventing the disease progression into morbid OSCC. Aims: This study aimed at estimating the alterations in salivary proteins and amylase activity in smokers with leukoplakia and controls to evaluate their role as a diagnostic and prognostic indicator. Setting and design: subjects for the case control study were taken from the outpatient department, of the dental school. **Materials and Methods:** Unstimulated whole saliva samples were collected from smokers with leukoplakia (Group I, n=15), smokers without leukoplakia (Group 2, n=15), and healthy nonsmokers (Group 3, n=15). The protein estimation was done by Biurette method and salivary amylase activity was measured by Maltose test and results were compared. Statistical analysis: SPSS Software Version 16.0, student's t-test were performed. **Results:** The results showed that salivary amylase and protein levels were not significantly altered in subjects with leukoplakia. **Conclusion:** Salivary amylase and protein levels in smokers with leukoplakia are not significant diagnostic and prognostic indicators.

Key words: Leukoplakia; Smokers; Salivary Amylase; Salivary Protein; Oral; Cancer.

Introduction

Saliva is the first body fluid to confront inhaled smoke and a synergistic, deleterious, interaction takes place between smoking and saliva resulting in the rapid destruction of biological macromolecules such as salivary enzymes and proteins giving it a possible pivotal role in the pathogenesis of oral squamous cell carcinoma(OSSC).[1,2] There are conflicting reports on the effects of smoking on salivary amylase activity and total proteins ranging from significant decrease to no effect.[3-5] Callegari and Lami observed that cigarette smokers exhibit a reduced serum type S iso-amylase and salivary amylase activity.[3] Mahjoub et al reported a decreased α-amylase activity and increased total protein concentration in smokers.[4]

Terup and Masonori reported no effect of smoking on salivary proteins or amylase activity.[5]The changes in the salivary biological molecules are attributed to the reaction between redox active metals in saliva and low reactive free radicals in cigarette smoke and a toxic effect on salivary glands by acting through a local effect or neuromuscular reflex.[2]Saliva is useful in the easy, noninvasive and cost effective diagnosis of a wide range of diseases. Decreased salivary amylase activity and increased total salivary proteins are reported in oral cancer[2,6] but not in leukoplakia, a common pre-malignant lesion. Therefore the present study was to test the hypothesis that the salivary protein and amylase activity are altered in patients with leukoplakia, which might open a new vista as a simple and cost effective diagnostic and prognostic indicator. Present study aimed at estimation of total salivary protein levels and amylase activity among non-smokers and smokers with and without leukoplakia.

Saliva is the first body fluid to confront inhaled smoke and a synergistic, deleterious, interaction takes place between smoking and saliva resulting in the rapid destruction of biological macromolecules such as salivary enzymes and proteins giving it a possible pivotal role in the pathogenesis of oral squamous cell carcinoma (OSSC).[1] The reports on the effects of smoking show a significantly altered salivary amylase activity and total proteins.[3-5]

Decreased salivary amylase activity and increased total salivary proteins are reported in oral cancer[2,6] but no reports are found on leukoplakia, a common pre-malignant lesion. The study aimed to test

the hypothesis that the salivary protein and amylase activity are altered in patients with leukoplakia, and has a role as a simple and cost effective diagnostic and prognostic indicator.

Methodology

The case control study consisted of the sample taken from the patients visiting outpatient department, in a dental school. The cases (Group 1) consisted of 15 male patients with leukoplakia diagnosed based upon WHO 2007 Criteria, having a habit history of bidi/cigarette smoking.

The Group 2 consisted of 15 each of healthy, age and sex matched subjects with the habit history of bidi/cigarette smoking but without leukoplakic lesion and Group 3 of healthy age and sex matched subjects with neither a leukoplakic lesion nor a habit history of bidi/cigarette smoking. Subjects using other forms of tobacco, or having any systemic disease (e.g. Sjogrens syndrome, scleroderma, autoimmune diseases), mental illness, any physical defect (surgery involving major salivary gland) ,on any medications/drugs affecting the salivary or having any symptoms of dry eyes or hyposalivation or hypersalivation were excluded from the study.

Ethical clearance from the institutional ethical committee of ITSCDSR was obtained and written informed consent was obtained from each subject prior to inclusion in the study.

A case history proforma was used to register the history and examination findings. Habit index for smoking was calculated as product of number of bidi/cigarette smoked per day and number of years for Group 1 and Group 2. The salivary parameters were analysed by a technician who was unaware of the group to which the sample belonged.

Unstimulated whole saliva was collected for a period of 5 minutes for all the subjects between 9 am and 12 noon according to the method of Navazesh et al.[7] Subjects were asked to refrain themselves from intake of any food or beverage, smoking or chewing gums for at least 90 minutes prior to salivary collection and avoid swallowing and oral movements during collection. The collected sample was stored in a freezer at -20° C and transported to the lab in an insulated box for estimation of protein content and salivary amylase activity. The protein estimation was done by Biurette method and salivary amylase activity was measured by Maltose test.

Statistical Analysis: SPSS Software Version 16.0 was used. Mean values were calculated and total proteins and amylase activity compared using student's t-test. Significance was set at $p < .05$.

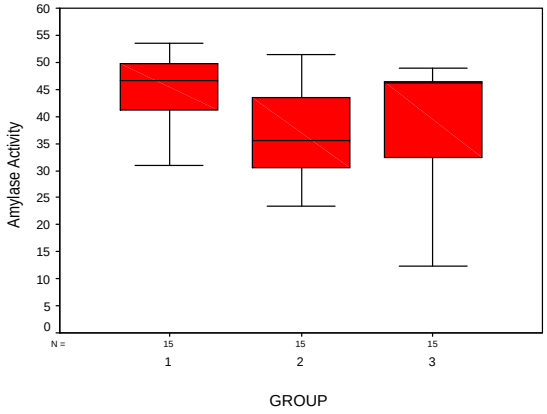
Results

Mean age of the population was 44.87yrs ranging from 30 to 65 yrs. Mean age in Group I was 47.33, Group II 42.20 and Group III 45.07. Difference in mean age among study groups was statistically insignificant. Difference in Mean Habit index for Group I [224] and Group II [176.80] was statistically insignificant. Mean salivary protein levels and amylase activity were calculated for all three study group (table 1 and 2) (Graph 1 and 2).

Table 1: Distribution of total protein levels in various study groups.

Groups	Total proteins P-value [sig </= 0.05]	Salivary amylase P-value [sig </= 0.05]
I – II	.62	.29
I – III	.16	.10
II – III	.47	.39

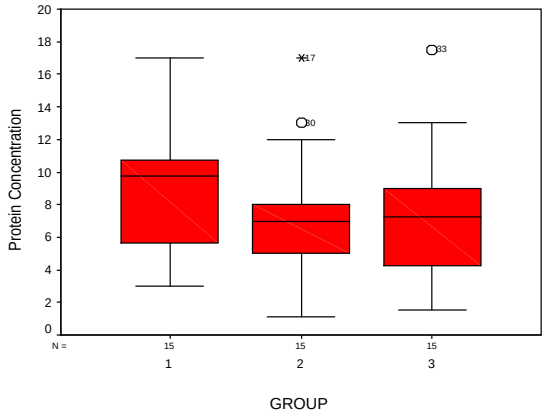
Student's t-test was applied for intergroup comparisons and significance was set at p-value < 0.05. No statistically significant difference were seen in the listed parameters among the case and the controls (p >.05) (Table 3).



Graph 1: Mean amylase activity for all three study group.

Table 2: Distribution of salivary amylase levels in various study groups.

Group I	Group II	Group III
Mean +/- SD	Mean +/- SD	Mean +/- SD
44.32 +/- 7.68	36.58 +/- 9.6	39.21 +/- 10.75



Graph 2: Mean salivary protein levels for all three study group.

Table 3: Comparison of mean total protein and salivary amylase levels among the study groups.

Group I	Group II	Group III
Mean +/- SD	Mean +/- SD	Mean +/- SD
8.5 +/- 3.68	7.27 +/- 4.1	7.32 +/- 4.45

Discussion

The study was conducted with the aim of estimating total salivary protein levels and amylase activity among non-smokers and smokers with and without leukoplakia. Mean Total protein levels were found to be highest among smokers with leukoplakia [8.50] when compared with non-smokers [7.32] and smokers without leukoplakia [7.27] however the difference was not statistically significant. This was in accordance with studies conducted by and Teruo and Masonori.[5] Studies have shown that smoking affects mucosa at cellular level therefore, any change in cell turnover, secretion/shedding may cause biochemical changes which can be reflected in saliva.[8]

In contrast to our study a significant increase in protein levels has been observed in studies conducted in vitro as well as in vivo by Nagler et al[1] and Weiner et al.[8] They hypothesized that aldehydes present in cigarette smoke may cause elevation of protein carbonyls by reacting with sulfhydryl groups of proteins.[1,8] However, the question as to whether salivary total proteins levels prove useful as a marker for detecting leukoplakia cannot be answered conclusively based on the present study but the clinical relevance of increased values of salivary proteins in leukoplakia patients is a food for thought for further research.

Mean Salivary amylase activity varied from 44.32 in smokers with leukoplakia to 39.21 in normal healthy controls, however, the difference was found to be non-significant among study groups. Teruo and Masanori[5] found that smoking prior to salivary measure did not significantly influence salivary amylase and the basal components of saliva.[5]In contrast to our study, other studies report a decrease in salivary amylase activity in smokers up to 34% probably due to aldehydes acting with thiol group.[1,9] However, large gender and inter-individual variations in basal levels of Salivary Amylase have been reported. Forthcoming studies have to increase the number of subjects investigated and take into account salivary flow rate as a possible confound. Association between salivary amylase and other markers of sympathetic and parasympathetic activity should be

investigated before final conclusion about usefulness of salivary amylase as a marker in leukoplakia can be drawn.

Conclusion: In this pilot study we made an attempt to evaluate the importance of saliva as a diagnostic fluid by measuring total protein levels and amylase activity in leukoplakia patients. Our results suggest the effect of smoking and resultant tissue damage on salivary protein levels and amylase activity is statistically insignificant and these parameters cannot be used as definite markers for leukoplakia. A detailed study with larger sample size and more advanced techniques for detecting molecular changes might possibly establish if there is any clinical relevance of salivary proteins and amylase activity as markers of pre-malignancy and malignancy.

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