



EFFECTS OF CIGARETTE SMOKE ON HUMAN SALIVARY TOTAL PROTEIN, ALBUMIN AND A-AMYLASE

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ABSTRACT

Malaria The purpose of this study is to evaluate the effect of salivary composition (Protein, albumin and α -amylase) before and after smoking. The samples were collected from 25 healthy people and tested. The salivary components of protein, albumin and amylase are before cigarette consumption (1.5020, 0.4480 and 1948.90) and after cigarette consumption (0.7270, 0.1120 and 1526.0). The chi square value shows very significant at 0.5% levels. The concentration of salivary components has been decreased after smoking.

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INTRODUCTION

Cigarette smoking is the most preventable cause of addiction, sickness and mortality in the world. Death attributed to cigarette smoking is estimated to rise from 5.4 million in 2005 to 6.4 million by 2015 (Fellows *et al.*, 2002). Chronic cigarette smoking is the single most important risk factor for lung and oral cancers, cardiovascular diseases, chronic obstructive pulmonary disease (COPD) and other tobacco related oral diseases, including periodontitis (Bergstrom, 2004; Freedman *et al.*, 2008). Cigarette smoke contains more than 60 carcinogens and around 4000 chemicals, including bacteria-derived endotoxins, which are toxic to cells (Hasday *et al.*, 1999; Pauly *et al.*, 2008). The risk of developing tobacco smoking-related diseases increases with the total exposure time to the cigarette smoke, which generally includes the number of cigarettes a person smokes each day and the number of years a person has been smoking (Lubin *et al.*, 2009). The oral cavity is the first organ in the human body to be exposed to cigarette smoke.

Tobacco smoke alters the normal homeostasis of the oral cavity, including saliva's antioxidant and other protective systems. This may lead to oral inflammatory diseases and oral cancers (Reznick *et al.*, 2003; Hershkovich *et al.*, 2004). Early tumorigenic activities have been detected in normal oral mucosa of heavy smokers who have no overt pre-cancerous or cancerous lesions (Ayan *et al.*, 2000). Mucosal changes in smokers may also arise from the drying effects of the mucosa, high intra-oral temperatures, intra-oral pH changes, local alteration of membrane barriers and immune responses, or altered resistance to bacteria, fungal and viral infections. Smoking-related cell damage may leave molecular

footprints in saliva, offering the potential for on-invasive early diagnosis of tobacco-related oral diseases.

Human saliva contains a large number of proteins and peptides that are easily accessible and may serve as a potential source of biomarkers to monitor changes that occur under pathological conditions. The value of saliva as a biological fluid for the detection of diagnostic and prognostic biomarkers has become increasingly well-established (Ghafouri *et al.*, 2003; Wong *et al.*, 2006; Kala Jessie *et al.*, 2010). The collection of human saliva is a simple, non-invasive and cost-effective approach for screening large populations. It is easy to handle and may be repeated without inflicting much discomfort to the subjects (Hofman, 2001; Wong *et al.*, 2006). The present study was carried out before and after the cigarette smoking effect on salivary composition of human beings.

MATERIALS AND METHODS

SAMPLE COLLECTION

Twenty five healthy, non-medicated, young males (Age: 21 ± 3 years; body mass: 55 ± 5 kg) participated in the scientific sample study. Each of the subjects performed a single exercise for 10 minutes, smoked a single cigarette and then consumed a cup of coffee. For each subject, the two testing sessions were held on consecutive days at the same time between 7.30 am and 12.00 am. Subjects refrained from food, beverage, and smoking for one hour preceding their test sessions.

The saliva was collected before and after smoking. The procedure for each testing session was the same. The subjects began a session by rinsing their mouths thoroughly several times with tap water and then resting quietly for 5 minutes. Saliva was first collected from behind closed lips (Navazesh and Christensen, 1982;

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Navazesh, 1993) (the Narazesh and Christensen-spitting method). Saliva was then expectorated at the end of each period into a container that had been ice-chilled for 5 minutes. All samples were centrifuged and the supernatant was used as a sample for Protein, albumin and α -amylase activity.

ESTIMATION OF TOTAL PROTEIN (BIURET METHOD)

The total protein in saliva is determined using the total protein kit from the crest bio-system. The unit is expressed as (gm/dl). Pipette out into clean dry test tubes labeled as blank (B), standard (S) and test (T). Mixed well and incubated at 37°C or at room temperature for 30 min. The total protein in the sample is determined with the help of a semi-auto analyzer (Gornall *et al.*, 1949; Layne and Ennis, 1957).

$$\text{mg Protein/ml} = \frac{(\text{mg Protein})}{(\text{ml Reagent D})}$$

ESTIMATION OF ALBUMIN (BROMOCRESOL GREEN METHOD)

Albumin in saliva is determined by using the albumin kit from the crest bio-system. Pipette out into clean dry test tubes labeled as blank (B), Standards (S) and test (T). It was mixed well and incubated at room temperature for 5min. Albumin in the sample is determined with the help of semi-auto analyzer (Duly *et al.*, 2003). Calculation for albumin is the following formula

$$\text{Globulin in g/dl} = (\text{Total protein}) - (\text{albumin})$$

$$\text{(g/dl)} \qquad \qquad \qquad \text{(g/dl)} \qquad \qquad \qquad \text{(g/dl)}$$

ESTIMATION OF AMYLASE (DIRECT SUBSTRATE METHOD)

Amylase activity in saliva was determined using the amylase kit from the crest bio-system. The amylase activity in saliva was expressed in terms of units per liter. Pipette out into clean dry test tube labeled as test (T). Sample was taken from the 1:100 dilutions. Mixed well and the amylase activity in the sample was determined with the help of semi auto analyzer (Bretaudiere *et al.*, 1981).

RESULTS

This study was performed in order to understand the relationship between the salivary components before and after smoking. The quantity and quality of salivary total protein, albumin, and α -amylase were observed among 25 healthy people. The salivary components were estimated before and after cigarette smoking, total protein (before 1.5020±0.2727 and after 0.7270±0.3519); albumin (0.4480±5.574, 0.1120±2.201) and α -amylase (1948.90±885.33, 1526.0±488.19). The χ^2 square test analysed the total protein 5.505, albumin 17.731 and α -amylase 1.323 and all the value are significant at 0.5% levels (Table 1, Graph 1-2).

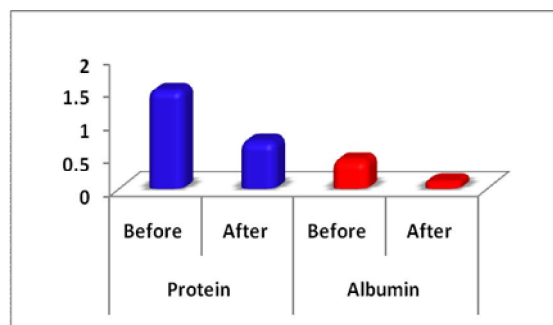


Fig. 1 Effect of smoking on salivary total protein and albumin levels in saliva (gm/dl)

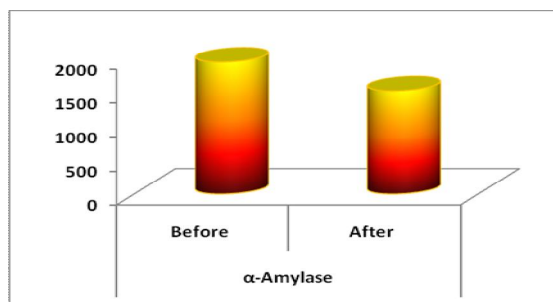


Fig. 2 Effect of smoking on salivary α -Amylase levels in saliva (gm/dl)

Table 1 Effect of smoking on salivary total protein, albumin and α - amylase levels in saliva (gm/dl)

Component	Time	Mean± Stdevi.	χ^2 value	Significant
Protein	Before	1.5020±0.2727	5.505	0.000*
	After	0.7270±0.3519		
Albumin	Before	0.4480±0.574	17.731	0.000*
	After	0.1120±2.201		
Amylase	Before	1948.90±885.33	1.323	0.202*
	After	1526.0±488.19		

The values are expressed as mean ± standard deviation for 25 healthy persons. * Significant

DISCUSSION

The quantity and quality of the salivary total protein, albumin, amylase and the quantity and quality of saliva secreted depends on the conditions for entrance through the secreted cells and synthesis in these cells, and on the modifications as primary saliva passes through the excretory ducts (Suddick and Down, 1980). These processes are regulated in a complex way, which includes control by the sympathetic and para-sympathetic nerve systems (Emmelin, 1981), Neuropeptides (Boyd *et al.*, 1991). The mechanism and control of salivary secretions have been reviewed recently (Turner and Sugiya, 2002; Noble, 2000). Parasympathetic stimulation produces copious saliva with low protein concentration, while sympathetic stimulation produces little saliva but which is high in protein concentration and may thus give a sensation of dryness (Carlson, 2000).

The result shows a decrease in total protein, albumin, and amylase in saliva after smoking as compared to before. Also, albumin decreases significantly relative to globulin. The result associated to smoke could also be the

result of parasympathetic stimulation of post-ganglionic neurons in response to nicotine, in the same manner as acetylcholine, because the membrane of these neurons all contains the nicotinic type of acetylcholine receptors. This study has demonstrated that some toxic components of tobacco smoke, unsaturated and saturated aldehydes, could interact with thiol rich components, leading to structural and functional modification of these molecules. Further, salivary enzymatic activities (include amylase) showed a significant inhibition following single cigarette, probably due to the interaction between aldehydes and -SH groups of the enzyme molecules (Oppenheim, 1970). The stimulated saliva mostly contained a significantly higher proportion of parotid saliva, but the distribution of parotid saliva was still extremely variable. These facts are important, considering that various areas of the mouth will be exposed to different fluid environments, which may have important implications for the site specificity of several oral diseases.

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