



EVALUATION OF SOME ANTIOXIDATIVE PARAMETERS IN SALIVA AMONG SMOKERS AND NON-SMOKERS HEALTHY SUBJECTS

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ABSTRACT

The use of saliva as diagnostic samples provides a non-invasive, cost-effective method of sample collection for oral and systemic diseases screening and its collection is far more practical and safe compared with blood sampling. Cigarette smoke (CS) contains a large amount of oxidative species and so smoking is a source of oxidative stress (OS). The aim of this study was determine some oxidative biomarker in saliva among (20) smokers and (20) non-smokers healthy subjects. The following salivary biomarkers uric acid, albumin and total proteins, Were evaluated by using analysis kits on automatic spectrophotometer. The kits were provided by Dia Mond-Diagnostic Medical international –Jordan. **Result:** statistical analysis revealed significant changes of the three parameters insmoker compared to the non-smokers healthy subjects. **Conclusion:** The results of this study suggested that OS caused a depletion of some antioxidant status in the oral cavity. Uric acid, albumin and total protein can be used and promising salivary biomarkers for monitoring the oral and/or systemic oxidative stress and decline in healthy persons who smoke.

KEYWORDS: saliva, uric acid, albumin, total protein, salivary biomarkers, smokers, non-smokers.

1. INTRODUCTION

The blood analysis serves as the most convenient indicator of the general condition of the human body. Blood data has been the key for laboratory diagnostic procedure for several decades in assessing the systemic diseases such as cancer, cardiovascular, kidney failure, diabetes, etc. however, other biological fluids are also utilize frequently for the diagnosis of such diseases^[1]

Recently, saliva could be offer some distinct advantages for diagnostic and research purposes^[2-4] because its collection is easy, safe non-invasive and cost – effective . the wide diversity of biochemical constituents of human saliva makes it suitable to be used as a biological fluid of diagnosis value . any alteration of biochemicals of the salivary fluid could be related to internal or metabolic disorder and/or associated with periodontal disease.^[5-6]

The salivary glands make saliva and release it in to mouth. There are three pairs major salivary glands (parotid, sublingual and submandibular) as well as numerous minor salivary glands lining parts of the mouth.^[7] Saliva is important biological fluid that provides various function , such as digestion of food and protection of the mouth and throat against microorganisms, because it has enzymes and antibodies.^[8-9] on other hand, there is a growing appreciation for the use of saliva as a diagnostic medium

in a variety of clinical, monitoring and testing for hepatitis, HIV, and candida.^[10]

Moreover, recent developments in salivary diagnostics that have been accomplished using genomic and proteomic approaches have been reviewed elsewhere and provides critical data to forensic investigations and to molecular medicine.^[11]

Whole saliva offers an attractive medium for detecting a range of candidate biomarkers, such as proteins, enzymes, antibodies, nucleic acids, hormones, minerals, and cytokines.^[12]

Cigarette smoking (CS) is a major cause of preventable morbidity and mortality. The risk of death in the smoker in the smokers measured by the number of cigarettes smoked daily, the duration of smoking, the degree of inhalation and the age of initiation^[13- 4]

it is well known that cigarette smoke contains over 4000 different chemicals, 400 of which are proven to be carcinogenic, it also contains various oxidants such as oxygen and nitrogen free radicals and volatile aldehydes, which are probably the major causes of damage to biomolecules.^[15] saliva is the first biological fluid that encounters inhaled cigarette smoke (CS) and has antioxidant defense system able to counter toxic

activities of free radical species (ROS) saliva contains enzymatic (CAT, SOD, GPX) and non enzymatic (uric acid, albumin, glutathione) etc . antioxidants^[16-18]

So, the aim of this study was to compare the levels of some antioxidative parameters (uric acid, albumin and total protein) in saliva of smokers and non smokers healthy subjects.

2. MARIAL AND METHODS

2-1. saliva sample

The present study includes fourty healthy volunteers, who were divided into two groups , smoking (20) and non-smoking^[20] their ages were ranged from^[19-23] years their main cigarette (20) smoked per day over the provious 6 months and the number of years have smoked. the study was approved and all volunteers gave their written informed consent to take part in this investigation.

About 5 ml of unstimulated saliva was collected from each subject. The pure saliva separated from all by centrifugation at 3000 rpm / 10 min. and each sample kept at 4°C until analysis of biochemical assay. All assays were performed at the laboratory of biomedical sciences in AL-Zarka–university college.

2-2.Biochemical assays

Unstimulated saliva contains a higher concentration of antioxidant than in stimulated saliva. Biochemical assays for saliva, uric acid, albumin and protein were perfomed in all subjects (control and smokers) using analysis kits on automatic biochemistry spectrophotometer.

The principle of the method for salivary uric acid, albumin and total protein is the colorimetric method according to the manufacture's instructions.

Statistical analysis

Result were expressed as the mean $\pm$ SD

Statistical evaluation was performed using two-tailed t-test anad analysis of variance (ANOVA) for comparison between group and less than $p < 0.05$ was considered statistically significant.

3. RESULTS

Fourty healthy volunteer were include in this study in the present study, estimated means and standard deviation for each parameter result were calculated. The parameter results were measured in saliva for both smokers and non-smokers groups. High significant changes were observed in uric acid, albumin and total protein values among smoker group compared to non-smokers group ($p < 0.001$) (Table).

Table (1): saliva uric acid, albumin and total protein in smokers and non-smokers healthy subjects (n=20+20)

Smoker	Uric acid mg/dl Mean $\pm$ SD	Albumin mg/dl Means $\pm$ SD	T.protein Means $\pm$ SD	P-value
	0.65 $\pm$ 0.123	0.30 $\pm$ 0.160	1.34 $\pm$ 0.290	0.01
Non-Smoker	0.85 $\pm$ 0.149	0.35 $\pm$ 0.110	1.42 $\pm$ 0.250	0.033 0.042

Data are means $\pm$ SD P value: distribution differences between mean values of smoker group, Vs non smoker group.

4. DISCUSSION

Tobbaco use is known as a serious global public health problem and is also an important risk factor for various related disease. This may due to the fact that cigarratte smoker (CS) contains a large variety of compounds include nicotine, aldehyde, penzopyrwne, carbon monoxide , many oxidants and oxygen and nitrogen free radicals. Wich are considered as the main causes of damage to biomolecules (e.g. protein, DNA and lipid) when exposed to CSF^[18-20]

Saliva is the first body fluid confronted to any external agent entering the oral cavity and gastrointestinal tract.

Host responses to oral disease including the production of different biochemicals released by stromal epithelial or inflammatory cells, important antioxidants associated with cell injury and cell death such as uric acid, albumin and protein.

The results of this study showed that the levels of these biomolecules in saliva samples of smokers group were significant decrease in relation to the non-smokers subjects. The levels of uric acid, albumin and total protein in human saliva are considered a good probe for distinguishing between smokers and non-smokers and its determination is useful for the evaluation of smoking behavior.

The scientific and biochemical research have provided improved methods to measure and monitor the level of biochemical molecules in saliva, in this respect the values of saliva as indicator for systemic disease had been explored and found application in clinical diagnosis.^[4-5]

Several studies have demonstrated that decrease of antioxidant levels in saliva correlate with the presence of different diseases and that tobacco smoke is one of the pollutants sharing in the incidence of these diseases.^[21]

Uric acid is known to be potent antioxidant.^[22]

Sculey etal.^[23] reported that uric acid is an important salivary antioxidant biomarker in monitoring the

oxidative stress (OS). Roche et al^[24] concluded that albumin has antioxidant properties due to scavenging activity towards free radicals. Previous study showed that chronic hepatitis patient, current smokers were more likely to have lower albumin levels than non-smokers.

Recent study using mass spectrometry has reported nearly 2000 different proteins in human saliva.^[25]

A significant number of salivary proteins with a primary role in host defense were identified and salivary immunoglobulins were predominant and act as the first line of defense and maintenance of mucosal integrity and general oral health.

Changes in these proteins alter the normal functional properties of saliva, such changes would be expected to reflect systemic or local illness. These proteins may serve as sensitive biomarkers for better diagnosis and treatment.^[28]

The finding of current investigation suggest that cigarette smoke lead to significant changes in salivary antioxidant mechanisms.

Several studies have demonstrated that decrease of antioxidant levels in saliva correlate with presences of various diseases or disorders.^[26-28]

Thus, salivary antioxidants were significantly lower in smokers compared with non-smoker volunteers, this representing a significant oxidative process that occurs in the oral cavity.

CONCLUSION

This results indicate the deleterious influence of cigarette smoking and its effects on the concentration of various biomarkers levels in saliva related to oxidant/antioxidant status which might be benefit in the clinical recovery of subject exposed to nicotine and understanding these effects may help to develop counsel-programm for student on smoking cessation.

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