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Effect of oral tobacco consumption on serum and saliva antioxoidant levels

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Abstract

Background & Objectives: A study was undertaken to determine the effect of different types of tobacco consumption habit on total antioxidant capacity of saliva and serum as well as the correlation between salivary and serum TAC in oral tobacco habitues.

Methods: A total of 60 subjects (15 smokers, 15 tobacco chewers, 15 oral snuff dippers and 15 control subjects) were involved. Serum and salivary samples were collected from all the groups, which were further subjected for biochemical analysis. The observations of the study were statistically analysed. ANOVA, Tukey's honestly significance difference test, and Fisher's test were employed according to which the hypotheses were tested.

Results: The mean TAC values, both in saliva and serum were reduced in all study groups (oral tobacco habitues) as compared to control group.

Interpretation & Conclusion: The level of salivary and serum TAC decreases secondary to tobacco consumption. From the clinical standpoint, it may be reasonable to conclude that salivary TAC can be a useful marker to assess the oxidative stress, as an indicator of increased risk of pathoses in tobacco habitues. Further, it can be used to motivate tobacco habitues to abstain from the habit.

Keywords: tobacco chewing; total antioxidant capacity; reactive oxygen species; free radicals; smoking.

Introduction

Oxygen is an important requirement for all mammalian energy needs. It is used to oxidize molecules rich in carbon and hydrogen to produce the different forms of energy needed for life. The reduction of molecular oxygen to water is accompanied by production of large number of free radicals (FR) and/or reactive oxygen species (ROS). The most important FR in biological systems are radical derivatives of oxygen. [1]

An imbalanced production of ROS plays a role in the pathogenesis of a number of human diseases such as atherosclerosis, neurodegenerative diseases, cancer, and allergy. When antioxidant, free radical scavenging systems are overwhelmed, inflammation, hypersensitivity, and autoimmune conditions may result.

Cigarette smoke contains several types of toxic components, including carbon monoxide, nicotine and benzopyrene. Cigarette smoke is a major exogenous source of free radicals. The obligatory use of the body reserve of antioxidants to detoxify the excess of these free radicals in smokers therefore results in an alteration in the level of different antioxidants.[2]

Tobacco is composed of N-nitrosamines and other alkaloids, which form highly reactive electrophiles and increase oxidative stress. Smokeless tobacco extract has been shown to be more toxic in vitro than pure nicotine and to increase oxidative stress as a result of reactive oxygen free radicals.[3]

Betel quid chewing has potential links to the occurrence of oral cancer. Reactive oxygen species produced during auto-oxidation of areca nut polyphenols in the betel-quid chewer's saliva, are crucial in the initiation and promotion of oral cancer. Antioxidants such as glutathione and N-acetyl-L-cysteine can potentially prevent areca-nut elicited cytotoxicity.[4]

Saliva is the first body fluid to encounter exogenous substances or gases that penetrate the human body. Smoking affects the antioxidants in the saliva, there by affecting the protective mechanism of saliva.[5] Saliva possesses a variety of defense mechanisms responsible for the protection of the oral mucosa from oxidative stress, including uric acid, vitamin C, glutathione and others. However, as antioxidants work in concert, total antioxidant capacity (TAC) is the most relevant parameter.[6]

Total antioxidant capacity, may be a marker of the antioxidant status of the body, and may reflect the level of supplementation with antioxidant vitamins and oxidative stress imposed on the organism. Other body fluids are analyzed less frequently for TAC.[7]

Decrease in the total antioxidant capacity affects the metabolism and immunosurveillance of the body resulting in many systemic effects.

So, the present study was undertaken to compare the antioxidant status in saliva and serum of oral tobacco habitués with healthy adults.

Materials And Methods

A total number of 60 subjects reporting to the Department of oral medicine and radiology, A.B Shetty Memorial Institute of Dental Sciences, Mangalore were enrolled in the study. The patients included in the study were informed about the study being conducted on them and there consent was taken for the same. A study Group comprised of 45 known oral tobacco habitués and control Group was comprised of 15 adults without any systemic diseases and any oral deleterious habits. A Study group was further divided into 3 groups according to the type of oral tobacco use. Study Group includes 15 smokers (group A), 15 tobacco chewers(group B) and 15 oral snuff dippers (group C).

The patient was asked not to smoke or consume any food 2 hours prior to the collection of saliva. Following a thorough mouth rinse using distilled water, saliva was allowed to accumulate in his or her mouth for 5 minutes. Accumulated saliva was collected by spit method. 2 ml of collected saliva was stored at a temperature of +4˚C in glass vials, which was then subjected to analysis using spectrophotometer.

Venous blood was collected from the antecubital vein with syringe and placed in vials containing 3% citric acid. Serum was then extracted and stored at temperature of +4˚C in glass vials, which was then subjected to analysis using spectrophotometer.

Estimation of total antioxidant capacity [8]

Reagents required:

1. Ethanol

2. Reagent solution (Mehta Dye Chem., Bengaluru, India)

1ml of reagent solution = 0.6M of H_2SO_4 + 28mM sodium phosphate + 4mM ammonium molybdate (30ml H_2SO_4 + 218.4mg sodium phosphate + 247.2 mg ammonium molybdate made up to 50ml)

Protocol

The phosphomolybdenum method was used for the quantitative determination of total antioxidant capacity. The assay is based on the reduction of molybdate to molybdenum and the subsequent estimation of a green phosphomolybdenum complex. An aliquot of 0.1ml of sample solution containing a reducing species (ethanol) was combined in an eppendrof tube with1ml of reagent solution was added. The tubes were capped and incubated in a thermal block at 95°c for 90min. (or water bath at 37°c for 90min). After 90min the samples were allowed to cool to the room temperature. The absorbance of the aqueous solution for each was measured at 695nm against a blank using spectrophotometer. A typical blank solution containing 1ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples.

Statistical analysis

Data obtained were analyzed using ANOVA, Tukey's honestly significance difference test, and Fisher's test.

Results

Table 1 illustrates the mean total antioxidant capacity of saliva and serum for oral tobacco habitues and control subjects. The data showed that mean total antioxidant capacity of both saliva and serum of non-habitues was more as compared to oral tobacco habitues. The values were very highly significant ($F=64.07$; $p<0.001$), ($F=44.181$; $p<0.001$)

Table 2 illustrates the positive correlation of salivary TAC with serum TAC which was shown to be statistically significant for the groups II & III. ($p=.004$)

Table 3 illustrates the negative correlation of salivary TAC and serum TAC with age in all the groups. ($r = -0.050$, $p = 0.703$ and $r = -0.051$, $p = 0.697$,respectively). It suggests that as the age advances the salivary and serum TAC are depleted due to an uncontrolled production of free radicals by aging mitochondria.

Discussion

Antioxidants counteract the harmful effects of free radicals resulting in protection of structural and tissue integrity. The antioxidant status of an individual is therefore of utmost importance. Imbalances between levels of free radicals / reactive oxygen species and levels of antioxidants have been suggested to play an important role in the onset and development of several inflammatory oral diseases.

Most of the research work on this particular topic was carried out to evaluate individual antioxidant components in either serum or saliva samples. But, it has been suggested that antioxidant systems act in concert rather

than alone and investigation of individual antioxidant may be misleading & a may be less representative of the whole antioxidant status.[9] Hence, the estimation of serum as well as salivary TAC was done to find out whether serum TAC values correlate with the salivary TAC values.

In the present study, mean salivary TAC of all study groups was significantly less than that of control subjects which is in agreement with the previous study done by Ziobrio et al [7], who investigated the TAC value of different body fluids in smokers and non-smokers. They concluded that the TAC values of saliva were higher than those of blood plasma and the saliva of smokers was characterized by a much lower TAC than that of non-smokers.[7] It could be due to constant exposure of saliva to free radicals which are generated on tobacco consumption, which leads to a depletion in the TAC of saliva.

The previous in vitro and systemic in vivo studies data showed that tobacco smoke compromises antioxidant defenses. The acute influence of smoking a single cigarette on concentrations of glutathione, uric acid, and total antioxidant activity measured in saliva has been addressed previously.[10] No statistically significant differences were found for uric acid or total antioxidant capacity before or after smoking or between smokers and nonsmokers. However, salivary glutathione content was significantly higher in smokers but there was a significant decrease in salivary glutathione after smoking one cigarette. The authors reported that individuals with already low presmoking glutathione concentrations are most prone to the noxious effects of cigarette smoking.

Charalabopoulos et al [11] investigated the possible effects of smoking on total antioxidant capacity of total saliva and plasma samples in young healthy males. They found no statistically significant differences in the salivary antioxidant defenses of non-smokers and smokers, either before or after smoking.

In the present study, mean serum TAC of all study groups was significantly less than that of control subjects which could be due to depletion of serum antioxidants caused by excessive free radicals production on tobacco consumption. The serum TAC of snuff dipper group was lowest among all the study groups, although not statistically significant. This difference in serum TAC of snuff dippers could be due to a finely ground nature of snuff which enhances its absorption and its subsequent effect on the tissues. The results of the present study are in accordance with the study done by Jha et al [12] who investigated erythrocyte glutathione (GSH) which is a most potent endogenous antioxidant, plasma α-tocopherol and ascorbic acid, which are 2 most important nutrient AOs and total antioxidant activity (TAA) which practically measures antioxidant strength contributed by nutrient and other AOs in the diet. It was noted that smokers had normal levels of erythrocyte GSH, plasma α-tocopherol and ascorbic acid but significantly lower levels of TAA. However, Charalabopoulos et al [10], in his study found that TAC of plasma was significantly higher in smokers compared to non-smokers in young healthy subjects. It indicated that young smokers do not manifest different salivary antioxidant defense than non-smokers which is contrary to the present study in which the serum TAC values in oral tobacco habitues is much lower as compared to control subjects.

A positive correlation was noticed between salivary TAC and serum TAC in smoker group and tobacco chewer group which probably reflects the fact that tobacco consumption has significant impact on both salivary and serum TAC level. However, the positive correlation was not statistically significant. Salivary TAC, in addition to systemic factors, is also affected by local factors. Probably in the absence of systemic diseases, smoking or other

factors like gingivitis tend to act locally and alter salivary TAC when compared to serum TAC.

In the present study, a negative correlation was found between serum TAC and age in all the groups which could be due to the uncontrolled production of free radicals by the aging mitochondria of the cells. This result is in accordance with Reddy KK et al [13] who measured antioxidant enzyme levels (glutathione S-transferase, superoxide dismutase and catalase) in healthy individuals with an age range of 20-80 years. It was concluded that the antioxidant enzyme levels were significantly less in very old age when compared to young. Further cigarette smoking had a positive relation with free radicals and DNA damage, and inverse relation with antioxidants. The data indicate that reduction of antioxidant enzyme levels would render the older people more susceptible to free radical stress and DNA damage.

Several studies have been conducted to assess whether saliva can be used as a diagnostic tool. However, it harbors its own limitations. Saliva, although considered to be a representative of serum, it is influenced by other local factors affecting oral cavity. Saliva sample collection must be performed in a meticulous manner and the saliva must be preserved in proper conditions for further analysis, in order to avoid various technique-related effects. The present study showed that saliva and serum can be used to monitor the total antioxidant level in tobacco habitues.

The present study adds to the existing literature as it is the first to examine salivary and serum total antioxidant status in oral tobacco habitués including smokers, tobacco chewers and snuff dippers. The comparison of salivary and serum TAC among the study groups was not statistically significant so further studies are required to be conducted to establish the exact relationship of salivary and serum TAC with type of oral tobacco consumption habit.

Conclusion

In conclusion, the findings of this study strengthen the need for extensive research to identify the potential relationship between depletion of TAC in saliva of oral tobacco habitues. Further research should be devoted to the possible benefits after supplementation with antioxidant supplements. However, this issue still remains open and needs to be further investigated and confirmed by other studies with a larger sample.

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Legends Tables

Table 1: Comparison of saliva TAC and serum TAC in study groups

Groups	N	Mean TAC of saliva	Mean TAC of serum
Control	15	0.06148	0.68713
Smokers	15	0.02498	0.42140
Tobacco chewers	15	0.02409	0.41813
Snuff dippers	15	0.02895	0.33507

P<0.001 vhs

Table 2:-Correlation of salivary TAC with serum TAC

Group	Saliva TAC	Serum TAC
Control	r p N	0.280 0.312 15
smokers	Saliva TAC r p N	0.718 0.003 hs 15
Tobacco chewers	Saliva TAC r p N	0.701 0.004 hs 15
Snuff dippers	Saliva TAC r p N	0.114 0.685 15

Table 3: Correlation of Age with salivary TAC and serum TAC

Age	Saliva TAC	Serum TAC
r p N	-0.050 0.703 60	-0.051 0.697 60