Comparison of salivary catalase and superoxide dismutase enzymes levels in betel quid chewers and non-betel quid chewers of healthy adults

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Abstract

The diagnostic value of salivary secretions to detect systemic diseases had long been recognized. Salivary assays present a lot of advantages when compare to blood assays. The widespread use of saliva is helping individuals, researchers, healthcare professionals and community health programs to better detect and monitor disease. The salivary antioxidant system plays a very important role in the anticarcinogenic capacity of saliva and includes various enzymes and molecules. The present study compared the salivary antioxidant enzymes activities of betel quid chewers and non-betel quid chewers of Myanmar adults. Salivary catalase and superoxide dismutase enzymes were measured by spectrophotometer. In this study, the mean salivary catalase enzyme activity of betel quid chewers was 0.2217±0.1104 U/ml whereas that of non-betel quid chewers was 0.8371±0.4405 U/ml. Mean salivary catalase enzyme activity of betel quid chewers was significantly lower than non-betel quid chewers ($p<0.001$). The mean salivary enzyme superoxide dismutase activities of betel quid chewers and non-betel quid chewers were 1.9792±1.7010 U/ml and 1.6499±1.3739 U/ml respectively. Mean salivary superoxide dismutase enzyme level of betel quid chewers was higher than non-betel quid chewers but not significant. Present results reveal that betel quid chewing habit causes the changes in salivary antioxidant enzyme activities. In the fact that decreased salivary catalase enzyme activity in betel quid chewers, it may be due to the overproduction of H2O2 which might lead to increase consumption of catalase. The elevated level of superoxide dismutase showed the increased defensive system of the body occurring to reduce the free radicals produced by betel quid. The present study confirms that the betel quid chewing habit can change the antioxidant activities of saliva so that it can be used to detect the antioxidant activity of the body defense mechanism.

Introduction

Saliva is complex fluid, produced by the salivary glands, whose important role is maintaining the well-being of the mouth. Oral homeostasis is dependent upon saliva and its content of proteins [1]. The components of saliva act as a "mirror of the body's health" and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public [2].

Habit of betel quid or areca nut chewing is one of health endangering behaviours which frequently coincide, raising the clear potential for long-term and serious consequences. Betel quid chewing becomes one of the major public health challenges in Asian as well as worldwide. The use of tobacco is a serious global public health problem, and is also an important risk factor for oral disease [3].

Sies in 1986 defined oxidative stress as an imbalance between oxidants and antioxidants on a cellular or individual level. This imbalance may be due to either an over-production of reactive oxygen species (ROS) or a deficiency in an antioxidant system. The continuous efflux of these reactive species from endogenous and exogenous sources results in continuous and accumulative oxidative damage to cellular components and alters many cellular functions. Such damage may afflict all types of biological molecules, including DNA, lipids, proteins and carbohydrates [4].

Increased generation of ROS may cause toxic effects by oxidative damage of proteins, lipids and DNA. Oxidative damage of these biomolecules contributes to disease development [5]. Clearly, thus, ROS are important in the initiation and promotion of cancer [6].

The term 'antioxidant' refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Human have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage [7]. The most efficient antioxidants are enzymes that catalyse the reduction of ROS: superoxide dismutase, catalase and glutathione peroxidise. The higher reactive oxygen (O2) is converted to hydrogen peroxide (H2O2) by superoxide dismutase (SOD), catalase (CAT), peroxidase (POx), or glutathione peroxidase (GSH-Px) can in turn convert H2O2 to molecular oxygen and water [8]. A recent study has indicated that a combination of antioxidants is a powerful adjunctive preventive treatment for cancer [9] since the total activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase is reduced in certain types of cancers [10, 11]. However, the invasive potential of cancer cells is also increased in the presence of abnormally high levels of manganese SOD (Mn-SOD) [12].

Catalase (CAT) is an intracellular antioxidant enzyme and is mainly located in peroxisomes and to some extent in the cytosol. Human erythrocyte CAT is tetrameric protein of 244 kDa, comprising four identical subunits of 59.7 kDa plus heme group and four NADPH molecules [13]. CAT is the most abundant antioxidant enzyme and it plays a crucial role in the protection against oxidative stress [14].

The enzyme catalase provides one of the most spatula examples of increase in reaction rates brought about by enzymes. This enzyme is required for life in an oxygen-containing

environment. In this environment, the process of the aerobic (oxygen-requiring) breakdown of food molecules produces hydrogen peroxide (H2O2). Because H2O2 is toxic to the cells, it must be destroyed. One molecule of catalase converts forty million molecules of hydrogen peroxide to harmless water and oxygen in every second. Reaction occurs forty million times in every second! [15]. Catalase uses two molecules of H2O2, one as electron donor and other as electron acceptor [16].

Catalase exerts dual function,

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

There are three types of superoxide dismutase including Fe-SOD, Mn-SOD and Cu-Zn SOD. SOD contains copper and zinc and is found in all body tissues as well as in some body fluids, in particular saliva. All forms of SODs act by a common mechanism of dismutation of superoxide ($O_2^{\cdot-}$) into hydrogen peroxide (H2O2) and molecular oxygen (O2).

$$2\ O2^{\cdot-} + 2\ H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Betel quid chewing habit can affect the salivary antioxidant enzymes activities such as catalase and superoxide dismutase which are strong inhibitors for oxidative injuries and critical biological macromolecules in the oral cavity. Nair et al., (1987) demonstrated that saliva inhibits the production of oxygen species, the superoxide free radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) from betel quid tobacco, the most potent inducer of oral cancer [17]. The habit of smokeless tobacco chewing is one of the known risk factors for oral cancer among the residents of southeast of Iran. Most likely, the antioxidant defense system in dealing with free radicals induced paan and prevention of oral cancer is important [18].

The objective of this study is to compare the salivary catalase and superoxide dismutase enzymes levels of healthy adult betel quid chewers and non-betel quid chewers. This study could lay the ground for research on the prevention of adverse effects of betel quid chewing habit.

Materials and Methods

Study population

Unstimulated whole saliva from total 72 subjects of adult Myanmar population

Sample size

36 volunteers of betel quid chewers and 36 volunteers of non-betel quid chewers (aged 18-35 years)

Collection of saliva

Unstimulated whole saliva was collected from each subject at 8 am to prevent any variations which may be attributable to the circadian rhythm. The subjects were asked not to eat or drink 2 hours prior to saliva collection. The betel quid chewers were also prohibited from betel quid chewing for two hour prior sample collection. The subjects were instructed to rinse the mouth using distilled water. The unstimulated saliva were collected for five to ten minutes in a container and kept at low temperature. The samples were carried in an ice-box from the site of sample collection to the laboratory. Following saliva collection, the samples were centrifuged to remove cell debris for 10 minutes at 1500 rpm, at 4•C. The supernatant were stored at - 20•C until tests were performed.

Type of study

Laboratory based cross-sectional, comparative study

Place of study

Department of Oral Biological Science, University of Dental Medicine, Yangon

Department of Biochemistry, Department of Medical Research, Yangon

Inclusion criteria

Healthy betel quid chewers and non-betel quid chewers aged between 18 – 35 years

Exclusion criteria

Subjects with the following condition were excluded:

- Additional vitamin supplementation and antioxidants
- Medication, e.g. NSAID
- Excessive alcohol consumption
- Infection
- Chronic pulmonary diseases
- Heart diseases e.g. ischaemic heart disease
- Renal diseases, e.g. nephritis
- Smoking
- Severe/Acute periodontal disease
- Diabetes mellitus

Study procedure

Subjects were recruited from University of Dental Medicine, Yangon and the Biochemistry Research Division, Department of Medical Research. The subjects were interviewed and selected according to selection criteria. The subjects were firstly explained the nature and procedure of the study. Then, the subjects' consents, personal data and history were taken. After that, saliva samples were collected according to the collection method [19]. The clear supernatants were obtained to determine superoxide dismutase, and catalase activities. All the samples were collected with code numbers and analyzed batch by batch in duplicate.

Assays of CAT and SOD activities

The required materials and chemicals (Sigma-Aldrich, USA) were purchased from Bio-Med Myanmar Co., Ltd. Salivary catalase enzyme activity was determined by Aebi, H. (1984) method [20] and then salivary superoxide dismutase enzyme activity was determined by Suzuki, K. (2000) method [21].

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 16 and Window version. All values were reported as mean ± SD. The statistical significance of differences in salivary antioxidant levels between betel quid chewers and non-betel quid chewers were estimated by Student's t- test (unpaired). In this study a p value (<0.05) was accepted as significant.

Results

The mean levels of salivary catalase enzyme activity of betel quid chewers was significantly lower than non-betel quid chewers (t-test, $p < 0.001$). Mean salivary SOD enzyme activity of betel quid chewers was higher than that of non-betel quid chewers. Statistical significance was checked by unpaired t-test and found not significant ($p = 0.369$).

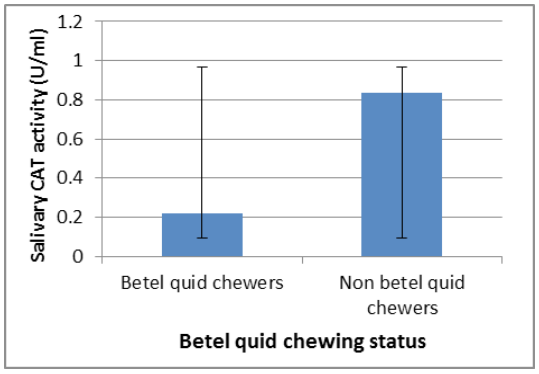

Table 1. Antioxidant enzymes levels of betel quid chewers and non-betel quid chewers

Biochemical Parameters	Betel quid chewers (mean ± SD)	Non-betel quid chewers (mean ± SD)	t test P values
CAT (U/ml)	0.2217±0.1104	0.8371±0.4405	-8.130 p<0.00*
SOD (U/ml)	1.9792±1.7010	1.6499±1.3739	0.904 0.369

* Significant level was p<0.001

p = p value (Significantly level p<0.05)

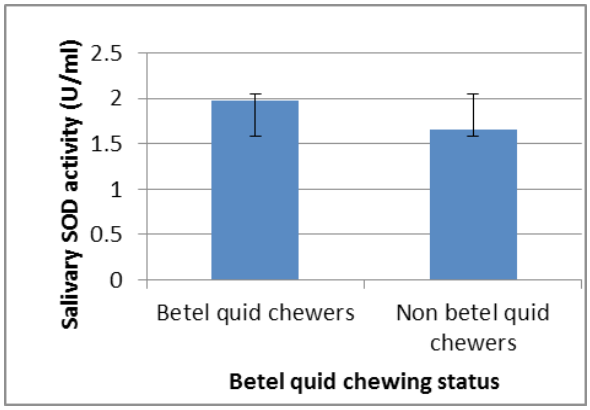

Figure 1&2. Salivary CAT and SOD activity in betel quid chewing status

Discussion

Betel quid chewing habit can affect the salivary antioxidant enzymes activities such as catalase and superoxide dismutase which are strong inhibitors for oxidative injuries and critical biological macromolecules in the oral cavity. The main objective of this study was to measure the activities of antioxidant enzymes in the saliva of betel quid chewers and non-betel quid chewers.

In the present study, the mean salivary catalase enzyme activity of betel quid chewers was significantly lower than non-betel quid chewers. Several studies demonstrated that cigarette smoke has inhibited the CAT activity. Significant reduction of erythrocyte CAT activity in smokers was found in the studies of Kacmez et al., 1997; Codandabany, 2000; Yildiz et al., 2002; and Hemalatha et al., 2006 (Cited in Yee, 2009) [22]. The present study is consistent with the results of these studies.

The results of the present study indicate that the exposure of betel quid chewing cause a statistically significant decrease in the levels of CAT in saliva of betel quid chewers than non-betel quid chewers. The decrease of CAT may be owing to the sufficient amount of catalase may not be available for the detoxification of H_2O_2 leading to elevated oxidative stresses produced by overproduction H_2O_2 during betel quid chewing. This study suggested that only salivary CAT activity was significantly decreased in betel quid chewers aged between 18 to 35 years. According to the Nair et al., (1987), saliva inhibits the production of oxygen species, the superoxide free radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) from betel quid tobacco, the most potent inducer of oral cancer. As a matter of fact, significant reduction of salivary catalase enzyme might be the protective usage of catalase enzyme in changing of toxic H_2O_2 to harmless oxygen (O_2) and water (H_2O). This may be due to the fact that CAT is by far the most important antioxidant enzyme and it plays a crucial role in the protection against oxidative stress produced by betel quid.

In this study, the mean levels of SOD were increased in betel quid chewers than non-betel quid chewers so that these results were inconsistent with the studies of Agnihotri and Abdolsamadi [23, 24]. In the study of Abdolsamadi et al. (2011) the activity of salivary SOD was significantly lower in smokers compared with non-smokers. They suggested

that this difference may be related to the age of subjects who were evaluated for salivary antioxidant status. Agnihotri et al. (2009) showed that the activity of SOD in saliva and gingival crevicular fluid of smokers was reduced in comparison with the control group. This may be due to the differences in the types of consumed tobacco, duration and consumption pattern, method of enzyme assessment, age of the subjects, dietary habit, research sample (saliva or blood), research method (in vivo or in vitro) and types of antioxidant agent.

This finding has been consistent with the research of Farhadmollashahi et al. (2014) who found that paan consumers have significantly increased activity of salivary SOD than non-consumers (18). Increased SOD activity in saliva resulted in overproduction of H2O2 leading to elevated oxidative stresses that was involved in a large number of diseases, including precancerous and neoplastic lesions of the oral cavity. The elevated level showed the defensive system of the body occurring to reduce the free radicals produced by betel quid.

Karincoaglu et al. (2005) studied antioxidant enzymes of SOD in saliva of patients with aphthous and healthy subjects and they argued that the salivary defense mechanisms which act through antioxidant system causes the whole body to send its stored antioxidants to the site of injury during aphthous occurrence, resulting in the increase of salivary antioxidant agents. Since and both, the activity of salivary SOD increases, however, during aphthous, the enzymatic changes precede the appearance of the lesion [25].

Several investigators also showed that the mean levels of enzyme SOD were significantly higher in the saliva or erythrocyte of smokers than non-smokers. In the studies of Zahraie et al., 2004; Duthie et al., 1991; Leonard et al., 1995; Kacmaz et al., 1997; Kocyigit et al., 2001; Ho et al., 2005 (Cited in Yee, 2009), the mean levels of SOD were significantly higher in the smoking group. The results of increased SOD levels in these studies are consistent with the present study. They demonstrated that erythrocyte SOD activity was also significantly higher in smokers compared with non-smokers. Their observation suggests oxidative stress induction following cigarette smoking. On the other hand, it has been shown that cigarette smoking causes stimulation of inflammatory response which leads to increased Cu-Zn SOD activity.

There might have been a recent surge in the studies where levels of SOD have been elevated in human saliva. A study revealed cupper/zinc SOD in human saliva might be useful for estimating the level of oxidative stress caused by cigarette smoke. A more recent study has indicated that decrease in concentrations of major antioxidants like SOD in saliva of the patients with cysts may increase the risk of neoplastic transformation especially in advanced age.

In this study, the salivary superoxide dismutase enzyme activity of betel quid chewers was higher than non-betel quid chewers but not statistically significance. This may be due to the small sample size that is difficult to exclude the extreme samples, the differences in the types of consumed tobacco, duration and consumption pattern, dietary habit. This finding suggested that the elevated level showed the defensive system of the body occurring to reduce the free radicals produced by betel quid chewing habit and on the other hand, the modulating antioxidant/oxidative processes of Piper betel.

Conclusion

The results of this study found that betel quid chewing habit was associated with a significant decreased in salivary CAT concentrations and increased in salivary SOD enzyme activity. Measurement of antioxidant enzymes in human saliva might be useful for estimating the level of oxidative stress caused by betel quid chewing.

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