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Correlation of Salivary Superoxide Dismutase Enzyme Activity with Frequency of Sudanese Toombak Consumption among Male Population in Khartoum State

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Abstract

Background: Sudanese Toombak is a sort of a locally-produced tobacco that is known for its high content of carcinogens. It is cheap, available, and simple to cultivate and manufacture. Toombak use is one of the most important risk factors for oral diseases such as cancer and keratoses. Its consumption has a direct correlation with DNA damage.

Objective: To correlate the salivary superoxide dismutase enzyme activity with frequency of Sudanese Toombak consumption among male population in Khartoum State

Materials and methods: This was a comparative, analytical, case control study conducted in Khartoum State during the period from February to May 2016. 100 apparently healthy adult male participants were investigated: 50 were representing the test group, and 50 were representing the control group. Whole saliva specimens were collected from each participant without oral stimulus. Saliva specimens collected were immediately centrifuged and subjected to superoxide dismutase enzyme measurement by spectrophotometry.

Results: The level of superoxide dismutase enzyme was found to be significantly decreased in Toombak-users as compared with non-Toombak-users (2.16 ± 0.76 versus 4.96 ± 2.21, $p = 0.02$). Also among Toombak-users, the superoxide dismutase enzyme has a negative weak correlation ($r = - 0.40$, $p = 0.00$) with the frequency rate of Toombak consumption per day; and has a negative weak correlation ($r = - 0.35$, $p = 0.01$) with the whole duration of Toombak consumption.

Conclusion: The superoxide dismutase enzyme activity had a significant decrease among Sudanese Toombak-users; and had a negative weak correlation with the frequency rate and the whole duration of Toombak consumption.

Key words: Sudanese Toombak, Salivary superoxide dismutase enzyme, Male population

Introduction

Saliva is a biological diluted solution of water, ions and proteins such as mucine, lysozyme and immunoglobulins. Its secretion is under autonomic control and can be triggered by multiple stimuli such as sight, smell, touch and even a thought of food. Water and mucus in saliva soften and lubricate food to make it easier to swallow. Also saliva begins chemical digestion in the mouth by secreting the salivary amylase enzyme and small traces of lipase enzyme[1].
An antioxidant is a molecule that inhibits the oxidation of other molecules, and it consists of vitamin A, vitamin C, vitamin E, catalase enzyme, and superoxide dismutase enzyme. Its function is to combat cancer and coronary heart diseases[2].
Superoxide dismutase is the antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to oxygen and to the less reactive hydrogen peroxide (H_2O_2)[3].
In humans both iron-containing hemoglobin and zinc-containing carbonic anhydrase play pivotal roles in binding oxygen and delivering it to the cells. Moreover, enzymes developed to protect cells from high levels of oxygen also contain metals. The superoxide dismutase (SODS) enzyme is a class of protective enzymes. Mammals have three different iso-enzymes of superoxide dismutase that catalyze conversion of superoxide to peroxide. The cytosolic form of superoxide dismutase contains copper/zinc (Cu/Zn) at its active site, as does the extracellular enzymes. However, the mitochondrial enzyme contains manganese (Mn) at its active site. Hydrogen peroxide is removed by catalase which is a hemo-containing enzyme present in high concentration in peroxisomes and to a lesser extent in mitochondria of liver cytosol. The superoxide radical is more toxic than previously believed. This radical is produced as a by-product of many oxidative reactions, but most of it probably arises as an abbreviation of the mitochondrial electron transfer element. It has been estimated that as much as 5% of the oxygen consumed in respiration might be converted to oxygen in young rat heart mitochondria, and it increases with the animal's age. Fortunately all aerobic cells contain the enzyme superoxide dismutase that scavenge and detoxify oxygen by catalyzing the dismutation reaction: $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$.
These enzymes are formed in species ranging from bacteria to mammals. They are found in mitochondria, in the cytosol of liver, in erythrocytes, and in other tissues. Superoxide dismutases are metallo-enzymes, but the metal requirement depends on the enzyme source[4].
The function of superoxide dismutase is to protect aerobic organisms against the potential deleterious effect of superoxide. The enzyme occurs in different compartment of the cells[5].
When a cell with damaged DNA divides, metabolism and duplication of cells become deranged and mutations can arise leading to risks of carcinogenesis. Reactive oxygen species, free radicals and reactive nitrogen species in Toombak snuff have been suggested to induce a gradual evolving process, because of direct localized absorption on contact with tissue[6].
On the other hand, Toombak contains oxidants and pro-oxidant agents that lead to dysplastic lesions which are then transformed into carcinoma lesions. This evidence emphasizes the role of Toombak on salivary antioxidants and predisposing ability to pathogenesis of oral cancer.
Free radical formation is naturally controlled by antioxidants that are capable of deactivating or stabilizing free radicals prior to injury of cells[7].
Biochemical laboratory tests can help in investigating cancer patients. Many cancer diseases are associated with abnormal production of enzymes, proteins, and hormones that are

measurable by specialized chemical processes[8].
Saliva specimens rather than blood specimens are collected to investigate the action of toombak due to the localization of Toombak in the oral tissues and its quick absorption in the oral cavity[9].
The aim of this study was to estimate the prevalence and the consumption frequency rate of the salivary superoxide dismutase enzyme in Sudanese Toombak (tobacco) users as compared with control patients.

Materials and methods

This was a comparative, analytical, case control, socially-based study conducted in Khartoum State. It was carried out during the period from February to May 2016. 100 apparently healthy adult male participants were investigated: 50 were representing the test group, and 50 were representing the control group. Each group was in the age range 25-65 years. All the test group participants were Toombak users and all the control group participants were non-Toombak users. Exclusion criteria were smokers, alcohol abusers, Toombak users with chronic and hereditary diseases; as well as individuals working in petrol-selling stations.
The purpose of the study was explained to all participants and their verbal consent was obtained. Information data collected were concealed. Clinical and demographical data were collected using a structural questionnaire. The software used for the analysis of data was Statistical Package for Social Sciences (SPSS) program (version 16.5).
Whole saliva specimens were collected from each participant without oral stimulus. In a sitting position, the participants were asked first to swallow their saliva, then to stay motionless to allow passive draining of saliva in a sterile container. Saliva specimens collected were immediately centrifuged and subjected to biochemical measurements.
Superoxide dismutase enzyme activity test was measured by spectrophotometry and reagents supplied by Fortress Diagnostics. The principle of the test depends on the ability of the superoxide dismutase enzyme to accelerate the dismutation of the toxic superoxide radicals produced during the oxidative energy processes resulting in hydrogen peroxide and molecular oxygen. Fortress method employs xanthine and xanthine oxidase enzyme to generate superoxide radicals which react with 2, 4-iodophenyl-3, 4-nitrofenol-5- phenyl-tetrazolium chloride to form a red formazan dye which was read in 505 nm. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of superoxide dismutase enzyme causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. Superoxide dismutase enzyme, uric acid, copper, and zinc were measured in saliva of all participants Toombak-users and non-Toombak users. Quality control for superoxide dismutase measurement in saliva was maintained.

Results

Table (1) shows a significant decrease in superoxide dismutase enzyme activity among Toombak-users as compared with non-Toombak-users (mean ± SD: 2.16 ± 0.76 versus 4.96 ± 2.21, p = 0.02).

Table (1): Superoxide dismutase enzyme activity in Toombak-users and non-Toombak-users

Variable	Toombak-users N=50 Mean ± SD	Non-Toombak-users N=50 Mean ± SD	p-value
Superoxide dismutase activity (U/g)	2.16 ± 0.76	4.96 ± 2.21	0.02

Fig. (1) is a scatter plot showing a negative weak correlation between the level of superoxide dismutase enzyme and the frequency rate of Toombak consumption per day among the test group (r = - 0.40, p = 0.00).

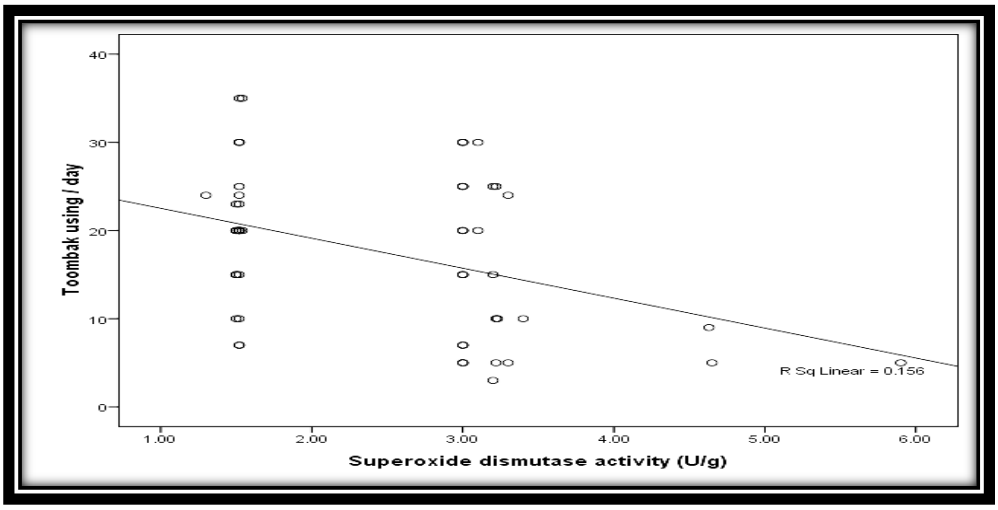

Fig. (1): Correlation of the activity of superoxide dismutase enzyme with the frequency rate of Toombak consumption per day among the test group

Fig. (2) is a scatter plot showing a negative weak correlation between the level of superoxide dismutase enzyme and the duration of Toombak consumption among the test group (r = - 0.35, p = 0.01).

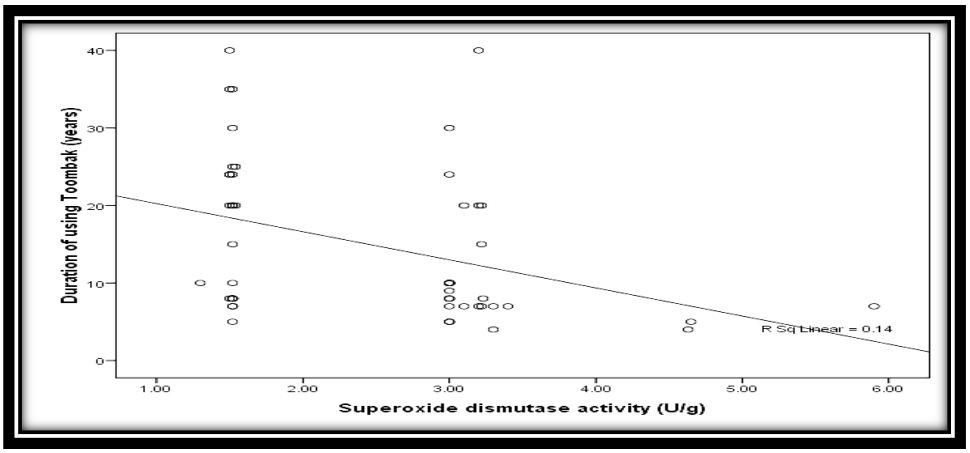

Fig. (2): Correlation of the activity of superoxide dismutase enzyme with the duration of Toombak consumption among the test group

Discussion:

Toombak consumption is a serious health problem and it is considered a major risk factor for oral cancer. The risk of disease is increased with increased intensity and duration of Toombak usage. In this study the level of superoxide dismutase enzyme was found to be significantly decreased in Toombak-users as compared with non-Toombak-users (2.16 ± 0.76 versus 4.96 ± 2.21, p = 0.02). Justification of this result is that the superoxide dismutase enzyme is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Toombak contains abundant amount of oxidants and superoxide (O_2^-) that leads to damage of oral endothelial cells. Superoxide dismutase enzyme is one of the most important antioxidants and contributes approximately to 70 % of the total salivary antioxidant capacity. This finding was similar to that reported by Abdolsamadi and his colleagues[10] who found a decrease in superoxide dismutase enzyme activity in Toombak-users as compared with control participants (6.24 ± 2.62 versus 8.07 ± 1.30).

Also in Toombak-users this study showed that the superoxide dismutase enzyme has a negative weak correlation (r = - 0.40, p = 0.00) with the frequency rate of Toombak usage per day. Furthermore, our study showed that the superoxide dismutase enzyme has a negative weak correlation (r = - 0.35, p = 0.01) with the duration of Toombak consumption.

From this study it may be recommended that periodic follow up of Toombak-users must be made since the superoxide dismutase enzyme is considered a high risk factor. Also it is recommended to use the superoxide dismutase enzyme as a biomedical marker in cases of oxidative stress. Health authorities need to make the population aware as regard the risk factors of Toombak consumption.

Conclusion: The level of superoxide dismutase enzyme showed a significant decrease in Toombak-users. In Toombak-users, the superoxide dismutase enzyme has a negative weak correlation, with the frequency rate of Toombak consumption per day. In Toombak-users the

superoxide dismutase enzyme has a negative weak correlation with the duration of Toombak consumption.

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