Assessment of Superoxide dismutase activity and total antioxidant capacity in adult male cigarette smokers in Nnewi metropolis, Nigeria

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Abstract

Background: Toxic constituents in cigarettes include carbon monoxide, benzopyrene and reactive oxygen species. Antioxidants are the body’s first line of defence against the damaging effects of free radicals and may offer protection against some harmful effects of cigarette smoking. Study design: This is a case control study. Objective: This study assessed the superoxide dismutase activity and total antioxidant capacity in adult male cigarette smokers. Method: A total of 100 subjects were recruited for this study. These comprised 50 adult male cigarette smokers and 50 adult male non-smokers as the control group. Serum superoxide dismutase activity and total antioxidant capacity were estimated using the semi-automated spectrophotometric procedure. Result: The mean serum SOD activity was significantly lower in smokers compared with the control group (P<0.05). However, no significant difference was observed in the mean serum TAC of smokers compared with the control (P>0.05). Furthermore, a negative correlation existed between the serum SOD activity of cigarette smokers and number of cigarette sticks smoked per day (r=-0.557, p=0.000). In addition, a negative relationship was observed between SOD activity and duration of smoking (r=-0.064, p=0.656). Moreover, an inverse relationship existed between total antioxidant capacity and number of cigarette sticks smoked per day (r=0.546, p=0.000) and between TAC and duration of smoking (r=-0.302, p=0.035). Conclusion: There is a reduction in Superoxide dismutase antioxidant enzyme activity in cigarette smokers.

Keywords: Smoking, Antioxidants, Superoxide dismutase.

INTRODUCTION

Despite frequent notice of irreversible consequences of smoking in public media and by other forms of advertising, the consumption of cigarette is growing dramatically in both developed and developing countries. Based on available statistics, even in previous years, almost one-third of population over age 30 years are smokers [1]. In addition, the consequences of smoking are not only temporary, rather, according to the existing evidence, there is a direct correlation between cigarette smoking and many cardiovascular and respiratory, as well as atherosclerosis problems along with morphological changes of vessel walls [2]. Cigarette smoke is a complex mixture of chemical compounds that are bound to aerosol particles or are free in gas phase. Researchers have estimated that cigarette smoke has 7,357 chemical compounds of different classes [3]. Of the more than 7,000 chemicals in tobacco smoke, at least 250 are known to be harmful, including hydrogen cyanide, carbon monoxide and ammonia [4]. Among the 250 known harmful chemicals in tobacco, at least 69 can cause cancer. These cancer causing chemicals include the following: acetaldehyde, aromatic amines, arsenic, benzene, benzene(α)-pyrene, cadmium, chromium, cumene, ethylene oxide, formaldehyde, nickel, polonium-210, polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines, vinyl chloride [5].

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals. The addition of one electron to dioxygen forms the superoxide anion radical (O2−). Types of ROS include the hydroxyl radical, the superoxideanion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage.
Reactive oxygen species are highly reactive due to the presence of unpaired valence shell electrons. ROS are formed as a natural byproducts of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (example UV or heat exposure) ROS levels can increase dramatically [6]. This may result in significant damage to cell structures and culminates into a situation known as oxidative stress. ROS are also generated by exogenous source such as ionizing radiation, cigarette smoke etc. Under normal circumstances, cells are able to defend themselves against ROS damage with enzymes such as superoxide dismutases, catalases, lactoperoxidases and glutathione peroxidases. Antioxidants present in the plant such as ascorbic acid (vitamin C), tocopherol (vitamin E) and phenolic acids play important roles as cellular antioxidants.

Superoxide Dismutase (SOD) catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen. SOD plays a critical role in the defense of cells against the toxic effects of oxygen radicals. It competes with nitric oxide (NO) for superoxide anions, which react with NO to form peroxynitrite. The measure of antioxidant capacity considers the cumulative action of all antioxidants present in the plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants [7].

Decrease in protective systems of anti-oxidants due to cigarette smoking may be a cause of many pathological conditions such as cardiovascular and respiratory disorders. Assessment of antioxidant markers may be relevant in the evaluation of risks of such pathologic conditions and associated oxidative stress induced by contents of cigarettes. Therefore this study is aimed at assessing the levels of superoxide dismutase activity and total antioxidant capacity in adult male cigarette smokers.

MATERIALS AND METHODS

Study site

The study was carried out in Nnewi metropolis. Nnewi is the second largest city in Anambra State, South Eastern Nigeria. It has an estimated population of over 391, 277. It is made up of four quarters namely; Otolo, Uruagu, Umudim and Nnewichi.

Research design

This is a case-control study that assessed the levels of superoxide dismutase activity and total antioxidant capacity in adult male cigarette smokers in Nnewi metropolis, Anambra state, Nigeria. A total of 100 subjects were randomly recruited for the study. These comprised 50 adult male smokers and 50 adult male non-smokers as controls.

Inclusion criteria

Adult male cigarette smokers with smoking history of at least one cigarette a day for not less than one year and adult male non-smokers.

Exclusion criteria

Subjects with type II diabetes, respiratory and cardiovascular diseases and other chronic diseases.

Ethical consideration and informed consent

The ethical approval for this research was obtained from the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi. Informed consent of the subjects was sought and obtained from the subjects prior to this study.

Collection of samples

Five millilitres (5ml) of venous blood was collected aseptically from each subject and was dispensed into a plain container. The sample was centrifuged at 5,000 rpm for 5 minutes and the serum separated for analysis.

Determination of superoxide dismutase (SOD) activity

The levels of SOD activity was determined by the method of Misra and Fridovich [6].

Determination of total antioxidant capacity

Total antioxidant capacity was estimated by the method as described by Benzie and Strain [9].

Statistical analysis

The statistical analysis was performed using Statistical package for Social Sciences version 20. Differences in mean values between groups were analysed using students’ t-test, while correlation studies were performed using the Pearson’s correlation co-efficient. Values were deemed significant if p < 0.05.

RESULTS

The mean serum levels of superoxide dismutase (SOD) activity was significantly lower in smokers compared with non-smokers (P<0.05). Conversely no significant difference was observed in the mean serum levels of total antioxidant capacity (TAC) in smokers and non-smokers (P>0.05) (Table 1).

Table 1: Comparison of superoxide dismutase (SOD) activity and total antioxidant capacity (TAC) in adult male cigarette smokers and non-smokers

<table>
<thead>
<tr>
<th>Subjects</th>
<th>SOD Activity (U/ml)</th>
<th>TAC (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers (n=50)</td>
<td>9.10 ± 4.00</td>
<td>1179.13 ± 261.11</td>
</tr>
<tr>
<td>Non-smokers (n=50)</td>
<td>12.73 ± 2.34</td>
<td>1262.62 ± 219.28</td>
</tr>
<tr>
<td>t value</td>
<td>5.419</td>
<td>1.700</td>
</tr>
<tr>
<td>P value</td>
<td>0.000</td>
<td>0.092</td>
</tr>
</tbody>
</table>

A negative correlation existed between the serum SOD activity of cigarette smokers and number of cigarette sticks smoked per day (r= -0.557, p=0.000) (Figure 1).

Figure 1: Correlation between SOD activity and number of cigarette sticks per day
A negative relationship was observed between SOD activity and duration of smoking in years \( r = -0.064, p=0.656 \) (Figure 2).

**Figure 2:** Correlation between SOD activity and duration of smoking in years

An inverse relationship existed between total antioxidant capacity and number of cigarette sticks smoked per day \( r = -0.546, p=0.000 \) (Figure 3).

**Figure 3:** Correlation between TAC and number of cigarette sticks per day

An inverse relationship was observed between total antioxidant capacity and duration of smoking in years \( r = -0.302, p=0.035 \) (Figure 4).

**Figure 4:** Correlation between TAC and duration of smoking in years

**DISCUSSION**

Cigarette smoking has been implicated as a significant risk factor for the establishment and progression of several diseases, including atherosclerosis, cancer and emphysema \[^{10,11}\]. Cigarette smoke is a complex mixture of chemical compounds, containing many free radicals and oxidants and may be associated with lower antioxidants concentrations, increased oxidative stress and damage as well as an increased risk of several chronic diseases.

In this study, the mean serum levels of superoxide dismutase (SOD) was significantly lower in smokers compared with non-smokers \( P<0.05 \) and no significant difference was observed in the mean serum total antioxidant capacity (TAC) of smokers compared with non-smokers \( P>0.05 \). This implies that cigarette smoke lowers the activity of SOD. The significant decrease in SOD from this study is in agreement with some previous studies \[^{11,12}\]. Agnihotri et al. \[^{13}\] showed that the mean levels of SOD activity in the gingival crevicular fluid (GCF) and saliva of smokers were decreased compared with the control group. Hamid-reza et al. \[^{14}\] observed that the mean levels of salivary superoxide dismutase, glutathione peroxidase and peroxidase were significantly lower in smokers than non-smokers. Manafa et al. \[^{15}\] had reported a significantly low concentration of serum zinc in sickle cell disease patients compared with that of HbAA group which they linked to oxidative stress in the sickled cells. Since zinc is an important metallic component of superoxide dismutase enzyme, this may possibly have contributed to a decrease in its activity in smokers. The decreased activity of antioxidant enzymes (SOD) in this study may also be due to increased utilization in scavenging the free radical generation associated with cigarette smoking.

In contrast to our findings, Zahraie et al. \[^{16}\] observed that erythrocyte Cu-Zn SOD activity was significantly higher in smokers compared with nonsmokers. They opined that this observation suggests oxidative stress induction following cigarette smoking. However, it is not exactly clear which constituent of the cigarette smoke is involved in this phenomenon. In some studies nicotine has been shown to be involved in oxidant production following cigarette smoking \[^{17}\]. In other studies, cadmium has been shown to be involved in free radical production \[^{18}\]. On the other hand, it has been shown that cigarette smoking causes stimulation of inflammatory response which leads to increased Cu-Zn SOD activity \[^{19}\]. Kocyigit et al. \[^{20}\] observed that erythrocyte Cu-Zn SOD activity following cigarette smoking. It is worth mentioning that in these studies Cu-Zn SOD activity has been determined in erythrocyte, while we assayed SOD activity in serum. The conflicting results in these studies may also have arisen from differences in smoking patterns, the numbers and ages of samples, the type of tobacco, the cigarette design including filtration, blend selection, paper and additives and the structure of the studies \( \text{in-vivo, in-vitro or animal study} \) \[^{20}\].

Measurement of total antioxidant capacity (TAC) is appropriate for evaluation of the total antioxidant defenses of blood, cells, and different kinds of tissues and organs. TAC is reduced by alcoholism, smoking, and exposure to radiation, herbicides, carbon monoxide, carbon tetrachloride, lead, arsenic, mercury, cadmium, aluminum, and other toxic elements. We observed a lower but insignificant mean TAC in smokers compared with non-smokers. In a study in Lodz, Poland, smokers presented lower plasma TAC values in comparison to non-smokers \[^{21}\]. Another research has also shown that workers exposed to dust from tobacco leaves (used in cigarette production) presented reductions in TAC from 15.5% to 31% \[^{22}\].

Lifetime smoking exposure is quantified in “pack years”, where one “pack year” is 20 cigarettes smoked/day for one year \[^{23}\]. Quantification of pack years smoked is important in clinical care where degree of tobacco exposure is closely correlated to risk of disease \[^{23}\]. There is a strong dose–response relationship between the number of pack years smoked and the risk \[^{24}\]. Richard \[^{25}\] reported that the act of
cigarette smoking, in particular the number of years participating in this activity, may manifest in impaired antioxidant capacity and elevated oxidative stress. Our study showed a negative correlation between the serum SOD activity of cigarette smokers and number of cigarette sticks smoked per day (r = -0.557, p<0.000). Moreno, a negative relationship was observed between SOD activity and duration of smoking in years (r = -0.064, p=0.656) (Figure 1 and 2). Furthermore, an inverse relationship existed between total antioxidant capacity levels and number of cigarette sticks smoked per day (r=0.546, p=0.00) and between TAC and duration of smoking in years (r=0.302, p=0.035) (Figure 3 and 4). Greabu et al. demonstrated that smoking a single cigarette rapidly reduces the concentration of plasma antioxidants. Agnihotri et al. showed that the mean levels of SOD activity in saliva of heavy smokers were lower than those in light smokers.

In disagreement with the result of this study, Duker et al. reported an increased SOD activity in long term smokers compared with short term smokers. Russo et al. stated that acute exposure to high levels of free radicals may down regulate the gene expression of antioxidant enzymes whereas chronic continuous exposure may increase the gene expression of these enzymes. Naga et al. reported a positive relationship between duration of smoking and SOD activity.

CONCLUSION

This study has been able to show that the mean serum SOD activity was significantly lower in smokers compared with the control group. However, no significant difference was observed in the mean serum TAC of smokers compared with the control. Furthermore, a negative correlation existed between the serum SOD activity of cigarette smokers and number of cigarette sticks smoked per day . In addition, a negative relationship was observed between SOD activity and duration of smoking. Moreover, an inverse relationship existed between total antioxidant capacity and number of cigarette sticks smoked per day and between TAC and duration of smoking. Therefore frequent SOD activity and TAC assessment as well as intake of balanced diet and antioxidant supplementation is recommended in smokers. Finally, further studies in oxidative stress in large populations of cigarette smokers may be required.

Conflicts of interest

The authors declare that no conflict of interest exists in this research.

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REFERENCES