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Evaluation of the Activities of Salivary Superoxide Dismutase and Glutathione Peroxidase in Cellular Phone Users

Fereshte Arbabi Kalati [1, 2] and Fateme Arbabi Kalati [2, 3, *]

[1]Periodontology Department, Zahedan University of Medical Sciences, Zahedan, IR Iran
[2]Oral and Dental Disease Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran
[3]Oral Medicine Department, Zahedan University of Medical Sciences, Zahedan, IR Iran

[*] *Corresponding author*: Fateme Arbabi Kalati, Oral and Dental Disease Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran. Tel: +98-5433414002, E-mail: arbabi@zaums.ac.ir

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Abstract

Background: Currently, use of cellular phones has increased dramatically. Therefore, there are major concerns about their effect on users' health. Only a limited number of studies have evaluated the effect of cellular phones on salivary activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx).
Objectives: The present study was performed to evaluate the salivary activities of superoxide dismutase and glutathione peroxide in cellular phone users.
Methods: The present cross-sectional study was carried out on 40 volunteers, with no oral diseases, who were assigned to the control (deaf subjects) and case (cellular phone users) groups. Salivary samples were collected from all subjects. The salivary activities of the two enzymes above were determined. Data were analyzed with the SPSS 21 software. Independent t-test was used to compare the two groups.
Results: The salivary levels of SOD in the control and case groups were 128.9 $\pm$ 35.6 U/mL and 82.37 $\pm$ 32.3 U/mL, respectively, indicating a statistically significant difference (P = 0.000). There was no significant difference in salivary levels of GPx between the two groups (P = 0.3).
Conclusions: Based on the results of the present study, use of cellular phones resulted in a decrease in the activity of a component of the anti-oxidative system, decreasing the capacity to eliminate free radicals by the saliva; this makes the individual susceptible to inflammatory and malignant conditions.

Keywords: Cellular Phone, Superoxide Dismutase, Glutathione Peroxidase

1. Background

During the past decade, use of cellular phones has increased significantly, becoming a cultural tool (1). There are some concerns regarding the deleterious effects of electromagnetic waves, radio frequencies, and macro waves of cellular phones and their telecommunication centers on human health (2). Dental science is increasingly emphasizing the important role of saliva in maintaining the ecologic balance of oral cavity (2). Saliva plays an important role in maintaining oral homeostasis and is considered the first line of defense against microbial invasion, protecting the oral cavity mucosa mechanically and immunologically (3) Shivashankara et al. (4), studied the salivary levels of amylase, malondialdehyde (MDA), lactate dehydrogenase (LDH), glutathione, and also total salivary protein levels, and reported that subjects using cellular phones to a great degree, in terms of duration and frequently of use, exhibited higher levels of MDA and LDH in their saliva yet exhibited insignificantly lower levels of total salivary proteins and GSH compared to subjects, who used cellular phones less frequently. Khalil et al. (5), studied the effects of exposure to radio frequencies (RF) of cellular phones and concluded that there was no relationship between exposure to RF and changes in salivary antioxidant levels. Bhargava et al. (6), studied bilateral parotid salivary flow rate in 142 subjects in a control group and in a group with extensive use of cellular phones, concluding that subjects using cellular phones for a long time exhibited an increase in salivary flow rate, an increase in blood flow to the gland, and hypertrophy of the parotid gland on the side in which the cellular phone was used more frequently. Arbabi Kalati et al. (7), evaluated the effect of the duration of use of cellular phone on total salivary anti-oxidative capacity and reported that this capacity decreased with the use of cellular

phones for more than one hour.

2. Methods

The protocol of the present cross-sectional study was approved by the ethics committee of Zahedan University of Medical Sciences. The study was carried out at the department of oral medicine, Faculty of dentistry, Zahedan University of Medical Sciences, in 2016. Deaf individuals were included in the present study as control subjects on a voluntary basis. The cases were selected from patients referring to the department of oral medicine, who had a history of cellular phone use for five years, and had dialogs on cellular phones for more than 20 minutes and less than one hour daily. The two groups were matched in terms of age and gender. Exclusion criteria consisted of use of medications and alcohol, smoking, chronic systemic conditions, a history of trauma to the head and neck region, pregnancy, subjective and objective xerostomia, a history of chemotherapy or radiotherapy of the head and neck region, age of under 18 years old, use of food supplements during the previous three-month period, periodontal diseases, and use of hands-free tools.

After obtaining informed consent from all the subjects, they were asked to refrain from eating and drinking for one hour before collection of salivary samples by spitting. To this end, the subjects were asked to collect their saliva in 15-mL falcon tubes for two minutes and then the tubes were collected. Each tube containing the salivary sample was coded and immediately centrifuged for 10 minutes at 2500 rpm to separate possible debris. Then, the pure salivary samples were stored at -70°C until the day they were tested. All the samples were collected from 9 to 11 am.

2.1. Determination of Salivary Levels of Superoxide Dismutase

The technique introduced by Kakar et al. was used to determine salivary SOD levels. The technique relies on the inhibition of the formation of the blue color of formazan tetrazolium by SOD in the reaction mixture containing phenazine methosulfate-NADH-NBT (8).

2.2. Determination of Salivary Levels of Glutathione Peroxide

The technique introduced by Amini et al. was used to determine the salivary levels of GPx. In this technique, GPx reduces H_2O_2, resulting in the oxidation of glutathione. Then the oxidized glutathione is reduced again by glutathione reductase and NADPH is converted to reduced glutathione and NADP+. Finally, oxidation of NADPH to NADP+ is measured by the spectrophotometry technique, at a wavelength of 340 nm. In this study, the salivary GPx levels were reported in U/mg of protein (8).

2.3. Statistical Analysis of Data

Data were analyzed with SPSS 21, using independent t-test.

3. Results

The mean age of the subjects in the case group (consisting of 25 females and 22 males) was 25.53 $\pm$ 2.7 years; the mean age in the control group (consisting of 18 males and 15 females) was 27.09 $\pm$ 4.8 years, with no significant difference between the two groups (P = 0.2).

As shown in Table 1, the salivary levels of SOD were significantly different between the two groups, with higher levels in the control group; however, the mean salivary levels of GPx were not significantly different between the two groups.

Table 1. The Mean Salivary Levels of SOD and GPx in Two Study Groups

Group	SOD (u/mL)	GPX (u/L)
Case (healthy)	82.37 $\pm$ 32.3	607.37 $\pm$ 60
Control (deaf)	128.9 $\pm$ 35.6	568.40 $\pm$ 167
P-value	0.000	0.3

4. Discussion

The results of the present study showed significant differences in salivary levels of SOD between the two study groups; however, no significant differences were detected in the salivary levels of GPx between the two groups.

Saliva is a biologic fluid, which is considered the first line of defense against free radicals and oxidative stresses. Evaluation of anti-oxidative activities is in fact an indirect evaluation of the effect of cellular phones on an individual's health (5).

Collection of salivary samples is an easy and non-invasive technique for the evaluation of the anti-oxidative system.

The SOD is responsible for preventing the effect of oxidative agents within the cells and cellular organelles, such as mitochondria and peroxisomes. The location of the activity of the majority of these enzymes is the mitochondria. One of the most important anti-oxidative mechanisms of the body against the attack mounted by reactive oxygen species is the presence and activity of SOD (9). Glutathione peroxidase is a general term for a family of enzymes with peroxidase activity; their main biologic role is to protect the organism against oxidative injuries (4).

Only a limited number of studies have evaluated the effect of cellular phone on the components of the anti-oxidative system. The results of a study by Hashemipour

et al. (10), showed that subjects using their cellular phones for longer durations exhibited lower salivary levels of peroxidase, consistent with the results of the present study.

In a study by Khalid et al. (2014), 109 males aged 18 to 37 years old were evaluated. It was reported that an increase in the duration of the use of cellular phones resulted in an increase in the activity of SOD, which is different from the results of the present study. Such a discrepancy between the results of these two studies might be attributed to the duration of the use of cellular phones because in the study above, the salivary levels of the enzyme were evaluated in terms of the duration and frequency of the use of cellular phones (9). In the present study, only subjects, who used their cellular phones for more than 20 minutes daily were included.

Some studies have evaluated other antioxidants. Khalil et al. (5), showed no changes in MDA, OXO-Dg-8, ORAC, and HORAC antioxidant levels with up to 30 minutes of dialog on the cellular phone. Arbabi Kalati (7), showed that individuals, who used their cellular phones for more than 20 minutes exhibited a general decrease in salivary antioxidant levels; however, the activities of the components of the anti-oxidative system were not evaluated.

Hamzany et al. (11), reported that use of cellular phones resulted in an increase in oxidative injuries, which might be attributed to a decrease in the activity of SOD in the anti-oxidative system, resulting in a decrease in the capacity of saliva to neutralize oxidative stresses.

Goldwein and Aframian (1), evaluated the effect of cellular phones on the total salivary protein levels and reported a decrease in their levels; since the anti-oxidative system is composed of proteins, it might be concluded that a decrease in the salivary levels of antioxidants has resulted in a decrease in salivary proteins.

No study to date has evaluated salivary GPx levels; therefore, it was not possible to make comparisons with other studies.

5. Conclusions

Based on the results of the present study, use of cellular phones resulted in a decrease in the activity of a component of the anti-oxidative system, decreasing the capacity of saliva to eliminate free radicals; this makes the subjects susceptible to inflammatory and malignant conditions. It is suggested for future studies to focus on salivary oxidative changes and long-term studies be undertaken to evaluate the effects of cellular phones on mucous membrane diseases.

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