SHORT-TERM USE OF CELL PHONES HAS NO SIGNIFICANT EFFECT ON THE SALIVARY OXIDANT/ANTIOXIDANT PROFILE

Ahmad M Khalil[1]*, Khalid M Abu Khadra[2], Ahmad M Aljaberi[3], Marwan H Gagaa[4], and Hamzah S Issa[5]

[1] Department of Biology, Faculty of Science, Taibah University, Almadina Almunawwarah, Kingdom of Saudi Arabia, [2] Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid , Jordan, [3] Department of Pharmaceutical Sciences and Pharmaceutics, Faculty of Pharmacy, Applied Science University, Amman, Jordan, [4] Department of Biotechnology and Genetic Engineering, Faculty of Science, Philadelphia University, Amman, Jordan, [5] Research and Development Department, Jordan Company for Antibody Production (MonoJo), Amman, Jordan

kahmad76@yahoo.com, khalidmohd99@yahoo.com, aljaberi@asu.edu.jo, mqaqaa@philadelphia.edu.jo, h.issa@monojo.com.jo

ABSTRACT

To investigate whether or not low intensity radio frequency electromagnetic field exposure (RF-EME) associated with cell phone use can affect human cells, the present study was carried out. Saliva samples collected before using a cell phone as well as at the end of 15 and 30 min calls were tested for two commonly used oxidative stress biomarkers. The 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) levels were determined by enzyme linked immunosorbent (ELISA) competitive assay. The malondialdehyde (MDA) levels were measured using the OxiSelect MDA Adduct ELISA Kit. The antioxidant capacity of the saliva was evaluated using the oxygen radical absorption capacity (ORAC) and the hydroxyl radical averting capacity (HORAC) assays. The mean 8-oxodG and the Bradford protein concentrations (ng/ml and mg/ml, respectively) peaked at 15 min. The levels of HORAC, ORAC and MDA progressively increased with time and reached maximum at 30 min. All measured parameters were not significant when values before and after talking are compared indicating that serious changes in the salivary oxidant/antioxidant profile may not be strongly correlated with exposure to RF-EME.

Key Words: Antioxidant Activity; Cell Phone; Oxidative Stress Biomarkers; Saliva.

1. INTRODUCTION

Concerns about possible biohazards of the electromagnetic radiation (EMR) resulting from the cell phone use have started since the emergence of mobile phone technology and are still growing. A typical cell phone operates at a power output of 0.25 W, which results in a specific energy absorption rate (SAR) of about 1.5 w kg^{-1} 0.1°C) [1] and an associated very low rise in brain temperature (maximum, 0.1°C) [1].The radio frequency (RF) energy produced by cell phones may affect the brain and other tissues in the head because hand-held cell phones are usually held close to the head [2].

The parotid glands are the largest salivary glands, situated in front of the ear, near the place used by cell phones during calls. These

glands are likely to be exposed to RF radiation during mobile phone use if the handset is held close to the ear. Contradictory data were obtained by researchers investigated the adverse health effect of the cell phone use. Previous studies have suggested the possible health effects involved in the use of cell phones. Some reported no evidence for association between the use of wireless phones and an increased risk for parotid gland tumors [3], [4], [5], [6]. However, one study suggested association between cell phone use and the development of salivary gland tumor based on the elevated risk of developing benign and malignant parotid gland among mobile phone user [7]. In another study, parotid glands respond by elevated salivary rates and decreased protein secretion reflecting the continuous insult to the glands [8]. More recently, functional and volumetric changes in the parotid glands associated with cell phone use have been reported [9]. Thus, currently the health community still has a vague idea as to the extent of the harmful effects of mobile radiation on the human body in general, and on the adjacent parotid gland physiologic function in particular.

This possible association validates the use of altered oxidative stress indices because of cell phones use as an indicator of increase incidence of certain tumors. Oxidative stress alteration because of mobile phone RF such as Lipid peroxidation (LPO), DNA damage, free radicals formation and other oxidative stress biomarkers were assessed in different animals tissue to prove that association [10], [11], [12], [13], [14], [15]. Controversial results were reported by researchers using different oxidative stress biomarkers after exposing animals to RF of cell phone [16], [17], [18]. For example, levels of LPO and 8-Oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) in liver of female rabbits increased [19] total antioxidant capacity and catalase activity in rat brain were affected [17], MDA rat plasma concentration was increased [16] and superoxide dismutase (SOD) activity in hippocampus of rats was significantly decreased [18]. In contrast, RF radiation did not lead to serious alterations in antioxidant and oxidative stress parameters in eye tissue and blood of rat [20]. In human, significant increase in all salivary oxidative stress indices were reported among cell phone users [21]. However,

oxidative damage was suggested after the statistically significant decrease in salivary SOD after mobile phone use [22].

In the present study, we further tested the antioxidant capacity of the saliva of cell phone users using the ORAC (oxygen radical absorption capacity) and HORAC (hydroxyl radical averting capacity) assays. In addition, the oxidative stress resulting from exposure to cell phone radiation has been explored using two biomarkers; 8-oxo-dG) and MDA.

2. MATERIALS AND METHODS

2.1. Subjects

Twelve normal healthy males (average age 22 years) with no prior history of periodontal disease volunteered in this study. All the participants completed a self administered questionnaire including questions about personal and present history, past history, and if they lived near high voltage power lines or mobile base stations.

2.2. Saliva Collection

Volunteers were asked not to eat, drink, or brush their teeth an hour before saliva collection. Each participant used the same device during experiment, a Nokia C3-00 (RM-614) cell phone with a dimension of 13.6 x 115.5 x 58.1 mm. The phone was in connection with an 1800 MHz Umniah Jordanian network (Irbid, Jordan) with 1.09 w kg^{-1} SAR value. None of the participants used hands-free accessories. All participants used the right ear. Unstimulated whole saliva samples were collected from all the participants (at a fixed time in the afternoon) for 10 min in sterilized tube and kept on ice during and after saliva collections. Saliva samples were taken from the participants three times; before they started cell phone call, after 15 min and 30 min directly at the end of the call. To remove cells and insoluble materials, the samples were centrifuged at 14 000 g for 20 min at 4 ºC to remove cells and insoluble materials. The supernatants were taken, aliquotted into storage vials and kept frozen at –80°C until chemical analysis.

2.3. Chemical Analysis for Oxidative Stress Indices

Protein concentration was determined according to the procedure described by Bradford (Bio-Rad, Hercules, CA, USA). For determination of 8-OxodG, a competitive enzyme-linked immunosorbent assay (ELISA) (Northwest Life

Science Specialties, USA) was used. Saliva supernatants (50 µl) were added to the precoated 8-OxodG protein conjugate microtiter plate, followed by the addition of 50 µl of the primary antibody, anti-8-OHdG monoclonal antibody solution. 100 µl of chromatic substrate (Chromogenic) was added to the plate after completing the usual ELISA procedure and according to the kit instruction. The intensity of color produced from each sample was recorded at an optical density of 490 nm using ELISA Plate-Reader (Awarness Technology Inc. Stat Fax 3200, Palm City, FL, USA). For each standard 96-well microplate 6 to 9 control samples were randomly placed among the unknown samples.

Lipid peroxides are unstable indicators of oxidative stress in cells. Lipid peroxides decompose directly to form malondialdehyde (MDA) which is a natural byproduct of cellular damage in animal and plants known as lipid peroxidation. MDA in saliva was evaluated in this study in order to monitor if there is an oxidative stress after using cell phone. The MDA assay was performed using the OxiSelect MDA Adduct ELISA Kit (Cell Biolabs, INC). Saliva samples were diluted to 10 µg/ml in 1X PBS, and 100 µl from each sample were added in duplicates to the 96-well Protein Binding Plate. The plate was incubated at 37ºC for 3 h, followed by two washes with 250 µl 1X PBS per well. After the second wash, wells were taped on paper towel to remove excess wash solution. Then, 200 µl of assay diluent were added per well and incubated for 1 h at room temperature (RT) on an orbital shaker. The plate was washed three times with 1X wash buffer before adding the diluted anti-MDA antibody and incubated for 1 h at RT on the orbital shaker. Finally, substrate solution was added to the plate after completing modified ELISA procedure according to the kit instruction. The absorbance of each sample was measured at 450 nm after the addition of stop solution using MultiSkan Go spectrophotometer (Thermo Scientific, Finland).

2.4. Antioxidant Capacity Assay

The antioxidant capacities of saliva were evaluated in order to monitor if there is an oxidative stress effect on saliva biomolecules. The hydroxyl radical antioxidant capacity (HORAC) assay and the oxygen radical

absorbance capacity (ORAC) assay were used to study the saliva antioxidant capacity during this study. The HORAC assay kit and ORAC activity assay kit (Cell Biolabs, INC.) were used. Both assays are based on the oxidation of a fluorescent probe by radicals such as hydroxyl for HORAC and peroxyl for ORAC by way of a hydrogen atom transfer process. According to the procedure principle, antioxidants present in the saliva work to block the radical oxidation of the fluorescent probe until the antioxidant activity in the saliva is depleted. The remaining radicals start to destroy the fluorescence of the fluorescent probe. The assay continues until completion. Saliva samples were analyzed based on the procedure and instructions provided by HORAC and ORAC kits. Immediately after the addition of Fenton reagent to each well, reading for sample and standard were started using fluorescent microplate reader (Ultra micropplate reader, Bio-Tech Instruments Inc.) with an excitation wavelength of 480 nm and an emission wavelength of 530nm. Wells were read every 5 minutes for a total of 60 minutes. Saliva antioxidant capacity correlates to the fluorescence decay curve which was represented as the area under the curve (AUC). The AUC was used to quantify the total radical antioxidant activity in a sample and compared to an antioxidant standard curve of water soluble vitamin E for ORAC or Gallic acid solutions for HORAC. All assays were performed blinded to the subject status and in a single series to avoid interassay variability [23], [24], [25].

2.5. Statistical Analysis

All analysis was carried out at least three separate experiments (triplicates in a blind manner). The results of three independent blind biochemical analyses were statistically evaluated as follows: Statistical comparisons of means between various time groups were performed using an analysis of variance (ANOVA) when both, the homogeneity of variance and normal distribution were demonstrated using Levene's and Shapiro–Wilk tests, respectively. Planned comparisons were performed to determine significant difference between groups. Moreover, trend analysis to assess for linear versus quadratic relationships were evaluated. All analyses were carried out using SPSS 17 (Statistical Package for social

sciences) software. Statistical significance was set at $p< 0.05$. Effect size of the overall ANOVA represented by ω was calculated according to the following equation:

$$\omega = \sqrt{\frac{SS_M - (df_M)MS_R}{SS_T + MS_R}} \quad (1)$$

Whereas effect size for the contrasts employed where calculated according to

$$r = \sqrt{\frac{t^2}{t^2 + df}} \quad (2)$$

3. RESULTS

None of the participants was exposed to diagnostic X-ray during the three months that preceded supplying the saliva sample. Further, no one was taking medications during the last three weeks prior to participation. In addition, all participants responded that they do not use a microwave or live near a mobile phone base station. Figure 1 depicts a summary of the data collected from the present experiments. There was no significant effect of talking time on the levels of Bradford protein, $F (2, 33) = 0.384$, $p>0.05$. Similarly, no significant effect of talking time on the levels of 8-oxodG, $F (2, 33) = 0.246$, $p>0.05$ was observed.

4. DISCUSSION

The objective of this study was to examine whether the use of cell phone influences two fundamental aspects of saliva; the antioxidant capacity and the oxidative stress as well as protein concentration in saliva. The main outcome was that none of the measured parameters was significant affected suggesting that serious changes in the salivary oxidant/antioxidant profile may not be strongly correlated with exposure to RF-EME. Saliva plays an important role in maintaining oral homeostasis and could constitute a first line of defense against free radical- mediated oxidative stress [26]. Saliva being non-invasive and easy to collect can be used to assess the oxidative/ antioxidative status of cells in persons acutely exposed to cell phone radiation. Antioxidant activity and oxidative stress indices were used indirectly to assess the adverse health effects of cell phone use [8], [9]. Biomarkers of oxidative stress can be measured in saliva and represent a promising diagnostic tool for many oral and systemic diseases [27]. It has been indicated that 8-OxodG [28], [29], and MDA [30] levels in

saliva appear to reflect the status of periodontal health.

To the best of our knowledge, the present study represents the first attempt to determine the possible effects of short-term exposure to RF radiation on the biochemical quality of whole saliva of persons using cell phone. Therefore, the results we obtained cannot be compared with similar previous studies. Our observations regarding 8-oxodG, although the effect of talking time on the levels of 8-oxodG was not significant, may suggest that reactive oxygen species could play a role in the mechanism that has been proposed to explain the biological side effects of cell phones. On the other hand, maximum concentrations of MDA were measured after 30 min use of the cell phone. This is consistent with previous observations that markers of oxidative DNA damage and lipid peroxidation may not be strongly correlated, and suggests that no single measure can be used as an indicator of in vivo oxidative stress [31]. An increase in the MDA concentration was found in the brain tissues of male Wistar-Albino rats exposed to 900 MHz electromagnetic field emitting mobile phone [18].

The ability of RF radiation associated with cell phone use to stimulate oxidative stress has been reported before [10], [18], [31], [32], [33]. In this study, small concentrations of 8-oxodG were detected. This low accumulation may be explained by the repair of DNA lesions leading to 8-oxodG in saliva as suggested before in the urine of rats exposed to RF-EME [14]. However, this contradicts with the finding that DNA damage was stable within subject over time in both leukocyte and buccal mucosa cells [34]. The advantage of the ORAC assay is that it combines both the inhibition time and inhibition degree of the radical generation, as it takes the oxidation reaction to completion and uses the area under the curve to quantify the antioxidant capacity [35]. We were unable of detecting any evidence for changes in the antioxidant capacity as a result of acute exposure to RF radiation following short call periods.

However, limitations of the present study include small sample size, which reduced our ability of inspiring deep confidence in the

results and short exposure period, which does not allow extrapolation to the long-term effect of RF-EME. Considering the widespread use of phones, it will be essential to evaluate the long-term effects on the human organs, as well as protective measures. It appears that further tests on large scale with different frequencies and exposure periods should be performed to clarify the importance and role of antioxidants in cell phone radiation-induced oxidative stress. Such experiments are being done in our laboratories.

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6. REFERENCES

[1]. Haramaki, N., Aggarwal, S., Kawabata, T., Droy-Lefaix, M. T., Packer, L : Effects of Natural Antioxidant Ginkgo Biloba Extract (EGb 761) on Myocardial Ischemia-Eperfusion Injury. Free Radical Biology and Medicine 16, 789 - -794 (1994).

[2]. Hardell, L., Carlberg, M., Hansson, M. K. : Pooled Analysis of Case-control Studies on Malignant Brain Tumours and the Use of Mobile and Cordless Phones Including Living and Deceased Subjects. International Journal of Oncology 38, 1465 - -1474 (2011).

[3]. Johansen, C., Boice, J. Jr., McLaughlin, J., Olsen, J. : Cellular Telephones and Cancer—a nationwide Cohort Study in Denmark. Journal of the National Cancer Institution 93, 203 - -207 (2001).

[4]. Auvinen, A., Hietanen, M., Luukkonen, R., Koskela, R. S.: Brain Tumors and Salivary Gland Cancers Among Cellular Telephone Users. Epidemiology 13, 356 - -359 (2002).

[5]. Lonn, S., Ahlbom , A., Christensen, H. C., Johansen ,C., Schüz, J. : Mobile Phone Use and Risk of Parotid Gland Tumor. American Journal of Epidemiology 164, 637 - - 643 (2006).

[6]. Söderqvist, F., Carlberg, M., Hardell, L. : Use of Wireless Phones and the Risk of Salivary Gland Tumours: A Case-control Study. European Journal of Cancer Prevention [Epub ahead of print] (2012).

[7]. Sadetzki, S., Chetrit, A., Jarus-Hakak, A., Cardis, E., Deutch, Y., Duvdevani, S., Zultan, A., Novikov, I, Freedman, L. Wolf ,M. : Cellular Phone Use and Risk of Benign and Malignant Parotid Gland Tumors a Nationwide Case-control Study. American Journal of Epidemiology 167, 457 - - 467 (2008).

[8]. Goldwein, O., Aframian, D. J. : The Influence of Handheld Mobile Phones on Human Parotid Gland Secretion. Oral Diseases 16,146 - -150 (2010).

[9]. Bhargava, S., Motwani, M. B., Patni, V. M. : Effect of Handheld Mobile Phone Use on Parotid Gland Salivary Flow Rate and Volume. Oral Surgery, Oral Medicine, Oral Pathology, Orral Radiology 114, 200 - -206 (2012).

[10]. Irmak, M. K., Fadillioğlu, E., Güleç, M., Erdoğan, H., Yağmurca, M., Akyol, O.: Effects of Electromagnetic Radiation from a Cellular Telephone on the Oxidant and Antioxidant Levels in Rabbits. Cell Biochemistry and Function 20, 279- -283 (2002).

[11]. Phillips, J. L., Singh, N. P., Lai, H.: Electromagnetic Fields and DNA Damage. Pathophysiology 16, 79 - - 88 (2009).

[12]. Khalil, A., Alshamali, A. : No Significant Cytogenetic Effects in Cultured Human Lymphocytes Exposed to Cell Phones Radiofrequencies (900MHz and 1800MHz. Jordan Journal of Biological Sciences 3, 21--27 (2010).

[13]. Khalil, A. M., Alshamali, A. M., Gagaa, M. H. : Detection of Oxidative Stress Induced by Mobile Phone Radiation in Tissues of Mice Using 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a Biomarker. The World Academy of Science and Engineering Technology 76, 599 - - 604 (2011).

[14]. Khalil, A. M., Gagaa, M.H., Alshamali, A.M.. : 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a Biomarker of DNA Damage by Mobile Phone radiation. Human and Experimental Toxicology 31, 734 - -740 (2012).

[15]. Karaca, E., Durmaz, B., Aktug, H., Yildiz, T., Guducu, C., Irgi, M., Koksal, M.G., Ozkinay, F., Gunduz ,C., Cogulu, O. : The Genotoxic Effect of Radiofrequency Waves on Mouse Brain. Journal of Neurooncology 106, 53 --58 (2012).

[16]. Elhag, M. A., Nabil, G. M., Attia, A. M. M. : Effects of Electromagnetic Field Produced by Mobile Phones on the Oxidant and Antioxidant Status of Rats. Pakistan Journal of Biological Sciences 10, 4271- - 4274 (2006).

[17]. Dasdag, S., Akdag, M. Z., Ulukaya, E., Uzunlar, A. K, Ocak, A. R. : Effect of Mobile Phone Exposure on Apoptotic Glial Cells and Status of Oxidative Stress in Rat Brain. Electromagnetic Biology and Medicine 28, 342 - -354 (2009).

[18]. Kerman, M., Senol, N.: Oxidative Stress in Hippocampus Induced by 900 MHz Electromagnetic Field Emitting Mobile Phone: Protection by Melatonine. Biomedical Research 23, 147 - -151 (2012).

[19]. Güler ,G., Tomruk, A., Ozgur, E., Sahin, D., Sepici, A., Altan, N., Seyhan, N. : The Effect of Radiofrequency Radiation on DNA and Lipid Damage in Female and Male Infant Rabbits. International Journal of Radiation Biology 88, 367 - - 73 (2012).

[20]. Demirel, S., Doganay ,S., Turkoz, Y., Dogan ,Z., Turan , B., Firat,, P. G. B. : Effects of Third generation Mobile Phone-emitted Electromagnetic Radiation on Oxidative Stress Parameters in Eye Tissue and Blood of Rats. Cutaneous and Ocular Toxicology 31, 89 - - 94 (2012).

[21]. Hamzany, Y., Feinmesser, R., Shpitzer ,T., Mizrachi, A., Hilly, O., Hod, R., Bahar, G., Otradnov, I., Gavish, M., Nagler, R. M. : Is Human Saliva an Indicator of the Adverse Health Effects of Using Mobile Phones? Antioxidants and Redox Signaling. [Epub ahead of print]. (2012).

[22]. Abu Khadra, K. M., Aljaberi, A. M., Gagaa, M. H., Khalil, A. M.: Oxidant/antioxidant Profile of Whole Human Saliva before and after Using Cell Phone. International Journal of Advanced Science and Engineering Technology (IJASET) (In Press).

[23]. Ou, B., Hampsch-Woodill, M., Prior, R. L.: Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the fluorescence Probe. Journal of Agricultural and Food Chemistry 49, 4619 - - 4626 (2001).

[24]. Ou, .B, Hampsch-Woodill, M. , Flanagan, J., Deemer, E. K., Prior, R .L., Huang, D. : Novel Fluorimetric Assay for Hydroxyl Radical Prevention Capacity Using Fluorescein as the_Probe. Journal of Agricultural and Food Chemistry 50, 2772 - - 2777 (2002).

[25]. Gomes, A., Fernandes, E., Lima, J. L. F. C. : Fluorescence Probes Used for Detection of Reactive Oxygen Species. Journal of Biochemical and Biophysical Methods 65, 45 - - 80 (2005).

[26]. Mandel, I. D. : The role of Saliva in Maintaining Oral Homeostasis. Journal of the American Dental Association (JADA) 119, 298 - - 304 (1989).

[27]. Kamodyová, N., Celec, P. : Salivary Markers of Oxidative Stress and Salivette Collection Systems. Clinical Chemistry and Laboratory Medicine 49, 1887 - - 1890 (2011).

[28]. Takane , M., Sugano, N., Ezawa, T., Uchiyama, T., Ito, K. : A Marker of Oxidative Stress in Saliva: Association with Periodontally-involved Teeth of a Hopeless Prognosis. Journal of Oral Science 47, 551 - - 554 (2005).

[29]. Badea, V., Balaban, D., Amariei, C., Nuca, C., Bucur, L.: Salivary 8-Hydroxy-2-deoxyguanosine as Oxidative Stress Biomarker for the Diagnosis of Periodontal Disease. Farmacia 58, 660 -- 670 (2010).

[30]. Al-Rawi, N. H., Jaber, F. A., Atiyah, K. M. : Assessment of Salivary and Serum Oxidative Stress and Antioxidants as Plausible Parameters in Prediction of Ischemic Stroke among Iraqi Samples. The Internet Journal of the Third World Medicine 7, 1 - -15 (2009).

[31]. Ilhan, A., Gurel, A., Armutcu, F., Kamisli, S., Iraz, M., Akyol, O., Ozen, S. : Ginkgo Biloba Prevents Mobile Phone-induced Oxidative Stress in Rat Brain. Clinica Chimica Acta 340, 153 - -162 (2004).

[32]. De Iuliis, G. N., Newey , R. J., King, B. V., Aitken, R. J. : Mobile Phone Radiation Induces Reactive Oxygen Species Production and DNA Damage in Human Spermatozoa in Vitro**.** PLoS One 4, e6446 (2009).

[33]. Desai, N. R., Kesari, K. K., Agarwal, A. : Pathophysiology of Cell Phone Radiation: Oxidative Stress and Carcinogenesis with Focus on Male Reproductive System. Reproductive Biology and Endocrinology 7, 114 (2009).

[34]. Borthakur, G., Butryee, C., Stacewicz-Sapuntzakis, M., Bowen, P. Exfoliated Buccal Mucosa Cells as a Source of DNA to Study Oxidative Stress. Cancer Epidemiology, Biomarkers and Prevention 17, 212 - - 219 (2008).

[35]. Prior, R. L.: Fruits and Vegetables in the Prevention of Cellular Oxidative Damage. The American Journal of Clinical Nutrition 78, 570S - - 578S (2003).

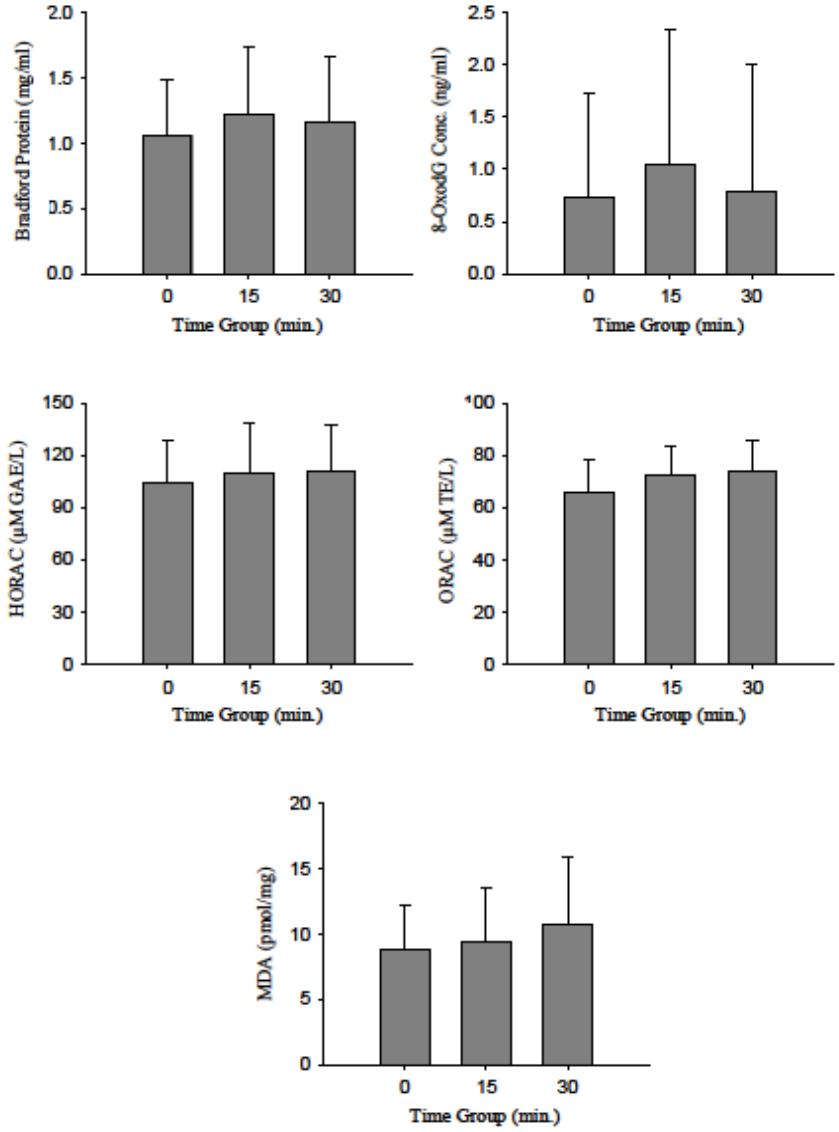

Figure1 Effect of talking time on the salivary levels of various antioxidants investigated.

Data represent mean ± standard deviation. Triplicate experiments were made and analyzed in a blind manner.