Antioxidant Profile of Saliva among Young Men Using Mobile Phones

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Abstract

Oxidative stress has been implicated as a mechanism of potential health effects that may result from exposure to Radio Frequency Electromagnetic radiation (RF-EMR). A cross sectional study was designed to investigate and analyze the biochemical effects of RF-EMR emitted from mobile phones at 835 MHz and 1900 MHz bands on some biochemical markers: Superoxide dismutase (SOD), albumin, amylase, uric acid and cytochrome C in the saliva of young men (18 -37 years; average age 27.74 ± 8.08). EMF caused a significant increase in the activity of SOD but a significant decrease in that of amylase in the saliva of people after using mobile phones. The increases in the activity of cytochrome C and the concentrations of albumin and uric acid were not significant. A true correlation between the salivary antioxidant biomarkers and the number of calling min., rather than the number of calls, was found. These oxidative changes may result in metabolic changes in the living cells up to oncogenic transformation. Thus, based on these findings, it is recommended that a long-term/or excessive use of mobile phones, especially by young individuals, should be avoided. This goal can be accomplished by telehealth technology promotion activities targeting the more sensitive ages, children and adolescents, since their developing brains absorb more EMR from a mobile phone. Such activities include: group discussions, public presentations and mass communication through available electronic and print media sources.

Keywords: Antioxidants; Human saliva; Mobile phone; Oxidative stress.

1. Introduction

Currently, of the world’s 7 billion people, 6 billion have mobile phones and the initial age of youngest users of the cell phone is estimated as three years old. Over time, the number of mobile phone calls per day, the length of each call, and the amount of time people use cell phones have increased (Khurana et al., 2009; Awadalla, 2013). Radiofrequency (RF) radiation is an important part of electromagnetic human exposure. This is due to the fact that in this frequency range, the electromagnetic energy penetrates skin depth in a way that the entire body is affected, not just the surface layers. Secondly, it is the frequency range where the outer membranes of mammalian cells are no longer barriers to electric fields, allowing access of the RF to subcellular structures (Goldsworthy, 2012; Awadalla, 2013). If a risk exists, it is likely to be greatest for regions with greatest energy absorption in close proximity to the head (Cardis et al., 2008; Awadalla, 2013; Bhargavi et al., 2013). Epidemiological studies of the association between exposure to routine mobile phone radio frequency-electromagnetic radiation (RF-EMR) and adverse health effects, including brain tumors, have been inconsistent (some, but not all, studies showed increased risk); the issue remains unresolved (Kundi, 2005; Ahlbom et al., 2009; Dubey et al., 2010; Yakymenko et al., 2011; Hardell et al., 2013). The parotid gland is one potential target of interest, since mobile phones are typically pressed up against the side of the face in front of the ear where the gland is located. In fact, an association between the mobile phone use and parotid gland tumors has been reported (Sadetzki et al., 2008; Czerninski et al., 2011; Duan et al., 2011; Bello et al., 2012; de Vocht et al., 2013). For example, the increase in the annual incidence
of head tumors was correlated with increased rate of mobile phone subscription (de Vocht et al., 2013).

One of the priorities in the RF-EMR research is to elucidate the underlying mechanisms of the biological effects of RF-EMR exposure. Despite the increasing number of reports concerning these effects in various biological systems (Sivani and Sudarsanam, 2012), no satisfactory mechanism has been proposed to explain these effects. One of such proposed mechanisms is the stimulation of oxidative stress. However, a comprehensive picture regarding the relationships between oxidative stress and the exposure to RF is still lacking. An oxygen damage of DNA in human spermatozoa (De Iuliiis et al., 2009), and saliva (Khalil et al., 2014), as well as in rat urine (Khalil et al., 2012) through formation of 8-Oxo-7, 8-dihydro-2'-deoxyguanosine (8-Oxo-dG) under non-thermal microwaves radiation has been demonstrated. The antioxidant capacity, measured by Oxygen Radical Absorption Capacity (ORAC), and Hydroxyl Radical Averting Capacity (HORAC) of human saliva did not significantly increase following a short-time mobile phone talk (Khalil et al., 2014). Mobile phone radiation induced a significant increase in the activity of superoxide dismutase (SOD) enzyme (Abu Khadra et al., 2014). In the latter study, the concentration of other salivary proteins, albumin and uric acid as well as the activity of the enzymes catalase and cytochrom C were not significantly altered.

The present study examines whether the human body responds to exposure to RF-EMR by investigating variations in the salivary profile among young mobile phone male users. This study will not only demonstrate whether the human body recognizes mobile phone radiation as an external stressor but it also provides information on whether molecules, proteins and genes respond (either activated or inactivated) to mobile phone radiation.

2. Materials and Methods

2.1. Design, Setting, and Participants

A randomized cross-sectional study was conducted between June 1 and October 31, 2012. To avoid gender effect and interlaboratory variations, the experiment was performed in one laboratory only on 109 healthy males recruited from North Jordan community. The age of participants ranged between 18 and 37 years (average 27.74 ± 8.08 years). The study was approved by the local institutional review board (Committee on Research Involving Human Subjects, Yarmouk University). All subjects were screened in a short personal interview in order to assure that they corresponded to our criteria of selection, including being nonsmokers, nondiabetic, and not suffering from significant dental, gingival, or chronic systemic inflammatory diseases. All were regular users of the mobile phone, making at least one phone call per week for a period of at least 6 months (less than 8 hours a month).

The mobile phones of all participants used a Global System for Mobile Communications (GSM), which operates in the 900 MHz or 1800 MHz bands, where the maximum power level is 1 watt or 2 watts at 1800 MHz and 900 MHz, respectively (Bhargavi et al., 2013). The goal of the study was explained to all, and each individual filled in a questionnaire that incorporates information on the intensity of mobile phone use, the total number of calls per day, the total number min. utilized per day when using mobile phone, and the calendar period of use. A written informed consent was obtained from all participants. Volunteers were not compensated for participation in the study. Volunteers were asked not to eat, drink, or brush their teeth 1h before the collection of saliva. Twenty ml of whole unstimulated saliva samples were collected for 10 min. in sterilized tubes and kept on ice during and after the collection. The sample collection procedure was done in the morning to avoid any diurnal variation in the assessed variables. Thereafter, the samples were centrifuged at 14000 g for 20 min. at 4 °C. The supernatant fraction was aliquotted into storage vials and kept at -80 °C until required for analysis.

2.2. Biochemical Analyses

After thawing, the saliva samples were centrifuged at 250 g for 5 min. before chemical analysis. Protein concentration was determined according to the procedure described in Bradford (1976). The SOD activity was assessed using SOD assay kit-WST (Fluka Analytical, St. Louis, MO, USA), which utilizes Dojindo’s highly water-soluble tetrazolium salt that produces water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O₂ is linearly related to the xanthine oxidase activity and the reaction is inhibited by SOD, which can be determined colorimetrically (Durak et al., 1996). The IC₅₀ (50% inhibition activity of SOD) was quantified by measuring the decrease in the color development at 440 nm using ELISA-Reader (Awareness Technology Inc., Palm City, FL, USA). Cytochrome C assay was used to detect the extracellular release of the superoxide radical anion, extracellular superoxide radicals reduce ferricytochrome to cytochrome²⁻ that can be measured at 550 nm, 100μM cytochrome C (Sigma-Aldrich, St. Louis MO, USA) in PBS/EDTA and 100 μl of saliva from each sample were added to each well of a 96-well plate. The plates were incubated at 37 °C for 1 h and the absorption was measured at 550 nm using ELISA-Reader (Awareness Technology Inc.)

Salivary amylase activity was measured by a quantitative colorimetric enzyme kinetic method using BioAssay Systems’ EnzyChromTM alpha-amylase assay method (BioAssay System, Hayward CA, USA) as described previously in Guilbault and Rietz (1976) and as modified later in Mashige et al. (1982). Alpha-amylase in the saliva hydrolyzes starch and the product is rapidly converted to glucose by alpha-glucosidase and hydrogen peroxide by glucose oxidase. The hydrogen peroxide concentration was determined with a colorimetric reagent. Saliva was diluted with double-distilled water. Then the diluted saliva and standard were transferred into transparent 96-well microplates in duplicates. The standard for the assay was prepared ranging from 5 to 326 U/L Amylase (Roche Diagnostics, Mannheim, Germany). The optical density was measured at 585 nm using ELISA plate-Reader (Awareness Technology Inc.). One unit of enzyme catalyzes the production of 1 mole of glucose per
min. under the assay conditions. Uric acid concentration was determined using uric acid assay kit (Biosystem S.A. Barcelona, Spain). Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide that in turn is oxidized by peroxidase enzyme to form a quinoneimine dye. Quinoneimine was quantified by measuring the absorption spectrophotometry at 505 nm which is proportional to the concentration of uric acid in the sample. The absorption was measured using spectrophotometer (Spectro UV-Vis Auto UV-2602, LaboMed, Inc. Culver City, CA, USA). The salivary albumin concentration was determined using albumin assay kit (Biosystem S.A). Albumin reacts with bromocresol green at slightly acidic pH. The color was quantified by measuring the absorption spectrophotometry at 630 nm. The intensity of the color formed is proportional to the albumin concentration in the sample.

2.3. Statistical analysis

The correlation between the participants’ age, number of calls performed per day and the total number of calling min. per day with the various salivary oxidative stress biomarkers was evaluated using SPSS 17 (Statistical Package for social sciences) software. Initially, the results of the five oxidative stress biomarkers were examined for normality of distribution. The Kolmogorov-Smirnov test revealed that only the amylase activity data demonstrated a normal distribution. In consequence to violation of this parametric assumption, the Spearman rho correlation coefficient as a non-parametric statistical test was calculated and used to evaluate the effect of mobile phone usage on the levels of the various salivary antioxidants of the participants. The correlation was considered significant if \( p \leq 0.05 \).

3. Results

Table 1 provides the baseline characteristics of the subjects volunteered in the mobile phone usage study whose data were used in the analysis. The Spearman rho correlation coefficient between number of calls per day and calling min. per day is 0.477, which is significant at the 0.01 level (2-tailed). However, as shown in Table 2, the levels of the salivary antioxidant biomarkers are related and correlated to the total usage of mobile phone and that is the total min. of calling time. In other words, it is not about the number of calls per day. A significant correlation was obtained between the number of calls per day and the SOD as well as the amylase levels. The correlation was much less than that between the total calling time and the level of these two enzymes: SOD and amylase. The true correlation is with the number of calling min. rather than the number of calls. It is just that the total calling min. per day (which is the true indicator) is related to the number of calls per day (\( R = 0.477 \)).

While increasing the calling time causes a progressive and a significant enhancement of SOD activity, it induces reductions in the activity of amylase (Figure 1). The increases in the cytochrome C activity as well as the elevations in the concentration of albumin and uric acid were not significant.

Table 1. Baseline Characteristics of Study Participants (N= 109). All Participants Were Males.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.74 ± 8.08</td>
</tr>
<tr>
<td>Number of calls per day</td>
<td>15.55 ± 8.19</td>
</tr>
<tr>
<td>Calling min. per day</td>
<td>67.47 ± 47.81</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>61.60 ± 24.39*</td>
</tr>
<tr>
<td>Albumin (µg/ml)</td>
<td>155.55 ± 86.04*</td>
</tr>
<tr>
<td>Amylase (U/ml)</td>
<td>84.07 ± 25.29*</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.75 ± 2.47*</td>
</tr>
<tr>
<td>Cytochrome C (Abs.)</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

Table 2. The Spearman's Rho Correlation Coefficient between the Investigated Factors and the Various Salivary Antioxidant Biomarkers.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of calls per day</th>
<th>Calling min. per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>Almin (µg/ml)</td>
<td>Amylase (U/ml)</td>
</tr>
<tr>
<td>0.01</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>0.36</td>
<td>0.08</td>
<td>-0.37</td>
</tr>
<tr>
<td>0.74</td>
<td>0.08</td>
<td>-0.81</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (2-tailed);
*Correlation is significant at the 0.05 level (2-tailed).
Figure 1. Effect of total calling usage of mobile phone on the salivary levels of various antioxidants investigated. Each data point represents mean of three separate readings. The solid line represents a linear regression fit of the data as a function of the cumulative calling minutes per day.

4. Discussion

Saliva is an important biological fluid that plays an important role in maintaining oral homeostasis and constitutes a first line of defense against free radical-mediated oxidative stress. Furthermore, many salivary proteins offer a great potential in clinical and epidemiological research, in oral as well as in general health studies (Fleissig et al., 2009; Goldwein and Aframian, 2010; Sathishkumar et al., 2010). The findings of the present study demonstrate that the exposure of human male subjects to RF-EMR emitted by mobile phone increases SOD activity in their saliva. The linear association between mobile phone-related increases in SOD activity may suggest that mobile phone induced free radical formation in human saliva. The mechanisms by which RF-EMR from mobile phones could affect the activity of salivary SOD are unclear. Some studies suggested that the exposure of certain cell types to RF-EMR could change gene and/or protein expression (Li et al., 2005; Gerner et al., 2010). In contrast, other studies did not show significant changes in gene expression following an exposure of cultured cells to RF-EMR (Gurisik et al., 2006; Remondini et al., 2006; Zeng et al., 2006). Further, the overproduction of Reactive Oxygen Species (ROS) in living cells has been implicated as the first step in tissue injury and the response of the cells under RF-EMR exposure (Burlaka et al., 2013). The main ROS that have to be considered are superoxide anion which is predominantly generated by the mitochondria, hydrogen peroxide produced from O₂ by the action of SOD and peroxynitrite, generated by the reaction of O₂ with nitric oxide. ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase in vivo (De Iuliis et al., 2009; Khalil et al., 2012, 2014; Ozgur et al., 2010) and in vitro (Zmyślony et al., 2004; Luukkonen et al., 2009). ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (Oktem et al., 2005). Disturbance of redox balance, uncontrolled activation of free radical processes, overproduction of ROS and/or suppression of antioxidant defense in cell are often the important signals of some hazardous changes in cell metabolism (Burlaka et al., 2013).

In the present study, no significant increases were observed, neither in the concentrations of albumin and uric acid nor in the activity of cytochrome C. In contrast, exposure of rats to EMR caused significant reductions in...
serum albumin levels in rats exposed for 3 and 6 months to RF-EMR from mobile phones at 900MHz (El-Bedawi et al., 2011). This discrepancy is likely to reflect differences in the species, the type of biological fluid and/or the exposure conditions, such as dose, time pattern, and frequencies of exposure used by different laboratories. In this regard, it has been indicated that the effect of EMR on living organisms depends on the frequency, intensity and duration of the exposure to phone radiation (Andersen et al., 2000; Sivani and Sudarsanam, 2012). Since amylase is the abundant protein in saliva, the decrease in amylase activity is intriguing in this study, a lower total parotid saliva protein concentration in dominant, compared with the non-dominant, mobile phone side has been found (Kelsh et al., 2011).

Furthermore, a higher saliva secretion rate from the parotid gland in the dominant mobile phone side usage has been reported (Goldwein and Aframian, 2010); it was suggested that a thermal effect or modified cutaneous blood flow may contribute to this result. Integrating exposure over time is further complicated by the fact that sources vary markedly over very brief time periods relative to the time periods of interest (Kelsh et al., 2011). Also, the type of location (urban, suburban, rural) where the phone is predominantly used also appears to influence power levels across the different technologies; the power level may be as low as 1 mW depending on the location of the mobile phone with respect to the base station. Therefore, such location data would capture additional exposure information that could improve the precision of exposure assessment for epidemiological research. Ideally, geographic differences in RF power output levels, the dose, time pattern, and frequencies (wavelengths) of exposure from all key sources should be estimated for each individual in the study (Awadalla, 2013).

In conclusion, the easy access and noninvasive collection make saliva a suitable fluid type to investigate surrogate biomarkers to detect the exposure to genotoxic agents or in intervention studies. Exposure to electromagnetic radiation from a mobile telephone can cause an increase in the activity of SOD and a decrease in the activity of amylase enzyme of the exposed people. This result, along with a previous one (Gerner et al., 2010), may suggest that human cells recognize mobile phone radiation as an external stressor and react to them through the activation of proteins and/or the synthesis of new protein molecules.

We have included a relatively large sample size (N = 109) to improve our ability to detect small effects that may have been missed in prior studies with smaller sample sizes (Abu Khadra et al., 2014). However, one restriction of this study is the lack of a properly matched control. Even from rural residential areas, it was not possible to get those so-called mobile phone non-users, or RF-EMR-“unexposed.” Continuation of the research on mobile phone radiation effects is needed to assess if these effects could have potential long-term harmful consequences and to improve the basis and the reliability of the safety standards. Based on this study, therefore, it is recommended that a long-term and/or excessive use of mobile phones should be avoided. This can be accomplished by a telehealth technology education program targeting the more sensitive ages: children and adolescents, since their developing brains absorb more EMR from a mobile phone (Gandhi et al., 1996). Such activities include: group discussions, public presentations and mass communication through the available electronic and print media sources.

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References


