Evaluation of selected biochemical parameters in the saliva of young males using mobile phones

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Abstract

The biochemical status in the saliva of 12 males before/after using mobile phone has been evaluated. Radio frequency signals of 1800 MHz (continuous wave transmission, 217 Hz modulate and Global System for Mobile Communications [GSM – non-DTX]) with 1.09 w/kg specific absorption rate (SAR) value were used for 15 and 30 min. Cell phone radiation induced a significant increase of superoxide dismutase (SOD); there was a statistically significant effect of talking time on the levels of SOD, F(2, 33) = 8.084, p < 0.05, ≥ 0.53. The trend analysis suggests a significant quadratic trend, F(1, 33) = 4.891, p < 0.05; indicating that after 15 min of talking the levels of SOD increased, but as talking time increased the SOD activity started to drop. In contrast to this, there was no statistically significant effect of talking time on the level of salivary albumin, cytochrome c, catalase or uric acid. Results suggest that exposure to electromagnetic radiation may exert an oxidative stress on human cells as evidenced by the increase in the concentration of the superoxide radical anion released in the saliva of cell phone users.

Introduction

Recently, the possible biological side effects which result from exposure to electromagnetic fields (EMF) or radio-frequency (RF) fields by the phone’s user and neighbors of stations have received considerable attention (Awadalla, 2013; Blackman, 2009; Kesari and Behart, 2010; Moulder et al., 2005). Because exposure to RF from phones is localized, if a risk exists it is likely to be greatest in regions with greatest energy absorption in close proximity to the head (Cardis et al., 2008). Controversial data regarding brain tumor development after electromagnetic radiation (EMR) exposure have been indicated in several studies; some, but not all, studies showed increased risk (Christensen et al., 2005; Jauchem, 2008; Kan et al., 2008; Khurana et al., 2009; Swerdlow et al., 2011). Another organ of interest is the parotid glands below the ear, near the place used by cell phones during calls. Several studies have found no association with parotid gland tumors overall for any measure of exposure investigated (Duan et al., 2011; Lonn et al., 2006; Shu et al., 2012). Contrary to this, reports from other studies have suggested that the incidence of the disease may be increasing with the distinct increase in the popularity of using the mobile phone (Bello et al., 2012; Carranza et al., 2011; Czerninski et al., 2011; de Vocht, 2011; Lahkola et al., 2007; Sadetzki et al., 2008). Increased malondialdehyde (MDA) concentration and decreased GSH concentration in blood of personnel exposed to microwaves have been observed (Garaj-Vrhovac et al., 2011). This indicates that such exposure may increase reactive oxidative species (ROS) production and that oxidative stress can be one of the possible mechanisms of DNA and cell damage indicating that some biological effects are likely to occur even at low-level EMR.

Saliva is a biological fluid that offers several opportunities in diagnosis, toxicology and in forensic science (Baum, 1993; de Almeida Pdel et al., 2008; Sathishkumar et al., 2010). It is rich in antioxidant biomarkers such as super oxide dismutase (SOD) cytochrome c, albumin, uric acid, 8-hydroxy-2′-deoxyguanosine (8-oxodG), 4-hydroxylalkenals, MDA, glutathione peroxidase GTH-Px, catalase (CAT), ascorbic acid and glutathione (de Almeida Pdel et al., 2008; Ergüder and Durak, 2006; Goldwein and Aframian, 2009; Moore et al., 1994; Ullmann et al., 2010). Little research has yet been conducted into the effects of RF fields of mobile phones on the constituents of human saliva.

More recently, Hamzany et al. (2013) reported significant increase in all salivary oxidative stress indices studied in mobile phone users. In contrast, they found decreases in the salivary flow, total protein, albumin and amylase activity. Using hydroxyl radical averting capacity (HORAC), oxygen radical absorption capacity (ORAC), MDA and 8-oxodG as biomarkers, no relationship between exposure to RF fields...
of mobile phones and changes in the salivary oxidant/antioxidant status (Khalil et al., 2013).

The present investigation is a continuation of our previous study (Khalil et al., 2013) and aimed at exploring the levels of selected biochemical parameters; albumin, cytochrome c, SOD and uric acid in the saliva of young Jordanian males under continuous short-effect of mobile phone radiation.

Materials and methods

Subjects and study design

The selection and characteristics of subjects (12 normally healthy males, average age 22 years) are as reported before (Khalil et al., 2013). All participants responded that they do not use a microwave or live near a mobile phone base station. The study was approved by the ethics committee on the human experimentation at Yarmouk University.

Participants were asked not to eat, drink or brush their teeth an hour before collection of saliva. All used the same Nokia C3-00 (RM-614) device with a dimension of 13.6 × 115.5 × 58.1 mm in connection with an 1800 MHz Umniah Jordanian network (Irbid, Jordan) with 1.09 SAR value. Saliva samples were collected and prepared as described previously (Khalil et al., 2013); before the start of the call as well as 15 min and 30 min immediately after using the cell phone.

Quantification of salivary biomarkers

SOD assay kit-WST (Fluka Analytical, St Louis, MO) was used to determine enzyme activity. The reaction is based on the reduction of the highly water-soluble tetrazolium WST-1 salt (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) with superoxide anions (O$_2^·-$) to form the water-soluble formazan dye. The rate of the reduction with O$_2^·-$ is linearly related to the xanthine oxidase activity. The degree of inhibition of the reaction by SOD is determined colorimetrically. The IC$_{50}$ (50% inhibition activity of SOD) was quantified by measuring the decrease in the color development at 440 nm using an ELISA-Reader (Awareness Technology Inc. Stat Fax 3200, Palm City, FL). One unit SOD activity is expressed as the enzyme amount causing 50% inhibition in WST-1 salt reduction rate.

Cytochrome c assay was used to detect the extracellular release of O$_2^·$-. Extracellular O$_2^·$- reduces ferricytochrome to cytochrome +2 that can be measured at 550 nm. Briefly, 100 μM cytochrome c (Sigma-Aldrich, St Louis, MO) in PBS/EDTA were added to 100μl of saliva sample/well of 96-well plate. The plate was incubated at 37°C for 1 h and the absorption was measured using the ELISA-Reader.

The salivary uric acid concentration was determined using a uric acid assay kit ( Biosystem S.A., Barcelona, Spain). Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide, the latter is in turn oxidized by peroxidase to form a quinoneimine dye. The quinoneimine concentration is proportional to the concentration of uric acid in the sample. Therefore, the uric acid is indirectly determined by assaying quinoneimine spectrophotometry at 505 nm (Spectro UV-Vis Auto UV-2602, Labomed Inc., Culver City, CA).

The salivary albumin concentration was determined using albumin assay kit (Biosystem S.A., Barcelona, Spain). 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.

Statistical analysis

Data from three independent blind biochemical analyses were statistically evaluated using SPSS 17 (Statistical Package for Social Sciences) software (SPSS Inc., Chicago, IL). Statistical significance was set at $p<0.05$. Comparisons of means of various time groups were performed by analysis of variance (ANOVA) when both the homogeneity of variance and normal distribution were demonstrated using Levene’s and Shapiro–Wilk’s tests, respectively. Log transformations of the data were used if needed to equalize variance and to provide more normally distributed measures.

Planned comparisons were performed to determine significant difference between groups. Moreover, trend analysis to assess for linear versus quadratic relationships were evaluated. The effect size ($\omega$) of the overall ANOVA was calculated according to the following equation:

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

Whereas effect size for the contrasts employed was calculated according to

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

Results

The levels of the various salivary antioxidants of the participants before they started the call, 15 min and 30 min of continuous use are presented in Table 1 and Figure 1. Except for salivary SOD, there were no significant differences in salivary albumin levels, $F(2, 33)=0.06, p>0.05$; cytochrome c, $F(2, 33)=0.088, p>0.05$; or those of uric acid, $F(2, 33)=1.15, p>0.05$ among the same subjects.

Although SOD levels were very similar after 15 min and 30 min of talking time, there was a significant effect of talking time on the levels of SOD, $F(2, 33)=8.084, p<0.05$, $\omega=0.53$; where $\omega$ is a measure of effect size. The current value represents a large effect as it is above the 0.5 threshold for a large effect. Trend analysis suggests a significant quadratic trend, $F(1, 33)=4.891, p<0.05$. This indicates that the levels of SOD increased after 15 min of talking but as talking time increased, the levels started to drop slightly. Nevertheless, this quadratic trend is not a substantive finding as an effect size of $\omega=0.28$ was obtained which is below 0.3 thresholds for a medium effect. Similarly, contrasts revealed saliva levels of SOD after talking on the phone significantly increased as compared to the samples taken before...
participants talked, $r(33) = 4.014$, $p < 0.05$ (one-tailed), $r = 0.573$. On the other hand, talking for a longer time was suggested to decrease the levels of SOD as compared to the levels after talking for 15 min only. That drop in SOD levels was found to be not significant with $t(33) = 0.236$, $p > 0.05$ (one-tailed), $r = 0.041$. In other words, the saliva levels of SOD increased after talking for 15 min and then reached a plateau remaining at constant comparable levels at 15 and 30 min.

**Discussion**

One of the most potential areas of research on the biological effect of EMR in humans is the assessment of the release of free radicals as well as antioxidant activity in plasma and other biological fluids after EMR exposure. Human saliva is an eminently accessible biological fluid with enormous and potential biomarkers that represent the secreted proteins of human tissue released upon exposure to physical or chemical hazards (Kaufman and Lamster, 2002). In this study, saliva was chosen as a safe and non-invasively obtained biological fluid to evaluate the release of free radicals after exposure to EMR. The levels of SOD and uric acid, beside other antioxidant substances that may be secreted in saliva, can be considered indicative of potential harmful effects of RF radiations. Among these substances, uric acid appears to be a major antioxidant in saliva as its level was found to be highly correlated to the total antioxidant activity of whole saliva (de Almeida Pdel et al., 2008). Nevertheless, the appearance or increases in other substances or antioxidant enzymes still suggest the formation of ROS. These ROS have been shown to increase dramatically after oxidative stress and they could directly lead to significant DNA damage (De Iuliis et al., 2009; Kaufman and Lamster, 2002).

<table>
<thead>
<tr>
<th>Anti-oxidant type</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± St. div.</td>
<td>Mean ± St. div.</td>
<td>Mean ± St. div.</td>
<td>$F(2, 33)$</td>
</tr>
<tr>
<td><strong>SOD (U/ml)</strong></td>
<td>70.92 ± 12.37</td>
<td>87.41 ± 9.46</td>
<td>86.33 ± 11.68</td>
<td>8.08</td>
</tr>
<tr>
<td><strong>Min–max</strong></td>
<td>48.7–88.0</td>
<td>66.0–98.0</td>
<td>66.0–106.0</td>
<td></td>
</tr>
<tr>
<td><strong>Albumin (mg/dl)</strong></td>
<td>0.12 ± 0.11</td>
<td>0.10 ± 0.095</td>
<td>0.11 ± 0.12</td>
<td>0.060</td>
</tr>
<tr>
<td><strong>Min–max</strong></td>
<td>0.02–0.35</td>
<td>0.0012–0.30</td>
<td>0.01–0.41</td>
<td></td>
</tr>
<tr>
<td><strong>Cytochrome c (Abs.)</strong></td>
<td>0.078 ± 0.12</td>
<td>0.046 ± 0.055</td>
<td>0.03 ± 0.026</td>
<td>0.088*</td>
</tr>
<tr>
<td><strong>Min–max</strong></td>
<td>0.002–0.377</td>
<td>0.001–0.194</td>
<td>0.005–0.075</td>
<td></td>
</tr>
<tr>
<td><strong>Uric acid (mg/dl)</strong></td>
<td>3.41 ± 1.90</td>
<td>4.58 ± 2.49</td>
<td>4.80 ± 2.80</td>
<td>1.150</td>
</tr>
<tr>
<td><strong>Min–max</strong></td>
<td>0.70–6.34</td>
<td>1.90–9.47</td>
<td>1.30–9.95</td>
<td></td>
</tr>
</tbody>
</table>

*Cytochrome c results were transformed into log(1000*abs) and ANOVA were performed on the transformed data.
Therefore, the salivary oxidant/antioxidant status after oxidative stress such as the exposure to RF radiation is believed to shed light on the aforementioned harmful effects of using mobile phones. It is worth noting that previous reports have already established a similar link between the salivary oxidant/antioxidant status after oxidative stress and the status of periodontal health (Borges et al., 2007; Takane et al., 2002).

As presented in Figure 1, there was no significant effect of talking time on the levels of cytochrome c, uric acid and albumin. Contrary to the results published before (Goldwein and Aframian, 2009), which reported increased salivary secretion rate and decreased total protein concentration because of the hand-held mobile phone use, the current results indicate a significant increase in SOD activity, after talking continuously for a period of 15 to 30 min. The increase in antioxidant activity is an indication of cellular response to oxidative stress in order to protect cells from the non-thermal damage of RF exposure. Therefore, the rise of the SOD activity is believed to be associated with ROS formation upon mobile phone use. SOD is a specific antioxidant enzyme that dismutate $O_2^-$ to form $H_2O_2$, which is scavenged by other antioxidant enzymes. It protects the cell against the toxic effect of superoxide radicals. However, it appears that the increase in SOD activity after 15 min of use is adequate to buffer cells from the non-thermal damage of EMR. This assumption is based on the fact that the increase of SOD activity was not followed by another fold of increase after 30 min of continuous phoning. It is known that cells respond differently to abnormal physiological conditions through producing different proteins and substances. The stress response induced by non-thermal radiation from the use of GSM mobile phone was demonstrated previously through many biomarkers. For example, it induced an increase in the proliferation and expression of stress proteins such as HSP70 in transformed human epithelial cells, Drosophila melanogaster, and rat brain (Fritze et al., 1997; Kwée et al., 2001).

In summary, the current findings suggest that the increase in SOD activity in human saliva indicates that exposure to RF fields generated by the mobile phones increases the level of ROS in the saliva. Although it is a small study, it could be considered to be a pilot study that leads to an active research in this area. The present study raises further questions to be answered by future research by using matching control speakers into a non-active cell phone (a “placebo” cell phone) to prove that just talking for half an hour itself is not associated with increased SOD. Is the same trend seen if subjects hold a non-energized cell phone or other object of similar size to their ear for the same period?

Finally, it would be interesting to assess oxidative/antioxidative status in other body tissues affected by the mobile phone radiations at various times after completion of the call, in order to determine the kinetics of the presumed return to baselines. This study was conducted on male volunteers and it would be interesting to compare the effects on gender basis and know if it would apply to female subjects as well. Doing additional novel studies is needed to correlate the outcome with confounding socio-demographic variables (age, gender, socio economic status, habits, addictions, etc.).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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