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ORIGINAL ARTICLE

The effect of radiofrequency radiations emitted from mobile phones on plasma oxidants and antioxidants in mobile phone users

AWANTI SM, INGIN JB, JEEVANGI SR, PATIL RB, PATIL GA, AWANTI BS

ABSTRACT

Introduction: Wide spread concerns have been raised about the possibility that exposure to the radiofrequency (RF) radiations emitted from mobile phones could affect people's health. The radiofrequency radiations emitted from mobile phones may affect biological systems by increasing free radicals which mainly enhance lipid peroxidation and by changing the antioxidant activities in human blood, thus leading to oxidative stress. The present study was designed to evaluate the effect of the exposure to the radiofrequency radiations emitted from mobile phones on the plasma parameters of oxidants and antioxidants in 50 volunteers using mobile phones. The plasma obtained, was used for the estimation of lipid hydroperoxide, total thiols and the ferric reducing ability of plasma (FRAP). There was a significant increase in lipid hydroperoxide ($p<0.05$) and a significant decrease in FRAP ($p<0.05$) and total thiols ($p<0.05$). FRAP correlated negatively with lipid hydroperoxide and positively with thiols. This decrease in antioxidants like FRAP and total thiols and an increase in oxidants like lipid hydroperoxide prove the existence of oxidative stress in mobile phone users.

Key words: FRAP (Ferric reducing ability of plasma), Total thiols, Lipid hydroperoxide, Oxidative stress, Antioxidants.

*Department of Biochemistry,
M.R Medical college, Gulbarga,Karnataka, INDIA
Corresponding author:Dr Sharanabasappa.M. Awanti, Associate Professor,Department of Biochemistry, M.R Medical college, Gulbarga,Karnataka, INDIA.
Telephone: 0091-9844030529 (mobile).
Email: drawanti73@yahoo.co.in

Introduction

There has been considerable public, scientific and media interest in the possible adverse effects which are associated with radiofrequency radiations emitted from these mobile phones on human health. The mobile phone combines the technology of the wireless communication with that of the telephone and specific frequencies called as microwave radiations as emitted by mobile phones. A mobile phone is a low power single channel two way radio and emits signals via radio waves, which comprise of radiofrequency energy, a form of electromagnetic radiation. Mobile phone systems operate within the radio frequency section (30 KHz – 300GHz) of the electromagnetic spectrum. The analogue phones operate between 450MHz – 900MHz and the digital phones operate between 900 MHz- 1800MHz. The specific frequencies used by cell phones are called as microwaves. The electromagnetic fields with frequencies in the microwave range do not have sufficient energy to induce DNA damage directly. However, exposure to a microwave field can cause an increase in the

temperature, thus resulting in "thermal" effects. For this reason, the current recommendations related to the exposure to radiofrequency radiations are based on the lowest exposure, which is known to result in observable effects due to heating [1] .

Recent reports have suggested that exposure to RF radiations is capable of altering protein levels which are indicative of the induction of cellular stress responses [2],[3] . Oxidative stress is a well characterized phenomenon that is inducible by a variety of agents, including heat [4] .Thus, the evaluation of oxidative stress allows the evaluation of both the thermal and the non thermal actions of RF radiations. Further, oxidative stress is known to arise through the production of oxygen radicals. It has been hypothesized that electromagnetic fields can alter the kinetics of enzyme-mediated reactions by a mechanism which is consistent of a radical pair mechanism [3],[6],[7]. Such effects could conceivably change oxidative stress because the cellular response to oxidative stress involves in part, enzymatic cascades that process oxidative free radicals. Thus, the goal of the present study was to determine whether RF radiations emitted by mobile phones are capable of altering parameters of oxidative stress and antioxidants.

Subjects and methods

The study was conducted on 50 volunteers who were using mobile phones for a period of 2-4 years and 25 age matched volunteers who never used mobile phones formed the control group. Informed consent was taken from all the subjects and it was approved by the institutional review board. The investigation confirms to the ethical guidelines for biomedical research on human subjects.

Under aseptic conditions, blood samples (5ml) were drawn into heparinized vacutainers. The blood was centrifuged at 2000g for 15 minutes at $4^{o}C$ for the clear separation of plasma and all assays were performed immediately.

All chemicals were purchased from Sigma Chemicals Co (St.Louis, MO, USA). Total thiols in plasma were measured by a spectrophotomteric method by using 5' 5' dithio-bis (2- nitrobenzoic acid) DTNB [8] ; 900µl of 0.2 M Na_2HPO_4 containing 2Mm of Na_2EDTA, 100 µl of Serum and 20 µl of 10Mm DTNB in 0.2 M Na_2HPO_4, which were taken in an eppendorf tube and warmed to $37^{o}C$. The solution was mixed in a vortex mixer and was transferred to a cuvette. The absorbance was measured at the end of 5minutes at 412nm by using a Genesys 10 UV spectrophotometer. Appropriate samples and reagent blanks were prepared and corrected absorbance values [absorbance of T-(absorbance of standard blank + absorbance of reagent blank] were used to calculate the concentrations of the thiols by using a calibration curve. The values were expressed in µmoles/L for plasma thiols.

The lipid hydroperoxide content of plasma was determined by using the Fox version II assay for lipid hydroperoxides (Fox_2) [9] . The Fox_2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8mg) in 10 ml of 250mmoles/L of H_2SO_4 to give a final concentration of 250µmoles/L of ferrous ion in acid. This solution was then added to 90ml of HPLC grade methanol containing 79.2mg of butylated hydroxyl toluene (BHT). Finally, 7.6 mg of xylenol orange was added with stirring, to make the final working reagent (250 µmol/L of ammonium ferrous sulphate, 100 µmol/L of xylenol orange, 25mmol/L of H_2SO_4 and 4mmol/L of BHT in 90% vol/vol methanol in a final volume of 100ml). The working reagent was routinely calibrated against solutions of H_2O_2 of known concentrations.

Aliquots (90µl) of serum were transferred to two test tubes.

Triphenylphosphine (TPP) in methanol (10 µl of 10mmol/L) was added to one to remove hydroperoxides. Methanol (10 µl) was added to the other test tube. This generated the blank and the test samples respectively. The aliquots were then vortex mixed and incubated at room temp for 30 minutes before the addition of 900 µl of FOX_2 reagent, with mixing. After incubation at room temperature for a further 30 min, the vials were centrifuged at 2000g for 10 minutes. The absorbance of the supernatant was then determined at 560nm. The hydroperoxide content in the serum sample was determined as a function of the mean absorbance difference of the samples with and without the elimination of the hydroperoxides by TPP. The concentration of hydroperoxides was calculated by using the extinction coefficient $4.5\times10^{-4}M^{-1}$ CM^{-1} and the results were reported as micromoles per liter (µmol/L).

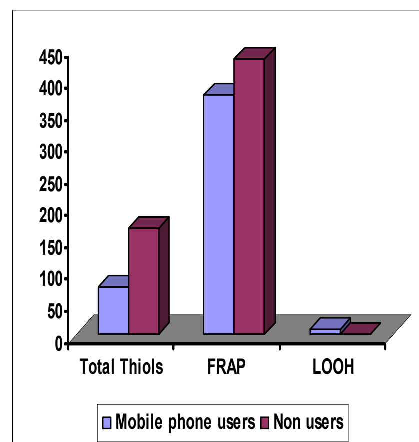
The FRAP assay was done according to Benzie and Strain [10] , with some modifications. The stock solution included 300Mm of acetate buffer (3.1g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10mM of TPTZ (2,4,6 tripyridyl-3-triazine) solution in 40mM HCL and 20Mm of $Fecl_2.6H_2O$ solution. A fresh working FRAP solution was prepared by mixing acetate buffer, TPTZ and $Fecl_2$ in a 10:1:1 ratio. 50 µl of plasma was made to react with the working FRAP solution. The solution was mixed in a vortex mixer, it was transferred to a cuvette and the absorbance was measured at the end of 6 minutes at 593nm by using a Genesys 10 UV spectrophotometer. The standard curve was linear between 25-80 µM.

Statistical analysis

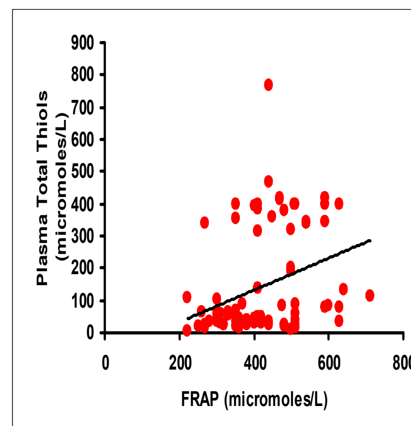
All the values were expressed as mean ± SEM. A p value less than 0.05 wasconsidered as significant. Statistical analysis was done by using SPSS (Statistical Package for Social Sciences, SPSS-10, Chicago, USA). An independent sample t test was used to compare the mean values. Pearson's correlation was used to correlate between the parameters.

Results

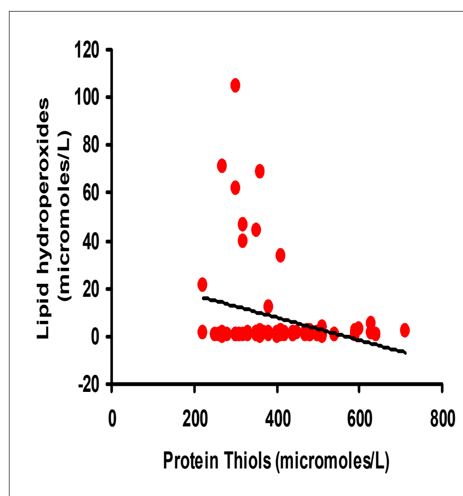
There was a significant increase in plasma lipid hydroperoxide levels ($p<0.05$) and a significant decrease in FRAP ($p<0.05$) and total thiols ($p<0.05$) in volunteers using mobile phones as compared to the non-users. Total thiols correlated negatively with lipid hydroperoxide ($r = -0.282$, $p<0.01$) and positively with FRAP ($r = 0.331$, $p<0.01$).



(Table/Fig 1) Total thiols, FRAP, Lipid hydroperoxide levels in mobile phone users and non users.



(Table/Fig 2) Correlation between total thiols and FRAP



(Table/Fig3) Correlation between Lipid hydroperoxide and Total thiols

Dicussion

Free radicals are a very reactive and unstable molecular species that can initiate chain reactions to form new free radicals. Although formed by means of a wide range of normal biochemical processes, they are potentially damaging. Several mechanisms are in place to neutralize their effects; which include a defence system of nutritional and indigenous antioxidants that generally hold the production of free radicals and prevent oxidative stress and subsequent tissue damage [11] . The balance between the oxidants and the antioxidants may be disturbed by an increase in free radical production or by a reduction in the antioxidants [12] .This imbalance between the oxidants and antioxidants can lead to oxidative stress in a series of peculiar and potentially damaging reactions [12] which are particularly susceptible to oxidative damage by free radicals like the polyunsaturated fatty acids and the acyl chain of phospholipids, which lead to lipid peroxidation. Uncontrolled lipid peroxidation is a toxic process, resulting in the deterioration of biological membranes [13] . Lipid peroxidation products eg; malondialdehye has been taken as a biomarker to oxidative stress in biological systems, which is relatively non-specific in biological samples like plasma [14] . Therefore, we have estimated lipid hydroperoxide along with antioxidants like thiols and FRAP. Radiofrequeny waves are a very important part of the electromagnetic spectrum, with respect to their applications and possible health consequences. Epidemiological studies still remain inconclusive with regard to the health effects of prolonged exposure to electromagnetic fields [15],[16],[17],[18],[19],[20]. Wireless communications, especially mobile phones, are rapidly gaining popularity, while only little is known about the effects of the radiofrequency fields of these phones on human health. The present study showed that decrease in FRAP and thiols and increase in oxidants like lipid hydroperoxide indicated increased oxidative stress in this population. The negative correlation of FRAP and total thiols with lipid hydroperoxide depicts the increased consumption of antioxidants in such an oxidative environment.

In conclusion, our study shows that decrease in antioxidants like FRAP and total thiols and increase in oxidants like lipid hydroperoxide prove the existence of the oxidative stress in mobile phone users. Whenever the balance of antioxidants is outweighed by prooxidant factors, as shown by the radiofrequency of mobile phones, oxidative stress may develop in cells. Therefore, these results support the theory of the interaction of the radiofrqency fields of mobile phones with biological systems, thus resulting in oxidative stress.

Note: This work has been presented in the International conference “Free Radical and Natural Products in health” and the 7th Annual meeting of SFRR-INDIA 2008.
I acknowledge that this particular work has not been published elsewhere.

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