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Original Article

Biochemistry Section

Effect of Cell Phone Use on Salivary Total Protein, Enzymes and Oxidative Stress Markers in Young Adults: A Pilot Study

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ABSTRACT

Introduction: The present study aimed to assess the levels of salivary enzymes, protein and oxidant-antioxidant system in young college-going cell phone users.

Materials and Methods: The cell users (students) were categorized in to two groups – less mobile users and high mobile users, based on the duration and frequency of cell use. Unstimulated whole saliva samples of the volunteers were analysed for amylase, lactate dehydrogenase (LDH), malondialdehdye (MDA) and glutathione (GSH).

Results: High mobile users had significantly higher levels of amylase (p = 0.001), LDH (p = 0.002) and MDA (p = 0.002) in saliva, when compared to less mobile users. The marginal decrease in salivary total proteins, GSH and flow rate were statistically not significant (p >0.05).

Conclusion: Significant changes in salivary enzymes and MDA suggest adverse effect of high use of cell phones on cell health.

Keywords: Amylase, Cell phones, Glutathione, Lactate dehydrogenase, Malondialdehdye, Oxidative stress, Saliva

INTRODUCTION

Globally, over the last two decades there has been a rapid growth in the number of people using hand held mobile phones (MPHs) [1,2]. Human beings are exposed to radiofrequency radiation emitted by the mobile phone device (which operates as a receiver and a transmitter) and this has created a need to investigate possible ill effects of mobile phone use on health of individuals [3,4].

Cell phones have been considered modern man's nemesis and reports indicate a wide ranging adverse effects of long term usage of cell phones on reproductive system, nervous system, cornea, gastrointestinal system and kidneys [2,5,6]. Although inconclusive, epidemiological data also indicate an increase in the development of brain tumours (glioma, acoustic neuroma, meningioma), parotid gland tumor, seminoma in long-term users of mobile phone [5-8].

The adverse effects of electromagnetic radiations have been proposed to be mediated by various mechanisms like increase in the generation of free radicals [9], alterations in gene expression [10], damage to DNA, loss of DNA integrity [11], and chromosomal instability [12]. Studies have reported increased lipid peroxidation, and decreased levels of antioxidants in the blood of regular cell phone users [9,13], and in the blood and tissues of experimental animals exposed to electromagnetic radiations for prolonged periods of time [14,15].

Saliva is an underused diagnostic tool, gaining lot of attention in last three decades, due to non-invasiveness of its collection, non-necessity of skilled persons and special equipments for its collection [16]. Researchers worldwide have attempted to evaluate the usefulness of salivary constituents as biomarkers of systemic diseases, malignancy, infectious diseases, drug toxicity, and hormonal imbalances [16].

There is paucity of studies on salivary biomarkers of diseases in general and salivary biomarkers of cell phone exposure in particular. A previous study has reported increased levels of salivary cortisol and amylase on exposure to electromagnetic radiations from the Global System for Mobile Communication (GSM) mobile base station [17]. Recently, a study has observed decreased salivary flow, total protein, albumin and amylase activity in mobile phone users [18]. In view of the potential diagnostic applications of saliva, and to understand the mechanisms of health effects of mobile phones, in this study we have attempted to assess the salivary levels of total proteins, lactate dehydrogenase (LDH), amylase, lipid peroxidation and glutathione (GSH) in both sparingly and high mobile phone users.

MATERIALS AND METHODS

This was a single-centre, investigator blinded purposive sampling study, and was conducted between April 2013 and September 2013 at Father Muller Medical College, Mangalore, India. Students in the age group of 18–24 years, studying various medical and paramedical courses, were the subjects. The study was two staged and was performed only after obtaining the necessary permission from the Institutional Ethics Committee. Additionally, as the study had to be performed during class hours in the forenoon period between break (9 to 11 A.M.), necessary permission was also obtained from the college administration before initiation of the study. The inclusion and exclusion criteria enlisted and represented as [Table/Fig-1].

In the first stage the students were informed by the investigators on the objective of the study and also that their participation was voluntary. Informed consent was obtained from all the study subjects. Two hundred and fifty seven volunteering students were requested to fill a preformed questionnaire that included aspects on the number of years on use of phones, the monthly frequency of phone usage and how long it was since the last time and for how long. Additionally, aspects on their health condition and habits (smoking, alcohol) were also requested. The completely filled questionnaires were analysed and only those students who met the inclusion criteria were selected. In the second stage, seventy one student volunteers (males = 40; females = 31) who met the inclusion criteria were categorized into two groups based on the duration and frequency of use as: 1) Less mobile phone users (using MPHs for less than two years and weekly use less than two hours) and 2) Heavy/more mobile users (using MPHs for four or more years and weekly mobile use two or more hours). The classification was done on analysing the filled questioners and confirming the fact by observing the volunteers mobile talk time usage inventory of the previous month.

Inclusion criteria	Exclusion criteria
Volunteers who were – • Using hand held mobile phones. • Young adults in the age group of 18 to 24 years. • Volunteers who used hand held mobile phones for less than 2 years in the less mobile users. • Volunteers who used hand held mobile phones for less than 4 years in the High mobile users.	Volunteers who were – • Smokers, tobacco chewers and alcoholics. • Had acute illness like fever, malaria, jaundice in the past one month • Were on medications (like antibiotics, anti malarial drugs, analgesics etc) for the past one month, • Regularly consumed anti-inflammatory medications, antioxidant supplements and multivitamins for the past one month. • Regular users of alcohol based dental products for gargling.

[Table/Fig-1]: Details on the criteria used for the selection of volunteers for the study

The next day the volunteers were requested for their saliva between 9-11 AM, and only after affirming that there was a minimum of one hour gap between the sample collection and last phone conversations and also that it was for less than two minutes. The volunteers were requested to salivate in to a pre weighed collecting tube, as per the method of Navazesh [19]. This was to ensure that the variability in salivary flow rate and composition, be minimized. The subject was asked to rinse the mouth with distilled water thoroughly to remove any food debris and then after 10 min, directed to expectorate into a sterile plastic container and by not exerting any form of force. Salivary flow rate (ml/min) was measured by the following formula [19]:

$$\frac{\text{= Weight of the container with saliva (g)} \\ \text{– Weight of the container without saliva (g)}}{\text{Duration of saliva collection}}$$

The duration of saliva collection was 10 minutes. The collected saliva samples were centrifuged at 3000 rpm for 10 minutes, and the supernatants were used for assay of biochemical parameters. The samples were analysed by investigators (ARS and SN) who were not aware of the cohorts. All the biochemical assays were done in UV-visible spectrophotometer of Shimadzu.

Estimation of Total Proteins

Salivary total protein levels were assayed by the method of Lowry et al., [20].

Estimation of Amylase

Amylase activity in saliva was assayed by the kinetic spectrophotometric method as previously described by Balcom et al., [21]. The reagent kit was obtained from Crest Diagnostics. The assay was based on hydrolysis of a 2 –chloro–4 nitro phenol salt to chloro nitrophenol (CNP).

Estimation of LDH

The assay for LDH in the saliva was performed by the kinetic spectrophotometric method described by Demetriou et al., [22]. The reagent kit obtained from Tulip diagnostics was used. The assay was based on LDH-catalyzed reduction of pyruvate with NADH to form NAD+. The rate of oxidation of NADH to NAD+ was measured as a decrease in absorbance at 340 nm.

Internal quality control programme using quality control specimen from Biorad was done to ensure precision and accuracy of values of LDH and amylase.

Estimation of GSH

The GSH levels were estimated by the method described by the Method of Beutler et al., [23]. GSH reduces 5, 5/ dithio, bis-nitrobenzoic acid (DTNB) to yellow coloured 5-thionitrobenzoic acid (TNB). Absorbance measured at 412nm is directly proportional to the concentration of GSH. GSH standards ranging in concentration from 25 to 100 mg/dl were run simultaneously, and the GSH level was calculated from the standard curve.

Estimation of MDA

MDA, the sensitive and convenient marker of lipid peroxidation, was assayed as thiobarbituric acid-reactive substances (TBARS), by the method of Ohkawa et al., [24]. MDA reacts with thiobarbituric acid at 100° C in acidic medium to form pink coloured complex. The colour intensity of MDA-TBA complex is measured at 535 nm. MDA standard (1, 1, 3, 3-Tetramethoxypropane) procured from Sigma-Aldrich was used for standardizing the assay and the standard curve was plotted using various concentrations.

STATISTICAL ANALYSIS

The values were expressed as mean with standard deviation. Significance of the difference of the values between the groups was evaluated by using Student's t-test and Karl-Pearson's Correlation analysis.

RESULTS

Results of this study are presented in [Table/Fig-2]. There was no statistically significant difference in the values between males and female MPH users and hence the results were pooled and presented in [Table/Fig-2]. The duration of mobile use in years was 1.54±0.48 and 4.88±2.13; while that of the time used per week were 2.96±0.40 and 8.04±2.79 respectively in the less and more mobile phone users and was statistically significant ($p<0.001$).

Salivary level of MDA, and activities of amylase and LDH were significantly higher in high mobile phone users when compared to less mobile phone users. There was no significant difference in the salivary flow rate in both groups of mobile users. The marginally lower values of salivary GSH and total proteins in high mobile phone users were statistically not significant, in comparison to less mobile phone users [Table/Fig-2].

	Less mobile users (n = 34)	High mobile users (n = 37)
Age, Years	19.55 ± 2.1	20.8 ± 1.34
Number of years of Cell Phone Use	1.54 ± 0.48	4.88 ± 2.13$^{\#}$
Cell Phone Use, Hrs/week	2.96 ± 0.40	8.04 ± 2.79$^{\#}$
Salivary flow rate (ml/min)	0.37 ± 0.04	0.34 ± 0.08^{NS}
Biochemical Parameters		
Total protein (mg/dl)	54.19 ± 12.51	50.83 ± 10.91^{NS}
LDH (IU/L)**	341.80 ± 11.52	360.89 ± 12.50$^{@}$
Amylase (IU/L)**	123.63 ± 8.22	147.24 ± 8.13*
GSH (µ moles/dl)	9.72 ± 1.05	9.39 ± 0.51^{NS}
MDA (n moles/dl)	28.44±4.44	33.59±4.37$^{\$}$

[Table/Fig-2]: Salivary oxidative stress and enzymes in mobile phone users
**One international unit (IU/L) of enzyme is the activity converting one micromole of the substrate to product in one minute
p-value = # < 0.001; $^{@}$0.002; * 0.001 and $^{\$}$0.02

DISCUSSION

The present study revealed significant changes in salivary levels of malondialdehyde, LDH and amylase in high mobile users in comparison to less mobile users.

Salivary amylase activity was significantly higher in high mobile users, indicating adverse effect of mobile phone use on cell health. Previous studies have reported increased salivary amylase in oral cancer [25]. Additionally, recent reports indicate it to be of use as a sensitive biomarker for stress-related changes in the body reflecting the activity of the sympathetic nervous system [26], and

in chronic stress [27]. Earlier studies have shown that non-ionic electromagnetic radiation emitted from base stations increased activity of the salivary amylase and support our observations [28]. However our reports contradict the observations of Hamzany and co investigators [18], with respect to the change in salivary amylase which may be due the difference in the age of the volunteers and use of deaf individuals as controls in the study.

The Enzyme LDH is a ubiquitous enzyme and has been studied as a general marker of cellular health. The source of salivary LDH could be any of the tissues in the body and reports suggest that the levels of salivary LDH are increased in oral cancer [29,30], and periodontitis [31]. Our study has observed that the levels of LDH were greater in the frequent MPH users than in the less mobile users. These observations support the earlier reports of Yuan et al., [32] who have also seen increased levels of LDH in the serum of volunteers occupationally exposed to very high frequency radiations. Together both these observations indicate that exposure to the radiofrequency radiation increases cell death and alter the homeostasis of the tissue.

Oxidative stress implicated in the etiopathogenesis of adverse effects of mobile phone use. Recent studies have shown that the levels of beta amyloid protein, protein carbonyl, and malondialdehyde levels were found to be higher in the brain of rats exposed to 900 MHz radiofrequency radiation indicating its deleterious effects [33]. In this study we observed increased levels of malondialdehyde in the saliva of volunteers using MPH and less in the sparingly MPH users. These observations are in agreement to recent publications from other investigators. Similar studies by Hamzany and co workers have observed that when compared with the hearing impaired volunteers, the levels of salivary MDA were increased in the mobile users [18], thereby substantiating our observations.

GSH, a sulfhydryl (-SH) intracellular molecule concentrated mainly in the cell cytosol and other aqueous phases of the living system is a potent antioxidant and a convenient cofactor for many enzymatic reactions. Our study observed marginal but statistically non significant decrease in salivary GSH in high mobile users. Previous studies with experimental animals have shown that the short term exposure to radiofrequency electromagnetic radiations resulted in decreased levels of GSH in plasma, testis and epididymis [34,35]. Findings of our study indicate that the oxidative stress generated by high mobile phone use is combated by antioxidant system, and is not high enough to elicit significant decrease in GSH. We have not assessed the effect of mobile use on enzymatic and non-GSH non enzymatic mechanisms in this study.

There was marginal but non-significant decrease in salivary flow rate and protein level in high mobile users compared to less mobile users. Decrease in salivary flow rate has been proposed to be partly responsible for the increase in salivary biochemical constituents in diseases [19]. The adverse effect of mobile use in the present study subjects, is not severe enough to cause any significant change in salivary flow rate and salivary proteins.

CONCLUSION

The findings of present study suggest adverse effect of mobile phone use on cellular health as indicated by increased salivary activities of LDH, amylase, and increased oxidative stress evident by higher salivary MDA level in high mobile users compared to less mobile users. Salivary biochemical parameters have served as sensitive indicators of health in mobile users. However, this was a preliminary project and has the limitations of small sample size and only with saliva. Additionally, we also could not ascertain the wave strength of the mobiles used and the effect of only hand held mobile phone use were considered. Future studies are planned to evaluate the effect of frequent mobile phone use on the auditory, biochemical, immunological and mutagenic parameters with a larger sample size. When this study was conducted only 2G and 3G mobile phones were prevalent and studies are also planned to investigate the affect of both 2G and 3G systems and the effect of wave strength on the individual users. Future studies are planned to carry out a large study with bigger sample size and with people of various age groups to arrive at a more confirmatory conclusion.

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