

Effect of Duration of Mobile Phone Use on the Salivary Flow and Total Antioxidant Capacity of Saliva and Salivary Immunoglobulin A Level: A Cross-sectional Study

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ABSTRACT **Introduction:** The objective of this study was to observe the effects of the duration of handheld mobile phone usage on the salivary flow, salivary immunoglobulin A (IgA) level, and salivary markers for oxidative stress. **Materials and Methods:** Eighty-one students were categorized into three groups based on their duration of mobile phone usage after age- and gender-matching. Students were grouped as: mobile phone usage <20 min/day (Group A), mobile phone usage 20–60 min/day (Group B), and mobile phone usage >60 min/day (Group C). Saliva was collected to evaluate salivary flow rate, salivary IgA level, and salivary markers for oxidative stress. **Results:** The salivary flow rate showed no statistically significant difference between the three groups ($P = 0.180$). There was no statistically significant difference in the salivary IgA between the three groups ($P = 0.237$). There was a statistically significant difference in the malondialdehyde (MDA) level between the three groups ($P = 0.042$). On pair comparison between the groups, group B and group C had a statistically significant difference ($P = 0.019$) in the MDA level. There was no statistically significant difference in the salivary thiol level between the three groups ($P = 0.237$). **Conclusion:** The duration of handheld mobile phone usage did not show any significant effects on the salivary flow rate, salivary IgA, and thiol levels. There was an increase in the salivary MDA concentration in subjects using handheld mobile phones for a longer duration, indicating higher oxidative stress in salivary glands exposed to mobile phone radiofrequency electromagnetic waves for a longer duration.

KEYWORDS: Mobile phones, oxidative stress, saliva

INTRODUCTION

Mobile phones are electronic telecommunication gadgets, which connect to a wireless network via electromagnetic waves. The first handheld mobile phone was demonstrated by John Mitchell and Cooper in 1973. From 1973 to 2016, the world over mobile phone subscriptions has grown to over seven billion, which is 96% of the world population. Mobile phones emit radiofrequency electromagnetic waves (RF-EMW) ranging from 800 to 2200 MHz, which is in the range of micro- and radiowaves of the EM spectrum. Although

non-ionizing in nature, there is a general apprehension regarding the detrimental ill-effects of its usage on general good being. ‘The International Agency for Research on Cancer (IARC), a World Health Organization (WHO)-specialized agency, identified that “radiofrequency

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electromagnetic fields” which are created by handheld cellular phones, belong to “Group 2B,” which suggests possible carcinogenic potential in the human body.^[1]

Various epidemiological studies have analyzed the harmful effects caused by the RF-EMW released by mobile phones on general health. The numerous adverse effects range from self-reported symptoms such as headache, dizziness, difficulties in concentration, depression, disturbance in sleep, effect on a reproductive system, which is due to increased reactive oxygen species being cytotoxic to sperm cells. There is also a possible role in cancer promotion and progression.^[2]

The RF-EMW emitted by mobile phones leads to a rise in adjacent skin temperature and thereby causes higher perfusion in the exposed salivary glands. This leads to an alteration in the flow rate and protein composition of saliva. Saliva as a diagnostic tool has been underused, gaining popularity in the last two decades due to non-invasiveness, and not a requirement of specialized skilled personnel for the sample collection. The alteration in physiologic properties of saliva leads to various harmful effects in the oral cavity such as the decay of teeth, mucosal inflammation, and difficulty in swallowing. Recent studies have mainly concentrated on the effect of mobile phone usage on the sympathetic and parasympathetic nervous system response, as measured using salivary flow rate and protein levels.^[3-5] The usage of handheld mobile phones for a long duration has been shown to cause higher oxidative stress in the oral cavity, thus increasing the risk of inflammatory diseases and oral cancer.^[6-11]

This study aimed to observe the effects of the duration of handheld mobile phone usage on the salivary flow, salivary immunoglobulin A (IgA) level, and salivary markers for oxidative stress.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Department of Oral Medicine and Radiology, Manipal College of Dental Sciences, Manipal, in association with the Department of Biochemistry, Kasturba Medical College, Manipal over 1 year from March 2015 to April 2016. Approval to conduct the study was obtained from the Institutional Ethical Committee (IEC 62/2014). Subjects were categorized into 3 groups of 27 participants in each group.

Group A: students with mobile phone usage <20 min/day;

Group B: students with mobile phone usage 20–60 min/day;

Group C: students with mobile phone usage >60 min/day.

SELECTION CRITERIA

Inclusion criteria

Students belong to the age range of 18–30 years.

Students using handheld mobile phone for at least 3 consecutive years in the study were included.

Exclusion criteria

1. Chronic systemic diseases;
2. Previous trauma and head and neck injuries;
3. Pregnancy;
4. History of chemotherapy or radiotherapy;
5. History of drug consumption and use of dietary supplements;
6. History of chronic smoking and alcohol consumption;
7. Subjective and objective signs of dry mouth;
8. Presence of >3 mm periodontal pockets;
9. Hands-free mobile phone usage;
10. Students not willing to participate.

Informed consent was acquired from all the students. Each participant was provided with a subject information sheet, and the details of the study were explained. In the first stage, the students were asked to record information about the number of years of handheld mobile phone use and the daily frequency of usage in a specially designed proforma. The demographic details and oral examination findings of each student were recorded. They were also requested to maintain a regular record of handheld mobile phone usage. The specific absorption rate (SAR) value, as well as the type of mobile phone “GSM (Global System for Mobile Communication) or CDMA (Code Division Multiple Access)” of each student, was recorded in the proforma. The SAR value of each specific mobile handset was ascertained using Google internet. Thus, 27 students were included in each of the three groups, with 81 being the total number of participating students. In the second stage, 81 students (females = 41; males = 40) who met the inclusion criteria were categorized into three groups after age- and gender-matching and based on their duration of mobile phone usage for 1 month.

SALIVA COLLECTION

The participants were requested not to drink or eat 1 h before saliva collection. All samples of saliva were collected in the morning hours assuring a minimum of 60 min difference between the saliva collection and the last usage of the phone during a conversation. The unstimulated saliva samples were collected in calibrated containers by the spitting method. The students were

asked to rinse the mouth with water thoroughly to remove any food debris, pool their saliva for 2 min, and then spit into a collecting tube.

ESTIMATION OF SALIVARY FLOW RATE

Salivary flow rate (mL/min) was measured by the following formula.

The volume of collected saliva is measured in milliliters, and the duration of saliva collection in minutes.

The duration of the saliva collection was 2 min. After the estimation of the salivary flow rate, the collected saliva samples were immediately transferred to the laboratory. Each saliva sample was centrifuged for 15 min (2500 rpm); the samples of saliva were then subjected to the necessary biochemical tests.

ESTIMATION OF SALIVARY IMMUNOGLOBULIN A LEVEL

An enzyme-linked immunosorbent assay (ELISA) method was used to determine the IgA levels in saliva. The ELISA kit specific for “quantitative estimation of IgA in human saliva” (SLV-4636 with 96 wells) was obtained from DRG International, USA. Salivary IgA ELISA is based on the simultaneous binding of IgA to two antibodies: one monoclonal antibody immobilized on microwell plates and the other polyclonal antibody conjugated with horseradish peroxidase (HRP).^[12] The normal levels of salivary IgA ELISA (SLV-4636) are = 40–170 µg/mL.

ESTIMATION OF SALIVARY MALONDIALDEHYDE LEVEL

Malondialdehyde (MDA) is the end-product of lipid peroxidation by reactive oxygen species; it is therefore used as a biomarker for oxidative stress. Estimation of MDA was done by Kei Satoh’s method.^[13] The pink-colored complex that occurred was measured by using a spectrophotometer at 532 nm. Normal range for MDA is <0.7 nmol/mL.

ESTIMATION OF SALIVARY THIOL LEVEL

The level of protein thiol indicates antioxidant status. Thiol levels in the saliva sample were estimated by a spectrophotometric method using dinitrobenzene (DTNB) Ellman’s method.^[14] In a spectrophotometric analysis, DTNB has a powerful absorbance at 412 nm.

The usual range of plasma-free protein thiol is 0.20–0.40 nM/mL.

STATISTICAL ANALYSIS

The sample size of 27 in each group was determined. The collected data were tabulated in a Microsoft Office 2013 Excel sheet. The analysis was done using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA). The analysis of variance test was applied for the mean age estimation in the three groups. A χ^2 test was done for the evaluation of the gender distribution. The Kruskal–Wallis test was utilized to compare values of the dependent variables: salivary flow rate, salivary IgA, MDA, and thiol level, in the three study groups. The *post hoc* Conover test was used to make pair comparisons between the three groups. A *P*-value less than 0.05 was considered as being statistically significant.

RESULTS

Eighty-one students (females = 41; males = 40) who met the inclusion criteria were categorized into three groups. Thus, 27 students were included in each of the three groups based on their duration of mobile phone usage.

There is no statistically significant difference found in the age and gender distribution between the three groups [*P* = 0.756 and (*P* = 0.952)], respectively. There was no statistically significant difference in the three groups with regard to the mean SAR value of mobile phones (*P* = 0.309) [Table 1].

The salivary flow rate showed no statistically significant difference between the three groups (*P* = 0.180). There was no statistically significant difference in the salivary IgA between the three groups (*P* = 0.237). The maximum concentration of salivary MDA level was in Group C, and the minimum concentration of salivary MDA level was in Group A. There was a statistically significant difference in the MDA level between the three groups (*P* = 0.042). On the *post hoc* comparison between the groups, Groups B and C had a statistically significant difference (*P* = 0.019). The minimum concentration of salivary thiol was in Group C. There was no statistically

Table 1: Student demographics and mean specific absorption rate (SAR) in three groups

Parameter	Group A	Group B	Group C	<i>P</i> -value
Age				
Mean age of years (range)	23.33 ± 2.47	23.37 ± 2.71	23.37 ± 2.04	0.756
Gender				
Males	13	13	14	0.952
Females	14	14	13	
Mean specific absorption rate (SAR)	0.82 ± 0.38	0.90 ± 0.47	0.70 ± 0.46	0.309

Values are given as mean ± SD

significant difference in the salivary thiol level between the three groups ($P = 0.237$) [Table 2].

DISCUSSION

The mean age of students was 23.33, 23.37, and 23.78 years in Group A, Group B, and Group C, respectively. There were 40 males and 41 females in the study, with no statistically significant difference in the gender distribution between the three groups ($P = 0.952$). It is well established in the literature that saliva exhibits qualitative and quantitative variations in many systemic conditions.^[4] Hence, in the present study, for achieving objective analysis of saliva (in terms of salivary flow rate, IgA levels, and markers for oxidative stress), various conditions that may alter these parameters were excluded.

The rate of absorption of energy by the body under the effect of the RF-EM field is called SAR. The unit of SAR is watts per kilogram (W/kg). Since September 2013, mobile handsets with a revised SAR value of a maximum of 1.6 W/kg are allowed in India by the Department of Telecom (DoT), Government of India.^[15] In the present study, the SAR values of all the mobiles being used by the students were noted. There was no statistically significant difference between the three groups ($P = 0.309$) [Table 1]. A survey comparing the SAR values of Samsung and Nokia smart mobile phones showed that in a fixed period, health risks with Nokia smartphones are higher.^[16]

The salivary flow rate was higher in Group B in comparison to Group A and that of Group C was marginally higher than that of Group A, but no statistically significant difference was present between the three groups ($P = 0.180$). The increase in salivary flow rate with the increase in the duration of mobile phone usage in Group B in comparison to Group A was in agreement with that observed by various studies.^[3,4,9-11] On the contrary, several other studies have noted a decrease in salivary flow rate with increased mobile phone usage.^[5-7] The increase in salivary flow rate was attributed to the effects of mobile phone usage

on the parasympathetic pathway, which controls the fluid component of saliva,^[17] and to the heating effect of mobile phone-emitted RF-EMW causing increased blood flow in the salivary gland, leading to the increased salivary flow rate in mobile phone users with increased duration.

The most important protein in the saliva is IgA. They act against bacterial antigens by preventing their mucosal adherence and subsequent metabolic reactions.^[18] In the present study, the salivary IgA concentration was higher in Group C in comparison to Groups A and B, with the median values being 37.64 $\mu\text{g/mL}$ in Group C, 37.34 $\mu\text{g/mL}$ in Group B, and 27.66 $\mu\text{g/mL}$ in Group A, but ($P = 0.237$) the intergroup comparison showed no statistical significance. Due to the heat generated by the emitted RF waves by the usage of phones, it leads to salivary gland damage and thereby increases levels of IgA.^[19] However, this result is not in concurrence with the study of Arbabi-Kalati *et al.*^[6] They found a decrease in the salivary IgA levels with higher duration of mobile phone usage. Hashemipour *et al.*^[10] have found no association between mobile phone usage and the levels of salivary IgA.

The high concentration of free radicals generated leads to oxidative stress which results in the molecular death of structures. The free radicals damage the structure of lipid molecule and thereby cause damage to protein structure and function. MDA is the resultant end-product of this metabolic reaction and is considered a biomarker for oxidative stress. There was a statistically significant difference observed in the MDA levels among the three groups, with a median and interquartile range of Group A being 0.10 nmol/mL (0.08–0.14), of Group B being 0.09 nmol/mL (0.07–0.12), and that of Group C being 0.13 nmol/mL (0.08–0.19) ($P = 0.042$). Also, there was a statistically significant difference between Group C and Group B ($P = 0.019$) and even a significant difference between Group A and Group B ($P = 0.057$). This finding is in agreement with all the previous studies that evaluated the effect of mobile phone usage on salivary MDA levels.^[7,9,11] Their main role is to prevent the intraprotein structure from

Table 2: Comparison of salivary flow rate, salivary immunoglobulin (IgA) level, thiol, and malondialdehyde (MDA) levels among the groups ($n = 81$)

Group	Salivary flow rate (mL/min)		IgA ($\mu\text{g/mL}$)		Thiol (nmol/mL)		MDA (nmol/mL)		P-value
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
Group A	0.33	0.16–0.66	27.66	16.80–46.13	0.24	0.10–0.35	0.10	0.08–0.14	0.180
Group B	0.40	0.33–0.66	37.34	20.90–86.29	0.13	0.09–0.24	0.09	0.07–0.12	0.251
Group C	0.34	0.20–0.50	37.64	28.19–57.24	0.13	0.08–0.30	0.13	0.08–0.19	0.246

Values are given as median (SD)

free radical species formed during oxidative stress. Chapple^[20] has observed that thiol has a protective role in gingival crevicular fluid free radical scavengers. The salivary thiol level in Group A was 0.24 nmol/mL (0.10–0.35), in Group B 0.13 nmol/mL (0.09–0.24), and in Group C was 0.13 nmol/mL (0.08–0.30). Comparison of thiol concentration among the three groups showed no statistically significant difference ($P = 0.237$); however as the duration of mobile phone usage increased, there was a reduction in the thiol level, as shown by the higher concentration of thiols in Group A in comparison to the other two groups. This aspect has not been evaluated in previous studies. The study was done by Arbabi-Kalati *et al.*,^[6] who found a reduction in antioxidant capacity of saliva with increased duration of mobile phone usage. This was due to the varied effect of mobile usage on the autonomic nervous system. While there is a decrease in sympathetic activity, there is an associated increase in parasympathetic activity of saliva also. Since the protein component of saliva is controlled through the sympathetic pathway, a decrease in sympathetic activity leads to the reduced antioxidant capacity of saliva.

CONCLUSION

The duration of handheld mobile phone usage did not reveal any significant changes in the salivary flow rate, salivary IgA, and thiol levels. There was an increase in the salivary MDA concentration in subjects using handheld mobile phones for a longer duration, indicating higher oxidative stress in salivary glands exposed to mobile phone RF-EMW for a longer period.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

DB, YC, KMP, RV designed the study. DB, YC performed data collection and investigation. SK performed the analysis of the samples. AK, DB performed the statistical analysis. DB, YC, MK wrote and reviewed the manuscript to be published. DB

designed the study, performed the data collection, data analysis and interpretation. YC, MK, SC wrote the manuscript. DB, YC, KMP, MK, RV, SC, SK, AK reviewed the manuscript to be published.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

All procedures have been performed as per the Declaration of Helsinki (revised in 2008). Approval to conduct the study was obtained from the Institutional Ethical Committee (IEC 62/2014) before the start of the study.

PATIENT DECLARATION OF CONSENT

All procedures have been performed as per the Declaration of Helsinki (revised in 2008).

DATA AVAILABILITY STATEMENT

The additional data of this study are available on request from Dr. Yogesh Chhapparwal at yogesh.chhapparwal@manipal.edu.

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