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Impact of Mobile Usage on Salivary Flow Rate and Protein Concentration A Hospital Based Original Research

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ABSTRACT:
Background: Electromagnetic radiations are used in today's common mobile phones to transmit signals. The salivary glands, in particular, may experience pathological alterations as a result of these radiations. The objective is to compare the salivary amylase levels and total antioxidant capacity of the parotid saliva on the dominant and non-dominant sides of mobile phone usage. Mobile phones are a ubiquitous and necessary device of today that generate electromagnetic radiation. Their technology has flawlessly merged with public expectations. However, frequent use of these devices exposes users to radiation, which might have harmful effects later on and cause irreversible alterations.
Keywords:*Smart mobile phone, Immunoglobulin A, Saliva, Salivary amylase, Total antioxidant capacity*

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INTRODUCTION

*'The effects of technology has unanticipated results. But they are always inevitable'*Technological progress is an growing phenomenon as with each passing day advancements in technology are providing us with new opportunities and solutions, which were inconceivable few years ago. Following the trend of rapidity, development of technology has led hard copy publications into e-books, radio players and televisions have become combination of broadband internet and Cable Television streaming services and the telecommunications has converged into mobile phones. We are living in an environment where knowingly or unknowingly, we are surrounded by electromagnetic radiation (EMR) produced by electrical appliances, power towers and lines, inbuilt wiring in buildings and a swing of other technologies. Going back to history just a few decades back the life when mobile phones didinot exist and only source of long distance communications was letters, telegrams, in person travelling, landline phones etc Then as the technology grew mobile phones were introduced in markets in 1980s, and with time they gained much popularity and their use was wide spread in the mid- 1990s. When we see today the popularity of this device is increasing exponentially every day and is not limited to adults but to individuals belonging to almost all the age groups. Since 2020 we survived the pandemic by staying at a big social distance just because the mobile and mobile networks connected us and created a complete virtual world for us. The number of mobile users worldwide is above 6,800,000,000 which are further increasing at a very fast rate with the new upgrades and inventions that the mobile companies bring to market. India stands second with over 900 million users in the world. The fact is that mobile phones are used at an enormous number by all the age-groups in today's scenario. It has been noted that the average person spends more then 90-120 min a day on their phone [1].

Although the Mobile phones provide a quick connectivity to people by calling. Several gaming options, choices in music, social media and hundreds of such other features on a single tap makes this seemingly harmless gadget, an inevitable accessory in today's world [2]. However, the rise in popularity of mobile phones is accompanied by a growing concern about the potential negative health implications connected with their excessive usage. The global health catastrophe in the shape of the COVID-19 pandemic has caused people to migrate their normal activities into mobile devices. Microwave frequency electromagnetic radiation is used by mobile phones (radiofrequency [RF] waves and microwaves). Depending on the area of the world, the radiation frequency and modulation norms range from 300 to 2100 MHz. 2 Code division multiple access (CDMA) and the Global System for Mobile Communication

(GSM) are two types of mobile phone technologies that are widely utilised today (CDMA). 3 Despite all of the mobile phone characteristics, the longer usage periods and close closeness to the oro-facial region have led to an increase in potential hazard pertaining to this region.
People have been forced to change their normal routines due to the COVID-19 outbreak and concerns about potential negative effects from absorbing these emissions. Mobile phone use is linked to a variety of health issues, including cognitive impairments and more ephemeral side effects like headaches, weariness, and tumors induction. Two methods, thermal and non-thermal, have the potential to have effects on biological systems at the tissue and cellular levels, whether short-term or long-term consequences.
The radiation frequency and modulation drastically in range of 300-2100 MHz, depending on different geographical region [2].
The area of the face where salivary glands are located is extremely close to where handheld mobile phones are held when in use. Due to these, salivary glands are particularly prone to any alterations related to the heat and radiation from mobile phones. Additionally, past studies evaluated and discovered a potential link between mobile device use and parotid gland tumours [4]. Human saliva is essential for maintaining oral homeostasis because it acts as the first line of defence against microbial invasion and shields the oral mucosa from mechanical and immunological harm. Saliva serves as a readily available, non-invasive diagnostic tool in the form of a biomarker for a variety of diseases and medical disorders, as more and more members of the dentistry and medical communities are discovering. Given the enormous number of mobile phone users, even minor negative health consequences could have significant public health repercussions. Thus, this idea prompted us to investigate the impact of mobile phones—handheld or hands-free—on the salivary glands by comparing users to those who hardly ever use them, and by measuring changes in the flow rate and protein concentration of entire saliva among mobile users. In order to determine the benefits of hands-free vs handheld mobile phones, it is important to compare any changes between hands-free and non-mobile users.

MATERIAL AND METHODS

The study was conducted in SGT Dental College & Research Institute , to estimate the salivary flow rate and protein concentration among mobile users after the approval by institutional ethical committee . The participants for the study were enrolled from amongst the patients attending the outpatient department of Oral Medicine and Radiology. The study protocol was explained to the participants following which an informed and written consent was obtained. A case history regarding medical history, lifestyle and habits practiced was recorded along with questionnaire concerning mobile phone use practiced. The Study comprised total 150 participants in which controls were
Controls = 50 individuals who are non mobile users (C)
Group 1 = 50 individuals who use handheld mobile phones (HH)
Group 2 = 50 individuals who use hands free mobile phones (HF)
For the study individuals aged between 19 to 50 years with a history of using mobile phones for minimum of 2 years were included and subject with habits of alcohol or smoking , patients who underwent or undergoing radiotherapy, pregnant females, patients with underlying systemic diseases with or without treatment and patients with past head or neck injury and trauma were excluded .

Fig 1: Armamentarium used for A) clinical examination. B) Saliva collection and storage C) Saliva collection

Fig 2: Armamentarium used for protein estimation A) Centrifuge machine B) Micropipette, normal saline and Biuret reagent C) Test tubes with rack D) Colorimeter

Saliva and flow rate assessment

Saliva was collected from the shortlisted participants who did not have any intake of food, chewing gums and beverages except water at least 2 hours before the saliva collection on the same day while other participants were asked to come on following day. Saliva (unstimulated) was collected to examine the glands in their resting state (the state in which the glands are for most part of the day) using sterile disposable containers by spitting method between 9 am – 12 noon. Participants were instructed to sit in upright position in dental chair and asked to relax for 5 minutes before salivary flow measurement (**Fig 4(C).** Saliva was collected for 3 minutes, and samples were stored at 4°C in a thermo cool transport box with ice packs after its collection. Saliva was stored at temperature of 4°C to prevent the degradation of unstable analytes present in it. As soon as the saliva was collected in a sterile disposable container, sample was assigned with a number and 20 samples in a slot were taken in a thermo cool box to Biochemistry laboratory of Faculty of Medical Sciences. Saliva was measured with the help of the disposable syringe and flow per minute was estimated in ml/min.

Saliva and protein Concentration assessment

Total protein concentration is assessed using Biuret test. Protein assessment was done following centrifugation of saliva using centrifugation machine (REMI catalogue No. R 8C) at 14000 RPM for 20 minutes so that the supernatant can be separated. Centrifugation produces supernatant as the centrifugal force in the machine leads to sedimentation of denser particles i.e., proteins**.** Thereafter from the centrifuged sample, 0.5ml of supernatant was taken in cuvette and mixed with 3 ml of biuret reagent and 2.5 ml of normal saline.Biuret Reagent was made in the Department of Biochemistry itself, composed of 45g of potassium sodium tartrate, 15g of copper sulfate and 5g of potassium iodide in 0.2 N sodium hydroxide making it a total solution of 1 liter**.** The violet-colored mixture, which was then formed, was kept for 15 minutes at room temperature for colorimetric analysis. Colorimeter (Rolex) is a device, which measures the intensity of colored product, was set at zero and the intensity of the violet-colored product was measured and laid between the range of 520- 540 nm which were directly proportional to protein concentration.

Statistical analysis

The readings of all the parameters were entered in Microsoft excel for Mac 2011 spread sheet in form of master chart. All the collected data were then subjected to statistical analysis (i.e., mean, standard deviation, P value) so that significance of the study can be known, for this SPSS (statistical package for social science) for the windows version 16.0 software was used as it is a comprehensive system for analyzing data by taking from almost any type of file to generate tabulated reports, charts, descriptive statistics, and statistical analysis. Among all the statistical tests one-way ANOVA followed by Post Hoc test were applied. The $p<0.05$ value was considered statistically significant for the study.

RESULTS

The study had equal gender predilection in every group with age ranging from 19 to 50 years, which was further divided into three groups as Group 1 (Handheld (HH) mobile users = 50), Group 2 (Hands free

(HF) mobile users = 50) and Group C (controls= 50) as shown in (Table 1, Graph 1).

Table 1 : Characteristics of study participants .

GROUP	NO. OF PARTICIPANTS	GENDER	MEAN AGE (IN YEARS)
CONTROL (C)	50	25 females 25 males	32.54
GROUP 1 (HH)	50	25 females 25 males	31.98
GROUP 2 (HF)	50	25 females 25 males	31.76

Table 1: Number of Males and Females with mean age of 32.4, 31.98, 31.76 in Control (C) , Group 1 (HH) and Group 2 (HF) respectively in total of 150 participants.

Following routine dental evaluation as per their needs of the study participants, the said participants were further required for saliva collection to evaluate the unstimulated whole salivary flow rate and total protein concentration utilizing Biuret method.

The levels of both the parameters were entered in Microsoft Excel for Mac 2011 spread sheet in form of master chart. The statistical analysis was performed with the Statistical Package for social sciences (SPSS) for Windows version 16.0 software. The test applied were One-Way ANOVA followed by Post Hoc test.

TABLE 2: Estimated mean of unstimulated whole salivary flow rate

		N	Mean	Std. Deviation	Minimum	Maximum
UNSTIMULATED WHOLE SALIVARY FLOW RATE (ml/min)	CONTROL (C)	50	0.44	0.13	0.2	0.6
	GROUP 1 (HH)	50	0.56	0.18	0.2	0.9
	GROUP 2 (HF)	50	0.47	0.13	0.3	0.7

Table 2: Mean values, standard deviation, and range of unstimulated salivary flow rate in Control (C), Group1 (HH) and Group 2 (HF)

The level of unstimulated whole salivary flow rate in Control group ranged from 0.2-0.6 ml/min having standard deviation of 0.13 with the mean value of 0.44 ml/min, in Group 1 ranged from 0.2-0.9 ml/min with standard deviation of 0.18 and the mean value measured to be 0.56 ml/min and in Group 2 ranged from 0.3-0.7 ml/min, with standard deviation of 0.13 having mean value of 0.47 ml/min. (Table 2, Graph 2). P values are assessed by intergroup comparison as expressed in Table 3.

Table 3: Comparison of unstimulated salivary flow rate between Control, Group 1, Group 2

Intergroup	Mean Difference	P- value
C- G1	-.11400*	<0.001
C-G2	-0.024	0.688
G1-G2	.9000*	0.007
* The mean difference is significant at the 0.05 level		

Control – C, Group 1 – HH (handheld) , Group 2 – HF (handsfree)

As per the observation from Table 3 that the p-value is <0.001, which is significant in case of C-G1 comparison. However, in case of C-G2 and G1-G2 the p values are 0.688 and 0.007 respectively which are not significant.

Table 4: Estimated levels of total protein concentration

		N	Mean	Std. Deviation	Minimum	Maximum
TOTAL PROTEIN CONCENTRATION (g/dl)	CONTROL (C)	50	0.29	0.06	0.19	0.44
	GROUP 1 (HH)	50	0.18	0.06	0.11	0.62
	GROUP 2 (HF)	50	0.29	0.08	0.13	0.62

Table 4 Mean values, standard deviation, and range of total protein concentration in Control (C), Group1 (HH) and Group 2 (HF)

The level of total protein concentration in Control group ranged from 0.19 – 0.44g/dl having standard

deviation of 0.06 with the mean value of 0.29 g/dl, in Group 1 ranged from 0.11 – 0.35 g/dl in having standard deviation of 0.06 and mean was found to be 0.18 g/dl and in Group 2 the range of total protein concentration is 0.13 – 0.62 g/dl having standard deviation of 0.08 with mean values measured to be 0.29 g/dl (Table 4, Graph 4). P- values are assessed by intergroup comparison as shown in Table 5.

Table 5: Comparison of total protein concentration between Control, Group 1, Group 2

Intergroup	Mean Difference	P- value
C- G1	-10680*	<0.001
C-G2	-0.0036	0.962
G1-G2	-11040*	<0.001
* **The mean difference is significant at the 0.05 level**		

Control – C , Group 1 – HH (handheld) , Group 2 – HF (handsfree)

As per the observation from Table 5 that the p-value is <0.001, which is significant in case of C-G1 and G1-G2 comparison was done, however in case of C-G2 the p value is 0.962 which is not significant.

DISCUSSION

The exposure of mobile technology has emerged into our world very rapidly and has caused vivid changes in our day to day living. There are about 1 billion mobile phones in use worldwide, particularly adolescent and children the cream of our society in there prime working years are more fascinated to make use of mobile phones, though now a days even elderly use it for getting rid of their loneliness and for daily needs, however exponential usage of mobile phone is matter of health debate among clinicians and technocrats in this ear.[1]

The first focus of epidemiologic research was on link between moblie use and an increased incidence of brain pathologies. Numerous researches conducted over the past few years have revealed that the radiation emitted by mobile phones may have an impact on brain function and behaviour, DNA and gene expression, tumor-causing cell growth and proliferation, and changes in hormones, proteins, and enzymes. Mobile phones have been classified as Group 2B agents that may cause human cancer by the World Health Organization (WHO) and the International Association for Research on Cancer as of June 1, 2011. 5 WHO has concluded that mobile phone use has the potential to lead to brain and auditory canal cancers, while Hardell et al. and Lonn et al. have found that use of mobile phones for more than 10 years is associated with a consistent pattern.of increased risk for acoustic neuroma and glioma. Exposure to radiation for more than 2 hours in a day is proven to have deleterious effect.[1]

Salivary glands, particularly the parotid gland, which is situated over the jawbone in front of the ear, are another interesting organ. When using a cell phone, this gland is more likely to be exposed to radiofrequency radiation. But increasing the exposure is the fact that, unlike the brain and auditory canal, the superior lobe of the parotid glands lacks the natural protection of the skull or any bony encompassment, are much closer to mobile phones when they are in use, and may therefore be more susceptible to negative effects. Therefore, salivary gland modifications in terms of flow rate and composition may be linked to radiation exposure. [6]

Total or whole saliva is a complex mixture of substances that includes fluids from the salivary glands, the gingival fold, oral mucosal transudate, nasal and pharyngeal mucous, non-adherent oral bacteria, food remnants, desquamated epithelial and blood cells, as well as traces of medications or chemical products.

The average daily salivary flow of a healthy person is between 1 and 1.5 litres. The normal unstimulated salivary flow ranges from 0.25 to 0.35 mL/min 38 and the total protein in whole saliva ranges from 0.3 to 0.6 g/dl having specific gravity of 1.007.[7] Salivary secretion is mediated by autonomic nervous system where parasympathetic pathway is responsible for waterish (serous) saliva whereas sympathetic pathway is responsible for generating mucous saliva.

There is a close connection between oral and systemic health because saliva is essential for preserving and maintaining the overall health and healthy oral tissues. Saliva has also been used as a source of non-invasive investigation to diagnose various systemic illnesses, monitor general health, and as an indicator of disease risk. We investigated whether hands-free devices are preferable to handheld mobile phones with regard to the effects from EMR caused by contact over the gland surface. As with every technological advancement, there are some elements of health concern. Previous studies noted the impact of handheld mobile phones on salivary gland physiology.This thought led us to study if hands free devices are better over handheld mobile phones pertaining to the effects from EMR caused due to contact over the gland surface. The sample population analysed in the literature to date (Goldwein et al 2009 and Bhargava et al 2012) has an age range of 19 to 33 years. In our study, participants from a larger age and gender range (19–50 years old, with an equal number of males and females in each group) were taken into account (Table1). Finding individuals for the Controls group in our study was difficult since they had to use their

mobile phones very little or not at all over the 2 year mitigation period. As we are all know, the depth of utility of mobile phones has multiplied over time regardless of socioeconomic level, age, or gender, defining the control group and requiring individuals participating in the study to be in this group.As we are aware, the depth of utility of mobile phones has increased to many folds over the period irrespective of socioeconomic status, age or gender, thus identifying the control group and obliging those for the study to be undertaken was the toughest task.

DURATION

One of the major criteria of our concern was the period of mobile usage pertaining to duration in years and daily usage in minutes per day as studies have revealed that longer the duration of mobile in contact to the skin surface during the conversation, as the mobile emits EMR and parotid is situated superficially near the skin surface without any natural protection, chances of alteration of quality and quantity of saliva being produced by the same is more. As far as apparent literature available, few studies have been conducted in the past pertaining to mobile usage duration in years for e.g., Bhargava S et al [12] recorded minimum of 3 years to maximum of 12.5 years recorded by Hamzany Y et al, [13] in even in our study it was recorded to be 4 years which is in accordance with the review. Another aspect which was taken up by Bhargava et al, including duration as well as hours of utility per day which was recorded to be reported 3 hours/day or 180 minutes congruently the mean duration of calls in our study recorded to be 1 hour 45 minutes / day or 105 minutes.

SALIVARY FLOW RATE

As we are aware under physiological norms the unstimulated saliva ranged between 0.25-0.35 ml/min, however as expected this could be altered following utility of mobile phones as prevalent in review of literature. In our study too as enlisted in Table 2 which clearly depicts the salivary flow rate in various group i.e. In Group 1(HH) i.e. handheld mobile users is shown to be 0.56ml/min, in Group 2 (HF) i.e. hands free mobile users is 0.47 ml/min and in Control group i.e.(C) is shown to be 0.44 ml/min. Following this observation, when salivary flow rate of each group was statistically analyzed to know the variations if any that can help towards an inference. This was done using SPSS software utilizing ANOVA followed by Post Hoc test for inter group comparison and mean difference at 0.05 or less was considered significant. The results suggested that when Control and Group 1 (C-G1) was compared p-value came out to be <0.001 which is highly significant suggesting that there is a correlation between the handheld mobile phones and salivary secreting rate. Interestingly when Control and Group 2 (C-G2) comparison was made p-value was 0.688 and Group 1 and Group 2 i.e. (G1-G2) was compared p-value was 0.007 which is not statistically significant as shown in Table 3 suggesting that there is not much of a alteration in the physiology and function of salivary glands in terms of salivary secreting rate when hands free mobile phones are used. Our finding of this increased salivary flow rate in handheld mobile users are in accordance with the findings of Goldwein et al in 2009 where they evaluated the physiologic changes in the salivary gland, in terms of rate of secretion, where a significantly higher salivary secretion rate was demonstrated but the only difference being they collected stimulated parotid saliva. Bhargava et al.2012 [12] focused on functional and volume related changes in the parotid glands due to mobile usage. The heavy mobile user group demonstrated a significant high salivary flow rate and increased volume of the parotid glands in handheld users [8]. A similar study conducted in 2013 by Hamzany et al [13] studied various salivary components between users of handheld mobile phones and non-users by taking unstimulated whole saliva and obtained significant, profound increase in salivary flow rate in mobile users as opposed to those who do not use mobile phones [9].

PROTIEN CONCENTRATION

Saliva with its constituents mainly protein play a major role in maintaining oral health status of an individual [10-11]. As we are aware, the normal protein concentration in saliva is 0.3 – 0.6 g/dl and any alteration in the concentration could bring about changes in the oral cavity as they play a vital role in maintaining oral health of an individual. Considering this fact when we evaluated the protein concentration of the divided groups as mentioned in Table 4 i.e. In Group 1(HH) mean value is 0.18g/dl and in Group 2 (HF) and Controls (C) mobile users are 0.29g/dl. It is clearly evident that there is alteration as far as values are concerned however when this observation was statistically analyzed with inter group comparison the following values came i.e. comparison between Control and Group 1 (C-G1) for which p- value being <0.001 suggests highly significant variation between Control and Group 1 which demonstrate that handheld mobile phones definitely effects the salivary protein levels. However, when Control and Group 2 (C-G2) comparison was done p-value was 0.962, which is not significant. Similarly when Group 1 and Group 2 i.e. (G1-G2) was statistically compared p-value was < 0.001 which is again

highly significant as shown in Table 5 suggesting that hands free mobile phones have not cause enough alteration in the quality (protein component) of saliva when compared with handheld mobile (Group 1) users, as we can clearly see from findings that mean value of protein concentration is almost same with that of observed in Control Group. In the dominant side using handheld mobile phones, the total protein concentration obtained was lower in the study. A similar study conducted in 2013 by Hamzany et al [14] who studied various salivary parameters between users and non-users of mobile phones and obtained significant decrease in salivary protein levels in mobile users.

Thus, from the above observations, it is clearly evident that radiofrequency or EMR affects superficial or underlying structures of biological system by thermal effect which causes secretory parenchymal tissue expansion which could be the reason for increased salivary flow rate in Group 1 (HH). As in Group 1 (HH) mobile phones are kept in proximity to face. However, In Group 2 (HF) the salivary flow rate was almost like that of controls. Additionally, According to Hamzany et al 2013, this increase in salivary flow rate can contribute to diluting effect mostly on salivary macromolecules which leads to decrease in protein concentration [9].

The autonomic parasympathetic and sympathetic nervous systems, collectively known as the parasympathomimetic pathway, are responsible for controlling salivary secretion. However, in people who use hand-held mobile devices, this secretory pathway is altered, causing the parasympathetic tone to be stimulated, which results in more watery saliva and the sympathetic one to be down-regulated, which generates more acidic saliva. The varied effects of electromagnetic radiation from mobile phone use on parasympathetic and sympathetic pathways on fluid vs. protein content in our investigation may be explained by these differences. Furthermore, it has been hypothesised by Andrzejak *et al*. [14] that the electromagnetic field produced by portable mobile phones has an impact on the autonomic nervous system by altering its function.

One of the fact worth notifying, in Hands free participants the salivary flow was altered but not to the extent of handheld users, however the gross difference in protein concentration was present relative to alteration when Control and Group 2(HF) were subjected to comparison. Therefore, based on results we can say that Salivary flow is affected by EMR or RF which induces pathophysiological changes in the salivary glands as it is held in proximity to the glands reflecting continuous insult to the glands. Moreover, we have included relatively larger sample size (150) to improve our ability to detect small effects that may have been missed in prior studies with smaller sample sizes [13]. Also, no previous studies have been documented pertaining to hands free in comparison with study population grouped under HH and HF. Here on it could be recommended from the currently reported results of our study that utility of mobile phones is inevitable, but a precautionary approach should be taken to the use of mobile phone technology as these effects could have potential long-term harmful consequences [13]. Our results can contribute by influencing the pattern of future mobile phone usage by using the earphones or other hands-free devices more as handheld mobile phones if used for longer duration can induce functional changes in the salivary glands. According to Jeevitha et al It is important to take into account the place where the phone will be used, the power levels of the various technologies, base station-related parameters, geographic variances in radiofrequency power output levels, dose, and exposure patterns [14].

CONCLUSION

Mobile phones are an essential part of a contemporary lifestyle and a boon for better communication. The portability and ease of use have caused an exponential rise in the number of mobile users over a short period of time. This dependency on mobile phones has increased specially during this COVID -19 era. It has become more of a necessity to survive from e- learning to job related work than just being a mere gadget for communication. It becomes a prime concern regarding the harmful effects of electromagnetic radiation emitted by these devices.

Through this study, we summarize and conclude that mobile phones being the central part of our day-to-day lives cannot be avoided but the use of mobiles with hands free accessories can prevent the deleterious effects of electromagnetic radiation on salivary glands to certain extent and many other vital organs. In our study, since the size of study sample was discrete with two parameters, further studies can be conducted with larger sample size and inclusion of a greater number of parameters to see the effect of electromagnetic radiation. As already stated by the WHO, mobile phones can be the future tobacco and categorized under group 2B agents having carcinogenic potential, therefore to prevent the abuse of this technology the usage of the same should be done with consideration. Therefore, it is recommended that solutions be prepared to lessen the psychological and physiological effects of excessive usage of technology during pandemic lockdown as further waves of pandemics are projected.[2] Further to prevent the unseen harmful health hazards which it may cause on us and generations to come, it is recommended

to use hands free accessories along with mobile phones, instead directly holding the phones nearer to the ear so that the radiation and thermal effect on tissues can be avoided as far as possible.

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