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THE INFLUENCE OF USING MOBILE PHONE ON PAROTID GLAND SALIVARY FLOW RATE AND LIPID PEROXIDATION LEVELS

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ABSTRACT

Increasing use of mobile phones creates growing concerns regarding harmful effects of non-ionizing electromagnetic radiation on human tissues located close to the ear, where phones are commonly held for long periods of time. The study was conducted on 200 healthy male and female individuals aged 18-30 years with a history of handheld mobile phone use ≥ 3 years. Group I (50 male, 50 female participants) was the heavy-user group, who used handheld mobile phone ≥ 2 hours daily on average. Group II, the control group, (50 male, 50 female participants) participants used mobile phone < 2 hours daily. Unstimulated parotid saliva flow rate from parotid glands on both sides was measured by Modified Schirmer test. Lipid peroxidation levels were biochemically analysed using calorimetric methods. Group I showed more significant rate of parotid salivation on the dominant side compared with the non-dominant side in contrast to Group II, whereas no significance was observed in antioxidant levels. These observations lead to the hypothesis that the use of mobile phones may modify salivary gland function.

KEYWORDS: Mobile Phones, Parotid gland, Modified Schirmer test, lipid peroxidation levels.

INTRODUCTION

Concerns about possible biohazards of the electromagnetic radiation (EMR) resulting from the cell phone use have started since the emergence of mobile phone technology and are still growing. A typical cell phone operates at a power output of 0.25 W, which results in a specific energy absorption rate (SAR) of about 1.5 w kg-1 0.1°C and an associated very low rise in brain temperature (maximum, 0.1°C) (Haramaki et al.1994).

But the long duration and proximity of mobile phones to human body during use has given rise to concerns of possible adverse effects resulting from absorption of these emissions by the tissues adjacent to the area of mobile phone handset use. The parotid glands are the largest salivary glands, situated in front of the ear, near the place used by cell phones during calls. This makes parotid glands vulnerable to changes, if any, resulting from mobile phone heat and radiation (Bhargava et al.2012).

Previous studies have suggested the possible health effects involved in the use of cell phones. Some reported no evidence for association between the use of wireless phones and an increased risk for parotid gland tumors (Johansen et al.2001); (Auvinen et al.2002); (Lonn et al.2006); (Söderqvist et al.2012). Contradicting literature exists regarding the potential of mobile phone emissions (thermal and radiation effects) to cause notable physiologic, structural, functional, or even carcinogenic changes in the human body (Ahmad et al.2013).

Radiofrequency waves are a very important part of electromagnetic spectrum with respect to their applications and possible health consequences. This possible association validates the use of altered oxidative stress indices because of cell phones use as an indicator of increase incidence of certain tumors. Oxidative stress alteration because of mobile phone RF such as Lipid peroxidation (LPO), DNA damage, free radicals formation and other oxidative stress biomarkers were assessed in different animals tissue to prove that association (Irmak et al.2002); (Phillips et al.2009); (Khalil et al.2010); (Karaca et al.2012).

Controversial results were reported by researchers using different oxidative stress biomarkers after exposing animals to RF of cell phone (Elhag et al.2006); (Dasdag et al.2009); (Kerman et al.2012).

This contradiction can be attributed to inaccurate reporting by the participants about their mobile phone use habits or by participation of the next of kin of

subjects who are unaware of actual use habits and participation of individuals after diagnosis of a disorder, because these subjects can have negative responses to questionnaires, implying an entirely different etiology then the actual cause.

In the present study, changes in the salivary flow rate of the parotid glands were evaluated thereby aiming to assess whether any adverse effects are associated with heavy use of mobile phones. In addition, the oxidative stress resulting from exposure to cell phone radiation has been explored using Salivary Lipid Peroxidation levels.

MATERIALS AND METHODS

The study was approved by the Institutional Research Ethics Committee and written informed consent was obtained from all participants. A total of 200 subjects aged 18-30 years were randomly selected from the outpatient department of institution with a history of handheld mobile phone use for ≥ 3 years.

Inclusion criteria

Individuals with caries-free dentition and clinically healthy periodontium and good oral hygiene with no clinical signs of gingival inflammation, bleeding on probing, gingival sulcus depth no more than 2mm, without any oral complaints or disorders, so as to avoid any oral condition which may possibly affect the salivation.

Exclusion criteria

- Individuals with history of
- Systemic Disorders
- Major Head, Neck and Facial Trauma
- Presence of salivary gland disorders
- Patients on medication or addictive habits (smoking, alcohol use) that may cause Xerostomia
- Pregnant women

They were divided into 2 groups: Group I was Heavy-user group (50 male, 50 female participants) who used handheld mobile phones for > 2 hours daily on average. Group II was Control group (50 male, 50 female participants) who used mobile phones for <2 hours daily. **(Table 1)** None of the participants used a hands-free device. Users of both Global Systems for Mobile (GSM) and Code division Multiple Access (CDMA) handsets were used in this study. These 2 systems differ in the manner of utilization of available radiofrequency spectrum.

The least use was reported as once in a fortnight for 10-20 minutes by a control group participant and the highest time of 6 hours daily by a group I participant. 92% of the subjects used GSM cellular services and 8% CDMA phones.

Participants began by answering about their mobile phone use habits and were informed 1 week in advance to make a note of their usual mobile phone use habits. Salivary flow rate was measured during a period of 9:00 a.m. and 1:00 p.m. Individuals were to refrain from intake of any food or beverages except water (chewing gum, intake of coffee prohibited) 2 hours before the test session. Subjects were asked to relax for 5 minutes before flow rate measurement. For individuals showing an increase of ≥ 1.5 mL/5 min in salivary flow rate, the Modified Schirmer test was repeated on 2 consecutive days and the average score was recorded (Navazesh et al.2008);(Chen et al.2005).

Unstimulated parotid saliva flow rate from parotid glands on both sides was measured with the use of a MODIFIED SCHIRMER TEST (Bhargava et al.2012) using a test strip of 4 cm in length made of filter paper calibrated in 1mm intervals from 5 to 35 mm along its length.(Fig-1) (Tear Touch Schirmer Strips; Manufactured by Madhu Instruments, New Delhi, India).

The subjects sat upright in a dental chair and were asked to swallow once to clear secretions in the mouth. After proper isolation, the wick end of Schirmer strip was held at the opening of the Stenson duct for 5 minutes using cotton pliers, and salivary flow rate was expressed as __ mm/5 min.(Fig-2) The side of head frequently preferred for mobile phones use was considered to be the dominant side and the side on which use was remarkably less, was considered non-dominant side. The dominant side salivary flow rate was compared with the non-dominant side in both groups.

Biochemical Analyses for **lipid peroxidation levels.** Unstimulated saliva samples were collected from each subjects after mouth had been rinsed with distilled water. The collection was carried out at the same time of day (between 09:00 a.m. and 01:00 p.m.) and in restful and quiet circumstances. Saliva samples were stored at −20◦C until analysis. LPO was assayed by measurement of MDA, an end product of fatty acid peroxidation, and reacts with thiobarbituric acid (TBA) to form a colored complex that has maximum absorbance at 532 nm. In the TBA test reaction, MDA or MDA-like substances and TBA react together for production of a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 90◦C for 15 min. The sample was mixed cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm (Hammouda et al.1995). The concentration of MDA was calculated by the absorbance coeffi cient of MDA-TBA complex 1.56×105 $cm^{-1}M^{-1}$ and expressed as nmoles of MDA per milliliter saliva.

All chemicals in this study were of analytical grade and purchased from Sigma (Stockholm) or Merck Chemicals Co. (Germany). All solutions were prepared in de-ionized water.

The paired t-test was used to evaluate the variations of salivary flow rate and lipid peroxidation levels. A p-value of ≤0.05 was taken as statistically significant.

RESULTS

Salivary Flow Rate

Group I showed increased parotid salivation on the dominant side compared with the nondomi- nant side. Even Group II showed more salivary flow rate on the dominant side than on the nondominant side but it was less as compared to group 1. Results obtained in group I were statistically significant (P<0.02) in contrast to group II which showed non significant results (P = 0.580). (Table 2.).

Salivary Lipid Peroxidation levels-

There was no significant effect of talking time on the levels of Salivary Lipid Peroxidation in the two groups. p>0.05 was observed amongst the two groups.(Table 3).

Table 1- Distribution of % of individuals according to duration of calls

	Duration of calls	% of individuals
Calls / day	<1 hr	**25**
	1-2 hr	**25**
	>2 hr	**50**

Table 2-Salivary flow rate on Dominant and Non-dominant side in Group 1 and 2 P value ≤ 0.05 is considered statistically significant

Participants	**GROUP 1**		**GROUP 2**	
	Males 50 + Females 50 = 100		**Males 50+ Females 50 = 100**	
Sides	**Dominant**	**Non Dominant**	**Dominant**	**Non Dominant**
Salivary flow rate (mean±standard deviation)	4.5 ±1.2 mm/5 min	3.2 ± 1.1 mm/5 min	3 ± 0.2 mm/5 min	2.4 ± 0.2 mm/5 min
P value	P<0.02		P = 0.580	

Table 3- Salivary lipid peroxidation levels in Group 1 and 2 P value ≤ 0.05 is considered statistically significant

	Salivary lipid peroxidation value (mean±standard deviation)	**P value**
Group 1	**1.5 ± .502 nmoles of MDA per milliliter saliva.**	**0.06**
Group 2	**1.3 ± .460 nmoles of MDA per milliliter saliva.**	

DISCUSSION

The aim of the study was to assess the difference in salivary flow rate and antioxidant levels of the parotid glands on the side adjacent to frequent cell phone use than the other non-dominant, less frequently used side. The main outcome was that measured antioxidant parameter was not significantly affected suggesting that serious changes in the salivary oxidant/antioxidant profile may not be strongly correlated with exposure to RF-EME.

The results presented are dependent on accurate reports of mobile phone usage. Rationale for using unstimulated saliva rather than stimulated was to examine the parotid glands in their resting state (the state in which the glands are for most part of the day, i.e., unstimulated). Therefore this study concentrated on the resting state of parotid glands only.

Group I showed more parotid salivation on the dominant side compared with the non-dominant side (P<0.02) with significance difference. In contrast non-significant difference was seen in group II salivary flow rate on the dominant side than on the non-dominant side (P = 0.580). Results are in accordance with studies conducted by Goldwein et al.2010 and Bhargava et al.2012.

Two emissions from the mobile phones, namely, Heat generated and Radiofrequency radiation, can be possibly implicated for causing changes in human body. Mobile radiation can modify cutaneous blood flow (Monfrecola et al.2003). Symptoms reported by handheld mobile phones users include a feeling of warmth on the ear and behind it, and a feeling of burning and tingling on the face. (Sandstrom et al.2001). Monfrecola et al found an elevation in skin perfusion when the mobile phone was switched on and in the proximity of the skin.

Consequently, the repetitive use of the handheld mobile phones causes an elevation in skin temperature and induces an increase in the perfusion of the tissue to cool it down. Furthermore, Handheld mobile phones generates heat in adjacent tissues, no greater than 0.1C for the highest powered models. (van Leeuwen et al.1999). Nevertheless, continuous use results in a warm sensation on the skin adjacent to the Handheld mobile phones location during transmission (Sandstrom et al.2001, Straume et al.2005). 44% participants in our study had actually experienced the heating sensation on and around the ear after cell phone use for long durations. This finding was in agreement with findings of Sandstrom et al. (2001) and Straume et al. (2005).

Our finding of increased salivary flow rate concurred with the findings of Goldwein and Aframian (2009) and we agree with their hypothesis that "enriched capillary blood flow adjacent to the parotid glands may result in an increase of perfusion because of blood vessel propagation over an extensive time of exposure to heat, leading to an increase in the salivary rate flow." Another rationale for increased salivary flow from the dominant side because of thermal effect may be attributed to secretory parenchymal tissue expansion. It has been shown previously that heat acclimation of rats for up to 28 days changes the ratio of weight to size in the salivary glands. (David et al.2008); (Horowitz et al.1978). We assume that thermal effects of mobile phones is the principle factor for causing the ipsilateral volume increase. Further studies should be conducted to assess the parenchymal volume of parotid glands to test this assumption.

Elevated ROS concentrations lead to oxidative stress that causes molecular damage to vital structures and functions. A lot of endogenous factors like inflammatory, exercise, psychological stress and exogenous factors like food, alcohol, cigarette smoke, environmental pollution and radiation cause the susceptibility to oxidative stress (Moller et al.1996, Yaser et al.2001). Free radicals are very reactive and unstable molecular fragments that have an unpaired electron and they can produce new free radicals by means of chain reactions. This molecules although formed as a result of normal biochemical processes, sometimes they may be damaging and interact with all the macromolecules including lipids, nucleic acids and proteins. There are some mechanisms to neutralize their effects, two of them nutritional and endogenous enzymatic antioxidant defenses that generally hold the production of free radicals and prevent oxidant stress and subsequently tissue damage (Yaser et al.2001, Halliwell et al.1994). As a consequence, free radical attack on unsaturated fatty acids of lipid structures leading to lipid peroxidation and damaging effects on proteins may occur. Lipid peroxidation products e.g. malondialdehyde has been taken as a biomarker for oxidative stress in biological system (Yaser et al.2001, Winklhofer.1994). This circumstance can lead to 'oxidative stress' i.e. a series of peculiar and potentially damaging biochemical reactions (Yaser et al.2001, Pompella et al.1997). Particularly susceptible to oxidative damage by free radicals are the polyunsaturated fatty acid acyl chains of phospholipids, which lead to lipid peroxidation.

Uncontrolled lipid peroxidation is a toxic process resulting in the deterioration of biological membranes (Moller et al.1996, Yaser et al.2001, Pompella et al.1997). In a study shown that ROS may generate various lesions in DNA such as base modifications, degradation products of deoxyribose, chain breaks. These various lesions have been characterized and it is possible to quantitate them in the DNA of cells which have been irradiated or treated by free radical generating systems.

Our observations regarding lipid peroxidation was not significant but still it may be suggested that reactive oxygen species could play a role in the mechanism that has been proposed to explain the biological side effects of cell phones.

This assay was utilized on whole salvia unstimulated flow in which the submandibular glands produce 60%, the sublingual glands 7%-8% and the parotid glands only 25% of total saliva. Consequently, the affected parotid gland (dominant) secrete just ~12.5% of total unstimulated saliva reducing the efficacy of the assay. The more reliable, accurate method will be to collect directly saliva from the parotid glands and to compare lipid peroxidation levels between the 2 glands' secreted saliva.

The World Health Organization (WHO) and the International Association for Research on Cancer declared mobile phones to be group 2B agents, possibly carcinogenic to humans. According to WHO, mobile phones have the potential to cause brain and auditory canal tumors, similar to conclusions offered by Hardell et al. (2007) that use of mobile phones for 10 years give a consistent pattern of increased risk for acoustic neuroma and glioma. (Hardell et al.2007).

However, limitations of the present study include small sample size, which reduced our ability of inspiring deep confidence in the results and short exposure period, which does not allow extrapolation to the long-term effect of RF-EME. Further studies with long exposure time should be carried out to evaluate and substantiate our findings and to reveal the pathophysiology underlying such changes.

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