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Mobile Phone Radiation and It's Effects on Salivary Amylase and Lysozyme: An Observational Study

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ABSTRACT

Introduction

In the recent decades, the use of mobile phone has increased drastically. This has raised much concern about the potential health risk to the individuals who are exposed to mobile phone emitted radio frequency radiation.

Aim and Objectives

The aim of the study is to assessthe alterations in the level of salivary amylase and lysozyme in mobile phone users for minimal, average, and long period of time per day.

Methodology

Based on inclusion and exclusion criteria, the study samples were selected. 5ml of unstimulated saliva was collected using spit method for 30 individuals.A two-site sandwich ELISA is employed to quantify lysozyme (LZM) in salivary samples.Salivary Amylase is evaluated using spectrophotometry method.The data were entered in Microsoft Excel and analyzed statistically using SPSS software, version 26; SPSS Inc., (Chicago, IL, USA). The normality of the data was assessed prior to analysis using the Shapiro-Wilk's test/Kolmogorov-Smirnov test.

Results

Salivary lysozyme level was lowest in high mobile users and the mean value of salivary lysozyme level is same for moderate and low mobile users. The mean salivary amylase level was highest in high mobile users and lowest in moderate mobile users.

Conclusion

This present study conclude that the radiations emitted by the mobile phones have detrimental effects on salivary gland causing upregulation in salivary amylase and downregulation in salivary lysozyme level.

Keywords: *Salivary amylase, Lysozyme, potential stress markers, Mobile phone, Saliva*

1. INTRODUCTION

The rapid advancement of mobile technology has significantly transformed the daily lives of individuals. Mobile phones, being the most efficient and convenient handheld communication tool, have become a vital component of wireless communication—essential for both professional and social interactions in today's fast-paced modern society. It is projected that between 2018 and 2025, the number of mobile phone users will grow at an average annual rate of 1.9%, reaching approximately 5.8 billion subscribers and accounting for 71% of the global population [1]. Mobile phone users are frequently exposed to ultra-high-frequency non-ionizing electromagnetic radiation (EMR), typically ranging between 300 and 3000 MHz. There are two primary mechanisms through which this exposure may impact health: firstly thermal effects resulting from an increase in the temperature of surrounding tissues during prolonged use; and second, non-thermal effects stemming from emissions by both mobile phones and base stations. In 2012, the International Agency for Research on Cancer (IARC) classified radiofrequency electromagnetic radiation as possibly carcinogenic to humans, designating it as a Group 2B agent.

EMR exposure from mobile phones is localized, with significant biological effects linked to continuous exposure and energy absorption, especially in regions such as the head and neck, which are in close contact during usage. These effects not only contribute to the risks of users but may also impact individuals living in close proximity to mobile phone base stations.

Several studies have explored the association between mobile phone usage and the development of brain tumors. Findings suggest that prolonged use typically over 10 years may elevate the risk, particularly among children. Some studies report a higher incidence of brain tumors such as meningioma and glioma in long-term mobile phone users compared to non-users.

The salivary glands, especially the parotid gland, are situated close to where mobile phones are typically held and have become a focus of interest. Multiple studies have indicated an increased incidence of salivary gland dysfunction in individuals who use mobile phones extensively over extended periods. However Contrary to this, various other studies stated no significant association exists between salivary gland disorder and mobile phone radiation exposure[2]

The effects of radiation on the salivary glands and their components have garnered particular interest, as alterations in salivary composition can lead to irreversible complications. These include an increased risk of oral infections, oral discomfort, and a heightened susceptibility to dental caries due to xerostomia. The parotid gland, located superficially under the skin near the anterior aspect of the ear, receives significant attention as it is in direct contact with mobile devices during usage. As a result, it is more likely to be affected by the heat and potentially harmful radiation emitted by mobile phones.

Human saliva serves as a valuable diagnostic fluid, containing various biomarkers such as genetic material, proteins, and enzymes. Among these, salivary amylase and lysozyme are of particular importance. Salivary amylase plays a key role in the initial digestion of carbohydrates within the oral cavity, while lysozyme contributes to the innate immune defense by targeting bacterial pathogens. With the widespread and increasing use of mobile phones, concerns have been raised regarding potential alterations in the production and concentration of these salivary enzymes, which may affect oral and systemic health.

Thus this present study focuses to assess the level of salivary amylase and lysozyme in mobile phone users, altered due to mobile phone radiation.

2. METHODOLOGY

The objectives were to

- To evaluate the activity of salivary lysozyme in individuals who use mobile phones for minimal, average, and long period of time per day.
- To evaluate the activity of salivary amylase in individuals who use mobile phones for minimal, average, and long period of time per day.

The subjects for this study were recruited from the Outpatient Department of Oral Medicine and Radiology. The study protocol was reviewed and approved by the Institutional Review Board. The purpose and procedures of the study were clearly explained to all participants, and informed consent was obtained prior to their inclusion.

Inclusion criteria

People who are above 18 years, People who are prolonged mobile users, Employees who use mobile phone as the primary work device without a Bluetooth device (hand-held) are selected.

Exclusion criteria

Patients with systemic disease, People above 75 years, Patients with salivary gland abnormalities or local lesions, Patients under certain medications which alter salivary amylase / lysozymeEg., antidepressants. People having adverse habits like smoking and alcohol and Patients with poor oral hygiene.

A total of 30 subjects were enrolled and categorized based on their mobile phone usage: 10 samples from low mobile users (less than 1 hour per day), 10 samples from moderate mobile users (more than 2 hours per day), and 10 samples from high mobile users (more than 4 hours per day).

Saliva container, Micropipette, Spectrophotometer, Salivary Amylase Kit, Microplate reader for ELISA, ELISA kit for salivary lysozyme were the armamentarium used5 ml of unstimulated saliva is collected using spitting method. Prior to saliva collection patients are refrained from any dental procedure atleast before 24. Saliva is collected in a 5ml sterile container, which has a wide top to make it convenient for spitting. Atleast 2 ml sample should be collected from each person.Saliva is usually stored in a refrigerator **at -20 degree C.**So, after collecting, the tubes are placed in a well insulated container packed with icecubes, and then taken to the lab for storing.

Salivary lysozyme is evaluated using the ELISA method. A two-site sandwich ELISA is employed to quantify lysozyme (LZM) in salivary samples. A specific antibody is pre-coated onto the microplate. Standards and samples are then pipetted into the wells, where any lysozyme present binds to the immobilized antibody. An HRP-conjugated human lysozyme detection antibody is subsequently added. After a washing step, a chromogenic substrate is introduced, leading to color development proportional to the amount of bound lysozyme. The intensity of the resulting color is then measured.

Salivary Amylase is evaluated using **spectrophotometry** method.CNP(ChloroNitrophenol) is a direct substrate for determination of alpha amylasee activity, which does not require the presence of ancillary enzymes.The rate of CNP formation can be monitored at 400-420nm in a spectrophotometer, and is proportional to the alpha amylase activity.

3. STATISTICAL ANALYSIS

The data were entered in Microsoft Excel and analyzed statistically .SPSS software, version 26; SPSS Inc., (Chicago, IL, USA). The normality of the data was assessed prior to analysis using the Shapiro-Wilk's test/Kolmogorov-Smirnov test. If data were found to be normally distributed, parametric test was used otherwise non parametric test was used. Descriptive statistics were used to calculate frequencies, percentages, and mean values. One way ANOVA/Kruskal Wallis test followed by Tukey's/ Dunn's post hoc were carried out to determine the difference between the groups. All statistical tests were performed at a significance level of 5% ($p < 0.05$).

4. RESULTS

Results of this study are presented in [Table-1/Fig-2]. Statistical analysis revealed **no significant difference** observed in**age group** and **gender** between the groups (p = 0.064) (p=0.303).

Demographic characteristics of the participants among high, moderate and low mobile users.

SD-Standard deviation. All values are expressed as a frequency with percentages (in parentheses) and mean 2 standard deviation (SD) The statistical test used. "One way ANOVA and "Chi-square test, level of significance p50.05 is considered statistically significant.

[Table 1]:

Demographic characteristics		**High mobile users (n=10)**	**Moderate mobile users (n=10)**	**Low mobile users (n=10)**	**p-value**
Age ¥	Mean ± SD	22.90 ± 1.29	23.30 ± 1.16	24.40 ± 1.71	0.064
Gender¶	Male	1 (10.0%)	4 (40.0%)	3 (30.0%)	0.303
	Female	9 (90.0%)	6 (60.0%)	7 (70.0%)	

[Table 2]:

Results of this study are presented in [Table 2/Fig-1].The mean salivary lysozyme level was lowest in high mobile users (0.09 ± 0.01) and the mean value of salivary lysozyme level is same for moderate and low mobile users (0.11 ± 0.02) (0.11 ± 0.01). And the mean salivary amylase level was highest in high mobile users (23.06 ± 29.20) and lowest in moderate mobile users (8.47 ± 6.22).

Parameters		High mobile users (n=10)	Moderate mobile users (n=10)	Low mobile users (n=10)	p-value
Age ¥	Mean ± SD 95%CI	0.09 ±0.01 0.08-0.10	0.11 ± 0.02 0.09-0.13	0.11± 0.01 0.10-0.12	0.006
Gender¶	Mean± SD	23.06 ± 29.20	8.47±6.22	11.86 ± 11.18	0.725
	95%CI	-7.59-53.70	2.72-14.22	2.51-21.21	

[Table 3]: Comparison of salivary lysozyme and amylase level among high, moderate and low mobile users One way ANOVA and ‡Kruskal Wallis test; level of significance: *$p \leq 0.05$ is considered statistically significant.

Results of this study are presented in [Table 3].The mean salivary lysozyme level was lowest in high mobile users (0.09 ± 0.01) and the mean value of salivary lysozyme level is same for moderate and low mobile users (0.11 ± 0.02) (0.11 ± 0.01). And the mean salivary amylase level was highest in high mobile users (23.06 ± 29.20) and lowest in moderate mobile users (8.47 ± 6.22).

Parameters		High mobile users (n=10)	Moderate mobile users (n=10)	Low mobile users (n=10)	p-value
Salivary lysozyme†	Mean ± SD	$0.09 \pm 0.01^{\alpha}$	$0.11 \pm 0.02^{\beta}$	$0.11 \pm 0.01^{\gamma}$	0.006*
	95%CI	0.08 – 0.10	0.09 – 0.13	0.10 – 0.12	
Salivary amylase‡	Mean ± SD	$23.06 \pm 29.20^{\alpha}$	$8.47 \pm 6.22^{\alpha}$	$11.86 \pm 11.18^{\alpha}$	0.725
	95%CI	-7.59 – 53.70	2.72 – 14.22	2.51 – 21.21	

Comparison of salivary lysozyme among high, moderate and low mobile users; The statistical test used: One way ANOVA; level of significance: *$p \leq 0.05$ is considered statistically significant, NS: Not significant.

5. DISCUSSION

The impact of handheld mobile phone usage duration on salivary flow, salivary immunoglobulin A (IgA) levels, and salivary indicators of oxidative stress was evaluated in a study by Boensal et al. (2012) [3]. Eighty-one students, matched for age and gender, were divided into three groups based on daily mobile phone usage: less than 20 minutes/day, 20–60 minutes/day, and more than 60 minutes/day. Saliva samples were collected to assess salivary flow rate, IgA levels, and oxidative stress indicators. The study found no statistically significant differences in salivary flow rate among the three groups.

In 2015 a study investigated salivary enzyme activity, protein content, and the oxidant-antioxidant system in young college students who used mobile phones. Participants were categorized into high and low mobile phone user groups based on the frequency and duration of phone use. The results revealed significantly higher salivary levels of amylase ($p = 0.001$) which is accordance to this present study, additionally the levels of lactate dehydrogenase was also altered in high mobile users compared to low mobile users[4].

Multiple studies have shown a negative correlation between mobile phone usage and cellular health, evidenced by elevated salivary amylase activity in heavy mobile users. One such study in 2015 [5] similarly divided students into low and high mobile user groups and found that high users had significantly higher levels of salivary amylase, LDH, and MDA. These findings support this present study and the theory that the potential of salivary biomarkers as indicators of physiological stress [6].

J. Klin et al. (2014) conducted a study to evaluate whether salivary alpha-amylase activity could serve as a reliable biomarker of chronic stress and to examine its association with stress-related mucosal conditions. One hundred participants underwent oral examinations, and unstimulated saliva was collected between 2:00 and 3:30 PM, at least one hour after food intake, to eliminate the effects of chewing and circadian rhythms. The study concluded that patients with long-term psychosocial stress exhibited elevated salivary alpha-amylase activity, supporting its role as a marker of

chronic stress [7]. Further corroborating evidence suggests that non-ionizing electromagnetic radiation from mobile base stations can also increase salivary amylase activity [8].

In another study, Hamzany et al. assessed salivary enzyme activity, protein levels, and oxidative stress in mobile phone users. Twenty individuals with an average of 12.5 years of mobile phone use and 29.6 hours of monthly usage were selected. The study found reductions in salivary flow rate, total protein, albumin, and amylase activity in mobile phone users compared to non-users. These results suggest that mobile phone usage may negatively affect salivary function and contribute to oxidative stress [9].

In 2022, a similar investigation assessed the effects of handheld mobile phone usage duration on salivary flow, IgA levels, and oxidative stress indicators. Eighty-one students, matched for age and gender, were divided into three groups: Group A (less than 20 minutes/day), Group B (20–60 minutes/day), and Group C (more than 60 minutes/day). Salivary analysis revealed that prolonged mobile phone usage was associated with significantly higher MDA levels, indicating increased oxidative stress in salivary glands exposed to prolonged radiofrequency electromagnetic radiation [10]. A study conducted in the year 2014 stated that there was a decrease in salivary lysozyme which is a first in line anti-bacterial enzyme in saliva which potentially could cause an impact in oral flora [11]. Concurrently, the observed decrease in salivary lysozyme levels in the same group indicates a compromised oral immune defense, potentially disrupting the microbial balance and increasing vulnerability to oral infections. These findings emphasize the need for increased awareness of the biological effects of excessive mobile phone use and underscore the importance of regular monitoring of salivary biomarkers as non-invasive indicators of stress and oral health.

6. CONCLUSION

The present study highlights the potential impact of prolonged mobile phone usage on salivary biomarkers, indicating significant physiological and oral health implications. Increased salivary amylase levels among high mobile phone users suggest heightened stress responses, which may contribute to elevated postprandial glucose levels and a greater risk of metabolic disorders such as diabetes. Concurrently, the observed decrease in salivary lysozyme levels in the same group indicates a compromised oral immune function, paving more vulnerability to infections of oral environment. This present study unveils that even though mobile phone emits only non - ionizing radiation but its long term chronic exposure do cause changes in vital components like amylase and lysozyme levels in saliva which may compromise the health of oral cavity and oral flora may become imbalanced. Further research with more sample size and parameters are needed to critically analyze the effect of mobile phone radiation which is the utmost need of the hour.

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