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Effects of Salivary Constituents In Stimulated And Unstimulated Saliva Among Mobile Phone Using Young Adults in Tamil Nadu-A Pilot Study

Research Article

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Abstract

Introduction: Hand-held mobile phones cause disruption in physiology which can impair the successful functioning of the oral cavity. Hence, this study aimed to evaluate the salivary constituents of stimulated and unstimulated saliva among young adult mobile phone users in Tamil Nadu.

Materials and Methods: A pilot study comprising 30 participants with age ranging from 17 to 27 years were recruited. Based on the approximate number of hours of use of mobile phones for talking per day, the participants were grouped as <1 hour,1-3 hours and >3 hours.

Results: Their salivary protein and malondialdehyde (MDA) levels were assessed from stimulated and unstimulated whole saliva. The collected data were analyzed using SPSS version 23.The mean salivary protein and MDA level of stimulated and unstimulated saliva was (2.050±0.670) & (1.950±0.112) found to be statistically less among the participants who use mobile phones >3 hours. Similarly, MDA levels in mobile users of >3 hours was (20.20±5.996) & (17.20±4.016) found to be increased which impairs salivary gland.

Conclusion: The salivary total protein & MDA levels will be altered based on the hours of use of mobile phones for speaking. There is decrease in salivary protein and increase in the salivary oxidative stress.

Keywords: Mobile Phone Usage; MDA Levels; Total Salivary Proteins; Young Adults.

Introduction

Oral health is intertwined with general health, contributing to everyone's overall well-being [1]. Use of hand-held mobile phones can disrupt the oral physiology by altering the salivary constituents, and not just pathological changes can impair the successful functioning of the oral cavity [2]. Various researchers have analysed the electromagnetic radiation released by hand-held mobile phones, and have found an elevated risk of malignant gliomas, acoustic neuromas, and tumours on the side of the head where the phone is regularly positioned. The salivary glands are the primary structures located close to where mobile phones are placed for speaking [3].

Saliva is an underutilised diagnostic method that has gotten a lot of attention in the last three decades due to its non-invasive nature, lack of need for skilled personnel, and lack of special equipment [4]. Saliva acts as a biomarker for many systemic disorders, cancer, infectious diseases, opioid toxicity, and hormonal imbalances by researchers all over the world [5]. Studies on salivary biomarkers of diseases in general, and salivary biomarkers of mobile phone exposure in particular, are scarce. The saliva plays an important role in preserving oral homeostasis as the first defensive line against microbial invasion which protects oral mucosa mechanically and immunologically [6].

The electromagnetic radiation from the mobile phones has an effect on the hemostatic system. As reactive oxygen species (ROS)

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degrade polyunsaturated lipids, malondialdehyde (MDA) is produced as a byproduct, which is a biomarker for oxidative stress [7]. MDA is a biomarker of oxidative stress since it is the end result of radical-initiated oxidative decomposition of polyunsaturated fatty acids. The functions of salivary proteins include homeostatic processes, lubrication, antimicrobial activity, and the control of demineralization/remineralization of teeth.

A previous study found that exposure to electromagnetic radiation from a Global System for Mobile Communications (GSM) mobile base station raised salivary cortisol and amylase levels [8]. Mobile phones have become an inseparable part of our lives in recent years. Every year, the number of prepaid cellular subscriptions continues to rise. Globally, there were over seven billion users. From 2000 to 2020, the global number of mobile users increased sevenfold, from 6.5 percent to 43 percent [9]. Due to the broad range of applications available on smartphones, the majority of the global population (especially college and university students) has been using them in recent years [10]. Smartphones, though helpful in many respects, have drawbacks such as decreased job effectiveness, personal focus, social nuisance and psychological addiction affecting oral and personal health [11]. Hence, this study was aimed to evaluate the salivary proteins and MDA levels of stimulated and unstimulated saliva of young adult mobile phone users in Tamil Nadu.

Material and Methods

The pilot study was conducted among students of the author's institution. Mobile phone users of age ranging from 17-27 years where a total of 17 males and 13 females participated. Students who use mobile phones for speaking and are willing to participate were included. Students with systemic illness were excluded. Ethical approval to conduct the study was obtained from the author's Institutional review board (IRB). Thirty students between the age group 17- 27 years who gave consent to participate after explaining the purpose of the study were recruited. The participants were requested to fill a preformed questionnaire which contained the demographic details, hours they spent talking through the mobile phone, type of mobile phone they were using.

Thirty students based on the number of hours they spend on talking through mobile phones were grouped as < one hour, 1-3 hours and more than 3 hours with 10 students in each group. Their salivary samples were collected during class hours in the forenoon time frame between break (9 to 11 A.M). This was done to reduce the amount of variability in salivary flow rate and composition. The participants were instructed to thoroughly rinse their mouth with distilled water to eliminate any food debris. They were asked to expel saliva into a sterile pre-weighted plastic container without exerting any force for 12 minutes, as per the method of Navazesh [12]. Their Stimulated salivary samples were collected by making the participants to chew sugarless chewing gums for 5 minutes and to spit the first formed saliva. They were asked to expel the next forming saliva into the pre-weighed sterile container for 5 minutes.

Salivary pH was evaluated using ELICO LI 120 digital pH meter. Protein concentration was determined by the method of Lowry et al., [13] with bovine serum albumin (BSA) as the standard.10µl of the saliva was taken in a clean test tube and made up to 1ml with distilled water. To this, 5ml alkaline copper reagent was added. The contents were mixed well and allowed to stand at room temperature for 10min. 500µL of 1N Folin-Ciocalteu reagent was then added and mixed well immediately. After 20min, intensity of the blue color developed and was read at 720nm against blank. For plotting the standard graph, a set of standards (25, 50, 75, 100 and 125µg) were taken in a series of test tubes, and made up to 1ml with distilled water and processed as that of the samples. The standard graph was drawn by plotting the concentration of standards on the X-axis and the optical density on the Y-axis. Concentration of protein in the sample was calculated by referring to the standard curve and expressed as µg/ml.

MDA levels in saliva was evaluated using the chemicals such as Tris HCl–KCl, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA) and 1,1,3,3 tetraethoxypropane (TMP) that were purchased from (Sigma Aldrich, USA). The level of MDA was assayed in the saliva of study subjects, as previously described by Stalnaya and Garishvili et al., [14]. Briefly, 0.3 ml of collected saliva was mixed with 3 ml of 0.025 M Tris–HCL and 0.175 M KCl buffer (pH 7.4). Then, 2.5 ml of diluted saliva was mixed with 1 ml of 17% (w/v) TCA and centrifuged at 4000 g for 10 min. The precipitate was pelleted by centrifugation and the supernatant reacted with 1 ml of 0.8% (w/v) TBA in a boiling water bath for 10 min. After cooling to room temperature, the absorption of the supernatant was recorded at 532 nm using a UV-Visible spectrophotometer (Thermo Fisher Scientific, UK). The arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 TMP). The results are expressed as micromoles per millilitre (mcmol/L). The collected data were analyzed using Statistical Package for Social Sciences (SPSS) software version 23. Normality of the data was assessed using Shapiro-Wilk's numerical test. The data was found to be normally distributed. Descriptive statistics was performed to present the mean salivary flow rate, salivary pH, total protein and MDA levels. Mean comparison was employed using One Way ANOVA with Tukey's post-hoc test. p value <0.05 was considered to be significant.

Results

The mean salivary flow rate, salivary pH, salivary total protein and salivary MDA levels of stimulated and unstimulated saliva shows statistical significance among the groups. Where p value <0.05. Tukey's post hoc test for salivary pH and salivary flow rate of both stimulated and unstimulated saliva showed statistical significance between the groups <1 hour & 1-3 hours, 1-3 hours and >3 hours, >3 hours and <1 hour with 95 % CI. where p value < 0.05. For salivary total protein there is no statistical significance between 1-3 hours and >3 hours group and for salivary MDA levels there is no statistical significance between <1 hour & 1-3 hours group where p value <0.05.

Discussion

The present study aimed to evaluate the salivary constituents of stimulated and unstimulated saliva among young adult mobile phone users. The results revealed that in contrast to less mobile users, salivary levels of total protein and malondialdehyde showed major differences in high mobile users. The total salivary protein levels are lower in participants using mobile phones for more than 3 hours of talking than the other groups with mean and SD

Table 1. Mean salivary flow rate, pH, total protein, and MDA levels in whole saliva.

Saliva		Group mean±SD			p value
		<1 hour	1-3 hour	>3hour	
Flow rate	stimulated	1.500±0.527	0.700±0.674	0.950±0.483	0.000*
	unstimulated	1.220±0.516	0.400±0.516	0.500±0.227	0.000*
pH	stimulated	8.00±0.816	6.800±0.421	4.400±0.966	0.000*
	unstimulated	7.700±0.674	6.500±0.707	5.100±0.737	0.000*
Total protein	stimulated	7.500±1.414	4.532±0.7276	2.050±0.670	0.0453*
	unstimulated	6.700±0.541	3.842±0.197	1.950±0.112	0.0326*
MDA	stimulated	10.38±2.246	15.00±1.886	20.20±5.996	0.0009*
	unstimulated	8.24±1.461	13.230±1.254	17.20±4.016	0.001*

(SD- standard deviation, p value <0.05)

Table 2. Tukey's post hoc test for salivary pH, flowrate, total protein and MDA levels in whole saliva.

Saliva			Mean difference	p Value	95% CI
pH	Stimulated saliva	<1hour vs 1-3 hours	1.2	0.004	0.34 to 2.05
		1-3 hours vs >3 hours	1.4	0	1.54 to 3.25
		>3 hours vs <1 hours	-2.6	0	-4.45 to -2.74
	Unstimulated saliva	<1hour vs 1-3 hours	1.2	0.002	0.41 to 1.98
		1-3 hours vs >3 hours	2.4	0	0.61 to 2.18
		>3 hours vs <1 hours	-3.6	0	-3.38 to -1.81
Flow rate	Stimulated saliva	<1hour vs 1-3 hours		0.001	-1.82 to -.57
		1-3 hours vs >3 hours	-2.1	0	-2.62 to -1.37
		>3 hours vs <1 hours	3.1	0	2.57 to 3.82
	Unstimulated saliva	<1hour vs 1-3 hours	-1.2	0	-1.57 to -.42
		1-3 hours vs >3 hours	-2	0	-2.67 to -1.52
		>3 hours vs <1 hours	3.2	0	2.52 to 3.67
Total protein	Stimulated saliva	<1hour vs 1-3 hours	2.95	0	1.84 to 4.05
		1-3 hours vs >3 hours	2.28	0	1.17 to 3.38
		>3 hours vs <1 hours	-5.23	0	-6.33 to -4.12
	Unstimulated saliva	<1hour vs 1-3 hours	2.97	0	-4.34 to 4.34
		1-3 hours vs >3 hours	2.33	1	-14.16 to -5.48
		>3 hours vs <1 hours	-5.3	0	5.48 to 14.16
MDA levels	Stimulated saliva	<1hour vs 1-3 hours	-0.06	0.999	-4.6297 to 4.5097
		1-3 hours vs >3 hours	-10.19	0	-14.7597 to -5.6203
		>3 hours vs <1 hours	10.25	0	5.6803 to 14.8197
	Unstimulated saliva	<1hour vs 1-3 hours	0	0	1.95 to 3.99
		1-3 hours vs >3 hours	-9.82	0	1.31 to 3.35
		>3 hours vs <1 hours	9.82	0	-6.32 to -4.28

of (2.050±0.670, 1.950±0.112) respectively, being the highest of other groups. Salivary total protein was significantly higher in high mobile users, indicating adverse effect of mobile phone use on cell health. The Previous studies have reported increased salivary protein levels in oral cancer [15]. Furthermore studies suggest that it can be used as a responsive biomarker for stress-related changes in the body that represent sympathetic nervous system activity [16]. Previous research has shown that non-ionic electromagnetic radiation released by base stations decreases salivary amylase activity, which supports our findings [17].

Cell phones are one of the most widely used devices that emit electromagnetic waves, and are readily available to half of the world's population [18]. Salivary flow was found to be decreased in people who spoke on the phone for more than 3 hours in this study. However, if you use your phone for more than an hour, your salivary flow will rise as well. Despite the increased salivary flow, total salivary protein was also decreased; this is in contrast to the findings of another study, which found that as cell phone use increased over time, salivary flow increased as well, where total salivary protein was also increased [19]. On the other hand, it may be due to the various effects of using a cellphone on the sym-

pathetic and parasympathetic nervous systems. The sympathetic and parasympathetic nervous systems regulate salivation; the parasympathetic nervous system regulates fluid secretion, while the sympathetic nervous system controls protein secretion. Using a cell phone increases parasympathetic activity while decreasing sympathetic activity; this may explain the findings of the current research.

Oxidative stress has been related to the etiopathogenesis of several chronic diseases [20]. There have been very few published studies at saliva MDA levels. Saliva is considered functionally equivalent to serum. Although the blood is the gold standard for doing many medical tests, changes in serum have been reported to be reflected equally in saliva. Therefore, the salivary evaluation of MDA could serve as an alternative. In our investigation Estimation of MDA levels in saliva of the participants showed elevated MDA levels in the group who talk through hand held mobile phones for more than 3 hours. This reflected in both stimulated and unstimulated saliva. MDA levels in saliva of children with SCA were found to be elevated, while they were normal in healthy controls, and these results were consistent with serum MDA levels obtained in a recent study [21]. Some studies have found elevated MDA levels in patients with periodontitis. Marton et al. (1993) discovered that the MDA content of chronic apical periodontitis tissues was higher than that of healthy tissue from the same people [22]. In the present study the estimation of salivary flow rate, total salivary protein and MDA levels in saliva samples from the 30 participants showed that the effect increases when usage of the mobile phone increases. Hence the null hypothesis was rejected stating that there is significant difference in salivary flow, total salivary protein and salivary MDA levels in relation to the mobile phone usage.
The limitations of the study are lack of elaborated details on demographic data and small sample size. Future studies should be carried out with a large population and with people of various age groups to arrive at a more confirmatory conclusion.

Conclusion

In conclusion the study proved that Speaking on the mobile phone over an hour will decrease total protein levels, increase salivary MDA levels than those speaking less than 1 hour. This may be due to an increase in electromagnetic radiation caused by hand held mobile phones which leads to increase in the risk of inflammatory diseases or oral cancer in people.

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