

Impact of mobile phone-emitted non-ionizing electromagnetic radiation on parotid gland function: A comprehensive study

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Abstract

Background and Objective: The rapid proliferation of mobile technology has intensified debates on the potential adverse effects of non-ionizing electromagnetic radiation (NIER) from mobile phones on various human organs and cells. This study aimed to evaluate the impact of mobile phone-emitted NIER on parotid gland function.

Methodology: This cross-sectional study included 104 student volunteers from the University campus, categorized based on their mobile phone usage and exposure duration. Saliva samples were collected from the dominant (test) and non-dominant (control) sides. Key metrics measured were salivary flow rate, pH, albumin, Ischemia-Modified Albumin (IMA), and the IMA/albumin ratio (IMAR). Data analysis was performed using one-way ANOVA and Fisher's LSD multiple comparisons, with $p \leq 0.05$ considered statistically significant.

Results: The salivary flow rate and pH were higher on both sides in all groups, correlating with increased mobile phone usage duration. Although albumin levels were lower on the dominant side, they increased with longer mobile phone use. Salivary IMA and IMAR were higher on the dominant side in subjects using mobile phones for ≤ 3 years compared to those using them for >3 years.

Interpretation and Conclusion: Consistent exposure to NIER and the heat generated by mobile phones adversely affects parotid gland function, as indicated by increased salivary flow rate, pH, and altered levels of albumin, IMA, and IMAR. Public health recommendations should encourage reducing long-duration conversations and using earphones to minimize NIER exposure. Further studies are needed to evaluate the long-term effects of NIER on parotid gland function.

Keywords

saliva, pH, albumin, mobile phone, ischemia-modified albumin (IMA)

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Introduction

Since their introduction in the 1980s, cell phones have spread worldwide, among all strata of society, irrespective of age and socioeconomic status, enabling telecommunications worldwide quickly and efficiently. They have changed

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from expensive items mainly used by the business elites to communication tools used by the general population. This use is now so extensive that in some countries, the number of phone subscriptions outnumbers the population. Indeed, cell phones have contributed significantly to our communication system and helped to improve the quality of life in all sectors. They have also proved lifesaving in certain circumstances, particularly life-threatening situations. However, the increasing use of mobile phones among young children and adolescents is alarming.¹ Recent reports have suggested the high prevalence of mobile phone addiction among adolescents in urban settings is a public health concern.²

Cell phones and their base stations operate by transmitting and receiving radiofrequency (RF) signals in the range of 800 MHz–2000 MHz, which falls within the microwave segment of the electromagnetic spectrum. This emitted radiation is non-ionizing, and the exposure is quantified as the rate at which RF energy is absorbed per unit mass of a biological body. The biological impact of non-ionizing electromagnetic radiation (NIER) is influenced by the level of radiation emitted, which varies based on the specific mobile phone technology employed.³ The absorption of NIER by the human body is measured using the Specific Absorption Rate (SAR), expressed in watts per kilogram (W/kg) or milliwatts per gram (mW/g). Different mobile devices have varying SAR ratings, and regulatory bodies in different countries impose maximum SAR limits for modern handsets. For instance, the Federal Communications Commission in the United States has set a maximum SAR limit of 1.6 W/kg, a standard also adopted in India.⁴ In contrast, Europe adheres to a higher limit of 2.0 W/kg.⁵

As cell phone use increased, concerns regarding the potential adverse health impacts of NIER emitted by cell phones on different human body organs have also been fueled. The vast majority of mobile phone users use it as a handheld device, generally holding it very close to the ear, enabling the nearby tissues to direct the effect of radiation on the ear and surrounding tissues. Many reviews and epidemiological studies have shown the possible adverse health consequences of handheld mobile phones (MPHs), including an increase in skin temperature, cutaneous blood flow in the area that is directly exposed, brain glucose metabolism, increased oxidative stress, and risk of tumor.^{6–11}

In humans, the parotid glands are the most prominent salivary glands, situated in front of the ear and behind the ramus of the mandible near the skin of the face. Earlier reports point to the possible adverse effects of MPHs on the parotid salivary gland, including tumors.^{12,13} These glands are exposed directly to NIER from mobile phones due to the anatomic location, that is, near the place where a handheld mobile phone comes in close contact while the devices are in use. Therefore, they are vulnerable to changes resulting from mobile phone heat and radiation, if any, and parotid saliva, which is loaded with cells and macromolecules, is an ideal candidate for evaluating the hazardous effects.¹⁴

The study was designed to investigate the parotid saliva of subjects who have been using MPH for a specific duration. For this purpose, we analyzed the parotid saliva of one hundred and four healthy subjects, from the side being predominantly used (dominant side), for parameters such as salivary secretion rate, salivary pH, salivary albumin, and Ischemia-Modified Albumin (IMA) (an early marker of oxidative stress). These parameters were compared with the opposite side (non-dominant side), which is not often used and serves as a control.

Materials and methods

Participants and settings

The present cross-sectional study was conducted in the Yenepoya (Deemed to be University) Campus. The study commenced after obtaining approval from the Institutional Ethics Committee of Yenepoya University (YUEC250/2016). Written consent was obtained before the commencement of the study.

To study the effect of non-ionizing electromagnetic radiation (NIER) emitted from cell phones on the parotid gland, we analyzed the parotid saliva of 104 healthy volunteers who have been using mobile phones for specific durations. The selection of the subjects for the study was done based on the information collected by a personal interview regarding the use of mobile phones.

Inclusion criteria: Subjects using mobile phones, for conversations, for specified a period of time as mentioned in below (conversation indicates, attending the calls by keeping the mobile handset in close proximity to ear).

Subjects chosen were divided into four groups (26 subjects in each group) according to the duration of mobile phone use.

Group A – Subjects using mobile phone for less than 1 hour /day for ≤ 3 years.

Group B – Subjects using mobile phone for more than 1 hour /day for ≤ 3 years.

Group C – Subjects using mobile phone for less than 1 hour /day for > 3 years.

Group D – Subjects using mobile phone for more than 1 hour /day for > 3 years.

Table 1. Mean ages (in years) in the study groups.

Study group	Total number of subjects(n)	Age range	Mean age $\pm$ SD
Group A	26	18–20	18.6 $\pm$ 0.9
Group B	26	18–20	18.8 $\pm$ 0.9
Group C ^a	26	21–24	21.7 $\pm$ 0.7
Group D ^a	26	21–24	22.4 $\pm$ 0.9

^aThe mean age of Groups C and D is higher, since these groups include subjects using mobile phone for >3 years.

After recording the details related to the use of mobile phones and the make of their mobile phones (with SAR limit of 1.6 W/kg),⁴ the purpose of the study and the procedure to be carried out were explained to the subjects selected for the study.

Exclusion criteria: Subjects suffering from any salivary gland disorders or on long-term medications that can alter salivary secretion, those who were using both right and left side alternatively while using mobile phones. Participants who are using headphones or loudspeakers while using the mobile phone, have habits of tobacco use or alcohol consumption, or suffer from systemic diseases or a history of recent exposure to radiation.

Sample collection and analysis

From each subject, 1.5–2 mL of un-stimulated parotid saliva was collected between 10 AM and 12 PM, under resting conditions at least 1 hour after food intake.^{15,16} Subjects were asked to thoroughly rinse their mouth with water, after which a sample of parotid saliva was collected (Supplemental Figure 1). The required amount of saliva for various analyses was collected into a calibrated test tube using a cannula placed into the parotid duct. The procedure was repeated for the collection of saliva from the opposite side.

The sample collected from the gland directly exposed to the effects of mobile phones, i.e., the side of maximum placement of mobile phone was considered as dominant side (test). In contrast, the same subject's side of remarkably less usage was considered as the non-dominant side (control). The volume of the saliva sample collected during the first 5 minutes was recorded to determine the salivary flow rate. Salivary pH was recorded using a pH meter.

The salivary albumin estimation was carried out using a dye, Bromocresol Green (BCG), developed based on the method introduced by Doumas *et al.*¹⁷ When albumin binds to BCG, it results in a shift in the absorption spectra of the dye. The formed dye albumin complex has an absorbance peak of 625 nm, which is proportional to the concentration of albumin in the sample. It was estimated using a photoelectric colorimeter at a wavelength of 630 nm. Standard solution: 6 gm of bovine albumin dissolved in 100 mL of normal saline containing sodium azide (0.1 g/dL) was used for the calibration curve.

Table 2. Comparison of salivary flow rate in the dominant side in the study groups.

Study groups	N	Salivary flow rate (mL/min)			F Value (p-value)
		Mean $\pm$ SD	Minimum	Maximum	
Group A	26	0.45 $\pm$ 0.11	0.3	0.7	7.976 (0.00)
Group B	26	0.40 $\pm$ 0.12	0.2	0.6	
Group C	26	0.44 $\pm$ 0.12	0.2	0.7	
Group D	26	0.54 $\pm$ 0.09	0.4	0.7	

SD; standard deviation; One-way ANOVA was used for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen's duct. The salivary flow rate and volume of the saliva sample collected during the first 5 minutes were recorded. The salivary flow rate (mL/min) was calculated as follows – Volume of saliva collected in 5 minutes/5. The dominant side is the side used for mobile phone usage in the subjects.

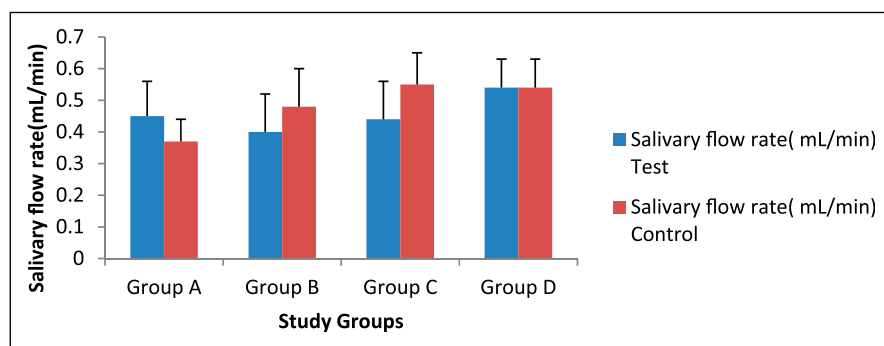


Figure 1. Salivary flow rate (mean ± SD) from the dominant and non-dominant parotid glands in the four study groups.

The level of albumin in the salivary sample was calculated using the formula as follows:

$$\text{Albumin} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of calibrator}} \times \text{Calibrator value}$$

Estimation of Ischemia-Modified Albumin (IMA)

IMA was assessed using the rapid and colorimetric method described by Bar-O *et al.* (2000),¹⁸ which measures the reduced cobalt binding capacity of albumin upon its conversion to IMA. This assay is based on the principle that ischemia induces structural changes in human serum albumin (HSA), leading to a decrease in its ability to bind exogenous Cobalt (II). The concentration of IMA is quantified by adding a known amount of Cobalt (II) to a salivary sample and determining the unbound cobalt (II) through a colorimetric assay using dithiothreitol (DTT). The intensity of color formation is inversely proportional to the level of cobalt bound to albumin.

Procedure

- Patient saliva (200 µl) was placed in two glass tubes marked “control” and “test.”
- 0.1% CoCl₂.6H₂O in H₂O (50 µl) was added.
- After gentle shaking, the tubes were left for 10 minutes in the dark to ensure sufficient cobalt-albumin binding.
- Dithiothreitol (DTT; 1.5 mg/mL H₂O) (50 µl) was added as a colorizing agent to the “test.”
- Distilled water (50 µl) was added to the “controls.”
- The reaction was quenched after 2 minutes by adding 0.9% NaCl (1.0 mL) to both test and control.
- The color intensities in both the “test” and “control” were analyzed at 470 nm using a spectrophotometer.

Table 3. Comparison of salivary flow rate in the non-dominant side in the study groups.

Study groups	N	Salivary flow rate (mL/min)			F Value (p-value)
		Mean ± SD	Minimum	Maximum	
Group A	26	0.37 ± 0.07	0.2	0.5	18.151 (0.00)
Group B	26	0.48 ± 0.12	0.3	0.7	
Group C	26	0.55 ± 0.10	0.2	0.7	
Group D	26	0.54 ± 0.09	0.4	0.7	

Min.: minimum; Max.: maximum. Statistical analysis: -Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensens duct. The salivary flow rate and volume of the saliva sample collected during the first 5 minutes were recorded. The salivary flow rate (mL/min) was calculated as follows: Volume of saliva collected in 5 minutes/5. The non-dominant side is the side that is less used for mobile phone usage in the subjects.

Table 4. Comparison of Salivary flow rate (mean $\pm$ SD) between the dominant side and non-dominant sides in the study groups.

Study groups	N	Mean Difference $\pm$ SD	95% confidence interval of Difference		T	Df	p-value
			Lower	Upper			
Group A	26	-0.08 ± 0.12	0.03	0.12	3.16	25	.004
Group B	26	-0.09 ± 0.10	-0.13	-0.05	-4.73	25	0.00
Group C	26	-0.10 ± 0.10	-0.15	-0.06	-4.92	25	0.00
Group D ^a	26	—	—	—			

^aThere was no difference in the flow rate (mean $\pm$ SD) values of dominant and non-dominant sides in Group D. Mean difference is the difference between the means of a subject's dominant and non-dominant sides. Statistical analysis: - Test used - Students paired "t" test. Level of significance - $p \leq 0.05$ was considered statistically significant.

IMA was calculated from the difference between samples measured with and without DTT. The results were reported as absorbance units (ABSU).

$$\text{IMA (ABSU)} = \text{Test} - \text{Blank}$$

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Prior to performing the one-way ANOVA, the normality of data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was assessed using Levene's test. The Shapiro-Wilk test indicated that the data for salivary flow rate and pH were normally distributed ($p > 0.05$), while the data for albumin, IMA, and IMAR were not ($p < 0.05$). Levene's test showed that the variances were homogeneous across groups for all variables ($p > 0.05$). Based on these results, one-way ANOVA was used for salivary flow rate and pH, and the Kruskal-Wallis test, a non-parametric alternative, was used for albumin, IMA, and IMAR. For significant ANOVA results, post-hoc comparisons were conducted using the Bonferroni correction to control for Type I error. For significant Kruskal-Wallis test results, Dunn's test with Bonferroni correction was used for post-hoc comparisons. A p -value of ≤ 0.05 was considered statistically significant for all analyses.

Results

To study the effect of non-ionizing electromagnetic radiation (NIER) emitted from cell phones on the parotid gland, we have analyzed the parotid saliva of one hundred and four healthy volunteers who have been using mobile phones for specific durations.

Study subjects were in four groups ($n = 26$ in each group) based on duration of use. Namely, Group A, less than 1 hour /day for ≤ 3 years; Group B, more than 1 hour/day for ≤ 3 years; Group C; less than 1 hour /day for > 3 years; and Group D,

Table 5. Comparison of Salivary flow rate of the dominant side between the study groups.

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
						Lower bound	Upper bound
Salivary flow rate mL/min	Group A	Group B	0.05	0.03	0.082	-0.01	0.11
		Group C	0.01	0.03	0.802	-0.05	0.07
		Group D	-0.09	0.03	0.003	-0.15	-0.03
	Group B	Group C	-0.46	0.03	0.14	-0.11	0.02
		Group D	-0.15	0.03	0.00	-0.21	-0.09
	Group C	Group D	-0.10	0.03	0.002	-0.16	-0.04

In one way, ANOVA showed a significant difference in salivary flow within the group ($p = 0.011$), Fischers LSD Multiple comparisons were done to compare the values between the groups (Tables 4 and 5). It showed a statistically significant ($p \leq 0.05$) difference in salivary Flow rate (ml/min) between Group A and Group D, Group B and Group D, and Group C and Group D.

Table 6. Comparison of pH of saliva from the dominant side in the study groups.

Study groups	N	pH			F Value (p-value)
		Mean $\pm$ SD	Minimum	Maximum	
Group A	26	7.34 $\pm$ 0.15	7.0	7.7	3.916 (0.011)
Group B	26	7.30 $\pm$ 0.22	6.8	7.6	
Group C	26	7.38 $\pm$ 0.15	7.1	7.7	
Group D	26	7.45 $\pm$ 0.16	7.2	7.8	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.005$ was considered statistically significant. The parotid saliva was collected from both the sides by a cannula placed into the stensens duct. Salivary pH was estimated using a pH meter. The dominant side is the side used for mobile phone usage in the subjects.

more than 1 hour/day for >3 years. Saliva samples collected from the side the subject has predominantly used were taken as test samples analyzed for salivary pH, salivary flow rate, salivary albumin, and Ischemia-Modified Albumin (IMA). The values obtained were compared with the control, i.e., the saliva collected from the non-dominant side of the same individual.

Out of 104 subjects who participated in the study, 81 were females, and 23 were males. The mean ages of the subjects included in the study were 18.6 ± 0.9 , 18.8 ± 0.9 , 21.7 ± 0.7 , and 22.4 ± 0.9 years for Groups A, B, C, and D, respectively (Table 1).

Salivary flow rate

The salivary flow rate in Group A ranged from 0.3 to 0.7 mL/min with a mean of 0.45 ± 0.11 mL/min. In Group B, it ranged from 0.2 to 0.6 mL/min with a mean of 0.40 ± 0.12 mL/min. In Group C and Group D, values were 0.2 to 0.7 mL/min with a mean of 0.44 ± 0.12 mL/min and 0.04 to 0.7 mL/min with a mean of 0.54 ± 0.09 mL/min, respectively. Post-hoc analysis with Bonferroni correction revealed that the salivary flow rate was significantly higher in the >5 years (Group C and D) compared to the ≤ 3 years (Group A and B; $p = 0.02$) (Table 2; Figure 1).

Similarly, when the salivary flow rate was recorded in control samples from the non-dominant side, Group A ranged from 0.2 to 0.5 mL/min with a mean of 0.37 ± 0.07 mL/min. In Group B, it ranged from 0.3 to 0.7 mL/min with a mean of 0.48 ± 0.12 mL/min. Group C ranged from 0.2 to 0.7 with a mean of 0.55 ± 0.10 mL/min, and Group D ranged from 0.4 to 0.7 mL/min with a mean of 0.54 ± 0.09 mL/min. The salivary flow rate from the non-dominant side is higher in Groups C and D (subjects using mobile phones for >3 years) in comparison with Groups A and B (subjects using mobile phones for ≤ 3 years) (Table 3; Figure 1).

The saliva from the parotid gland of the dominant side is shown (blue bar), and the saliva from the parotid gland of the non-dominant side in the same subject served as control (red bar). The difference between the dominant side's salivary flow rate (mean $\pm$ SD values) was higher than the non-dominant side in Group A. Group B and Group C showed a higher salivary flow rate on the non-dominant side compared to the dominant side. However, Group D did not show an appreciable difference in salivary flow rate values between the dominant and non-dominant sides.

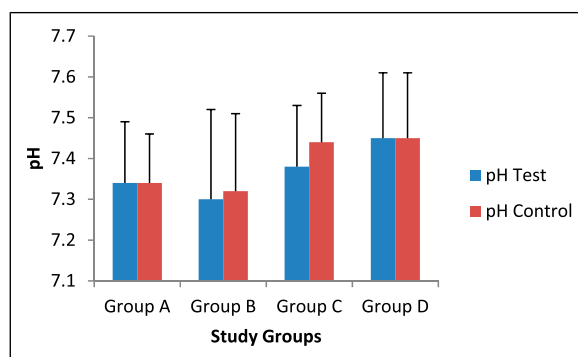


Figure 2. Salivary pH levels (mean $\pm$ SD) from the parotid glands of the dominant (test) and non-dominant (control) sides in the study groups.

Table 7. Comparison of pH of saliva from the non-dominant side in the study groups.

Study groups	N	Mean $\pm$ SD	Minimum	Maximum	F Value (p-value)
Group A	26	7.34 $\pm$ 0.12	7.1	7.6	4.891 (0.003)
Group B	26	7.32 $\pm$ 0.19	7.0	7.6	
Group C	26	7.44 $\pm$ 0.12	7.2	7.7	
Group D	26	7.45 $\pm$ 0.16	7.2	7.8	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen's duct. Salivary pH was estimated using a pH meter. The non-dominant side is the side that is not used for mobile phone usage in the subjects, which serves as a control for this study.

Comparison of the salivary flow rate in dominant and non-dominant sides of subjects in each of the study groups, that is, A, B, and C, showed that the differences in the flow rate on the two sides were of statistical significance (paired “*t*” test; $p < 0.05$; Table 4). However, in Group D, there was no difference in the salivary flow rates of the dominant and non-dominant sides.

Intergroup comparison (ANOVA) of the salivary flow rate in the dominant side showed only Group D was significantly different from the other three groups, namely, A, B, and C ($p < 0.05$). Meanwhile, intergroup comparisons between Groups A, B, and C were insignificant ($p > 0.05$). Table 5)

pH of the saliva

The salivary pH in Group A ranged from 7.0 to 7.7 with a mean value of 7.33 ± 0.15 . The salivary pH in Group B ranged from 6.8 to 7.6 with a mean value of 7.29 ± 0.22 . The salivary pH in Group C ranged from 7.1 to 7.7, with a mean value of 7.38 ± 0.15 . The salivary pH in Group D ranged from 7.2 to 7.8, with a mean value of 7.45 ± 0.16 . Post-hoc analysis with Bonferroni correction revealed that the pH of the saliva from the dominant side is higher in Groups C and D (who have been using mobile phones for >3 years) in comparison with Groups A and B (who have used mobile phones for <3 years) (Table 6 and Figure 2).

Similarly, when the salivary pH from the non-dominant side were recorded, Group A showed value ranging from 7.1 to 7.6 with a mean of 7.34 ± 0.12 , Group B showed value ranging from 7.0 to 7.6 with a mean of 7.32 ± 0.19 , Group C showed value ranging from 7.2 to 7.7 with a mean $\pm$ SD value of 7.44 ± 0.12 . Group D showed values ranging from 7.2 to 7.8 with a mean of 7.45 ± 0.16 . The pH of the saliva from the non-dominant side is higher in Groups C and D (who have been using mobile phones for >3 years) in comparison with Groups A and B (who have used mobile phones for <3 years) (Table 7; Figure 2).

Table 8. Comparison of Salivary pH (mean $\pm$ SD) between the dominant side and non-dominant sides in the study groups.

Study groups	N	Mean difference $\pm$ SD	95% confidence interval of difference		t	df	p-value
			Lower	Upper			
Group A	26	-0.02 $\pm$ 0.09	-0.06	-0.01	-1.30	25	0.20
Group B	26	-0.02 $\pm$ 0.15	-0.08	0.03	-0.77	25	0.45
Group C	26	-0.06 $\pm$ 0.12	-0.11	-0.01	-2.69	25	0.01
Group D ^a	26	—	—	—	—	—	—

^aThere was no difference in the pH (mean $\pm$ SD) values of dominant and non-dominant sides in Group D. Mean difference is the difference between the means of a subject's dominant and non-dominant sides. Statistical analysis: - Test used - Students paired “*t*” test. Level of significance $p \leq 0.05$ was considered statistically significant.

Table 9. Comparison of Salivary pH levels of test samples between different study groups.

Parameter	(I) Group	(J) Group	Mean difference (I-J)	Std. Error	p-value	95% confidence interval	
						Lower bound	Upper bound
Ph	Group A	Group B	0.04	0.05	0.43	-0.06	0.14
		Group C	-0.05	0.05	0.34	-0.14	0.05
		Group D	-0.11	0.05	0.02	-0.22	-0.02
	Group B	Group C	-0.09	0.05	0.08	-0.02	0.01
		Group D	-0.16	0.05	0.002	-0.26	-0.06
	Group C	Group D	-0.07	0.05	0.14	-0.17	0.02

A comparison between the groups was carried out using Fischers LSD Multiple comparisons (Table 9). A statistically significant difference in pH was found between Group A and Group D, Group B and Group D ($p \leq 0.05$).

The saliva from the parotid gland of the dominant side is shown (blue bar), and the saliva from the parotid gland of the non-dominant side in the same subject served as control (red bar). The difference between the pH of saliva (mean $\pm$ SD) of the non-dominant side was higher than the dominant side in Groups A, B, and C. However, Group D did not show an appreciable difference in pH values between the dominant and non-dominant sides.

Comparison of pH in saliva in dominant and non-dominant sides of subjects in each of the study groups, that is, Groups A and B, showed that the difference in pH on the two sides was not statistically significant (paired “t” test; $p < 0.05$; Table 8). Group C showed that the differences in the pH on the two sides were statistically significant (paired “t” test; $p < 0.05$; Table 8). However, in Group D, there was no difference in the saliva pH of the dominant and non-dominant sides.

Intergroup comparison (ANOVA) of pH in saliva on the dominant side showed only Group D was significantly different from the two groups, namely, A and B ($p < 0.05$). Meanwhile, intergroup comparisons between Groups D and C were insignificant ($p > 0.05$) (Table 9). None of the other comparisons were of statistical significance.

Salivary albumin

When the salivary albumin was estimated in different study groups, Group A showed a value between 0.23 and 0.39 mg/mL with a mean of 0.29 ± 0.04 mg/mL. In Group B, the salivary albumin level recorded was from 0.20 to 0.28 mg/mL with a mean of 0.23 ± 0.02 mg/mL. In Groups C and D, values were 0.26 to 0.37 mg/mL with a mean of 0.31 ± 0.03 mg/mL and 0.22 to 0.37 mg/mL with a mean of 0.32 ± 0.04 mg/mL, respectively. The salivary albumin from the dominant side is higher in Groups C and D (who have been using mobile phones for >3 years) in comparison with Groups A and B (who have used mobile phones for ≤ 3 years) (Table 10 and Figure 3).

Likewise, when the salivary albumin was recorded in control samples, Group A ($n = 26$) ranged from 0.23 to 0.33 mg/mL with a mean $\pm$ SD value of 0.28 ± 0.03 mg/mL. The salivary albumin in Group B ($n = 26$) ranged from 0.23 to 0.32 mg/mL with a mean $\pm$ SD value of 0.25 ± 0.02 mg/mL. The salivary albumin in Group C ($n = 26$) ranged from 0.28 to 0.39 mg/mL with a mean $\pm$ SD value of 0.33 ± 0.03 mg/mL. The salivary albumin in Group D ($n = 26$) ranged from 0.27 to 0.39 mg/mL with a mean $\pm$ SD value of 0.35 ± 0.03 mg/mL. The salivary albumin from the non-

Table 10. Comparison of salivary albumin levels in the dominant side in the study groups.

Study groups	N	Mean $\pm$ SD (mg/ml)	Minimum	Maximum	F Value (p-value)
Group A	26	0.29 ± 0.04	0.23	0.39	34.203 (0.00)
Group B	26	0.23 ± 0.02	0.20	0.28	
Group C	26	0.31 ± 0.03	0.26	0.37	
Group D	26	0.32 ± 0.04	0.22	0.37	

Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a canula placed into the stensens duct. Salivary albumin was estimated using a dye bromocresol green method. The dominant side is the side used for mobile phone usage in the subjects.

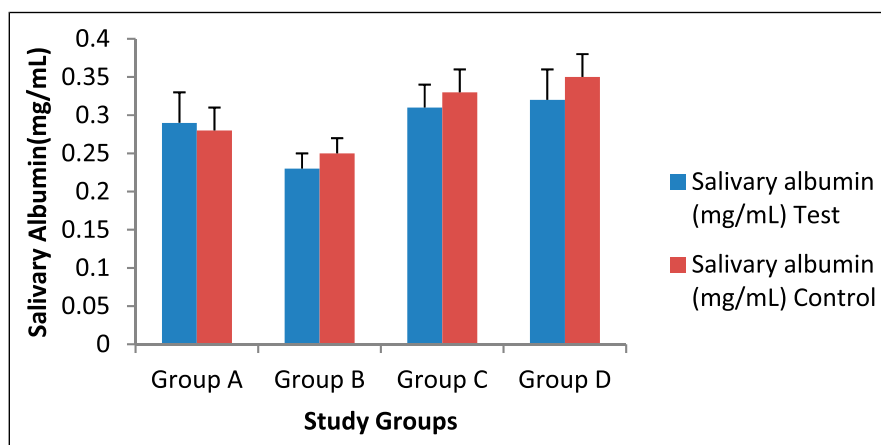


Figure 3. Salivary Albumin (mean $\pm$ SD) from the dominant and non-dominant parotid glands in the four study groups.

dominant side is higher in Groups C and D (who have been using mobile phones for >3 years) in comparison with Groups A and B (who have used mobile phones for <3 years) (Table 11; Figure 3).

The saliva from the parotid gland of the dominant side is shown (blue bar), and the saliva from the parotid gland of the non-dominant side in the same subject served as control (red bar). The difference between the salivary albumin (mean $\pm$ SD values) of the non-dominant side was higher than the dominant side in Groups B, C, and D. However, Group A showed an increase in salivary albumin values on the dominant side compared to the non-dominant side.

Comparison of the salivary albumin in dominant and non-dominant sides of subjects in each of the study groups, that is, Groups A, B, C, and D, showed that the difference in salivary albumin on the two sides was of statistical significance (paired “t” test; $p < 0.05$; Table 12).

Intergroup comparison (ANOVA) of salivary albumin levels in the dominant side showed that Groups A and B were significantly different from the other two groups, namely, C and D ($p < 0.05$). Meanwhile, intergroup comparisons between Groups C and D were insignificant ($p > 0.05$) (Table 13).

Ischemia-Modified Albumin (IMA)

The salivary IMA in Group A ranged from 0.139 to 0.68 ABSU with a mean value of 0.44 ± 0.13 ABSU. In Group B, it ranged from 0.15 to 0.64 ABSU with a mean of 0.44 ± 0.14 ABSU. The salivary IMA value in Group C ranged from 0.156 to 0.691 ABSU with a mean of 0.39 ± 0.028 ABSU. Group D showed values ranging from 0.16 to 0.69 ABSU with a mean of 0.41 ± 0.15 ABSU. The salivary IMA levels from the dominant side are higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for >3 years) (Table 14; Figure 4).

Similarly, when the salivary IMA was recorded in control samples, Group A ($n = 26$) ranged from 0.15 to 0.55 ABSU with a mean $\pm$ SD value of 0.43 ± 0.12 ABSU. The salivary IMA in Group B ($n = 26$) ranged from 0.28 to 0.68 ABSU with a

Table 11. Comparison of salivary albumin in the non-dominant side in the study groups.

Study groups	N	Mean $\pm$ SD (mg/mL)	Minimum	Maximum	F Value (p-value)
Group A	26	0.28 ± 0.03	0.23	0.33	55.981 (0.00)
Group B	26	0.25 ± 0.02	0.23	0.32	
Group C	26	0.33 ± 0.03	0.28	0.39	
Group D	26	0.35 ± 0.03	0.27	0.39	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a canula placed into the stensens duct. Salivary albumin was estimated using a dye bromocresol green method. The non-dominant side is the side not used for mobile phone usage in the subjects.

Table 12. Comparison of salivary albumin (mean $\pm$ SD) levels between the dominant side and non-dominant sides in the study groups.

Study groups	N	Mean difference $\pm$ SD	95% confidence interval of difference		T	df	p-value
			Lower	Upper			
Group A	26	0.01 $\pm$ 0.02	0.003	0.02	2.47	25	.021
Group B	26	-0.02 $\pm$ 0.03	-0.03	-0.01	-3.534	25	0.02
Group C	26	-0.01 $\pm$ 0.03	-0.02	-0.00	-2.70	25	0.01
Group D	26	-0.03 $\pm$ 0.02	-0.04	-0.02	-6.929	25	0.00

Mean difference is the difference between the means of a subject's dominant and non-dominant sides. Statistical analysis: -Test used - Students paired "t" test. Level of significance $p \leq 0.05$ was considered statistically significant.

mean $\pm$ SD value of 0.45 ± 0.14 ABSU. The salivary IMA in Group C ($n = 26$) ranged from 0.16 to 0.69 ABSU with a mean $\pm$ SD value of 0.40 ± 0.15 ABSU. The salivary IMA in Group D ($n = 26$) ranged from 0.18 to 0.71 ABSU with a mean $\pm$ SD value of 0.43 ± 0.15 ABSU. The salivary IMA from the dominant side is higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for > 3 years) (Table 15; Figure 4). Dunn's post-hoc tests showed that IMA levels were significantly higher in the ≤ 3 years group compared to the > 5 years group ($p = 0.03$).

The saliva from the parotid gland of the dominant side is shown (blue bar), and the saliva from the parotid gland of the non-dominant side in the same subject served as control (red bar). The difference between the salivary IMA (mean $\pm$ SD values) in ABSU of the non-dominant side was higher than the dominant side in Groups B, C, and D. However, Group A showed an increase in salivary IMA values on the dominant side compared to non-dominant sides.

Comparison of the IMA in dominant and non-dominant sides of subjects in the study groups, that is, Groups A and C, showed that the difference in IMA on the two sides was not statistically significant (paired "t" test; $p > 0.05$; Table 16). However, IMA in dominant and non-dominant sides of subjects in the study Groups B and D showed statistical significance (paired "t" test; $p < 0.05$; Table 16).

Intergroup comparison (ANOVA) of IMA in the dominant side showed that Groups A, B, C, and D were not significantly different between them ($p > 0.05$) Table 17.

IMA/albumin ratio in saliva (IMAR)

Group A's salivary IMA/albumin ratio ranged from 0.50 to 2.56 with a mean of 1.51 ± 0.50 . The same in Group B was between 0.60 and 2.91, with a mean of 1.87 ± 0.58 . In Groups C and D, salivary IMA/albumin ratios were from 0.43 to 2.38 with a mean of 1.25 ± 0.52 and 0.45 to 2.17 with a mean of 1.06 ± 0.06 , respectively. The salivary IMA from the dominant side is higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for > 3 years) (Table 18; Figure 5).

Table 13. Comparison of Salivary Albumin of test samples between the study groups.

	(I) Group	(J) Group	Mean difference (I-J)	Std. Error	p-value	95% confidence interval	
						Lower bound	Upper bound
Salivary albumin (mg/mL)	Group A	Group B	0.06	0.01	0.00	0.04	0.08
		Group C	-0.02	0.01	0.02	-0.04	-0.00
		Group D	-0.03	0.01	0.01	-0.04	-0.01
	Group B	Group C	-0.08	0.01	0.00	-0.10	-0.06
		Group D	-0.09	0.01	0.00	-0.10	-0.07
	Group C	Group D	-0.00	0.01	0.84	-0.02	0.02

When the result obtained from salivary albumin estimation in different groups was subjected to one-way ANOVA, a significant difference within the group was noted ($p < 0.05$) (Table 10). The comparison of the results between the groups using Fischers LSD Multiple comparisons (Table 13) showed that the difference is statistically significant between all groups except Groups C and D ($p \leq 0.05$).

Table 14. Comparison of salivary Ischemia-Modified Albumin (IMA) in the dominant side in the study groups.

Study groups	N	Mean $\pm$ SD	Minimum	Maximum	F Value (<i>p</i> -value)>
Group A	26	0.44 $\pm$ 0.13	0.14	0.68	0.618 (0.605)
Group B	26	0.44 $\pm$ 0.12	0.15	0.64	
Group C	26	0.39 $\pm$ 0.16	0.16	0.69	
Group D	26	0.41 $\pm$ 0.15	0.16	0.69	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen duct. The salivary IMA was estimated by the Albumin Cobalt Binding Assay. The dominant side is the side used for mobile phone usage in the subjects.

Similarly, when the salivary IMA/albumin ratio was recorded in control samples, Group A ($n = 26$) ranged from 0.50 to 2.27 with a mean $\pm$ SD value of 1.54 ± 0.47 . Group B ($n = 26$) ranged from 0.62 to 2.86 with a mean $\pm$ SD value of 1.8 ± 0.62 . Group C ranged from 0.51 to 2.39 with a mean $\pm$ SD value of 1.23 ± 0.48 . Group D ranged from 0.51 to 1.98 with a mean $\pm$ SD value of 1.24 ± 0.46 . The salivary IMAR from the dominant side is higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for >3 years) (Table 19; Figure 5).

The saliva from the parotid gland of the dominant side is shown (blue bar), and the saliva from the parotid gland of the non-dominant side in the same subject served as control (red bar). The difference between the non-dominant side's salivary IMAR (mean $\pm$ SD values) was higher than the dominant side in Group B. Groups A and C did not show appreciable differences in salivary IMAR value between the dominant and non-dominant sides. Group D showed increased salivary IMAR values on the dominant side compared to the non-dominant side.

Comparison of the IMAR in dominant and non-dominant sides of subjects in the study groups, that is, Groups A and C, showed that the difference in IMA on the two sides was not statistically significant (paired "t" test; $p > 0.05$; Table 16). However, IMA in dominant and non-dominant sides of subjects in the study Groups B and D showed statistical significance (paired "t" test; $p < 0.05$; Table 20).

Discussion

While recording the pH of saliva, we got a reading of 7.33 ± 0.15 in saliva from the dominant side of subjects in Group A and 7.3 ± 0.22 , 7.4 ± 0.15 , and 7.5 ± 0.16 in test samples of Groups B, C, and D, respectively. The variation in pH between Group A and Group D, Group B, and Group D was statistically significant when subjected to Fischers LSD Multiple comparisons ($p \leq 0.05$). The pH recorded for the non-dominant side was 7.34 ± 0.15 , 7.3 ± 0.22 , 7.4 ± 0.15 , and 7.5 ± 0.16 , respectively,

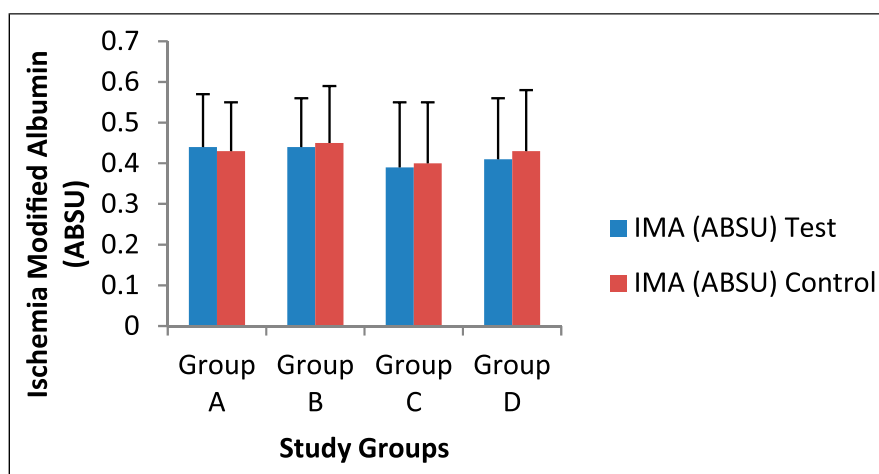
**Figure 4.** Ischemia-Modified Albumin (mean $\pm$ SD) from the parotid glands of dominant and non-dominant in the four study groups.

Table 15. Comparison of salivary Ischemia-Modified Albumin (IMA) in the non-dominant side in the study groups.

Study groups	N	Mean $\pm$ SD	Minimum	Maximum	F Value (p-value)
Group A	26	0.43 $\pm$ 0.12	0.15	0.55	0.399 (0.754)
Group B	26	0.45 $\pm$ 0.14	0.28	0.68	
Group C	26	0.40 $\pm$ 0.15	0.16	0.69	
Group D	26	0.43 $\pm$ 0.15	0.18	0.71	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen duct. The salivary IMA was estimated by the Albumin Cobalt Binding Assay. The non-dominant side is the side that is not used for mobile phone usage in the subjects.

for Groups A, B, C, and D. Normally, the pH of parotid saliva ranges from 5.45 to 6.06 (average 5.81) which can rise to two pH units on stimulation.^{16,19} In this study, the salivary pH recorded by all the participants of all groups was within the range of stimulated saliva. This is in direct correlation with the findings about flow rate. It is a proven fact that the saliva's pH largely depends on the flow rate.²⁰ Since bicarbonate is the chief determinant of the buffering capacity of saliva and the bicarbonate concentration is strongly dependent on secretion rate, there is a direct correlation between salivary pH and flow rate.^{21,22}

The mean salivary albumin in Group A was 0.29 ± 0.04 ; Group B was 0.23 ± 0.02 , and Group C was 0.31 ± 0.03 , and Group D was 0.32 ± 0.04 mg/mL with a statistically significant difference between all groups ($p < 0.05$) except Groups C and D ($p > 0.05$). In the control sample, the same were 0.28 ± 0.03 mg/mL, 0.25 ± 0.02 mg/mL, 0.33 ± 0.03 mg/mL, and 0.35 ± 0.03 mg/mL, respectively, in Groups A, B, C, and D. It is interesting to note that the albumin level was lesser in the test samples collected from the predominantly exposed dominant side compared to non-dominant side, except in Group A in which the participants have been using mobile phones for less than 1 hour per day for less than 1 year. The probable reason could be the conversion of albumin to IMA in countering the oxidative stress condition created by the consistent use of mobile phones. This is reasonably possible as albumin is a protein with antioxidant properties and contributes to most of the antioxidant protection in saliva.²³ However, we have observed a relative increase in salivary albumin levels, with an increase in the duration of mobile phone use. Our findings are consistent with those of Hashemipour *et al.* (2014) and Shivashankara *et al.* (2015), who related this divergent effect on fluid versus protein concentrations to the different impacts of MPH use on parasympathetic and sympathetic pathways.^{9,24} The electromagnetic field generated by MPH may affect the autonomic nervous system by modulating the function of the circulatory system and leading to electromagnetic hypersensitivity.^{10,25}

The mean value of salivary IMA 0.44 ± 0.13 , 0.44 ± 0.136592 , 0.39 ± 0.028 , and $0.41 \text{ ABSU} \pm 0.15$ in dominant side and 0.43 ± 0.12 , 0.45 ± 0.14 , 0.40 ± 0.15 , and $0.43 \text{ ABSU} \pm 0.15$ in non-dominant sides of Groups A, B, C, and D, respectively. The salivary IMA from the dominant side was higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for > 3 years). When the same was compared between the dominant and non-dominant sides, the dominant side was higher in all the groups than the non-dominant side except for Group A participants who used mobile for a shorter duration than other groups. The differences in Groups B and D were

Table 16. Comparison of Salivary IMA (mean $\pm$ SD) between the dominant side and non-dominant sides in the study groups.

Study groups	N	Mean difference $\pm$ SD	95% confidence interval of difference		T	Df	p-value
			Lower	Upper			
Group A	26	0.00 $\pm$ 0.02	-0.01	0.01	0.82	25	0.41
Group B	26	-0.01 $\pm$ 0.02	-0.02	-0.00	-2.37	25	0.02
Group C	26	-0.01 $\pm$ 0.04	-0.03	-0.01	-1.38	25	0.18
Group D	26	-0.02 $\pm$ 0.02	-0.02	-0.01	-5.42	25	0.00

Mean difference is the difference between the means of a subject's dominant and non-dominant sides. Statistical analysis: - Test used - Students paired "t" test. Level of significance $p \leq 0.05$ was considered statistically significant.

Table 17. Comparison of Salivary Ischemia-Modified Albumin (IMA) of the dominant side between different study groups.

IMA (ABSU)	(I) Group	(J) Group	Mean difference (I-J)	Std. Error	p-value	95% confidence interval	
						Lower bound	Upper bound
	Group A	Group B	-0.00	0.04	0.96	-0.08	0.07
		Group C	0.04	0.04	0.27	-0.03	0.12
		Group D	0.03	0.04	0.50	-0.05	0.11
	Group B	Group C	0.02	0.04	0.25	-0.03	0.13
		Group D	0.04	0.04	0.47	-0.05	0.10
	Group C	Group D	-0.02	0.04	0.67	-0.06	0.10

Comparison of the salivary IMA levels between the groups using Fisher's LSD Multiple comparisons did not show a statistically significant difference in Salivary IMA between any group ($p > 0.05$).

statistically significant ($p < 0.05$). A similar finding was observed concerning the salivary IMA/Albumin rates. The dominant side was higher in Groups A and B (who have been using mobile phones for ≤ 3 years) in comparison with Groups C and D (who have used mobile phones for > 3 years). When comparing the dominant and non-dominant sides, the salivary IMA/albumin ratio was higher on the dominant side across all groups, except in Group D.

Ischemia-Modified Albumin (IMA) is a novel protein many studies have proved to be a marker of oxidative stress. Reactive oxygen species (ROS) modify the N-terminal region of albumin, leading to increased levels of IMA. It is plausible that higher levels of ROS generation and oxidative stress result in a more pronounced elevation of IMA levels. Even though IMA level was studied as an early marker of oxidative stress, no attempt has been made to estimate IMA in mobile phone-induced oxidative stress. From the results obtained in the study, it may be inferred that, as the duration of mobile phone use increases, the parotid gland starts expressing the impact of oxidative stress, which is more on the dominant side than the non-dominant side. Our observations are consistent with the earlier reports by Hamzany *et al.* (2013) and Shivashankara *et al.* (2015),^{24,26} who have demonstrated increased oxidative stress parameters in mobile users.

IMA detection was done by the albumin cobalt binding assay offers rapid results but has limitations such as the change in pH, structural changes in albumin, presence of denaturing agents, and fatty acids can hinder the binding of albumin to bind metal ions that can lead to inaccurate results of the assay.^{27,28} Incorporating methodologies such as Albumin-Adjusted Ischemia-Modified Albumin (AAIMA) and utilizing buffer systems to stabilize salivary pH can enhance the robustness of studies examining oxidative stress biomarkers. AAIMA provides a more accurate assessment by accounting for variations in albumin levels, thereby offering a standardized measure of oxidative stress. Additionally, buffer systems in saliva, particularly the bicarbonate buffer, play a crucial role in maintaining pH stability, which is essential for reliable biomarker analysis.^{28,29}

The use of $p \leq 0.005$ as the threshold for statistical significance was a deliberate choice to ensure the reliability and robustness of the results, especially given the potential for multiple comparisons in the analysis. While we acknowledge that a stringent p -value may increase the risk of Type II errors (failing to detect real effects), this approach was selected to minimize the risk of Type I errors (incorrectly rejecting the null hypothesis), which could lead to misleading conclusions

Table 18. Comparison of salivary IMA/albumin ratio (IMAR) from the dominant side in the study groups.

Study groups	N	Mean $\pm$ SD	Minimum	Maximum	F Value (p-value)
Group A	26	1.50 $\pm$ 0.50	0.5	2.6	7.102 (0.00)
Group B	26	1.87 $\pm$ 0.58	0.6	2.9	
Group C	26	1.25 $\pm$ 0.51	0.4	2.4	
Group D	26	1.49 $\pm$ 0.58	0.4	2.2	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen's duct. The salivary IMA was estimated by the Albumin Cobalt Binding Assay. The dominant side is the side used for mobile phone usage in the subjects. The salivary IMA/albumin ratio (IMAR) was calculated as follows: Salivary IMA levels (ABSU)/salivary albumin level (mg/mL).

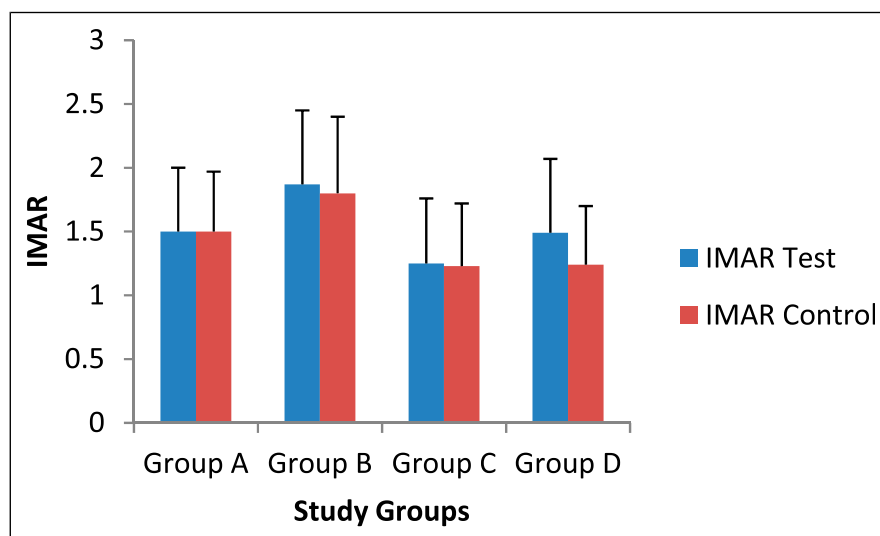


Figure 5. IMA/albumin ratio (mean ± SD) from the dominant and non-dominant parotid glands in the study groups.

about the effects of mobile phone radiation on salivary biomarkers. In this study, multiple statistical tests, including paired *t*-tests, one-way ANOVA, and Fisher's Least Significant Difference (LSD) test for post-hoc comparisons, were performed. Each of these tests contributes to the cumulative Type I error rate. By adopting a more conservative threshold, we aimed to control this error rate and ensure that any significant findings were not due to random chance. To balance this approach, future studies could mitigate the risk of Type II errors by increasing the sample size, enhancing statistical power, and exploring alternative methods for adjusting for multiple comparisons (e.g., Bonferroni correction or false discovery rate). This rigorous approach aligns with the study's aim of generating credible and clinically relevant insights into the impact of electromagnetic radiation on salivary function.

Careful measures were taken to reduce errors in the study during collection, transportation, storage, and analysis. However, the major limitation of this study is that, under its current design, we did not hold onto a minimum time gap between mobile use and saliva collection, which could have influenced the levels of various parameters, mainly the result of the instant effects of NIER. This study has several critical limitations that may have influenced its outcomes. Despite rigorous inclusion and exclusion criteria, there is a possibility that participants did not consistently use a single side of the face as intended. External variables such as diet, hydration, stress, and lifestyle—each of which can substantially influence salivary biomarkers and oxidative stress levels—were not controlled. Several potential confounders, such as diet, hydration, stress levels, and lifestyle factors, were not standardized in this study. Specifically, we did not control for participants' dietary intake prior to saliva collection, which could influence salivary biomarker levels. Future studies should aim to minimize the influence of these confounders through standardized protocols. Additionally, the sample population consisted

Table 19. Comparison of salivary IMA/albumin ratio (IMAR) from the non-dominant side in the study groups.

Groups	N	Mean ± SD	Minimum	Maximum	F Value (p-value)
Group A	26	1.50 ± 0.47	0.5	2.6	7.344 (0.00)
Group B	26	1.80 ± 0.60	0.6	2.9	
Group C	26	1.23 ± 0.49	0.4	2.4	
Group D	26	1.24 ± 0.46	0.4	2.2	

Min.: minimum; Max.: maximum. Statistical analysis: - Test used - One-way ANOVA for intergroup comparison. Level of significance $p \leq 0.05$ was considered statistically significant. The parotid saliva was collected from both sides by a cannula placed into the stensen's duct. The salivary IMA was estimated by the Albumin Cobalt Binding Assay. The dominant side is the side used for mobile phone usage in the subjects. The salivary IMA/albumin ratio (IMAR) was calculated as follows: Salivary IMA levels (ABSU)/salivary albumin level (mg/mL).

Table 20. Comparison of Salivary IMAR (mean $\pm$ SD) between the dominant side and non-dominant sides in the 4 study groups.

Study groups	N	Mean difference $\pm$ SD	95% confidence interval of difference		T	df	p-value
			Lower	Upper			
Group A	26	0.03 $\pm$ 0.16	−0.03	0.10	1.11	25	0.28
Group B	26	−0.07 $\pm$ 0.13	−0.12	−0.26	2.67	25	0.01
Group C	26	−0.02 $\pm$ 0.15	−0.08	0.04	0.57	25	0.57
Group D	26	−0.08 $\pm$ 0.13	−0.13	−0.02	2.95	25	0.01

Mean difference is the difference between the means of a subject's dominant and non-dominant sides. Statistical analysis: - Test used - Students paired "t" test. Level of significance $p \leq 0.05$ was considered statistically significant.

exclusively of university students, limiting the generalizability of our findings. Future research should include a more diverse demographic sample to better represent the broader population. Another limitation is the variability in mobile phone models used by participants. We did not collect data on the specific phone models or their corresponding SAR values, which could influence the extent of NIER exposure. Furthermore, mobile phone usage duration was self-reported, and we did not validate this information with phone records, which may introduce inaccuracies. Future research should consider recording the specific phone models and SAR values and employ methods to objectively measure mobile phone usage duration, such as cross-referencing with phone records. Moreover, variability in mobile phone models, radiation types, and usage modes (e.g., voice calls, 4G data, and Wi-Fi) introduces additional confounding factors. The sample population, consisting exclusively of university students, restricts the generalizability of the findings. Future studies incorporating participants from diverse age groups, health conditions, and lifestyles are warranted to achieve broader applicability. Furthermore, while the study highlights potential long-term health risks, such as tumorigenesis, no direct evidence was provided, and claims about such outcomes should be cautiously made or supported by longitudinal evidence. The reliance on self-reported mobile phone usage introduces potential inaccuracies due to recall bias. Incorporating smartphone usage logs in future research could significantly enhance exposure precision. Additionally, the relatively short duration of mobile phone use among participants limits the study's ability to extrapolate findings to chronic exposure scenarios. These limitations highlight the need for more comprehensive, multicentric, and longitudinal studies. Such investigations could further elucidate the interaction between non-ionizing electromagnetic radiation and salivary gland function, complementing the broader research trajectory championed in related fields like the impact of external factors on physiological responses.

Within the limitations of this study, it can be concluded that consistent use of mobile phones emitting NIER and heat generated have adverse effects on the parotid gland, manifesting as an alteration in the physical and chemical properties of parotid saliva. It is necessary for further research in this direction using more sensitive techniques and more standardized conditions to confirm the results obtained in this study and substantiate that IMA can be an early marker for mobile-related oxidative stress. Since it is a known fact that oxidative stress is a potential contributor to the risk of developing cancer.^{30,31}

Within the limitations of this study, it can be concluded that prolonged use of mobile phones emitting NIER and associated heat adversely impacts the parotid gland, resulting in alterations to the physical and chemical properties of parotid

Table 21. Comparison of salivary IMA/albumin ratio (IMAR) of the dominant side between the study groups.

	(I) Group	(J) Group	Mean difference (I-J)	Std. Error	p-value	95% confidence interval	
						Lower bound	Upper bound
IMA/albumin ratio	Group A	Group B	−0.37	0.15	0.02	−0.66	−0.07
		Group C	0.26	0.15	0.09	−0.04	0.55
		Group D	0.19	0.15	0.20	−0.10	0.49
	Group B	Group C	0.62	0.15	0.00	0.32	0.92
		Group D	0.56	0.15	0.00	0.26	0.85
	Group C	Group D	−0.07	0.15	0.65	−0.36	0.23

Comparison of the result between the groups using Fischers LSD Multiple comparisons (Table 21) showed a statistically significant difference in IMA/albumin ratio (IMAR) between all groups ($p < 0.05$) except Group C and Group D ($p > 0.05$).

saliva. Further research employing more sensitive techniques and standardized conditions is essential to validate these findings and confirm the potential of IMA as an early biomarker of mobile phone-induced oxidative stress. Given the well-established role of oxidative stress as a potential contributor to cancer risk, these findings warrant careful consideration.³² While our findings indicate increased levels of IMA, a marker of oxidative stress, in the saliva of mobile phone users, it is important to emphasize that oxidative stress is associated with cellular damage and is a factor in the development of many diseases. However, this study does not establish a direct causal relationship between mobile phone-emitted NIER and cancer. Further, longitudinal studies are needed to investigate potential long-term health effects. The observations of the current study, it may be predicted that mobile phones emitting NIER may predispose to parotid tumors in the long run. So, it may be advisable to encourage the public to avoid long cell phone conversations and promote the widespread use of earphones during cell phone conversations to avoid close contact of NIER to ears and adjoining tissues and organs. We also did not record the time interval between the participants' last mobile phone use and saliva collection. This variability in the time gap could influence salivary biomarker levels. Future studies should consider documenting the time of mobile phone usage prior to saliva collection to control for this potential confounding factor. The current study is the reliance on self-reported data for determining the 'predominant side' of mobile phone usage, which may introduce recall bias. Participants were asked to identify the side of the head they most frequently used for voice calls. Future studies should consider incorporating objective tracking methods, such as smartphone usage logs, to enhance the accuracy of side classification.

Conclusion

The study found that the parotid gland's salivary flow rate and pH were relatively high on both the dominant and non-dominant sides across all groups, correlating with increased mobile phone usage duration. Albumin levels were lower in saliva from the predominantly exposed side than the non-exposed side, except for participants with minimal mobile phone use. However, a relative increase in albumin levels was observed with more extended mobile phone use. Salivary Ischemia-Modified Albumin (IMA) levels on the dominant side were higher in subjects who had used mobile phones for ≤ 3 years compared to those who had used them for more than 3 years. Additionally, the IMA/albumin ratio was consistently higher on the dominant side in all groups except for those with the most extended usage duration. These findings suggest prolonged exposure to mobile phone-emitted non-ionizing electromagnetic radiation may adversely affect parotid gland function.

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Supplemental Material

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