

Cell Phone Use and Parotid Salivary Gland Alterations: No Molecular Evidence

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Abstract

Background: The association between cell phone use and the development of parotid tumors is controversial. Because there is unequivocal evidence that the microenvironment is important for tumor formation, we investigated in the parotid glands whether cell phone use alters the expression of gene products related to cellular stress.

Methods: We used the saliva produced by the parotid glands of 62 individuals to assess molecular alterations compatible with cellular stress, comparing the saliva from the gland exposed to cell phone radiation (ipsilateral) to the saliva from the opposite, unexposed parotid gland (contralateral) of each individual. We compared salivary flow, total protein concentration, p53, p21, reactive oxygen species (ROS), and salivary levels of glutathione (GSH), heat shock proteins 27 and 70, and IgA between the ipsilateral and contralateral parotids.

Results: No difference was found for any of these parameters, even when grouping individuals by period of cell phone use in years or by monthly average calls in minutes.

Conclusion and Impact: We provide molecular evidence that the exposure of parotid glands to cell phone use does not alter parotid salivary flow, protein concentration, or levels of proteins of genes that are directly or indirectly affected by heat-induced cellular stress. *Cancer Epidemiol Biomarkers Prev*; 1–4. ©2014 AACR.

Introduction

There is no consensus on the association between the use of cell phones and parotid or other tumor types (1–4). Molecular studies in healthy patients may show early alterations in tissue homeostasis associated with cell phone use, and currently there are no such data. We evaluated the effects of cell phone use on a number of physiologic, biochemical, and molecular parameters of the saliva produced by the parotid, comparing in each individual the saliva from the side where he/she predominantly held the cell phone (ipsilateral) during phone calls with the saliva from the opposite gland (contralateral).

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doi: 10.1158/1055-9965.EPI-13-1357

Materials and Methods

Participant recruitment

Sixty-two individuals with no systemic diagnosed disease or history of head and neck trauma were recruited after ethical approval and signed consent. Those who predominantly held the cell phone to one side of the head were included; those who reported using the cell phone randomly on both sides of the head, individuals having conditions that cause hyposalivation or who used hands-free devices were excluded. Participants completed a questionnaire that addressed questions of cell phone use profile (Table 1).

Saliva collection

Clinical examination excluded clinical alterations of the parotids. Saliva from both parotids of each individual was simultaneously collected. Salivary flow and total protein concentration were obtained for all 62 samples. The number of subjects included in the assays ranged from 43 to 48, depending on saliva availability.

The saliva collection was in the morning and individuals did not eat/drink, or brush their teeth an hour before. Saliva was collected using a collector connected to a dental vacuum suction device. Salivary flow was stimulated with 2% citric acid in a total collection of

Table 1. Sociodemographic characteristics and cell phone use profile of the subjects

	Number (%)
Age (y) mean (SD)	24.32 (\pm 4.88)
Sex	
Male	25 (40.3)
Female	37 (59.7)
Ipsilateral side of cell phone use	
Right	53 (85.5)
Left	9 (14.5)
Period of cell phone use	
5–10 years	38 (61.3)
More than 10 years	24 (38.7)
Monthly cell phone use in minutes ^a	
Up to 60 minutes	10 (16.1)
Up to 120 minutes	10 (16.2)
Up to 200 minutes	8 (12.9)
More than 200 minutes	34 (54.8)
Cell phone manufacturer	
Nokia	19 (30.6)
Samsung	17 (27.4)
Apple/iPhone	10 (16.1)
Motorola	6 (9.7)
Other	10 (16.1)
Phone calls profile (self-reported)	
Short calls and a few times per day	9 (14.5)
Short calls but several times per day	30 (48.4)
Long calls	23 (37.1)

^aCell phone use was categorized according to the duration of the monthly subscription.

10 minutes. Saliva was stored as described (5), except for salivary glutathione (GSH).

Molecular analysis of saliva

Samples were coded and experiments were carried out blind. Total protein was measured using the Bradford method. Saliva was processed according to the manufacturer's protocol and the total protein concentration was used to correct the values for each sample. p53 and p21 levels were detected by ELISA (DuoSet; R&D Systems and 900-161; Enzo Life Sciences) and the total protein was concentrated (ProteoExtract-kit; CalBiochem). GSH was measured using the Bioxytech GSH-400 kit (Oxis Research), HSP70 and HSP27 using ELISA kits (EKS-715 and 960-076; Enzo Life Sciences), and IgA with DKO078 (DiaMetra). The evaluation of reactive oxygen species (ROS) was carried out by the standard DCFH-DA protocol.

Statistical analysis

The data were analyzed using the SPSS (version 21.0) and GraphPad Prism (version 5.0). Descriptive statisti-

cal methods were used for the evaluation of data. The tests of Shapiro–Wilk and Kolmogorov–Smirnov, Wilcoxon, and *t* test were used. A significance level of $P < 0.05$ was used.

Results

The characterization of the subjects and the cell phone use profile are listed in Table 1.

We compared the parameters of the ipsilateral with the parameters of the contralateral side. There was no difference in the salivary flow, total protein concentration, or salivary levels of p53, p21, ROS, GSH, HSP70, HSP27, and IgA between the ipsilateral and contralateral parotids ($P > 0.05$), as illustrated in Fig. 1.

No differences in the parameters were found by grouping individuals according to monthly cell phone use (more vs. less than 200 minutes/month) or according to exposure period in years (more vs. less than 10 years) ($P > 0.05$).

Discussion

Although radiofrequency electromagnetic fields emitted by cell phones cannot break chemical bonds in the human body, they penetrate exposed tissues, producing heat (6). The phone battery may contribute to the heating (7). The most significant surface temperature increase during cell phone use occurs in the ear region (6), where the parotid gland is very superficially located.

Cell culture and animal experimentation have their limitations in fully reproducing the conditions of a given human tissue microenvironment. Thus, molecular studies in healthy patients naturally exposed to a supposed "carcinogenic" source can point to early alterations in tissue homeostasis induced by cell phone use. Of course, it is not possible to obtain human normal parotid samples of both parotid glands. However, saliva formation depends on the functional state of the glands that produce it (8), making the saliva a valuable research tool.

If there is a link between cell phone use and tumor development, it most likely arises from the cellular stress caused by the heating of the tissue. We compared the levels of specific proteins that may indicate cellular stress and found no difference between the ipsilateral and contralateral parotids.

On the basis of our findings, we conclude that in the population studied, the heat induced in the parotid glands region by cell phone use does not alter parotid salivary gland flow, salivary protein concentration, or levels of p53, p21, ROS, GSH, HSP70, HSP27, and IgA in the saliva produced by these glands.

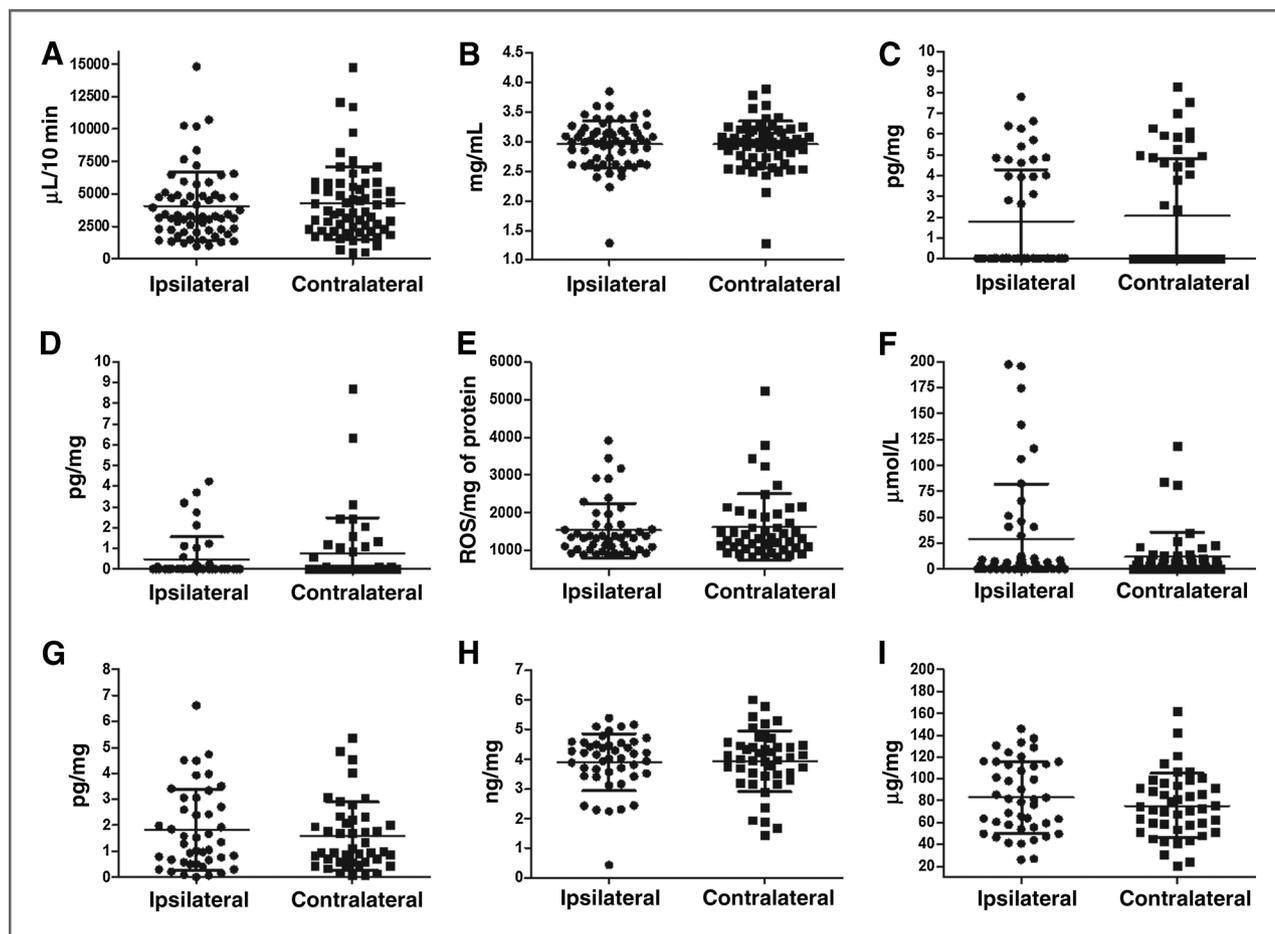


Figure 1. Parotid salivary parameters according to exposure to the cell phone (ipsilateral and contralateral sides). No significant difference was observed in the mean values of the parameters investigated when comparing both sides ($P > 0.05$). A, salivary flow; B, total protein concentration; C, p53 levels; D, p21 levels; E, DCF-fluorescence (reactive oxygen species); F, GSH levels; G, HSP70 levels; H, HSP27 levels; and I, IgA levels.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: F.T.A. de Souza, R.S. Gomez, C.C. Gomes
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F.T.A. de Souza, E.F. Ferreira, A.P. Duarte,
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F.T.A. de Souza, J.F. Correia-Silva, E.F. Ferreira, E.C. Siqueira, R.S. Gomez, C.C. Gomes
Writing, review, and/or revision of the manuscript: F.T.A. de Souza, R.S. Gomez, C.C. Gomes
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.T.A. de Souza, E.C. Siqueira, M.V. Gomez

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Acknowledgments

The authors thank Pró-Reitoria de Pesquisa da Universidade Federal de Minas Gerais, Brazil.

Grant Support

This work was supported by grants from the National Council for Scientific and Technological Development (CNPq)/Brazil, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)/Brazil, and L'Oreal Brasil For Women in Science. R.S. Gomez, E.F. Ferreira, M.V. Gomez, and C.C. Gomes are research fellows at CNPq.

Received December 31, 2013; revised March 18, 2014; accepted March 24, 2014; published OnlineFirst April 21, 2014.

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Cancer Epidemiol Biomarkers Prev Published OnlineFirst April 21, 2014.

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