

Habits of Mobile Phone Use and Modulation of Selected Inflammatory Salivary Markers

Ahmad M Khalil*, Khaled M Al-Qaoud and Israa F Alemam

*Department of Biological Sciences, Yarmouk University, Irbid-Jordan.

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ABSTRACT

Objectives: This study investigated habits of mobile phone use, awareness on health effects of radiofrequency radiation (RFR) and modulation of selected inflammatory humoral markers; immunoglobulin A (IgA), interleukin-33 (IL-33) and myeloperoxidase (MPO) by mobile phone use among a sample of the Jordanian Yarmouk university students.

Materials & Methods: One hundred volunteers (21.41±2.92 years) were randomly recruited and interviewed to fill questionnaire prior to collection of unstimulated saliva samples. Participants were divided into groups based on daily call time and history of phone use. The immunological response to RFR exposure was recorded by ELISA sandwich technique for quantitating the salivary levels of IgA. Salivary MPO ODs was measured by colorimetric assay.

Results: It was revealed that participants were aware about mobile phone/radiation hazards as reflected by the notion that majority of them used precautionary measures and having a habit of putting mobile phone away from body to minimize unwanted effects. No significant correlations were observed between salivary IgA and MPO levels on one hand and intensity and duration mobile use on the other hand.

Conclusions: The possibility that prolonged and frequent exposure to RFR from mobile phone use may cause damage in the immune system cannot be excluded.

Keywords: Immunoglobulin A, Mobile phone, Myeloperoxidase, Radiofrequency radiation, Saliva biomarkers

INTRODUCTION

There is a great concern over harmful effects of electromagnetic and radiofrequency waves generated by mobile phones and their telecommunication stations. On average, mobile phones radiate a power in the range of 0.2-0.6 watt/kg, 40% of this energy is absorbed in the head and neck region [1]. Recently, a significant positive correlation between duration of mobile phone use and severity of neck pain has been demonstrated [2]. Parotid gland is the biggest salivary gland and its anatomic location, in front of ear and behind ramus, makes an ideal candidate for influence by exposure to mobile phones [3,4]. Increase in the parotid gland volume in the dominant side than the nondominant side of mobile phone usage concurred with the findings of earlier studies [3-6]. One study [7] reported an association between exposure to mobile phone radiation for more than 1 h daily and possible development of parotid tumor. Histopathological changes in the parotid [8, 9] and the thyroid [10] glands of rats were linked to increased exposure duration RFR similar to that emitted by mobile phones.

Changes in the saliva and parotid gland as a result of exposure to mobile phone radiations [11]. Dentistry science underlines increasingly the significance of saliva in maintaining oral homeostasis and in protecting oral mucosa

mechanically and immunologically [12]. Few *in vitro* human [13] or *in vivo* animal [14] studies have focused mostly on effects of exposure to Global System for Mobile communications-radiofrequency electromagnetic fields (GSM-REF) on endocrine responses or immune system. Previous studies showed that a significant decrease in antioxidant profile increases the risk of inflammatory diseases of the oral cavity such as gingivitis, periodontitis, and mucositis in individuals utilizing cell phones for longer durations [15]. When the integrity of the dental pulp is threatened, pulp cells, especially fibroblasts, produce various pro-inflammatory cytokines and vascular endothelial growth factor (VEGF) [16]. The cytokine expression profile of the salivary gland in heavy cell phone users was investigated [17,18].

Corresponding author: Ahmad M Khalil, Department of Biological Sciences, Yarmouk University, Irbid-Jordan, Tel: +962 7 9992 3290; E-mail: kahmad76@yahoo.com

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In comparison to contralateral parotids in subjects using cell phones for more than 10 years, differences in IL-10 levels in ipsilateral parotids as well as increases in the salivary flow rate and alteration of the cytokine expression profile were reported [17]. In spite of the increasing popularity of mobile phones, the immune response to RF fields in humans is still unknown [18,19]. Intensive use of mobile phones has negative impact on bladder tissue as well as the other organs [19]. Therefore, minimizing level of mobile phone use makes it easy to be kept under control of diseases in which inflammation is an etiologic factor [19].

Saliva is readily available from most individuals, can be non-invasively collected, easily stored and processed. Recently, markers of inflammation in human saliva have been a subject of active research [20-23]. The potential use of saliva as alternative to frequent serum sampling to study inflammatory biomarkers has been suggested. Due to limited studies conducted in this field, the present research was carried out to explore the degree of awareness of phone users at Yarmouk University (Jordan) and to investigate whether frequency and duration of using mobile phone alter the health status by measuring levels of salivary inflammatory markers; IgA and MPO.

MATERIALS & METHODS

Subjects

This cross-sectional, descriptive-analytical study was approved by the Ethics Committee of Yarmouk University. A random sample of 100 healthy volunteer students from Yarmouk University in the age range of 18-30 years was recruited. Each participant was interviewed by the same person to explain the study's aim and standardize data collection regarding the knowledge, attitude and habits of using mobile phones by completing a specially constructed questionnaire. The subject was asked to sign a consent form and he/she was assured that the provided data would be confidential and only for scientific purposes with no identifying information. Exclusion criteria consisted of chronic systemic diseases, previous head and neck injuries, and history of chemotherapy or radiotherapy. Participants having any oral lesions, signs of inflammation or infections, and tooth decay were excluded. So that any change in examined immunological parameters will probably be due to exposure to RFR. Participants were divided into three groups based on total time of daily calls; <30 min/day, 30-60 min/day and >60 min/day. The subjects were further classified in three categories based on the number of years using phone; < 5 years, 5-10 years and >10 years.

Collection of saliva

This was done in the university lab settings. Before providing saliva sample, the subject was requested not to eat, drink or brush his/her teeth one hour before collection. All samples were collected daily in the morning between 10 and 12 am. A sample of 1.5-2.0 ml of unstimulated saliva (saliva

in rest position without stimulated salivary gland) was collected for 15 min by spitting method into a sterile wide test tube provided by laboratory to the researcher. Salivary samples were kept on ice during and after sample collection. Samples were centrifuged for 20 min at 14000 g at 4 °C to isolate probable debris. Then, the pure sample of saliva for each subject was kept at -80°C for further analysis within a month period.

Measurement of IgA level

Immunoglobulin A level in saliva was estimated by using enzyme immunoassay (ELISA) technique according to the Human IgA ELISA kit (Abcam, UK), IgA assays were run in duplicates. Saliva samples were added to pre-coated 96 well microplate, followed by addition of antibody cocktail and incubated for 1 h. After washing, TMB substrate was added to each well and incubated for 10 min. Followed by stop solution. OD was obtained at 450 nm using multi scan microplate reader (Thermo, USA).

Interleukin 33 estimation

Interleukin 33 was estimated by ELISA test according to the Human IL-33 ELISA kit (Abcam, UK). IL-33 assays were run in duplicates. Saliva samples were added to pre-coated 96 well microplate, incubated for 2 h, followed by addition of biotin conjugated antibody, incubation for 1 hour, then the streptavidin-HRP was added and followed by TMB substrate. OD was obtained at 450 nm using multi scan microplate reader (Thermo, USA).

Determination of MPO level

Salivary MPO OD was measured using 3, 3'-diaminobenzidine (DAB, Bioworld, USA) as a substrate according previous work [24]. Serial dilution of Horse Radish Peroxidase HRP was used as a standard sample (5 mg/ml of HRP was prepared). The optimum absorption of 5 mg/1024 ml was at 340 nm). Each saliva sample (10µL) was pipetted into 100 µL of the 0.5 mM DAB solution (5 mg DAB in 50 mL of 0.1 M potassium dihydrogen phosphate pH 4.5). 50 µL of 6 % H₂O₂ was added to initiate the reaction. Absorbance was measured at 340 nm.

Statistical Analysis

The collected questionnaires were revised for completeness and logical consistency. The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS; Chicago, Illinois, USA). Descriptive statistics was used to study the samples. One-way Analysis of Variance (ANOVA) test for numerical variables was used to compare between more than two independent groups. Statistical significance was defined as $p \leq 0.05$.

RESULTS

This study included 100 healthy volunteers (45 males and 55 females) with a mean age of 21.41±2.92 years (range of 18-31 years). Sixty-nine of the respondents were between the

age group of 18 and 21 years (**Table 1**). **Table 1** summarizes habits of mobile phone usage among the study participants. The most favorite mobile phone brand was Huawei (45%). Mobile phone radiations ranged from 0.29 to 1.16 watt/kg. Ninety percent of students used right ear as the dominant side for mobile phone usage, only 10% used left as the dominant side for mobile phone usage and 8% were bilateral users. Most participants used precautionary measures to keep phone away from their head such as earphones (51%) and handheld devices (46%). Results indicated that more female participants used earphone than handheld device; 30 out of 55 (54.55%) and 25 out of 55 (45.45), respectively. Among males, the use of these two modes was equal; 21 out of 45 (46.67%). Very small margin of males; 3 (6.76%) and

none of the females used the speaker mode (Gender differences data are not shown). The phone is left in silent position in 64% of the cases. Sixty eight percent of people used to keep phone away from body (near table or bed), 26% kept it in hand and few (6%) had the phone in the trousers/shirts pocket. The majority of the study group (74%) lived near phone base station and they were mostly (68%) nonsmokers. When participants were asked about their knowledge of phone health side effects, 89% responded by yes. The health risks associated with mobile phones include increased chances of anxiety or depression, lack of sleep, brain tumors and low sperm counts, headache, and hearing loss.

Table 1. General demographics and habituation analysis of mobile phone usage. Total number of investigated subjects (N=100).

Parameter	Participants (%)			
	Females (55%)	Males (45%)		
Gender	Females (55%)	Males (45%)		
Age (year)	18-21 (69%)	22-25 (19%)	26-29 (9%)	26-29 (9%)
Brand/ model of phone	Huawei (45%)	iPhone (20%)	Samsung (18%)	others (13%)
SAR (Watt/kg)	0.320-1.160	1.100-1.150	0.290-0.380	
Dominance ear	Right (90%)	Left (10%)		
Mode of phone Use	Always/often active 20%	Sometimes vibrate 16%		Never/Seldom silent 64%
Precautionary measures	Hand held set (46%)	Earphone (51%)		others 3%
Use while charging	Yes (39%)	No (34%)		Sometimes (27%)
Place of phone at home	Trousers/ Shirts pocket 6%	Near table/ bed 68%		Hand 26%
Health problems	Neuropsychiatric (40%)	Ear/Oral (40%)		None (20%)
Awareness of phone health side effects	Yes 89%	No 11%		

In the present study, fifty-seven percent of the study group used mobile phone for less than 30 min/day. Only 16% reported calls longer than 60 min/day. Thirty-seven students used phone less than 5 years, about half of the sample (47%) used mobile phone for a period between 5 and 10 years, the rest (16%) had a history longer than 10 years (**Table 2**). The results of ANOVA test analysis are presented in **Table 2** and depicted in **Figure 1**. It was found that although shortest time use (<5 years) had lowered levels of salivary IgA, the differences were not statistically significant (P-value = 0.76). The IgA concentrations were 32.63±3.60, 36.34±3.39 and 35.89±7.00 ng/ml in an increasing order of phone use; <5 years, 5-6 years and >10 years, respectively (**Table 2 and Figure 1**). Similarly, total daily speaking time had no significant (P-value = 0.69) effect on IgA concentration; 30.38±3.48 (>60 min), 36.38±5.80 (30-60 min), and 35.46±2.96 (<30 min).

In an attempt to correlate between IgA and IL-33 on one hand and the mobile phone use on the other, we were unable of recording any picogram of IL-33 in the saliva of different studied groups. The IL-33 kit was tested with serum of a positive patient known by high score of C reactive protein (CRP) and matched with a healthy individual. Unfortunately, no absorbance readings were obtained at 450nm. This confirmed that the IL-33 kit was not sensitive enough to detect a probably extremely low salivary IL-33 level.

The MPO data are summarized in **Table 3** and **Figure 2**. Results indicate that salivary MPO values were not significantly affected (P-value = 0.84), by the intensity of daily phone. Similarly, duration (year) of phone use had no significant (P-value=0.98) effect on the MPO value.

Table 2. Immunoglobulin A concentration in the saliva of mobile users according to time of calls (min/day) and duration of mobile phone use (years). Total number of participants 99.

Parameter	Number of Participants	Immunoglobulin A (ng/ml) ± SEM	P-value*
History of phone use (Year)			
<5	37	32.63±3.60	0.76
5-10	46	36.34±3.39	
>10	16	35.89±7.00	
Time of Phone Use (Min/day)			
<30	57	35.46±2.96	0.69
30-60	26	36.38±5.80	
> 60	16	30.38±3.48	

*ANOVA test; 95% Confidence Interval; Significance Level $P \leq 0.5$

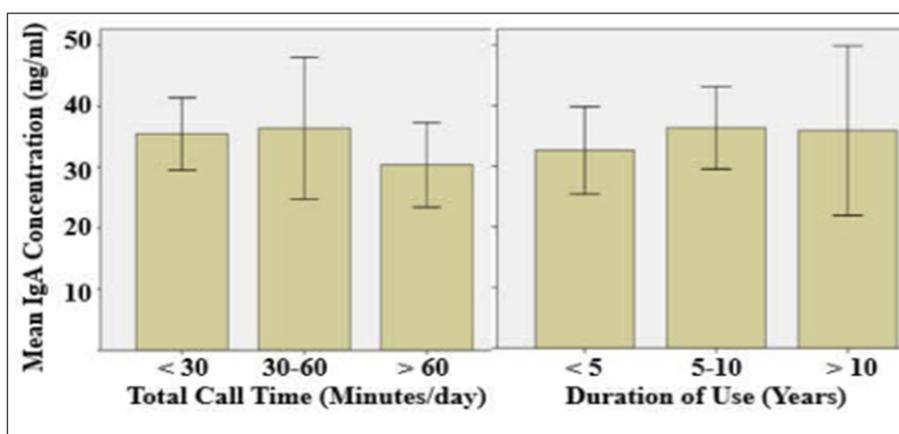


Figure 1. Histograms of immunoglobulin A concentration (ng/ml) in relation to total daily minutes of calls and total years for phone use. Error bars indicate the standard error of the mean (SEM) within each group. Error bars ± 2 SE. No significant differences observed between different groups ($p > 0.05$)

DISCUSSION

The present study is useful to the general population particularly to the students as the perceived health risk did not significantly deter students from using mobile phone. It showed that 89% students (mean age 21.41 ± 2.924 years) reported a very good knowledge of adverse health impact of mobile phone radiation on their heads. Therefore, in addition to keeping their phones away from their bodies, they followed precautionary measures such as use of handheld sets or earphones. The third method was to reduce mobile using frequency to less than 30 min a day (57%). A study in Malaysia [25] reported an overall 62% perception of mobile phone hazard among 200 Medical School students. Another study [26] found that 50% of 400 Saudi final year medical students and medical interns had poor awareness of cell phone use and its health hazards. An average knowledge

about physical hazards related to mobile phone usage; about 72% of an Indian studied group [27]. About half of 145 higher secondary school students in Nepal showed low level of knowledge on the mobile phone hazards [28]. More recently it was revealed [29] that only 60.7% of a sample of 150 Egyptian nursing students had knowledge of mobile phone cancer hazards.

Placing the phone at a distance of about 0.05 m from the body during conversation has been highly recommended [30]. The latter study revealed that the intensity of measured field strength is about three times higher while dialing the network compared to when the call has been established in the network. As a consequence, it is advisable for a call to be established on the network before placing the phone to the ear.

Table 3. Myeloperoxidase (MPO) ODs (340 nm) in the saliva of mobile users according total time of calls (min/day) and duration of mobile phone use (years). (Total number of participants N=100).

Parameter	Number of Participants	MPO Optical Density 340 nm ± SEM	P-value*
History of phone use (Year)			
<5	37	0.010±0.005	0.98
5-10	47	0.010±0.005	
>10	16	0.009±0.003	
Time of Phone Use (Min/day)			
<30	57	0.012±0.005	0.84
30-60	27	0.008±0.006	
> 60	16	0.006±0.005	

*ANOVA test; 95% Confidence Interval; Significance Level $P \leq 0.5$

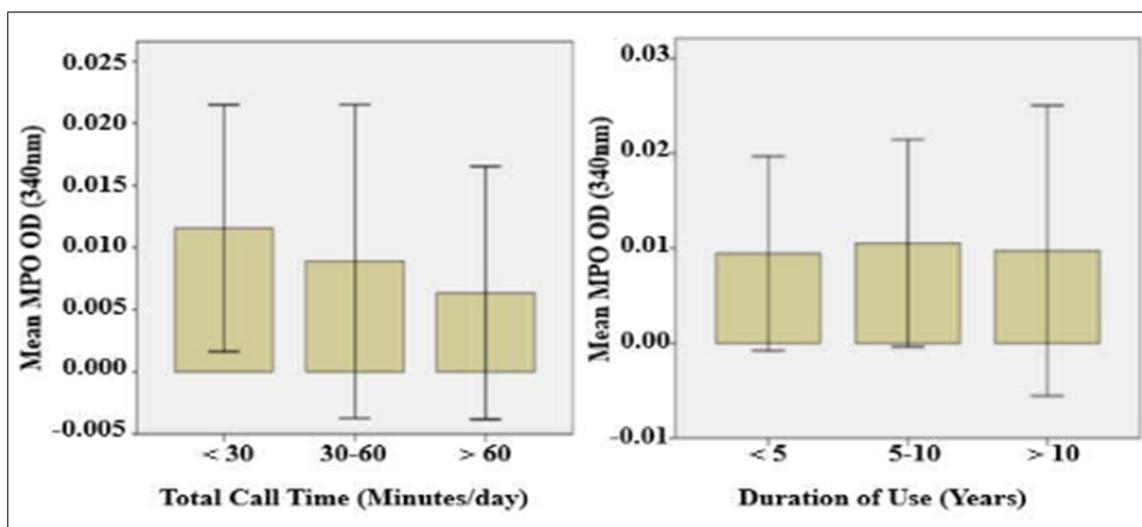


Figure 2. Histograms showing the myeloperoxidase optical density (340nm) in relation to daily call time and history of phone use. Error bars indicate the standard error of the mean (SEM) within each group. Error bars ± 2 standard errors (SE). No significant differences observed between different groups ($p > 0.05$).

In our study, no relationship was observed between age or gender and preference side of mobile phone use; 90% used right ear as the dominant side. This is in agreement with a previous study [6], where 39 out of 50 (78%) used right dominant.

Previous studies correlated EMF exposure to disturbance in the immune system including increased oxidative stress, enhanced phagocytic activity and increased production of chemokines [31-35]. This increases risk of inflammation and can predispose mobile phones users for longer durations to oral diseases such as gingivitis, periodontitis, and mucositis

[15,35,36]. Decrease in salivary flow rate has been proposed to be partly responsible for the increase in salivary biochemical constituents in diseases [37]. Conversely, increase of the salivary flow rate in mobile-phone users was suggested to have a diluting effect on the saliva components [4].

In the present study, all confounding factors that could cause immune toxicity were excluded (tobacco, alcohol, recent medication, systemic factor etc.). Therefore, any change observed in the examined immunological parameters was expected to be the immediate result of phone use. To make

sure that the observed alterations are attributed to radiation induced effects, people with gingivitis or people with periodontitis were not included in the study. We found that about 5 years of mobile use and more than 60 min of close exposure to EMF emitted by the phone had lowered, but not significantly, the concentrations of salivary IgA. Critical analysis of the data with the inflammation may relate the absence of the positive effect to the fact that most of the participants were using earphone (51%) or handheld set (46%) as a precautionary measure i.e. they were not keeping their phones near the ear while calling.

These results do not depart from those reported in literature, where levels of salivary flow, concentration of protein and of IgA in saliva [6,13,38-40] and blood [41] of people were not significantly affected by exposure to RF radiation. Contrary to this, speaking on mobile phone over an hour decreased total antioxidant capacity of saliva and salivary IgA [42]. Three studies on the effect of use of mobile phones on the level of salivary anti-inflammatory cytokine (IL-10) reported three different results; decrease [17], increase [43] and no change [44]. The proinflammatory cytokine IL-1 β values in subjects who used mobile phones for more than 10 years presented higher differences between ipsilateral versus contralateral parotids [17].

Likewise, in vitro human studies gave no convincing evidence that exposure to RF field initiate adverse modifications in immune cells or cytokines characteristic of human disease [12,45-47]. It was proposed [48] that pulse-modulated microwaves may represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes of cultured human blood at relatively low levels of exposure. A small but significant downregulation of expression of CD95 gene, which regulates immunologic response in lymphocytes, was found [49] in cells taken from older (88 ± 2 years), but not younger (26 ± 5 years) donors. Results from experiments with RFF exposure at 2.45GHz SAR at 10 W/kg have shown very little or no effects on either chemotaxis or phagocytosis in neutrophil-like human HL-60 cells [50].

Although it is better to limit the discussion on one model organism as the findings may be totally different (or to an extent) in two different species, it may deserve mentioning some of the similar studies. Animal studies reached no definite conclusions regarding the immunologic effects of mobile phone and microwave radiation; no change was detected in humoral response of young rats exposed in utero and postnatal to non-ionizing radiofrequency field regardless of the types of biomarker and SAR levels [51]. In contrast, exposure of rats to EMF resulted in significant decrease in immunoglobulin levels (IgA, IgE, IgM, and IgG); total leukocyte, lymphocyte, eosinophil and basophil counts [52]. The presence of more inflammatory cells especially large and small lymphocytes, which are characteristic of chronic

inflammation, has been shown recently in gingival tissues of rabbits exposed to mobile phone radiation [53].

In the present work, the salivary IL-33 concentration was measured by ELISA, however, no absorbance was detected. This may due to the fact that IL-33 is detected in serum of people with chronic diseases, or that the IL-33 kit was not sensitive enough to detect a probably extremely low salivary IL-33 level [54]. The salivary MPO ODs values measured in this study were not significantly affected neither by time of call per day, nor by the duration (year) of phone use. Up-to-date, no experimental human studies describing changes in the salivary MPO levels due to the exposure RFR have been encountered in the published literature. The available literature on the effect of RF fields on MPO using laboratory animals is scarce. In line with our data are those found by others, who demonstrated no significant ($p > 0.05$) alterations in MPO concentrations in livers [55] and submandibular glands [56] of rats exposed to 100 and 500 μ T extremely low frequency magnetic field (ELF-MF) (2 h/day, 7 days/week, for 10 months) corresponding to the safety standards for public and occupational exposure [56,57]. In contrast, significant increases in MPO were observed in various organs, such as rat kidney and guinea pig's liver after RFR exposure [58,59].

One point of strength of the present study is the fact it was conducted on both sexes. However, it is limited by small sample size and only with saliva from relatively young volunteers. The disadvantage of epidemiological studies; small sample size and a lack of prospective data acquisition should be kept in mind. For this reason, it is difficult to evaluate the results on a person basis. Another methodological issue concerns the different mobile phone types assessed in the study. Therefore, it is recommended that future studies should plan to examine whether or not the reported results herein represent adaptive response to radiation stress and to evaluate effect of frequent mobile phone use on salivary flow rate and other immunological parameters. To arrive at a more confirmatory conclusions sample size should be larger and with people of various age groups from different geographical regions. A sample of deaf people may serve as negative control for the mobile phone users.

CONCLUSION

Salivary IgA and MPO levels were lower, but not significantly, in saliva from people whose daily use exceeded 60 min as compared with those observed in shorter period callers. Likewise, duration of phone use had no significant effect on the IgA and MPO values. However, higher IgA concentrations were noticed in saliva of subjects who used phone longer than 5 years as compared to scores of the shortest time less than 1 year. Whether or not the slight alterations in the immune system relevant to RFR have any clinical implications deserves further investigation.

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