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Exposure to non-ionizing electromagnetic fields emitted from mobile phones induced DNA damage in human ear canal hair follicle cells

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ABSTRACT

The aim of this study was to investigate effect of radiofrequency radiation (RFR) emitted from mobile phones on DNA damage in follicle cells of hair in the ear canal. The study was carried out on 56 men (age range: 30–60 years old)in four treatment groups with n = 14 in each group. The groups were defined as follows: people who did not use a mobile phone (Control), people use mobile phones for 0–30 min/day (second group), people use mobile phones for 30–60 min/day (third group) and people use mobile phones for more than 60 min/day (fourth group). Ear canal hair follicle cells taken from the subjects were analyzed by the Comet Assay to determine DNA damages. The Comet Assay parameters measured were head length, tail length, comet length, percentage of head DNA, tail DNA percentage, tail moment, and Olive tail moment. Results of the study showed that DNA damage indicators were higher in the RFR exposure groups than in the control subjects. In addition, DNA damage increased with the daily duration of exposure. In conclusion, RFR emitted from mobile phones has a potential to produce DNA damage in follicle cells of hair in the ear canal. Therefore, mobile phone users have to pay more attention when using wireless phones.

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Radiofrequency radiation; mobile phone; DNA damage; single strand break; ear canal hair follicle cells

Introduction

Recently, electromagnetic pollution has become one of the most controversial issues. Along with being indispensable to our daily lives, the biological effects of radiofrequency radiation (RFR), which is emitted by mobile phones, have become a major debate in recent years. Scientific studies on the relation between mobile phone use and various diseases especially brain tumors have been causing concerns in public. The World Health Organization (WHO) defines RFR from wireless communication devices "low-powered radiofrequency transmitters, operating at frequencies between 450 and 2700 MHz with peak powers in the range of 0.1–2 W (WHO, 2014).

Being indispensable communication devices in our daily lives, mobile phones can now be used both as a modem and a computer. With the newly added capabilities such as gaming, internet access, etc., mobile phones have emit more RFR than previous phone. Because of the new features, children and young adults in particular use smart phones more extensively, which has resulted in excessive RFR exposure). There are 6.9 billion mobile phone subscribers around the world and 84% of people use mobile broad band services and 47% of people have internet access (World Health Organization (WHO), 2014; ITU, 2016), which makes this issue worthy of attention.Because of the controversies on the effects of RFR on human health, many *in vitro* and *in vivo* studies on the biological effects of RFR from mobile phones have been performed particularly in the past 10 years. Some of them indicated a relationship between mobile phone use and brain tumors, when the phone has been used for at least 10 years (Dasdag et al., 2004; Hardell et al., (2006); Hardel and Carlberg (2009); Hardell and Carlberg (2015).

Studies on the subject generally suggest that RFR can produce various biological effects such as DNA damage, DNA breaks, oxidative stress, lipid peroxidation, various tissue cancers particularly brain tumors, abnormalities in chromosomes, brain neurodegeneration and premature aging (Lai and Singh 1995, 1996, 1997a, 1997b, 2004, 2005; Lai, 2012; IARC Working Group, 2011). All these results have led to classification of RFR as a Group 2B human carcinogen (possibly carcinogenic agent) by the International Agency for Research on Cancer (IARC), an agency of WHO (IARC Working Group, 2011). However, the fact that most of the studies are animal

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experiments and human experiment studies are limited in number causes WHO not to be able to make a definite decision on the issue. New human studies are needed. Therefore, the present human study is intended to make contributions to these concerns.

The ear is one of the organs most exposed to RFR when talking in the mobile phone. To know the effects of RFR on ear canal hair follicle cell, this may give us a perspective about what kind of effects of the radiation could have on other organs. People generally have their ear canal hairs removed due to aesthetic concerns and this situation makes ear hairs one of the most adequate biological materials to investigate in ethical terms. Therefore, getting to know the effects of RFR on follicle cells of hair in the ear canal of humans would considerably contribute to understanding the current concerns on the subject. The issue whether or not RFR causes any DNA damage is one of the most important and controversial research subjects. Therefore, this study investigated whether the RFR from mobile phones produces DNA damage in ear hair follicle cells. Another aspect of the present study is that it is the first human study to investigate the effects of RFR on the DNA of ear hair cells. The study having these aspects is an original human experimental study that can make considerable contributions to the discussions about mobile phones and health problems. The aim of the study was, therefore, to investigate whether DNA damage was produced in follicle cells of ear canal hairs of people who were exposed to RFR from mobile phones for different daily durations of time.

Material and method

Subjects

In the first phase of the study, a volunteer form was prepared and a volunteer-signed survey was conducted in which participants could determine their habit of using mobile phones or other wireless communication devices. Participants using smart phones with similar SAR values, were used. The head peak SAR values of the smart phones used by the participants, ranged between 0.45–0.97 W/kg. Participants' smartphone usage habits were also identified. In the study, follicle cells of hair in the ear canal were collected by the same person for standardization. Subjects who had ear diseases or undergone any ear surgery were excluded from the study. However, we paid maximum attention to standardizing parameters such as high temperature, smoking, chemical exposure, radiation and occupational exposure, drugs, age of the subjects in the groups. The study was carried out on 56 men (age range: 30–60 years old) with 14 subjects each in the control and three exposure groups. Although the age range was given as 30–60, the age ranges in each group were comparable to each other. No significant difference was found between all groups in terms of subjects' age. The median age in years of the subjects are as follow:control: 40, (age range:29–67), group 2:42 (age range:33–64), group 3:40 (age range:30–52), group 4:40 (age range:28–51). All the parameters were controlled by a professor of Biostatistics professionally. Therefore, we eliminated the parameters that could affect the results of this study. The groups in this study were designed as follows: people do not use mobile phones (Control group), people use mobile phone for 0–30 min/day (Group 2), people use mobile phones for 30–60 min/day (Group 3) and people use mobile phones for more than 60 min/day (Group 4) during a10 year period. All the subjects in the control group were under the same conditions except RF exposure. Comet Assay was performed by researchers who were unaware of which group the hair samples were from. The study was approved by the Ethics Committee of the Medical Faculty of Istanbul Medeniyet University, Report No: 2016/0044.

Chemicals

Hank's Balanced Salt Solution was obtained from Sigma (St. Louis, Missouri, USA), 0.05% Trypsin EDTA was obtained from Gibco (Toronto, Canada), and other chemicals were obtained from Merck (Darmstadt, Germany). For chemical and biochemical examinations, ultrapure water received from two-way water purification system (Purelab ELGA, High Wycombe, UK) was used. All reagents and chemicals were of analytical grade or higher purity.

Collecting and storing human ear hair roots

Hair roots (follicle) from a subject were obtained using a forceps. Ear hairs were taken from the side of the head that the subject generally used mobile phones and placed into a 1.5-ml Eppendorf microfuge tube with the roots towards the bottom of the container. Samples were placed in −80°C immediately and stored until analysis.

Preparation of cell suspension from human ear hair roots

About six or seven hair roots from each subject were placed into a 1.5-ml Eppendorf microfuge tube (Greiner bio-one, Monroe, NC, USA) with the roots towards the bottom of containing 50 µl of Hanks solution (1× Hanks' Balanced Salt Solution, 20 mM EDTA and 10% DMSO) and incubated at 4°C. The Hanks solution was then

drained from the tube. 50 µl of 0.05% Trypsin-EDTA was added to the hair sample for enzymatic digestion at room temperature for 45 min. The reaction was stopped by adding 50 µl of PBS (phosphate buffer saline) and vortexed for 5 min to separate the cells from the hair follicles. The cell suspension consisted of different types of hair follicle stem cells.

DNA damage assay (Comet Assay)

DNA Comet Assay or single cell gel electrophoresis (SCGE) is a rapid, simple and highly sensitive fluorescence microscopic method used to detect DNA damage at single cell level in live populations. DNA damage in the ear hair cells was investigated by using single cell gel electrophoresis (Comet) method under high alkaline conditions as described by Singh (1996) with some modifications. In brief, each microscope slide was pre-coated with a layer of 1% normal melting point agarose in distilled water and was dried thoroughly at room temperature. Next, a 50 µl cell suspension was mixed with 100 µl of 0.5% low-melting agarose prepared with PBS and was dripped onto the first layer and covered with a coverslip. The cell preparation was allowed to solidify for 15 min at 4°C in a moist box. The coverslip was removed and the slide was immersed in freshly prepared cold lysis buffer containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH:10 for 1 h at 4°C. The slide was removed from the lysis buffer, drained, and placed in a horizontal electrophoresis unit filled with fresh alkaline electrophoresis solution, containing 300 mM NaOH and 1 mM EDTA, pH: 13, for 20 min at room temperature to allow the DNA to unwind. Electrophoresis was performed for 30 min at 8°C at 25 V and was adjusted to 300 mA. Subsequently, the slide was washed with a neutralizing solution of 0.4 M Tris-HCI, pH 7.5 to remove the alkali ions and detergents and was air-dried. After drying, the slides was stained with 50 µl of ethidium bromide (1 µg/ml) and covered with a coverslip. All steps were performed under dim light to prevent further DNA damage (Haines et al., 1998; Singh et al., 1998).

Assessment of DNA damage

Observations were made at a magnification of 200x using a fluorescent microscope (Olympus, BX51, Tokyo, Japan). The images of 100 randomly chosen nuclei were analyzed by Comet Assay Software Project (CASP-1.2.2, Windows 2010) (Sarıozkan et al., 2012). To quantify the DNA damage, we calculated seven parameters: head length, tail length, comet length, head DNA, tail DNA, tail moment (TM) and Olive tail moment (OTM).

Damage was detected by a trial of fragmented DNA that migrated from the nuclei of ear hair follicles cells, causing a 'comet' pattern, whereas nuclei without a comet, were not considered damaged (Verit et al., 2006).

Statistical method

Whether or not the data are from a normally distributed population was assessed by using the Shapiro-Wilks test. Logarithmic transform was then used to make the data normally distributed. Then, one-way analysis of variance (ANOVA) followed by the post-hoc Tukey Test to determine significant difference between two treatment groups was performed. A difference at $p < 0.05$ is accepted as statistically significant. Data analysis was performed using STATA MP/11.

Results

Significant changes in the parameters of DNA damage, which were classified as head length, tail length, comet length, percent head DNA, percent tail DNA, tail moment, and OTM, were detected in the hair follicle cells of the subjects who used mobile phone for 10 years with different durations per day when compared with those of the control.

Head length

It was shown in statistical terms that the head lengths measured in the subjects who were exposed to radiation emitted from mobile phones decreased compared to the control group ($p < 0.05$), however, no significant difference, though different from the control, was observed between Group 2 and Group 4 ($p > 0.05$) (Figure 1; Table 1).

Tail length

The tail length measured in subjects exposed to mobile phone radiation showed a statistically significant increase compared to the control group ($p < 0.05$). The most prominent increase was in Group 4, and no significant difference, though different from the control, was observed between Group 2 and Group 3 ($p > 0.05$) (Figure 2; Table 1).

Comet length

No significant difference was observed in comet length of ear canal hair cells in Group 2 and Group 3 subjects exposed mobile phone radiation when compared to the control group ($p > 0.05$). However, a statistically significant increase was observed between Group 4 and the other groups (Figure 3; Table 1).

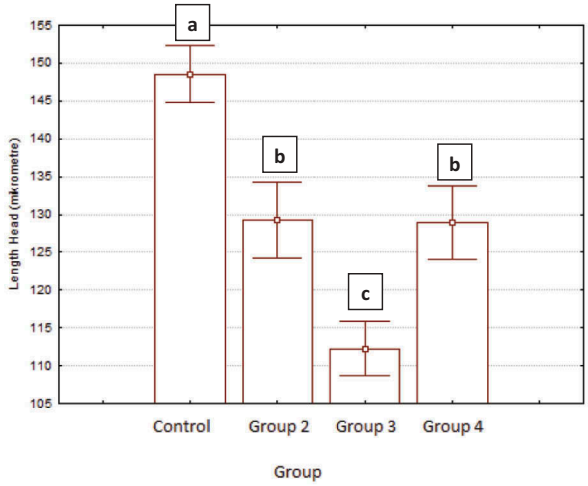

Figure 1. Statistical comparison of head length. Statistical significant difference was found between control and the other groups ($p < 0.05$). However, no significant difference was observed between Group 2 and Group 3 ($p > 0.05$). Statistical difference is observed between groups labeled by different letters ($p < 005$), whereas no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

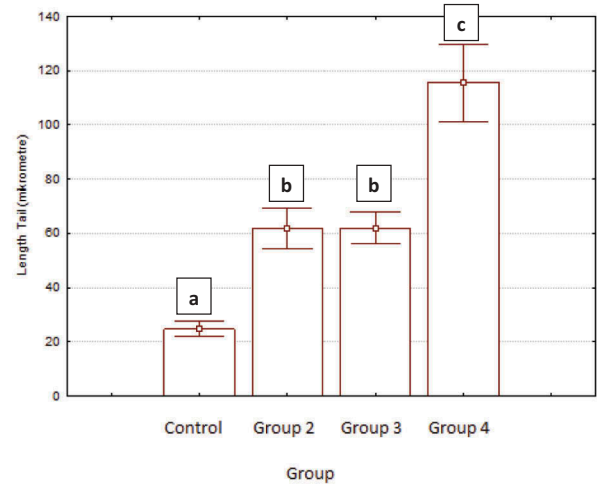

Figure 2. Statistical comparison of tail length. Tail length of Groups 2, 3, 4 increased when compared to the control group ($p < 0.05$). The most prominent increase was in Group 4, and no significant difference was observed between Group 2 and Group 3 ($p > 0.05$). Statistical significant difference was observed between groups labeled by different letters ($p < 005$), however, no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

Percent head DNA

Percent DNA in the comet head measured in subjects exposed to mobile phone radiation decreased significantly compared to the control group ($p < 0.05$). It was observed among the experimental groups with different daily durations of exposure to the radiation that a prominent decrease emerged in the percent head DNA as the exposure duration increased ($p < 0.05$). Considering the findings, we can state that decrease in percent head DNA and daily duration of exposure to the radiation are positively correlated to one another (Figure 4; Table 1).

Percent tail DNA

Percent tail DNA measured in the subjects exposed to mobile phone radiation increased prominently when compared with the control group ($p < 0.05$). Considering the findings, we can state that increase in percent tail DNA and daily durations of exposure to the radiation are positively correlated to one another (Figure 5; Table 1).

Tail moment

Tail moment measured in the subjects who were exposed to mobile phone radiation was found to be significantly higher than the control group ($p < 0.05$). The most prominent increase was observed in Group 4, which had the longest daily duration of exposure to the radiation (Figure 6; Table 1).

Olive Tail Moment

OTMs measured in the subjects who were exposed to mobile phone radiation were found to be significantly higher than the control group ($p < 0.05$). The most

Table 1. Summary of statistical analysis of DNA damage parameters in all groups.

Parameters	Control (Group 1) Mean ± S.E.M. (n = 14)	Group 2 Mean ± S.E.M. (n = 14)	Group 3 Mean ± S.E.M. (n = 14)	Group 4 Mean ± S.E.M. (n = 14)
Head length (µm)	148.558 ± 1.921[a]	129.248 ± 2.557[b]	112.274 ± 1.802[b]	128.947 ± 2.441[c]
Tail length (µm)	24.681 ± 1.441[a]	61.716 ± 3.728[b]	61.946 ± 3.012[b]	115.566 ± 7.180[c]
Comet length (µm)	173.238 ± 2.616[a]	190.964 ± 5.706[a]	173.389 ± 4.503[a]	244.247 ± 8.692[b]
Percent head DNA	97.476 ± 0.111[a]	91.222 ± 0.395[b]	88.247 ± 0.437[c]	83.955 ± 0.640[d]
Percent tail DNA	2.521 ± 0.111[a]	8.777 ± 0.395[b]	11.840 ± 0.437[c]	15.592 ± 0.552[d]
Tail moment	0.701 ± 0.070[a]	6.602 ± 0.697[b]	8.166 ± 0.669[b]	20.685 ± 2.113[c]
Olive tail moment	1.627 ± 0.103[a]	6.945 ± 0.487[b]	7.800 ± 0.425[b]	14.380 ± 1.211[c]

Statistical difference is observed between groups labelled by different letters (p<005), whereas no significant difference was observed between groups labelled by the same letter (p>0.05).

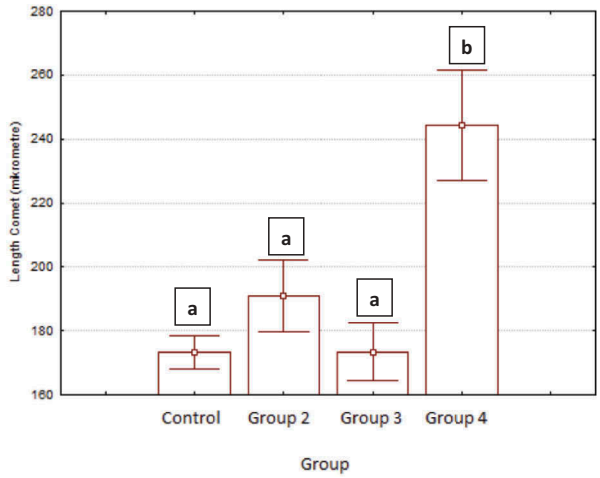

Figure 3. Statistical comparison of comet length. No significant difference was observed in comet length of ear canal hair cells in Group 2 and Group 3 subjects exposed to mobile phone radiation compared to the control group ($p > 0.05$). However, a statistically significant increase was observed between Group 4 and the other groups. Statistical significant difference was observed between groups labeled by different letters ($p < 005$), however, no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

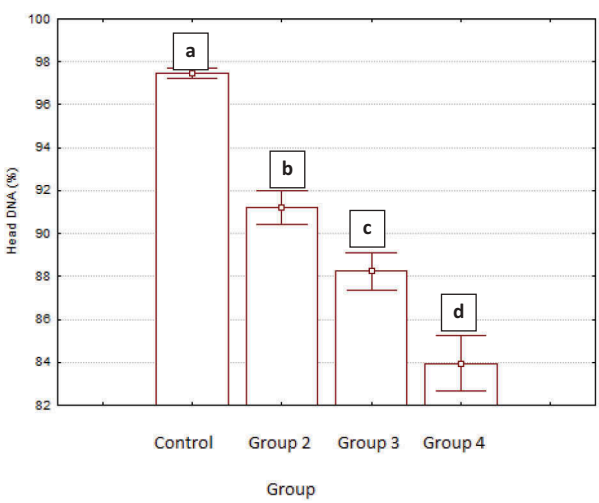

Figure 4. Statistical comparison of the percent head DNA. Percent DNA in the comet head measured in subjects exposed to mobile phone radiation decreased prominently compared to the control group ($p < 0.05$). Statistical significant difference was observed between groups labeled by different letters ($p < 005$), whereas no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

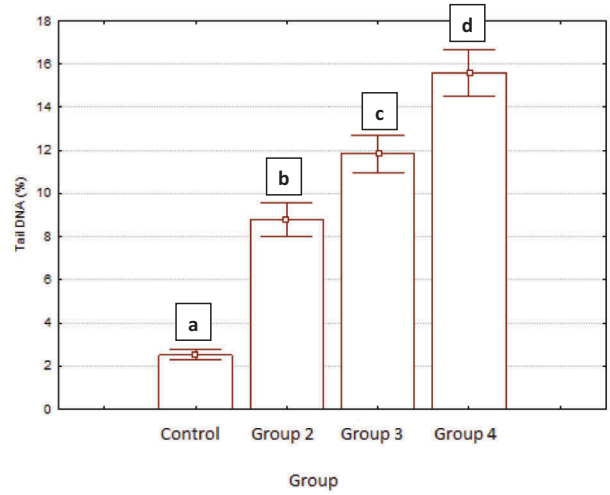

Figure 5. Statistical comparison of the percent tail DNA. Percent tail DNA measured in subjects exposed to mobile phone radiation increased prominently compared to the control group ($p < 0.05$). A linearly correlation was observed between the two factors. Statistical significant difference was observed between groups labeled by different letters ($p < 005$), whereas no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

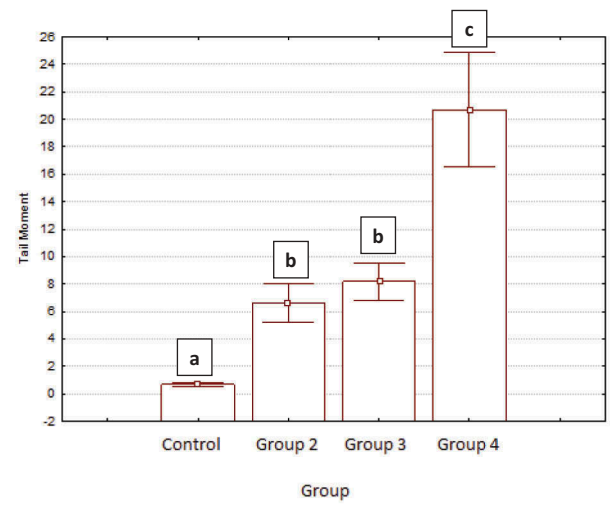

Figure 6. Statistical comparison of Tail moment. Tail moment measured in the subjects who were exposed to mobile phone radiation was found to be significantly higher than the control group (p < 0.05). The most prominent increase was observed in Group 4, which had the longest duration of daily exposure. Statistical significant difference was observed between groups labeled by different letters (p < 005), whereas no significant difference was observed between groups labeled by the same letter (p > 0.05).

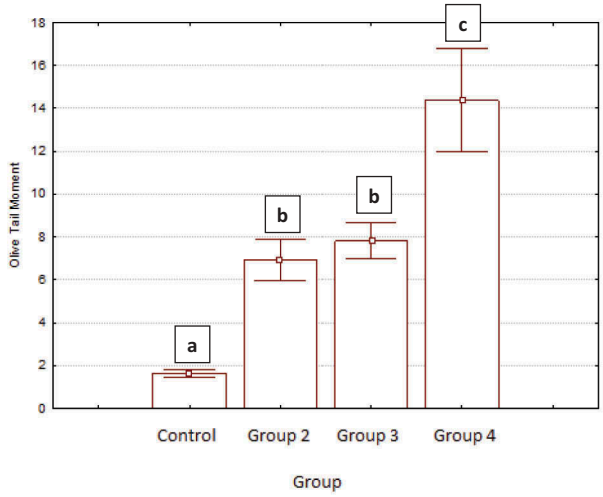

Figure 7. Olive tail moments (OTMs) measured in subjects exposed to mobile phone radiation were found to be significantly higher than the control group ($p < 0.05$). The most prominent increase was observed in Group 4 having the longest daily duration of exposure. Statistical significant difference was observed between groups labeled by different letters ($p < 005$), whereas no significant difference was observed between groups labeled by the same letter ($p > 0.05$).

prominent increase was observed in Group 4 that had the longest daily exposure to the radiation (Figure 7; Table 1).

Discussion

Theoretically, the energy of RFR used in mobile phone systems is insufficient to break the bonds between biomolecules. However, it is suggested that the radiation may cause damages in biological systems, DNA in particular, in various ways (Lai & Singh, 1995; 1996; Lai & Singh, 1997a; Lai, 2012; Akdag et al., 2016). The biological effects which are dependent on exposure to RFR from mobile phones vary according to the type of phone (smart phone or not), position of phone antenna, and the type of the tissue being exposed to (Cardis et al., 2008).

Genotoxic and non-genotoxic effects of RFR emitted from mobile phones and base stations have been shown in *in vitro* situations. The genotoxic effects are transformative alterations like DNA strand breaks, micronuclei formation, mutation, DNA damage, etc. (Challis, 2005). And the non-genotoxic effects cover cell proliferation, DNA synthesis, ion channels, cell differentiation, apoptosis, immune system, reactive oxygen species, signal transfer, and gene expressions that include mRNA and proteins. Other studies are *in vivo* animal experimentations and the number of human experiments is limited. And human studies are mostly epidemiologic studies. There are some retrospective studies that suggest that there is a relation between RFR exposure and diseases such as cancer-related deaths (brain tumors, childhood leukemia and lymphoma, breast cancer, and melanoma), spontaneous abortion, and Alzheimer's disease (Challis, 2005). There are also studies that correlate RFR exposure with physiological and psychological problems (obesity, headache, sleeping disorders, anxiety, brain waves and electrocardiogram), melatonin, and immune system changes (Miyakoshi, 2013). The fact that the majority of the above-mentioned studies are animal experimentation makes a limited contribution as to the assessment of the effects with regards to humans. This is why the present study sought to determine whether RFR produces DNA damage in follicle cells of hair in the ear canal in humans.

There are studies that suggest that RFR produces DNA damage, however, there are also other studies that suggest exactly the opposite. As mobile phones today are extensively used, the number of studies suggesting a possible relation between RFR exposure and brain tumors has been increasing (Carlberg & Hardel, 2017; Hardel & Carlberg, 2015).

Concerns about harms of mobile phone radiation have led scientists to conduct intensive research on organs like ear and brain which absorb the majority of the radiation. Some studies suggest exposure to RFR from mobile phones can increase incidence of cancer and DNA damage in brain cells (Kesari et al., 2013). When these damages reach to an unrepairable level or they are not repaired in the right way, such situations might lead to mutation and apoptosis. It is estimated that each cell in the human body is subjected to around 2×10^4 DNA damages per day (Madabhushi et al., 2014). It is suggested that some DNA bases which have undergone alterations might be harmful in terms of the genome integrity. Phillips et al. (2009) reported that DNA is continuously damaged by endogenous and exogenous factors, but is repaired by DNA repair enzymes. They also stated if the balance of damage and repair was lost, it would lead to accumulation of DNA damage and this accumulation might eventually lead to cell death, aging and cancer

In fact, Diem et al. (2005) observed in a study that a 16 and 24 h of constant and pulsed RF exposure caused genotoxic effect. Liu et al. (2013), in their study, examined the effects of RF exposure on GC-2 cells, a mouse spermatocyte-derived cell line, with 1800 MHz frequency at 1, 2, or 4 W/kg SAR in the talking mode on the phone for 5 and 10 min per day. It was determined that DNA migration extension was affected at 4 W/kg. And it was observed that the same SAR (4 W/kg) increased the level of 8-oxoguanine. Furthermore, along with these increases, an increase in reactive oxygen species was also observed. However, they also found that there was no break in DNA plasmid, therefore RFR did not have any direct genotoxic effects on

DNA, but it might lead to oxidative damage on DNA (Liu et al., 2013). Dasdag et al. (2015) showed in their study that one-year exposure to a 2.4-GHz (Wi-Fi) RFR might cause changes in testis and sperms. Dasdag et al. (2015a) also indicated that 2.4-GHz (Wi-Fi) RFR exposure might change expression of some miRNAs. Another article by Dasdag and Akdag (2016) reported that RFR from wireless communication devices led to oxidative stress. The same article emphasizes that human experimentations should be conducted to shed light on the issue. Gandhi et al. (2015) examined the leucocytes in individuals living in close proximity to base stations to determine the genetic effects of RFR exposure. They took blood samples from 63 individuals living within 300 meters distance to a base station, and the samples of DNA were examined by using the comet assay. The study assessed DNA migration length, damage frequency, and damage indexes. It was observed that those living in close proximity to base stations had a significant increase in their DNA migration length, damage frequency, and damage indexes ($p = 0.000$). It was observed that females had a higher level of damage than males ($p = 0.004$). As a result, the study determined that those living in a distance of 300 m to a base station had more DNA damage ($p = 0.000$). Also, it was found that the duration of mobile phone usage, distance of residence and the intensity of migration played a role in terms of the actual damage (Gandhi et al., 2015).

The results of data in the study by Gandhi et al. (2015) support the results of our present study, because our study, too, suggests that there is a linear relationship between exposure time and DNA damage. Garajvrhovac et al. (1992) observed chromosomal aberrations (dicentric, ring, and polycentric chromosomes) in peripheral blood leucocytes and increases in micronuclei of those who work with radar systems (10–50 mW/cm^2) (Garajvrhovac et al., 1992). The results of their study support also those of our study. Gandhi et al. (2015) observed that chromosome aberrations increased in those who have used mobile phones for a period of 2 years with 4–5 h per day, and that these changes increased even further in those who smoke and drink (Gandhi & Singh, 2005). The findings of the study by Gandhi et al. (2015) are also similar to our findings.

In spite of the above-mentioned studies, there are also other studies that indicated exposure to RFR did not lead to any health problems. For instance, the study on human leucocyte cell culture by Zeni et al. (2008) suggests that RFR exposure at the safety limits caused no cytotoxic or genotoxic effect at particular stages of the cell cycle (Zeni et al., 2008).

The results of the study by Zeni et al. (2008) are different from those of our study. We consider that the difference was caused by the use of different biological materials. In our study, the distance to RFR source was almost zero, in other words, mobile phones are generally at a touching distance to the ear that the exposure of ear hairs is much higher compared to the exposure in the study with the cell culture.

Only 3 studies out of 16, which were examined in a review study published by Verschaeve (2009) indicated that RFR caused no DNA damage, whereas 13 other studies in the same review study reported contrary results, in other words, those studies reported that there was a relationship between RFR and DNA damage. Therefore, the results of those 13 studies also support our results (Verschaeve (2009).

Examining the effects of 1.8-GHz (3 W/kg) RFR on the epithelial cells of human eye lens, the study by Lixia et al. (2006) showed that RFR causes DNA breaks. The group of researchers stated that those results actually emerged in the first 30 min of exposure. Considering this duration of exposure, it is understood that the findings are similar to those of our study, because we found statistically that a mobile phone usage by our experimental groups for 30 min per day produced DNA damage in follicle cells of ear hairs.

Kesari et al. (2014) asserted that the RFR from 3G (2100 MHz) mobile phones triggered large scale DNA strand breaks in the brain. Investigating whether or not an 1800-MHz RFR caused DNA damage in human lens epithelial cells, the study by Yao et al. (2008) showed 1800-MHz (3 and 4 W/kg) RFR exposure triggered DNA damage in epithelial cells of lens in the human eye. The results of Yao et al. (2008) supported our results.

Esmekaya et al. (2011) state that 1800 MHz RFR exposure for 6, 8, 24, and 48 h produced cellular damages in nucleus, chromatin, and mitochondrial cristae alterations, and morphological changes in human lymphocytes. Deshmuck et al. (2013) examined the effects of 900, 1800, and 2450 MHz RFR exposure for 30 days and reported that RFR produced DNA strand breaks in brain tissues of rats. Nikolova et al. (2005) reported a short period of 6 hrs of RF exposure caused a temporary increase in double strand breaks. As can be seen, even a short term RFR exposure can cause DNA breaks.

It is clear that the results of the both studies above supported our findings. In spite of all these findings, the mechanisms of RFR by which causing DNA damage have not been understood completely. Regarding the damages, various mechanisms are suggested. One of the suggested mechanisms is thermal effects. Indeed, Tahvanainen et al. (2007) reported that a 35-min exposure to mobile phone radiation (900 MHz or 1800 MHz), at maximal allowed

antenna powers, could increase the temperature in the ear canal by 1.2 – 1.3 °C. Skin temperature underneath a cell phone in use could reach 2–3°C which is mainly caused by heat conduction from the handset instead of from the radiofrequency energy emitted by the phone (Anderson & Rowley, 2007; Paredi et al., 2001; Straume et al., 2005). However, such an increase in temperature has been shown not to significantly affect DNA strand breaks in cells (Mitchel & Birnboim, 1985). Thus, the increase in DNA strand break in hair follicle cells observed after cell phone use was probably not caused by heating and was likely a 'nonthermal' effect of the radiation. However, we did not collected hair follicles of ear canal immediately after exposure. Therefore, the results of our study indicate directly non thermal effects of mobile phones. In some studies also stated that the effects of RFR on DNA arised from non-thermal effects and that those effects might lead to structural and functional changes in proteins (Güler et al., 2016; Ongel et al., 2009). These researchers suggested that RFR is a type of energy and may change the protein-receptor interaction, thus causing the signal transmission to be affected. They also emphasized that changes might emerge as protein conformation due to these reasons. Other researchers stated that the damage was related to free radicals of oxygen (Lai and Singh, 2004; Ongel et al., 2009)

Çam and Seyhan (2012) showed in their study that a short term (15 and 30 min) 900- MHz RFR exposure from mobile phones caused DNA single strand breaks in the roots of hairs around the ears. Diem et al. (2005) and Paulraj and Behari (2006) reported in their studies that chronic RFR exposure caused increases in DNA strand breaks in brain cells of the rat. On the contrary, Stronati et al. (2006) showed in an *in vitro* study that exposure to a 935-MHz RFR at SAR of 1 or 2 W/kg for 24 h did not cause any DNA strand breaks in human lymphocytes. A series of studies conducted with human lymphocytes cultured for various time periods reported that no chromosome aberration, micronuclei, or sister chromatid change was observed after exposure with a 900-MHz RFR (SAR 0.2–10 W/kg) (Nikolova et al., 2005; Scarfi et al., 2006; Zeni et al., 2003). Verschaeve et al. (2006) reported that long term (2 h/day, 5 days/week, 2 years) exposure to a 900-MHz RFR with 0.3 and 0.9 W/kg SAR did not lead to any significant increase in DNA strand breaks in rats. Takahashi et al. (2002) exposed rats to a 1500-MHz RFR at 2.0, 0.67, or 0 W/kg SAR for 4 weeks with 5 days a week and 90 min a day, and they did not found any mutagenic effect in the brain of the rat. Trosic et al. (2011) stated that a 915-MHz RFR exposure (2.4 W/m^2, 0.6 W/kg; daily 1 h for 2 weeks) caused a prominent increase in DNA tail lengths in the kidney and liver in the rat, but there was no significant effect on their brain cells. Akdag et al. (2016) reported that their long-term (12 months) 2.4-GHz RFR exposure produced significant DNA damage in testicular tissue of rats, however, no significant DNA damage was observed in the brain, kidney, liver, and skin tissues of the rat. As stated in this study, it is observed that different tissues could have different effects from the same RFR exposure.

In this study, we applied the Comet assay to reveal whether or not exposure to radiation from mobile phones leads to DNA damage in follicle cells of hair in the ear canal. Parameters including head length, tail length, comet length, percent head DNA, percent tail DNA, tail moment and OTM were examined, and the results obtained from the three RFR-exposed groups were compared to control group and to one another. It was observed that DNA damages in the three groups with RFR exposure were statistically significant ($p < 0.05$). Our findings are in parallel with those of the studies by Liu et al. (2013); Dasdag and Akdag (2016); Gandhi and Singh (2005), Lixia et al. (2006); Deshmuck et al. (2013); Nikolova et al. (2005); Çam and Seyhan (2012); Diem et al. (2005); Paulraj and Behari (2006), and Akdag et al. (2016). Also, in our study, not some but all parameters of DNA damage measured, which are head length, tail length, comet length, percent head DNA, percent tail DNA, tail moment, and OTM were statistically significant in the exposure groups compared to the control group.

In conclusion, the findings of the present study indicated that exposure to radiation from mobile phones can lead to DNA single-strand breaks, therefore, to DNA damage. The results of this study indicated a positive correlation between duration of exposure and DNA damage. We consider that the result of this study might be important in terms of the balance involved in DNA damage and repair mechanisms.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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