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Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations

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ABSTRACT

Radiofrequency radiations (RFRs) emitted by mobile phone base stations have raised concerns on its adverse impact on humans residing in the vicinity of mobile phone base stations. Therefore, the present study was envisaged to evaluate the effect of RFR on the DNA damage and antioxidant status in cultured human peripheral blood lymphocytes (HPBLs) of individuals residing in the vicinity of mobile phone base stations and comparing it with healthy controls. The study groups matched for various demographic data including age, gender, dietary pattern, smoking habit, alcohol consumption, duration of mobile phone use and average daily mobile phone use. The RF power density of the exposed individuals was significantly higher ($p < 0.0001$) when compared to the control group. The HPBLs were cultured and the DNA damage was assessed by cytokinesis blocked micronucleus (MN) assay in the binucleate lymphocytes. The analyses of data from the exposed group ($n = 40$), residing within a perimeter of 80 m of mobile base stations, showed significantly ($p < 0.0001$) higher frequency of micronuclei when compared to the control group, residing 300 m away from the mobile base station/s. The analysis of various antioxidants in the plasma of exposed individuals revealed a significant attrition in glutathione (GSH) concentration ($p < 0.01$), activities of catalase (CAT) ($p < 0.001$) and superoxide dismutase (SOD) ($p < 0.001$) and rise in lipid peroxidation (LOO) when compared to controls. Multiple linear regression analyses revealed a significant association among reduced GSH concentration ($p < 0.05$), CAT ($p < 0.001$) and SOD ($p < 0.001$) activities and elevated MN frequency ($p < 0.001$) and LOO ($p < 0.001$) with increasing RF power density.

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Antioxidants; genotoxicity; humans; micronucleus; power density

Introduction

The mobile phone base stations are one of the essential parts of mobile telecommunication as they transmit the signals in the form of radiofrequency radiations (RFRs) that are received by the mobile phones, acting as a two-way radio, i.e. transceiver (Kwan-Hoong, 2005), generally operating in the frequency range of 900 MHz to 1.9 GHz (Levitt and Lai, 2010). The ever-increasing subscription of mobile phones has led to a phenomenal increase in the mobile phone base stations required to cater to the needs of increasing demand of the mobile subscribers. For decades, there has been an increasing concern on the possible adverse effects of RFR on humans living near mobile phone base stations despite the fact that RFR spectrum are of low frequency (ARPANSA, 2011). There has been a link between the RFR exposures and several human health disorders including cancer, diabetes, cardiovascular and neurological diseases (Bortkiewicz et al., 2004; Eger et al., 2004; Havas, 2013; Lerchl et al., 2015; Wolf and Wolf, 2004). The International Agency for Research on Cancer (IARC, 2011) has classified RFR as a possible carcinogen to humans (group 2B), based on the increased risk for glioma, a malignant type of brain cancer associated with wireless phone use (Hardell et al., 2013).

RFR may change the fidelity of DNA as the increased incidence of cancer has been reported among those residing near mobile phone base stations (Abdel-Rassonl et al., 2007; Bortkiewicz et al., 2004; Cherry, 2000; Eger et al., 2004; Hardell et al., 1999; Hutter et al., 2006; Wolf and Wolf, 2004). RFR emitted from mobile base stations is also reported to increase the DNA strand breaks in lymphocytes of mobile phone users and individuals residing in the vicinity of a mobile base station/s (Gandhi and Anita, 2005; Gandhi et al., 2014). Exposure of human fibroblasts and rat granulosa cells to RFR (1800 MHz, SAR 1.2 or 2 W/kg) has been reported to induce DNA single- and double-strands breaks (Diem et al., 2005). Irreversible DNA damage was also reported in cultured human lens epithelial cells exposed to microwave generated by mobile phones (Sun et al., 2006). The adverse health effects of RFR are still debatable as many studies indicated above have found a positive correlation between the DNA

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damage and RFR exposure; however, several studies reported no significant effect of RFR on DNA strand breaks and micronuclei formation in different study systems (Li et al., 2001; Tice et al., 2002; McNamee et al., 2003; Maes et al., 2006). The potential genotoxicity of RFR emitted by mobile phone base stations can be determined by micronucleus (MN) assay, which is an effective tool to evaluate the genotoxic or clastogenic effects of physical and chemical agents. This technique has also been used to quantify the frequencies of radiation-induced MN in human peripheral blood lymphocytes (HPBLs) (Fenech and Morley, 1985; Jagetia and Venkatesha, 2005; Prosser et al., 1988; Yildirim et al., 2010).

Besides its effect on DNA damage and association of cancer in individuals living near mobile phone base station, the deep penetration of RFR within the living cells may cause overproduction of free radicals particularly reactive oxygen species (ROS), thereby inducing adverse effects in living cells (Yakymenko et al., 2015). ROS amount is also reported to increase during infections, exercise, exposure to pollutants, UV light, ionizing radiations, etc. (Kunwar and Priyadarsini, 2011). Uncontrolled generations of ROS can lead to their accumulation causing oxidative stress in the cells. Any chronic exposure to conditions that increase the oxidative stress leads to an increased risk of cancer, and elevated levels of cancer have been demonstrated in populations with increased residential exposure to RFR (Dart et al., 2013; IARC, 2011). The change in the activities of antioxidants such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) may be regarded as an indicator of increased oxidative stress (Kerman and Senol, 2012). Since lipid peroxidation (LOO) is a free-radical oxidation product of polysaturated fatty acids, detection and measurement of LOO is the evidence which is frequently cited to support the involvement of free-radical reactions in toxicity and disease progression (Gutteridge, 1995). The increasing use of mobile phones and installation of more mobile base stations stimulated us to obtain an insight into the genotoxic effects of RFR using MN assay and alteration in the antioxidant status in the PBLs of the individuals residing in the vicinity of the mobile phone base stations.

Methods

Chemicals

RPMI-1640 medium, phytohemagglutinin, acridine orange, bovine serum albumin (BSA), GSH reduced, nicotinamide adenosine dinucleotide (NADH), nitrobluetetrazolium (NBT) and *n*-butanol were purchased from HiMedia laboratories Pvt Ltd. (Mumbai, Maharashtra, India). Methanol, acetic acid, Folin–Ciocalteu reagent, potassium tartarate, hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), hydrochloric acid (HCl) and potassium chloride (KCl) were purchased from MERCK (Mumbai, Maharashtra, India). Cytochalasin B, thiobarbaturic acid (TBA) and phenazinemethosulphate (PMS) were purchased from Sigma Aldrich Chemical Co (Bangalore, Karnataka, India) and 5,5′-dithio-2-nitrobenzoic acid (DTNB) was procured from Tokyo Chemical Industry (Tokyo, Japan).

Power density measurement from mobile phone base stations

Six mobile phone base stations, operating in the frequency range of 900 MHz ($N = 2$) and1800 MHz ($N = 4$), erected in the thickly populated areas of Aizawl city were selected for the present study. Both dish and sectored antennas of each base station are arranged equilaterally that provide 360° network coverage. The power output of all the base stations is 20 W, with their primary beam emitting radiation at an angle of 20°. Power density measurements (using HF-60105V4, Germany) were carried out in the bedroom of each participant where they spent most of the time and hence have the longest constant level of electromagnetic field exposure. Power density measurement was carried out three times (morning, midday and evening), and the average was calculated for each residence around each base station. The main purpose of the measurement of power density was to ensure that RFR emission from each site did not exceed the safe public limits and to determine any difference in power density between selected households that were close to (within 80 m) and far (>300 m) from the mobile phone base stations. The safety limits for public exposure from mobile phone base stations are 0.45 W/m^2 for 900 MHz and 0.92 W/m^2 for 1800 MHz frequency as per Department of Telecommunications, Ministry of Communications, Government of India, New Delhi guidelines (DoT, 2012).

Selection of subjects

The study was carried out in Aizawl city (23°43′37.58′′N and 92°43′3.49′′E), Mizoram, India, during 2015 and 2016. Since the city is located in the hilly region, some residences are located horizontally with the top of the towers from which RFR are emitted, making it possible to get an exposure at a short distance of 1–20 m, despite being erected on the rooftop or in the ground. A minimum of two individuals were sampled from each household and at least five individuals were sampled around each mobile base station. Individuals sampled around each base station were matched for their age and gender (Table 1). The exposed group consisted of 40 healthy

Table 1. Composition of base stations and the demographic characteristics of the exposed group.

	Components				Gender of volunteers	
Base station	Disc antenna	Sectored antenna	Power density (mW/m^2)	Average age (years) of volunteers	Male	Female
1	3	10	3.90–6.52	28.8	3	4
2	6	10	5.12–7.32	30.0	3	3
3	3	9	2.80–6.55	28.2	4	4
4	11	6	3.58–7.52	28.9	2	4
5	6	4	4.56–5.43	28.6	3	2
6	6	4	3.58–6.53	27.6	3	5

individuals who fulfilled the inclusion criteria of being above 18 years of age and residing in the vicinity of mobile phone base stations (within 80 m radius). The control group comprised of 40 healthy individuals matched for age and gender who had been living at least 300 m away from any mobile phone base stations. None of the participants have occupational exposure to RFR, and there were no electric transformer, high tension electric power line and radio and television transmitters close to (at least 500 m) their residences. Sampling was also done only from those residences who did not use microwave oven for cooking, Wifi devices and any other major source of electromagnetic field as they are known to cause adverse effects (Atasoy et al., 2013; Avendaño et al., 2012). The study was approved by the Human Ethics Committee, Mizoram University, Aizawl, India, and only those individuals who gave their voluntary written consent were included in the study.

Questionnaire used

A questionnaire was prepared to collect information on demographic data such as family and exposure histories, lifestyle such as smoking habit (≤10 cigarette in a day), alcohol consumption (three to four times a week) and dietary pattern, duration of stay near mobile phone base stations, duration of mobile phone use and average daily mobile phone use.

Blood sample collection and lymphocyte culture

The blood samples were collected by venipuncture from each volunteer of both groups in individual heparinized tubes. The lymphocyte culture was carried out according to the method described earlier (Jagetia et al., 2001). Briefly, the blood was allowed to sediment and the buffy coat containing nucleated cells was collected in individual sterile glass tubes. Usually 10^6 nucleated cells were inoculated into sterile glass tubes containing RPMI-1640 medium, supplemented with 10% fetal calf serum and phytohemagglutinin as the mitogen. The cells were allowed to grow for the next 44 h and cytochalasin B was added at a final concentration of 5 µg/ml to block the cytokinesis (Fenech and Morley, 1985). The cells were harvested at the end of 72 h after initiation of lymphocyte culture by centrifugation. The cell pellet was subjected to mild hypotonic treatment so as to retain the cell membrane and fixed in freshly prepared Carnoy's fixative (methanol: acetic acid, 3:1). The cell suspension was dropped onto precleaned coded slides to avoid observer's bias and stained with acridine orange. Usually a total of 1000 binucleate cells (BNCs) with well-preserved cytoplasm were scored from each individual using a fluorescence microscope (DM 2500, Leica MikrosystemeVertrieb GmbH, Wetzlar, Germany). Scoring of MN frequencies was performed based on the criteria of Fenech et al. (2003).

Biochemical estimations

The antioxidants were measured in the plasma of the study groups. Protein contents were measured by the method of Lowry et al. (1951) using BSA as the standard.

Glutathione

GSH contents were measured using the method given by Moron et al. (1979). Briefly, 80 µl of plasma was mixed with 900 µl of 0.02 M sodium phosphate buffer and 20 µl of 10 mM DTNB and incubated for 2 min at room temperature. The absorbance of the sample was read against blank at 412 nm in a UV-Visible spectrophotometer (SW 3.5.1.0. Biospectrometer, Eppendorf India Ltd., Chennai), and the GSH concentration was calculated from the standard curve and expressed in µmol/mg protein.

Superoxide dismutase

The SOD activity was measured by the method of Fried (1975). Briefly, 100 µl each of plasma and 186 µM PMS were mixed with 300 µl of 3 mM NBT and 200 µl of 780 µM NADH. The mixture was incubated for 90 s at 30°C and 1 ml of acetic acid and 4 ml of *n*-butanol were added to stop the reaction. The blank consisted of all the reagents, and distilled H_2O was added instead of plasma. The absorbance of test and blank was measured at 560 nm using a UV-VIS spectrophotometer, and the

enzyme activity has been expressed in units (1U = 50% inhibition of NBT reduction)/mg protein.

% inhibition = (OD of blank − OD of test/OD of blank) × 100
SOD unit = 1/50 × % inhibition.

Catalase

The CAT activity was determined using the modified protocol of Aebi (1984). Briefly, 200 µl of 3% H_2O_2 was mixed with 50 µl each of plasma and 150 µl of 50 mM phosphate buffer (pH 7.0). The absorbance was recorded at 240 nm in a UV-VIS spectrophotometer. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance. The enzyme activity has been expressed in units/mg protein. The catalytic activity of CAT at a time interval of 15 s was calculated by the following formula,

$$K = 0.153\,(\log A_0/A_1)$$

where A_0 is the absorbance at 0 s and A_1 is the absorbance at 15 s.

Lipid peroxidation

The LOO was estimated by the method of Beuege and Aust (1978). Briefly, plasma was mixed with 10% TCA, 0.8% TBA and 0.025 N HCl in a 1:2 ratio. The mixture was boiled for 10 min in a boiling water bath. After centrifugation, the absorbance of the supernatant was recorded at 540 nm UV-VIS spectrophotometer.

Statistical analyses

The data are expressed as mean ± standard error of the mean. Student's "*t*" and Chi-square tests were used for comparison of demographic variables of the exposed and control groups. Pearson`s correlation analysis was performed to determine the relationship between power density and the distance of residences from the base stations. Mann Whitney *U* test was applied to determine the significance between the control and exposed group for MN frequencies. Student's "*t*" test was performed to determine the significance between the groups for antioxidants. Multiple linear regression analyses were carried out for the prediction of MN frequency and antioxidants status separately from the demographic characteristics. SPSS Ver.16.0 software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. A *p*-value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics of both exposed and control groups are depicted in Table 2. The groups matched for most of the demographic data such as age, gender, dietary pattern, smoking habit, alcohol consumption, mobile phone usage, duration of mobile phone use and average daily mobile phone use (Table 2). A highly significant variation ($p < 0.0001$) was observed for the distance of household from the base station (40.10 ± 3.02 vs. 403.17 ± 7.98 in m) between exposed and control groups. The data of RF

Table 2. Demographic data of the exposed and control groups.

		Exposed group		Control group			*p*-value
Characteristics	Category	*N* (%)	M±SEM	*N* (%)	M±SEM	t/χ^2-value	(t/χ^2-value)
Age (years)	20–30	26 (65)	28.6 ± 0.85	29 (72.5)	28.6 ± 0.85	1.074/–	0.286/–
	31–40	14 (35)		11 (27.5)			
Gender	Male	18 (45)		21 (52.5)		–/0.450	–/0.502
	Female	22 (55)		19 (47.5)			
Diet	Vegetarian	5 (12.5)		7 (17.5)		–/0.392	–/0.531
	Nonvegetarian	35 (87.5)		33 (82.5)			
Smoking habit	Yes	16 (40)		14 (35)		–/0.213	–/0.644
	No	24 (60)		26 (65)			
Alcohol consumption	Yes	7 (17.5)		9 (22.5)		–/0.312	–/0.576
	No	33 (82.5)		31 (77.5)			
Mobile phone usage	User	37 (92.5)		35 (87.5)		–/0.556	–/0.456
	Nonuser	3 (7.5)		5 (12.5)			
Duration of mobile phone use (years)	≤5	9 (24.32)	6.32 ± 0.265	11 (31.42)	5.91 ± 0.296	1.032/–	0.306/–
	>5	28 (75.68)		24 (68.58)			
Daily mobile phone use (hours)	≤3	24 (64.86)	3.054 ± 0.229	25 (71.42)	2.800 ± 0.156	1.145/–	0.256/–
	>3	13 (35.13)		10 (28.58)			
Distance from the base station (m)	1–20	8 (20)	40.10 ± 3.02		403.17 ± 7.98	42.046/–	0.0001/–
	21–40	12 (30)					
	41–60	13 (32.5)					
	61–80	7 (17.5)					
Power density (mW/m^2)	Range	2.80–7.52	5.002 ± 0.182	0.014–0.065	0.035 ± 0.002	27.247/–	0.0001/–
Duration of residing near the base station (years)	5–10	33 (82.5)	7.85 ± 0.419	–	–	–	–
	11–15	7 (17.5)					

power density were collected from 23 houses, each of the exposed group staying within a perimeter of 80 m and those of control group staying at least 300 m away from mobile phone base stations. The RF power density of the exposed group (2.80–7.52 mW/m^2; average 5.002 ± 0.182 mW/m^2) was significantly higher ($p < 0.0001$) when compared to the control group (0.014–0.065 mW/m^2; average 0.035 ± 0.002 mW/m^2). The highest power density was recorded at a distance of 1–20 m (6.44 ± 0.31 mW/m^2), which is significantly higher ($p < 0.0001$) than those at a distance of 21–40 m (4.79 ± 0.33), 41–60 m (4.48 ± 0.22) and 61–80 m (4.61 ± 0.10). No significant variation was observed for the RFR power density among the distance ranges of 21–40 m, 41–60 m and 61–80 m (Table 1). Nevertheless, there was a highly significant negative correlation between distance from the base station and the power density ($r = -0.509$, $p < 0.0001$).

The MN frequency and LOO were significantly ($p < 0.0001$ for MN and LOO) higher in the exposed group as compared to that of control group, while antioxidants were significantly ($p < 0.01$ for GSH; $p < 0.001$ for CAT and SOD) lower for the exposed group compared to controls irrespective of their demographic characteristics (Tables 3 and 4). On consideration of the demographic characteristics, smokers had significantly higher MN frequency ($p < 0.001$) and LOO ($p < 0.01$) and significantly lower GSH ($p < 0.01$) and SOD ($p < 0.01$) than nonsmokers within each study group. Similarly, alcoholics compared to nonalcoholics had significantly higher MN frequency ($p < 0.01$) and significantly lower GSH ($p < 0.01$) within the exposed group and significantly higher MN frequency ($p < 0.001$) and LOO ($p < 0.01$) within the control group. The smokers of the exposed group had significantly higher MN frequency ($p < 0.001$) and LOO ($p < 0.01$) and significantly lower CAT ($p < 0.001$) and SOD ($p < 0.05$) activities than the smokers of control group. Alcoholic among exposed group also had significantly higher MN frequency ($p < 0.05$) and significantly lower GSH ($p < 0.05$) concentration and CAT ($p < 0.01$) and SOD ($p < 0.05$) activities than the alcoholic of control group. MN frequency and antioxidant status with LOO showed no significant variations between the ages, genders and dietary pattern within the exposed group. Among controls, males compared to females had significantly ($p < 0.05$) higher MN frequency (Table 3).

There was no significant variation in the MN frequency and antioxidant status between mobile phone user and nonuser of exposed group, while individuals who have been using mobile phone for more than 5 years had significantly higher MN frequency ($p < 0.01$) and lower GSH ($p < 0.05$) than those using for less than 5 years. Similarly, exposed group with average daily mobile phone use of above 3 h showed a higher MN frequency ($p < 0.05$) than those having the average daily use of less than 3 h (Table 4). Among the control group, features of mobile phone usage showed no variation in MN frequency and antioxidant status. Significantly lower levels of antioxidants ($p < 0.05$ for GSH; $p < 0.001$ for CAT; $p < 0.01$ for SOD) and higher

Table 3. Function of the demographic characteristics on MN frequencies and the antioxidant status of exposed and control groups.

				GSH	CAT	SOD	LOO	MN/1000 BNC
	Characteristics	Category	*N*	(M±SEM)	(M±SEM)	(M±SEM)	(M±SEM)	(M±SEM)
EXPOSED GROUP	Age (years)	20–30	26	4.604 ± 2.68**	0.022 ± 0.001***	1.832 ± 0.11***	0.646 ± 0.064***	38.15 ± 1.65**
		31–40	14	3.882 ± 2.09	0.021 ± 0.001***	1.791 ± 0.11**	0.755 ± 0.101*	43.71 ± 2.64**
		Total	40	4.351 ± 1.95**	0.021 ± 0.001***	1.823 ± 0.08***	0.677 ± 0.054***	40.10 ± 1.46***
	Gender	Male	18	4.209 ± 3.08*	0.020 ± 0.001***	1.802 ± 0.12**	0.667 ± 0.072**	40.77 ± 2.71*
		Female	22	4.467 ± 2.54	0.023 ± 0.001***	1.834 ± 0.11***	0.686 ± 0.080**	39.54 ± 1.51***
	Dietary pattern	Vegetarian	5	4.360 ± 4.26*	0.019 ± 0.001**	1.913 ± 0.18**	0.650 ± 0.040***	40.20 ± 2.87***
		Nonvegetarian	35	4.350 ± 2.17*	0.022 ± 0.001***	1.807 ± 0.09***	0.682 ± 0.053***	40.08 ± 1.63***
	Smoking habit	Yes	16	3.713 ± 2.28[a]	0.022 ± 0.001***	1.645 ± 0.11*	0.892 ± 0.102[a]**	46.50 ± 1.65[a]***
		No	24	4.777 ± 2.56**	0.021 ± 0.001***	1.932 ± 0.11***	0.535 ± 0.039**	35.83 ± 1.69***
	Alcohol consumption	Yes	7	3.394 ± 2.35[a]*	0.021 ± 0.001**	1.792 ± 0.22*	0.683 ± 0.119	49.71 ± 3.12[a]*
		No	33	4.554 ± 2.16*	0.022 ± 0.001***	1.823 ± 0.08***	0.676 ± 0.061**	38.27 ± 1.47***
CONTROL GROUP	Age (years)	20–30	29	5.380 ± 1.54	0.038 ± 0.001	2.534 ± 0.09	0.389 ± 0.037	31.89 ± 1.64
		31–40	11	4.023 ± 3.82	0.036 ± 0,002	2.492 ± 0.21	0.482 ± 0.062	35.09 ± 1.96
		Total	40	5.007 ± 1.79	0.037 ± 0.001	2.526 ± 0.09	0.415 ± 0.032	32.77 ± 1.31
	Gender	Male	21	5.067 ± 2.70	0.038 ± 0.002	2.434 ± 0.11	0.385 ± 0.049	35.23 ± 1.99[a]
		Female	19	4.940 ± 2.38	0.037 ± 0.001	2.622 ± 0.14	0.447 ± 0.040	30.05 ± 1.49
	Dietary pattern	Vegetarian	7	5.473 ± 2.53	0.039 ± 0.003	2.845 ± 0.17	0.378 ± 0.066	29.85 ± 1.95
		Nonvegetarian	33	4.908 ± 1.08	0.037 ± 0.001	2.453 ± 0.10	0.423 ± 0.038	33.39 ± 1.52
	Smoking habit	Yes	14	3.996 ± 2.66[a]	0.036 ± 0.002	2.181 ± 0.17[a]	0.522 ± 0.055[a]	39.78 ± 1.70[a]
		No	26	5.551 ± 1.53	0.040 ± 0.001	2.717 ± 0.08	0.356 ± 0.036	29.00 ± 1.30
	Alcohol consumption	Yes	9	4.416 ± 2.91	0.036 ± 0.002	2.212 ± 0.23	0.546 ± 0.073[a]	42.44 ± 2.29[a]
		No	31	5.178 ± 2.07	0.038 ± 0.001	2.616 ± 0.09	0.376 ± 0.033	29.96 ± 1.15

*Significant ($p \leq 0.05$) between the exposed and control groups.
**Highly significant ($p \leq 0.01$) between the exposed and control groups.
***Very highly significant ($p \leq 0.001$) between the exposed and control groups.
[a]Significant ($p \leq 0.05$) along the demographic characteristics within group.

Table 4. Function of mobile phone usage and residence near base stations on MN frequencies and antioxidants status on exposed and control groups.

				GSH	CAT	SOD	LOO	MN/1000 BNC
	Characteristics	Category	*N*	(M±SEM)	(M±SEM)	(M±SEM)	(M±SEM)	(M±SEM)
EXPOSED GROUP	Mobile phone usage	User	37	4.336 ± 2.07**	0.020 ± 0.002***	1.852 ± 0.08***	0.66 ± 0.051***	40.21 ± 1.55***
		Nonuser	3	4.534 ± 6.04	0.022 ± 0.001***	1.394 ± 0.10*	0.890 ± 0.205*	38.66± 1.37**
	Duration of mobile phone use (years)	≤5	9	5.006 ± 3.26[a]	0.023 ± 0.002**	1.834 ± 0.23**	0.673 ± 0.109*	34.77 ±3.23[a]
		>5	28	4.145 ± 2.24**	0.021 ± 0.001***	1.863 ± 0.08***	0.656 ± 0.058**	41.96 ±1.66***
	Daily mobile phone use (hours)	≤3	24	4.410 ± 1.26*	0.023 ± 0.001***	1.902 ± 0.11***	0.653 ± 0.068**	37.87 ±1.99[a*]
		>3	13	4.233 ± 1.73*	0.020 ± 0.001***	1.765 ± 0.13***	0.674 ± 0.073**	44.53 ±2.02***
	Distance from the base station (m)	1–20	8	3.884 ± 2.20**	0.018 ± 0.002***	1.654 ± 0.18***	0.720 ± 0.154**	43.00 ± 3.94**
		21–40	12	4.174 ± 3.72*	0.020 ± 0.001***	1.762 ± 0.13***	0.674 ± 0.106**	41.69 ± 2.49**
		41–60	13	4.692 ± 3.23	0.022 ± 0.001***	1.903 ± 0.15**	0.600 ± 0.069*	39.00 ± 1.24*
		61–80	7	4.631 ± 6.44	0.025 ± 0.002**	2.016 ± 0.17*	0.494 ± 0.084	36.71 ± 2.57
	Duration of residence near the base station (years)	5–10	33	4.406 ± 2.25*	0.024 ± 0.001***	1.872 ± 0.08**	0.642 ± 0.055***	40.03 ± 3.13**
		11–15	7	4.092 ± 2.54*	0.021 ± 0.001***	1.814 ± 0.12**	0.781 ± 0.170***	40.42 ± 1.66**
	Power density (mW/m^2)	≤4 mW/m^2	7	4.554 ± 2.22*	0.025 ± 0.002**	1.915 ± 0.16*	0.660 ± 0.122**	39.14 ±0.21*
		>4 mW/m^2	33	4.308 ± 2.32**	0.021 ± 0.001***	1.807 ± 0.09***	0.681 ± 0.061***	40.30 ± 1.59***
CONTROL GROUP	Mobile phone usage	User	35	5.145 ± 1.86	0.037 ± 0.001	2.550 ± 0.09	0.417 ± 0.035	32.28 ± 1.40
		Nonuser	5	4.038 ± 4.21	0.041 ± 0.004	2.282 ± 0.25	0.456 ± 0.022	31.80± 1.22
	Duration of mobile phone use (years)	≤5	11	5.528 ± 2.24	0.036 ± 0.003	2.553 ± 0.10	0.372 ± 0.062	31.09 ± 1.88
		>5	24	5.039 ± 2.31	0.037 ± 0.001	2.568 ± 0.13	0.438 ± 0.043	32.83 ± 1.87
	Daily mobile phone use (hours)	≤3	25	5.258 ± 1.99	0.038 ± 0.001	2.524 ± 0.11	0.436 ± 0.041	30.10± 2.46
		>3	10	5.027 ± 3.75	0.036 ± 0.001	2.655 ± 0.19	0.371 ± 0.070	33.16 ± 1.70

*Significant ($p \leq 0.05$) between the exposed and control groups.
**Highly significant ($p \leq 0.01$) between the exposed and control groups.
***Very highly significant ($p \leq 0.001$) between the exposed and control groups.
[a]Significant ($p \leq 0.05$) along the demographic characteristics within group.

MN frequency ($p < 0.001$) and LOO ($p < 0.001$) were observed in the exposed group residing in the vicinity of the base stations for 5–10 years and 11–15 years when compared to the control group. None of the parameters showed a significant variation among the exposed group residing for 5–10 years and 11–15 years in the vicinity of the base stations (Table 4).

As a function of distance from the base stations, MN frequency and LOO within the distance of 1–20 m ($p < 0.01$ for MN and LOO), 21–40 m ($p < 0.01$ for MN and LOO) and 41–60 m ($p < 0.05$ for MN and LOO) were significantly higher in the exposed group than that of the control group. There were no significant variation in MN frequency and LOO between the exposed group residing within 61–80 m away from mobile stations and the control group. GSH, CAT and SOD were significantly lower in the exposed group residing within a distance range of 1–20 m ($p < 0.01$ for GSH; $p < 0.001$ for CAT; $p < 0.001$ for SOD), 21–40 m ($p < 0.05$ for GSH; $p < 0.001$ for CAT; $p < 0.001$ for SOD), 41–60 m ($p < 0.001$ for CAT; $p < 0.01$ for SOD) and 61–80 m ($p < 0.01$ for CAT; $p < 0.05$ for SOD) than individuals residing at least 300 m away from the base stations. However, GSH contents did not differ between the exposed group residing between 41 and 80 m from the base stations and controls (Table 4). The individuals exposed to a power density of ≤4 mW/m^2 and >4 mW/m^2 showed a higher MN frequency ($p < 0.05$ for ≤4 mW/m^2; $p < 0.001$ for >4 mW/m^2) and LOO ($p < 0.01$ for ≤4 mW/m^2; $p < 0.001$ for >4 mW/m^2) and lower GSH ($p < 0.05$ for ≤4 mW/m^2; $p < 0.01$ for >4 mW/m^2), CAT ($p < 0.01$ for ≤4mW/m^2; $p < 0.001$ for >4 mW/m^2) and SOD ($p < 0.05$ for ≤4 mW/m^2; $p < 0.001$ for >4 mW/m^2) (Table 4).

Multiple linear regression analyses revealed a significant association with low GSH concentration and age ($p < 0.05$), smoking habit ($p < 0.001$), daily mobile phone use ($p < 0.05$) and increasing power density ($p < 0.05$). A similar association has been reported with reduced CAT activity with increasing power density ($p < 0.001$) and alleviated SOD activity with smoking habit ($p < 0.05$) and increasing power density ($p < 0.001$) (Table 5). The analyses also showed a significant relationship between higher MN frequency with smoking habit ($p < 0.001$) and increasing power density ($p < 0.001$) and higher LOO with smoking habit ($p < 0.001$), alcohol consumption ($p < 0.05$) and increasing power density ($p < 0.001$) (Table 5). The parameter of mobile phone usage was not included in the multiple linear regression analysis due to multicollinearity with the duration of mobile phone use and average daily mobile phone use. Similarly, distance from the base stations showed multicollinearity with power density in the preliminary analysis; therefore, the former is also excluded in the multiple linear regression analysis.

Discussion

Mobile phone base stations have become an integral part of telecommunication, which use RFR to transmit the signals. These electromagnetic waves are generated by

Table 5. Multiple linear regression in the exposed and control groups.

	Characteristics	Durbin–Watson	Model-*F*	*B*-value	*t*-value	*p*-value
GSH	Age	2.22	6.62***	−0.24	−2.10	**0.043**
	Gender			0.11	1.09	0.283
	Dietary pattern			−0.10	−0.99	0.328
	Smoking habit			0.44	−3.86	**0.001**
	Alcohol consumption			−0.06	−0.47	0.640
	Duration of mobile phone use			−0.09	−0.69	0.492
	Daily mobile phone use			0.22	2.06	**0.039**
	Power density			−0.18	−1.97	**0.041**
CAT	Age	2.10	11.19***	−0.09	−0.94	0.352
	Gender			0.03	0.29	0.774
	Dietary pattern			0.01	0.12	0.907
	Smoking habit			−0.01	−0.07	0.950
	Alcohol consumption			0.03	0.29	0.771
	Duration of mobile phone use			0.01	0.08	0.944
	Daily mobile phone use			−0.07	−0.77	0.447
	Power density			−0.72	−8.93	**0.001**
SOD	Age	2.23	4.94***	0.01	0.11	0.911
	Gender			0.00	0.01	0.993
	Dietary pattern			−0.12	−1.22	0.237
	Smoking habit			−0.32	−2.70	**0.012**
	Alcohol consumption			0.01	0.10	0.923
	Duration of mobile phone use			0.11	0.81	0.426
	Daily mobile phone use			−0.07	−0.61	0.551
	Power density			−0.46	−4.74	**0.001**
LOO	Age	1.82	6.53***	0.22	1.96	0.052
	Gender			−0.13	−1.30	0.208
	Dietary pattern			0.11	1.13	0.262
	Smoking habit			0.47	4.12	**0.001**
	Alcohol consumption			−0.15	−1.25	0.210
	Duration of mobile phone use			−0.01	−0.05	0.965
	Daily mobile phone use			0.02	0.15	0.886
	Power density			0.37	3.99	**0.001**
MN	Age	2.17	11.10***	0.09	0.87	0.390
	Gender			−0.05	−0.58	0.572
	Dietary pattern			0.03	0.38	0.718
	Smoking habit			0.44	4.41	**0.001**
	Alcohol consumption			0.28	2.62	**0.013**
	Duration of mobile phone use			−0.04	−0.34	0.733
	Daily mobile phone use			0.06	0.58	0.562
	Power density			0.36	4.45	**0.001**

Values in bold are significant ($p < 0.05$).

electric charges that are rapidly accelerated to and fro in the transmitting antenna. Although RFR are nonionizing electromagnetic radiations, yet there has been a great concern about their deleterious effects on the human body as it is assumed that RFR could produce some of the biological effects akin to those produced by ionizing radiations such as X or γ-rays. Because of its adverse health effects reported worldwide, the presence of mobile base stations in the residential areas could be an electromagnetic threat, which is silently creeping in the lives of residents staying near the mobile base stations. We have therefore attempted to obtain an insight into the adverse effects of RFR in the inhabitants residing in the vicinity (within 80 m) of mobile base stations emitting RFR for mobile connectivity.

The frequency of nonspecific health symptoms such as nausea, loss of appetite, visual disturbance, irritability and depression were found to be significantly higher in the population living close (within 100 m) to mobile phone base stations as compared to those living away from these stations (Santini et al., 2002, 2003). Besides the nonspecific health symptoms of fatigue, headache, dizziness and muscle pain self-reported by the volunteers in the earlier study (Pachuau et al., 2015), the present study showed a significant increase in MN frequency and decreased antioxidants among inhabitants residing close to the base station/s when compared to controls. A number of studies have reported an increase in the DNA damage/micronuclei in different study systems. The human PBLs exposed to RFR have shown an increased frequency of micronuclei earlier (d'Ambrosio et al., 2002; Garaj-Vrhovac et al., 1992; El-Abd and Eltoweissy, 2012; Tice et al., 2002; Zotti-Martelli et al., 2000). Various studies conducted in other systems have also revealed an increased micronuclei frequency after exposure to RFR (Balode, 1996; Busljeta et al., 2004; Gandhi and Singh, 2005; Trosic et al., 2002, 2004). Our results are in agreement with a recent study where buccal mucosa cells showed increased micronuclei in mobile phone users (Banerjee et al., 2016). However, some of the studies did not find any increase in the MN frequency after RFR exposure both *in vitro* and *in vivo* (Bisht et al., 2002; Scarfi et al., 2006; Vijayalaxmi et al., 1997, 1999, 2001; Zeni et al., 2003, 2008), and such reports emphasized on the lack of thermal effects from RFR

(Vijaylaxmi and Obe, 2004), whereas the observed effect in the present study may be due to the interaction of RFR with various cellular macromolecules by producing ROS. This contention is supported by the fact that RFR-exposed individuals showed increased LOO and alleviated GSH contents, CAT and SOD activities in the present study. A similar effect has been observed earlier in the CAT activity in the rats exposed to low level of RFR (Achudume et al., 2010). Also, RFR emitted from cell phones led to oxidative stress in human semen (Agarwal et al., 2009). RFR (2.45 GHz) has been reported to cause a significant increase in the LOO of exposed Wistar rats (Aweda et al., 2003). The present study also revealed the induction of LOO by RF radiation, which could possibly react with DNA and produce lesions in it. The increased LOO has been reported in the plasma of rats with a decline in GSH and other antioxidants earlier (Aydin and Akar, 2011).

The highest measured power density was 7.52 mW/m^2. Most of the measured values close to base stations (Table 1) are higher than that of the safe limits recommended by Bioinitive Report 2012 (0.5 mW/m^2), Salzburg resolution 2000 (1 mW/m^2) and EU (STOA) 2001 (0.1 mW/m^2). However, all the recorded values were well below the current ICNIRP safe level (4700 mW/m^2) and the current Indian Standard (450 mW/m^2). Although cigarette smoking increased the MN frequency and decreased the antioxidants, the statistical analysis also revealed a close correlation between the power density and MN frequency and antioxidant status. Thus, the effects of RF radiation cannot be ignored as unrepaired DNA damage and oxidative stress are associated with several diseases such as cancer and several age-related diseases (Bernstein et al., 2013; Dart et al., 2013). The persistence of low level of DNA damage could have negative effect on human health.

The exact mechanism of action of RFR in micronuclei induction and reduced antioxidant status is not apparent. The possible putative mechanism of generation of DNA damage may be the production of endogenous free radicals due to continuous exposure. RFR has been reported to produce different free radicals earlier (Avci et al., 2009; Burlaka et al., 2013; Barcal et al., 2014; Kazemi et al., 2015). Cells possess a number of compensatory mechanisms to deal with ROS and its effects. Among these are the induction of antioxidant proteins such as GSH, SOD and CAT. Enzymatic antioxidant systems function by direct or sequential removal of ROS, thereby terminating their activities. An imbalance between the oxidative forces and antioxidant defense systems causes oxidative injury, which has been implicated in various diseases, such as cancer, neurological disorders, atherosclerosis, diabetes, liver cirrhosis, asthma, hypertension and ischemia (Andreadis et al., 2003; Comhair et al., 2005; Dhalla et al., 2000; Finkel and Holbrook, 2000; Kasparova et al., 2005; Sayre et al., 2001; Sohal et al., 2002). Because of the significant decrease in endogenous antioxidants and increased LOO among the exposed group, the extra burden of free radicals is unlikely to get neutralized, and these surplus ROS may react with important cellular macromolecules including DNA forming either DNA adducts or stand breaks, which may be later expressed as micronuclei once the cell decides to divide. The decline in the antioxidant status may be also due to the suppressed activity of Nrf2 transcription factor which is involved in maintaining the antioxidant status in the cells.

The present study has reported that RFR increased the frequency of MN and LOO and reduced GSH contents, CAT and SOD activities in the plasma of the exposed individuals. The induction of MN may be due to the increase in free-radical production. The present study demonstrated that staying near the mobile base stations and continuous use of mobile phones damage the DNA, and it may have an adverse effect in the long run. The persistence of DNA unrepaired damage leads to genomic instability which may lead to several health disorders including the induction of cancer.

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Declaration of interest

The authors report no declarations of interest.

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