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Genotoxicity evaluation of human populations exposed to radio frequency radiation

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Abstract

In the modern day life, mobile telephones have become an integral part for mutual communication. Virtually most people are exposed to radio frequency radiation. In the current study a group of 55 subjects with regular cell phone usage and an equal number of well-matched control individuals were studied. The possible genetic changes in lymphocytes of cellular telephone users were assessed with the comet assay, micronucleus test and chromosomal aberrations analysis. In addition micronucleus test on buccal epithelial cells was also carried out in the same subjects. The results suggested that in the mobile phone users there was a statistically significant increase in mean DNA damage when compared to controls. Similar result was observed with micronucleus test and chromosomal aberrations analysis also ($P < 0.05$). Age, smoking and alcohol consumption did not have any significant effect on genetic damage when analyzed by comet assay. However, no association of gender, age, smoking and alcohol consumption on micronucleus frequency was observed. Smoking and alcohol consumption had significant effect by the chromosomal aberration test ($P < 0.05$). The results of this study indicated that the genetic damage of peripheral lymphocytes and buccal epithelial cells in the mobile telephone users increased significantly, as compared with controls.

Keywords: Chromosomal aberrations, Comet assay, Genetic effect, Micronucleus test, Mobile telephones

Introduction

The growth in the usage of mobile phones, microwave ovens, base stations, radar installations, telecommunications and broadcast facilities has resulted in world wide human exposure to radio frequency (RF) fields. Among these, mobile phones form the largest used device. All mobile phones systems use microwaves (electromagnetic fields of frequencies between 300 MHz and 300 GHz) as carrier waves. A mobile handset represents a significant source of RF field exposure, because of the presence of the signal transmitting antenna close to head, neck, and hand of the user.

The effect of radio frequency radiation (RFR) exposure on the genetic material of cells is considered very important since damage to the DNA of somatic cells can be linked to cancer development whereas damage to germ cells can lead to damage to the next generation. A large number of studies on exposure to RFR report no increased genetic damage but yet, many investigations do suggest the damage (Verschaeve, 2005). Therefore, the potential risk to health caused by them is of great concern. Of special concern is the increased chance of cancer. There are reports of greater risk of cancer (Mild *et al.*, 2003; Kundi *et al.*, 2004; Hardell *et al.*, 2007) as well as negative findings (Lonn *et al.*, 2005, 2006; Schuz *et al.*, 2006). A number of laboratory experiments have been performed in the past three decades to assess possible genotoxic effects of a broad range of RF fields in a variety of short-term tests using different end points. Studies carried out with RFR using Ames test gave negative results (Chang *et al.*, 2005). The genotoxicity of RFR was investigated in vitro using chromosomal aberrations (CA), micronuclei (MN), DNA damage and sister chromatid exchanges (SCE). No effect of RF signal was observed (Stronati *et al.*, 2006). Similarly, in vitro exposure to RFR did not affect MN frequency in human lymphocytes (Scarfi *et al.*, 2006).

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However, some in vitro studies demonstrated damage to chromosomes, induction of MN and DNA damage (Mashevich *et al.*, 2003; Tice *et al.*, 2002; Zhang *et al.*, 2006). Controversial results were also reported with *in vivo* studies of RF exposure. Absence of genotoxicity was found in mice with micronucleus test (MNT) and in rats with comet assay (Lagroye *et al.*, 2004a; Gorlitz *et al.*, 2005). Nevertheless some investigations revealed significant genotoxicity. These include DNA damage (Paulraj and Behari, 2006) and induction of MN (Trosic *et al.*, 2004). The potential genotoxicity of RFR have also been assessed in human epidemiological studies that have demonstrated both positive (Garaj-Vrhovac, 1999; Gadhia *et al.*, 2003; Markova *et al.*, 2005; Gandhi and Anita, 2005; Gandhi and Singh, 2005) and negative reports (Garson *et al.*, 1991; Maes *et al.*, 1995, 2006). Therefore, there is a need to evaluate different exposed population with a variety of genotoxic parameters.

In the current investigation, the genotoxic effect of RF exposure was evaluated in cellular phone users utilizing alkaline single cell gel electrophoresis assay, also known as the comet assay. This assay has been found to be a very sensitive method for measuring DNA damage (Singh *et al.*, 1988). To substantiate our results the MNT in buccal cells and peripheral blood lymphocytes (PBL) was carried out. The non-invasive nature of MNT in exfoliated cells makes it an attractive candidate for the biomonitoring of human populations (Stich *et al.*, 1982). In human population studies, the frequency of MN is most commonly determined in cultured PBL after being stimulated to proliferate by phytohaemagglutinin (PHA). The most frequently applied methodology uses the cytokinesis-block micronucleus technique in which scoring is limited to cells that have divided once since mitogen-stimulation (Fenech and Morley, 1985). The CA analysis is a traditional method used to assess exposure to genotoxins that is internationally recognised. The influence of confounding factors like gender, age, smoking, alcohol consumption and duration of exposure on the differences in genotoxicity was also analyzed.

Materials and Methods

Study population

Biological samples were obtained from 55 individuals exposed to RFR from mobile phones. The subjects were recruited on the basis of their frequency and duration of cell phone usage. Questionnaires were used to obtain data on their gender, age, estimated duration of exposure to RF fields, therapeutic radio graphics, recent illnesses, lifestyle etc. All the RFR exposed subjects were sales executives and reported that they used cell phones on a regular basis. Individuals with 3-8 years of cellular phone usage were considered to be suitable for the study. The billing records for cell phone users revealed that the average duration of exposure as 4.56 ± 1.20 (years) and 5.56 ± 1.08 h per day. All of them used hand held models and had no additional accessories like radio or camera. All the users were right handed and used phones both indoors and outdoors. Blood and buccal smear samples from 55 subjects who had no exposure to RFR were used as control group. They were recruited from the general population of the same area. The selection of these subjects was also based on a questionnaire, which declared that the control subjects did not possess or used any mobile phones. They never used microwave ovens, digital cameras, laptop or personal computers, pagers and digital cordless phones. None of the study subjects had excessive exposure to any potential genotoxic compound. The subjects who smoked more than 5 cigarettes per day and who took at least 5 glasses/day of an alcoholic drink, for at least one year were considered as smokers and alcohol drinkers in exposed and control groups. It was assumed that the difference between exposed and control group was not significant except for exposure to cellular phone radiation. None of the subjects reported any recent illness, had not been taking any medicines nor had they been exposed to X-rays for about 8-10 months before sampling. All subjects were informed the purpose of the study. Samples were coded to avoid bias. All slides were scored by one person, to avoid inter scorer variability. The ethical committee of our institute approved the research procedures utilized in this study. The general characteristics of the study subjects are shown in Table 1.

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Collection of Samples

Blood (2ml/ subject) and buccal smear samples from 6 control and 6 exposed subjects were taken every 7 days. Sampling was carried out over a period of 3 months. The samples were transported on ice to the laboratory and were processed within 2 h.

Comet assay

40 μl of blood was taken from the collected samples for the comet assay, which was carried out according to Singh *et al.* (1988) with slight modifications. Cell viability determined by the trypan blue exclusion technique ranged from 92 to 96% (data not shown). Slides were prepared in duplicate per subject. The method followed has been described earlier (Chandrasekhar *et al.*, 2006). Analysis was performed using a 400 X magnification with Olympus BX 51 fluorescent microscope equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. A total of 100 individual cells were screened per subject (50 cells from each slide). The length of the DNA migrated in the comet tail, which is an estimate of DNA damage was measured using an ocular meter and calculated as: comet tail length (μm) = (maximum total length)-(head diameter).

Micronucleus test (buccal epithelial cells)

The MNT was carried out on the buccal epithelial cells of 55 exposed and 55 controls (Chandrasekhar *et al.*, 2006). Buccal cell samples were obtained by rubbing the inside of the cheeks of study subjects with a toothbrush. The cells were collected in sample bottles containing 20 ml of buffer solution (0.1 M EDTA, 0.01 Tris-HCl and 0.02 M NaCl, pH 7) and transported to our institute for processing. After three washes in the buffer solution by centrifugation at 400 g for 10 min, 50 ml of cell suspension was dropped onto preheated (55°C) slides and allowed to air dry for 15 min on a slide warmer. The slides were made in triplicates for each subject. The slides were fixed in 80% cold methanol for 30 min, air-dried overnight at room temperature, and stored at -20°C until use. The slides were stained with a DNA-specific dye, DAPI (1 μg/ml). A total of 5000 buccal epithelial cells were screened per subject and the frequency of MN per 1000 cells was calculated (Albertini *et al.*, 2000).

Micronucleus test (PBL)

The MNT was conducted according to the method of Fenech and Morley, (1985). Briefly, 0.5 ml of whole blood was mixed with 4.5 ml of RPMI-1640 medium supplemented with 20% fetal calf serum, 100 U/ml penicillin and 100μg/ml streptomycin.0.2 mg/ml PHA was added to stimulate the culture. Cultures were incubated in duplicates at 37°C for 72 h. Cytochalasin-B was added at the 44th h of culture growth at a final concentration of 5μg/ml to arrest the cells at cytokinesis. The cultures were harvested by centrifugation after 72 h. The lymphocytes were subjected to mild hypotonic treatment with 0.075 M KCl for 5 min, then fixed in fresh fixative solution (3:1, methanol:acetic acid). This fixation was repeated twice. Few drops of cell suspension was smeared on pre-cooled microscopic slides and air-dried. The slides were stained using 10% Giemsa of pH 6.8 for 10 min. One thousand binucleated lymphocytes (500 cells per culture) were scored at 400X magnification.

Chromosomal aberrations assay

The CA analysis was conducted following a standard protocol with slight modifications (Richardson *et al.*, 1991). 0.8 ml aliquot of venous blood was taken from the 2ml blood collected from each subject. Whole blood was cultured in F-10 medium supplemented with 20% fetal bovine serum, 0.5 ml PHA, 5000 IU/ml penicillin and 1000 IU/ml streptomycin. Each culture was incubated in 5% CO_2 and 95% air incubator at 37°C for 48h. Metaphases were obtained by adding 0.2μg/ml colchicine to the cultures 3 h before harvesting. The cells were collected by centrifugation, resuspended in a pre-warmed hypotonic solution (0.075 M KCI) for 15 min at 37°C and fixed in acetic acid: methanol (1:3, v/v). Chromosome preparations were stained with 3.3% Giemsa. The slides were analyzed at 1000X magnification using a light microscope and 200 metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid deletions, chromatid rings, dicentrics, quadriradial figures and acentric fragments.

Statistical analysis

The samples were coded at the time of preparation and scoring. They were decoded before

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statistical analysis for comparison. As the distribution of mean DNA tail length, MN and CA did not significantly differ from the normal distribution, untransformed data were used. The significance of the differences between control and exposed subject endpoint means were analyzed using student's t-test (mean DNA tail length, MN and CA). Mean and standard deviation (S.D) were computed for the scores and the statistical significance ($P < 0.05$) of effects (gender, age, smoking, alcohol consumption and exposure) was determined using analysis of variance (ANOVA). All calculations were performed using Graph Pad Prism 4 Software package for windows. P values less than 0.05 were considered significant. Multiple linear regression analysis was performed to assess the association between end points and the independent variables.

Results and Discussion

Within the last decade, a large proportion of the world's population has adopted the use of cellular phones. However, the advent of mobile telephones has been accompanied by an upsurge in public and media concern that RF emissions from mobile phones are a cause of cancer. In general, induction of genetic damage such as chromosomal and DNA lesions correlate strongly with carcinogenic activity. Thus genetic damage is considered as a relevant biomarker for cancer risk. Hence, the goal of this investigation was to study the genome damage in humans exposed to RF through use of mobile phones in India. The study was carried out utilizing comet assay, CA test and MNT in buccal cells and PBL. The distribution of subjects with respect to age, gender, smoking, alcohol consumption and duration of exposure, is

Table 1. Characteristics of the study population

	Controls (n=55)	Exposed (n=55)	t, χ^2	*P*
Age (years) (mean+SD)	33.05±5.91	32.74± 5.61	t=0.28	0.77
Years of exposure (mean+SD)		4.56±1.20		
Per day exposure (hours) (mean+SD)		5.56±1.08		
Gender				
Male: n (%)	30 (54.54)	30 (54.54)	t=0.00	1.00
Female: n (%)	25 (45.45)	25 (45.45)		
Smoking				
Yes: n (%)	22 (40.00)	20 (36.36)	χ^2=0.93	0.33
No: n (%)	33 (60.00)	35 (63.63)		
Alcohol consumption				
Yes: n (%)	17 (30.90)	14 (25.45)	χ^2=1.50	0.21
No : n (%)	38 (69.09)	41 (74.54)		

Table 2. Inter-group comparison of mean DNA damage (comet tail length in mm), micronucleus frequency (buccal cells and lymphocytes) and chromosomal aberrations in controls and exposed subjects.

Group	n	DNAdamage (μm) (mean±S.D)	MN/1000 BN (mean±S.D)		CA(%) (mean±S.D)
			Buccal cells	Lymphocytes	
Control	55	7.45±0.98	1.63±0.55	3.36±1.09	1.32±0.47
Exposed	55	13.68±2.13*	3.03± 0.94*	5.29±1.04*	2.41±0.87*

MN/1000 BN: Micronuclei /1000 Binucleated cells. *P < 0.05

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given in Table 1. The two groups studied had similar demographic characteristics and did not differ with respect to any of the confounding factors except for exposure to RFR. The comet assay has become an important tool in human biomonitoring studies. It enables the assessment of genetic damage in a great variety of cells. In conventional human biomonitoring studies, lymphocytes are preferred for the measurement of genotoxic effects. Even though long-term diseases are not expected to affect blood cells, lymphocytes are regarded as sentinel cell types being early warning signals for adverse health effects (Faust *et al.*, 2004). In the current study a significant increase in the level of DNA damage was observed in RF exposed humans (13.68 vs. 7.45 μm; $P<0.05$; Table 2) compared to controls. Male subjects showed higher DNA damage than females in both exposed and control group ($P<0.05$; Table 3). Age had no significant effect on basal DNA damage in

Table 3. Mean DNA damage (comet tail length in mm) as a function of age, gender, smoking, alcohol consumption and exposure in controls and exposed subjects.

Group	Control		Exposed	
	n	(mean+S.D.)	n	(mean+S.D.)
Gender				
Male	30	8.30±0.48*	30	15.55±1.06*
Female	25	6.75±0.70	25	12.11±1.40
		P<0.0001		P<0.0001
		t=9.35		t=10.08
Age				
<35	28	7.29±0.99	28	13.32±2.10
>35	27	7.62±0.97	27	14.04±2.14
		P=0.22		P=0.21
		t=1.22		t=1.26
Smoking				
Yes	22	7.63±0.88*	20	14.71±1.93*
No	33	7.19±1.09	35	12.82±2.24
		P=0.10		P=0.02
		t=1.65		t=2.35
Alcohol consumption				
Yes	17	7.62±0.86*	14	14.10±1.92*
No	38	7.07±1.17	41	12.45±2.30
		P=0.05		P=0.01
		t=1.95		t=2.63
Years of exposure				
<5			20	12.45±1.54
>5			35	15.82±1.03*
				P<0.0001
				t=8.66
Daily exposure (hours)				
<6			22	12.95±2.41
>6			33	14.77±1.61*
				P=0.001
				t=3.39

*P<0.05

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exposed and control subjects. Smoking and alcohol consumption was found to have significant effect on damage to DNA in both controls and exposed. The mean comet tail length for exposed increased with duration of exposure ($P<0.05$; Table 3). Studies of mobile phone users utilizing the comet assay are limited and contradictory. DNA damage was estimated using the comet assay in PBL of 24 individuals using mobile phones. Mean comet tail length in mobile phone users was significantly higher than that in control group (Gandhi and Anita, 2005). Our results are in line with this investigation. In contrast, 49 subjects professionally exposed to RFR were assessed for genotoxicity by the comet assay, SCE and CA tests. The study revealed no evidence of RFR induced genetic effects (Maes *et al.*, 2006).

MN arise in mitotic cells from chromosomal fragments or chromosomes that lag behind in anaphase and are not integrated into the daughter

Table 4. Frequency of micronuclei (MN/1000 BN) in buccal cells as a function of age, gender, smoking, alcohol consumption and exposure in control and exposed subjects.

Group	Control		Exposed	
	n	(mean+S.D.)	n	(mean+S.D.)
Gender				
Male	30	1.68±0.62	30	3.20±1.04
Female	25	1.60±0.49	25	2.90±0.84
		P=0.60		P=0.24
		t=0.52		t=1.18
Age				
<35	28	1.60±0.49	28	2.82±0.90
>35	27	1.66±0.62	27	3.25±0.94
		P=0.69		P=0.08
		t=0.39		t=1.75
Smoking				
Yes	22	2.00±0.43*	20	3.15±0.98
No	33	1.39±0.49	35	2.97±0.92
		P<0.0001		P=0.50
		t=4.65		t=0.67
Alcohol consumption				
Yes	17	2.00±0.50*	14	3.28±0.99
No	38	1.47±0.50	41	2.95±0.92
		P=0.0008		P=0.25
		t=3.57		t=1.152
Years of exposure				
<5			20	62±0.80
>5			35	3.75±0.71*
				P<0.0001
				t=5.15
Daily exposure (hours)				
<6			22	2.42±0.56
>6			33	3.95±0.57*
				P<0.0001
				t=9.81

*P<0.05. MN/1000 BN: Micronuclei /1000 Binucleated cells

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nuclei. MN harboring chromosomal fragments may result from: direct DNA breakage, or replication on a damaged DNA synthesis. MN harboring whole chromosomes are primarily formed from failure of the mitotic spindle, kinetochore, or other parts of the mitotic apparatus, alterations in cellular physiology, and mechanical disruption. Thus an increased frequency of MN cells is a biomarker of genotoxic effects that can reflect exposure to agents with clastogenic or aneugenic modes of action (Albertini *et al.*, 2000).

The MNT was conducted in exfoliated buccal cells and PBL in mobile phone users in the present study. The results showed that the subjects exposed to RFR through use of mobile phone display significant genotoxic activity in both buccal epithelial cells and PBL when evaluated by the MNT. The exposed subjects had significantly higher frequency of MN in buccal epithelial cells when compared to controls (3.03% vs. 1.63%; $P<0.05$: Table 2). Gender and age did not affect the MN frequency in exposed and control subjects. Smoking and alcohol consumption

Table 5. Frequency of micronuclei (MN/1000 BN) in lymphocytes as a function of age, gender, smoking, alcohol consumption and exposure in control and exposed subjects.

Group	Control		Exposed	
	n	(mean+S.D.)	n	(mean+S.D.)
Gender				
Male	30	3.46±1.10	30	5.56±1.32
Female	25	3.24±1.09	25	5.06±0.69
		P=0.44		P=0.08
		t=0.76		t=1.77
Age				
<35	28	3.25±1.09	28	5.14±1.00
>35	27	3.46±1.10	27	5.44±1.08
		P=0.49		P=0.29
		t=0.69		t=1.06
Smoking				
Yes	22	3.95±0.95*	20	5.45±0.94
No	33	2.97±1.01	35	5.20±1.10
		P=0.0007		P=0.39
		t=3.69		t=0.84
Alcohol consumption				
Yes	17	3.88±0.92*	14	5.42±0.93
No	38	3.13±1.09	41	5.24±1.09
		P=0.01		P=0.57
		t=2.45		t=0.56
Years of exposure				
<5			20	4.91±1.01
>5			35	5.95±0.75*
				P=0.0002
				t=3.97
Daily exposure (hours)				
<6			22	4.60±0.49
>6			33	6.31±0.77*
				P<0.0001
				t=9.96

*P<0.05

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did not have any effect in exposed group. The control subjects showed a significant difference in MN frequency between smokers and non-smokers, alcohol drinkers and never drinkers ($P<0.05$). The frequency of MN among exposed subjects increased with the duration of exposure as seen in Table 4. The results of MN frequency in PBL of subjects exposed to RFR increased significantly as compared to controls (5.29% vs. 3.36%; $P<0.05$; Table 2). No effect on MN frequency was seen with gender, age, smoking and alcohol consumption in exposed subjects. Smoking and alcohol consumption increased the MN frequency in control subjects. Duration of exposure to RFR for ≥5 years had a significant effect over an exposure of <5 years (5.95% vs. 4.91%; $P<0.05$). ≥6 h per day exposure to RFR caused a rise in MN frequency than exposure to less than 6 h (6.31% vs. 4.60%; $P<0.05$; Table 5). Previous investigations reporting genotoxic effect with MNT are consistent with our data. Assessment of cytogenetic damage in 25 mobile phone users was

Table 6. Chromosomal aberrations frequency (%) as a function of age, gender, smoking, alcohol consumption and exposure in controls and exposed subjects.

Group	Control		Exposed	
	n	(mean+S.D.)	n	(mean+S.D.)
Gender				
Male	30	1.43±0.50	30	2.64±0.95
Female	25	1.20±0.40	25	2.23±0.77
		P=0.06		P=0.08
		t=1.86		t=1.74
Age				
<35	28	1.32±0.47	28	2.39±0.83
>35	27	1.33±0.48	27	2.44±0.93
		P=0.92		P=0.82
		t=0.09		t=0.21
Smoking				
Yes	22	1.33±0.47	20	2.48±0.95
No	33	1.31±0.47	35	2.30±0.73
		P=0.90		P=0.45
		t=0.11		t=0.75
Alcohol consumption				
Yes	17	1.34±0.48	14	2.46±0.89
No	38	1.29±0.46	41	2.28±0.82
		P=0.73		P=0.51
		t=0.34		t=0.65
Years of exposure				
<5			20	2.40±0.88
>5			35	2.45±0.88
				P=0.84
				t=0.20
Daily exposure (hours)				
<6			22	2.36±0.85
>6			33	2.50±0.91
				P=0.57
				t=0.56

*$P<0.05$

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carried out by analyzing CA in cultured lymphocytes and buccal mucosal cells for MN. The results revealed a significantly increased number of MN in buccal cells and cytological abnormalities indicating genotoxic response from cell phone use (Gandhi and Singh, 2005). Likewise, there was a marked difference in the frequency of MN in PBL among mobile phone users (Gandhi and Anita, 2005). Similarly, 12 subjects occupationally exposed to RFR were evaluated for genome damage in PBL using MNT. Results showed an increase in frequency of MN compared to controls (Garaj-Vrhovac, 1999). The CA assay was also used to study the genotoxicity associated with human exposure to RFR. In the exposed subjects a significant increase in CA indicating chromosomal damage was observed when compared to controls (2.41 vs. 1.32; $P<0.05$; Table 2). In the exposed subjects, no significant difference was observed with respect to gender, age, smoking, alcohol consumption and duration of exposure when compared to controls ($P>0.05$; Table 6). The exposed group had significantly greater mean for chromatid breaks (0.70 ± 0.59); chromosomal breaks (0.83 ± 0.37) and acentric fragments (0.89 ± 0.45) in comparison to controls. Results of this investigation revealed that mobile phone users showed a significant increase in the level of CA. Other studies on RF exposed humans with CA have been reported. The results however were controversial. Examination of CA from people occupationally exposed to RFR showed that the number of CA was significantly higher in the exposed subjects (Lalic *et al.*, 2001). A study examined CA and SCE in blood lymphocytes of 24 mobile phones users. There was a significant increase in CA and SCE among mobile phones users than that in control group (Gadhia *et al.*, 2003). The effects of non-thermal microwaves (MWs) of Global System for Mobile Communication (GSM) at different carrier frequencies on PBL from healthy and hypersensitive persons were studied. The changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependence. MWs from GSM mobile telephones were found to affect chromatin conformation. Effects of MWs from mobile telephones on PBL were reported to be dependent on carrier frequency. The same

Table 7. Multiple linear regression analysis of endpoints.

End point	Independent variables	Frequency ratio (95% CI)	R2(%)	P	F
DNA mean tail length (μm)	Age	31.02 (20.99 to 41.06)		0.72	
	Years of exposure	3.80 (1.66 to 5.94)		0.47	
	Per day exposure	2.48 (0.74 to 4.22)		0.0007*	
			0.03	0.56	0.67
MN frequency (MN/1000 BN) (Buccal cells)	Age	28.15 (23.10 to 33.19)		0.06	
	Years of exposure	1.91 (1.09 to 2.72)		<0.0001*	
	Per day exposure	2.78 (2.17 to 3.39)		<0.0001*	
			0.09	0.17	1.71
MN frequency (MN/1000 BN) (lymphocytes)	Age	28.99 (21.10 to 36.88)		0.33	
	Years of exposure	0.90 (-0.45 to 2.27)		<0.0001*	
	Per day exposure	0.57 (-0.05 to 1.20)		<0.0001*	
			0.03	0.56	0.68
CA frequency (%)	Age	32.22 (27.69 to 36.76)		0.80	
	Years of exposure	4.47 (3.50 to 5.44)		0.84	
	Per day exposure	5.56 (4.68 to 6.43)		0.99	
			0.03	0.57	0.67

*$P<0.05$

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response was observed in PBL of all the subjects (Markova *et al.*, 2005). On the other hand, CA analysis was performed on PBL of radio linemen employed by Telecom Australia. There was no statistically significant difference in CA (Garson *et al.*, 1991). Cytogenetic study of subjects exposed to RFR showed no statistically significant increase in CA frequency in PBL compared with the control subjects (Maes *et al.*, 2006). Blood samples from maintenance workers exposed to microwaves were investigated for CA. The RFR did not induce significant genotoxic defects in the studied population (Maes *et al.*, 1995).

Gender, smoking, alcohol consumption and duration of exposure had a significant effect on DNA damage in the exposed subjects in the present study. Similarly, in a study of digital mobile phone users there was a significant increase in CA among the users who were smokers-alcoholics as compared to non-smokers–non-alcoholics (Gadhia *et al.*, 2003). In contrast, smoking habit had no significant effect on the induction of genotoxicity in an investigation of subjects professionally exposed to RFR (Maes *et al.*, 2006). In case of MNT in buccal cells and PBL of our study, the MN frequency did not increase with age, gender, smoking and alcohol consumption in RF exposed subjects. Likewise, no significant influence of sex on DNA tail length or MN frequency in RF exposed humans has been reported (Gandhi and Anita, 2005). In the current investigation confounding factors did not have any effect on CA frequency. Multiple linear regression analysis using the different independent variables showed that age had a positive but not statistically significant association with any of the end points (Table 7). Years of exposure correlated positively with DNA damage, MN and CA frequency. But the results were found to be statistically significant only for MN frequency in buccal cells and PBL. Per day exposure to RFR had a statistically significant association with DNA damage and MN frequency only. The effect was not significant with CA analysis.

Even-though many of the investigations on genetic and cytogenetic effects of RFR indicated that these electromagnetic fields are not capable of inducing any kind of genetic effect, the literatures available remain sufficiently controversial to exclude any potential RFR genetic hazard. It is assumed that only large-scale research under well-controlled conditions and allowing the generation of results with sufficient statistical power may lead to a better risk estimate.

Conclusion

The results of this study indicated that the genetic damage of peripheral lymphocytes and buccal epithelial cells in the mobile telephone users increased significantly, as compared with controls. The data highlights the potential dangers of prolonged exposure to radio frequency radiation and the widespread use of mobile phones requires caution.

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