

Increased frequency of micronucleated exfoliated cells among humans exposed *in vivo* to mobile telephone radiations

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Abstract

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any *in vivo* effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex–age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35 years in exposed subjects along with the 9.84 ± 0.745 micronucleated cells (MNCs) and 10.72 ± 0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75 ± 0.774 MNC and 4.00 ± 0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17 ± 2.750 and in exposed subjects is 13.06 ± 1.793 . The value of means of KH in exposed subjects (1.84 ± 0.432) is slightly higher than in controls (1.42 ± 0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65 ± 0.276) as compared to controls (0.50 ± 0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72 ± 0.374) in comparison to controls (0.67 ± 0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0–1, 1–2, 2–3 and 3–4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

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1. Introduction

With the advancement of technology, the use of portable hand held cellular mobile telephones is enormously increasing day by day. India ranks fourth behind China, USA and Russia, which have approximately 450 million, 233 million and 160 million subscribers, respectively [1]. In India 110.5 million global system for mobile telecommunication (GSM) and 45.87 million of code division multiple access (CDMA) phones make a total of 156.37 million subscribers of mobile phones at the end of January 2007. It is projected to a mammoth 250 million mobile phones in India and 3.2 billion in world at the end of 2007 [1,2].

Given the immense number of users of mobile phones, even a small adverse effect on health could have a major public health implication [3].

Exposure to radio frequency (RF) field in the frequency range of 300–2100 MHz emitted by cell phones elicit a host of biological responses and therefore do represent an unnatural stressor to the biological system no matter how small [4]. This type of exposure is likely to cause biological effects in living organisms [5,6] although at present no definitive association can be obtained with the incidences of cancer or of other genetic and non-genetic pathological conditions [7]. India mainly has two system of digital mobile telephony, i.e. GSM with a carrier frequency around 900 MHz and CDMA with a carrier frequency of 1800 MHz [8]. In case of biological system the extent of RF field exposure depends on the amount of energy deposited in tissue, and it is measured by specific absorption rate (SAR), i.e.

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the amount of energy absorbed per unit time per unit mass of tissue and is expressed in W/kg and its maximum level for modern handsets have been set by governmental regulatory agencies, e.g. Telephone Regulatory Authority of India (TRAI), Federal Communications Commission of USA (FCC) and Radiation Protection Bureau, Canada, in many countries [4]. The SAR limit recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) is 2.0 W/kg. This value has been averaged over 10 g of body tissue. The guidelines incorporate substantial margin of safety to assure the protection of all the persons regardless of age and health [9,10]. During the past 50 years, a number of biomarkers have been used in environmental carcinogenesis research in the pretext of refining the reliability of the exposure assessment and providing tools for early detection of disease related changes and their association with environmental and genetic factors [11,12]. About 90% of human cancers are carcinomas, perhaps because most of the cell proliferation in the body occurs in epithelia, or because epithelial tissues are most frequently exposed to various forms of physical, chemical and radiational damage that favors the development of cancer. The buccal epithelial cells are taken in the present study, as these cells are in more proximity to the exposure while using mobile phone devices.

Micronucleus assay is a multi-endpoint test of genotoxic responses to clastogens [13]. The frequency of micronucleus (MN) is extensively used as a biomarker of genomic instability, genotoxic exposure and early biological effect in human biomonitoring studies [14]. It is used as an indicator of genotoxic exposition since it is associated with chromosome aberrations [15]. MN may contain (i) acentric chromosome (fragments formed by unpaired double stranded breaks or by misrepair of various DNA lesions) or (ii) lagged chromosomes that have failed to segregate into daughter macronucleus during mitosis [16].

Keeping in view the above facts, the aim of the present study was to investigate the genotoxic effects, if any, of low frequency electromagnetic radiation emitted by mobile phones on humans under study. We used the exfoliated micronucleus assay for the study of presence of any nuclear anomalies like micronuclei, karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells in buccal epithelial cell of the subjects under investigations.

2. Materials and methods

2.1. Subjects

For the present investigation 109 individuals, 60 females and 49 males, irrespective of age/sex/cast had been taken. Subjects exposed to mobile phone radiations (user) and healthy controls, matched in respect to age, sex and socio-economic status, smoking and alcoholic habits and drug intake, if any but unexposed to any chemical or radiational agent (non-user) and selected at random, comprised the materials for the present investigations. Subjects under investigation were all healthy and interviewed to assess their habits, according to the proforma designed for the purpose. None of the subject had family history of any genetic anomaly or major illness nor had they undergone irradiation examination and none have been on drugs for the last 6 months. Informed consent was taken from all the participants and the study was approved from the institutional ethics committee (via letter no. IEC/07/143). During the present study we found

that persons were using mobile phone models from different manufacturers, i.e. Nokia, L.G., Samsung, Tata, Reliance, Motorola, etc. The SAR value for these handsets in our investigation ranged from 0.34 in Nokia 9210 (Type RAE-3N) to 0.95 in Nokia N70 (Type RM-84) as provided by the manufacturer's manual [17].

2.2. Cytological preparations

The study of exfoliated buccal epithelial cells was performed by the standard technique of Tolbert et al. [18], with slight variations made for the requirements of this investigation. Oral mucosa was washed before the buccal cell sample was collected. The buccal epithelial cells were then swabbed with a moistened wooden tongue depressor. Two slides were prepared for each individual by smearing the cells on to pre-cleaned microscope slides. The air dried samples were hydrolyzed in 1N HCl at 60 °C for 8 min and then they were stained with 2% aceto-orcein stain (HIMEDIA, acetic acid RM 5564, orcein RM 277). Counterstaining was carried out in 0.1% fast green solution (HIMEDIA, RM 4266). Coded slides were analyzed using an Olympus CX-41 trinocular research microscope at 1000× magnification. At least 1000 cells from each individual were examined and the number of cells with nuclear anomalies was scored. The criterion of Tolbert et al. was followed for scanning cells for micronuclei and other nuclear anomalies. In order for the cell to be considered micronucleated, the putative micronucleus is required to meet the following criteria: (a) rounded, smooth perimeter suggestive of membrane; (b) less than third the diameter of the associated nucleus, but large enough to discern shape and color; (c) staining intensity similar to that of nucleus; (d) same focal plane as nucleus. In addition to MN other nuclear anomalies are also studied in sample smears are binucleates, or the presence of two nuclei with in a cell, BE or nuclei that appear to be broken but still connected to main nuclei with a thin band, KH or nuclear disintegration involving loss of integrity of the nucleus and KL or nuclear dissolution, in which aceto-orcein negative, ghost-like image of the nucleus remains. In addition to these there were other phenomenon occurring in the cells such as pycnosis and chromatin, which were observed during scoring of the anomalies. However, these were not counted in the anomalies. Pycnosis and condensed chromatin are considered as a part of normal epithelial cell differentiation and maturation.

2.3. Statistical analysis

Statistical analysis was done using Student's *t*-test [19] along with the Pearson correlation and Spearman's correlation analysis (non-parametric correlation) with the help of statistical software origin 6.1 and SPSS 11.5.

3. Results

The main characteristics of the exposed and matched control subjects (average age, exposure time, sex, smoking habits, drinking habits and dietary habits) are summarized in Table 1.

A total of 109 subjects with average age of 23.82 years (range 18–32 years) have been studied. Out of these 49 (45%) were males and 60 (55%) were females. Age-sex-matched controls (24) are also included within 109 subjects. The mean duration of exposure to mobile phone was 2.35 years (range 9 months to 7 years) with an average daily exposure time of 61.26 min (range 10–540 min).

The results of the cytological observations on MNC, TMN, KL, KH and BE in terms of mean, standard errors (S.E.), standard deviations (S.D.) of means and range both in exposed and control subjects are shown in Table 2.

The mean frequency of MNC (9.84 ± 0.745) and TMN (10.72 ± 0.889) shows a significant difference at 0.01 level in exposed and controls. The MNC and TMN in non-smoker exposed group is 9.500 and 10.333, respectively, whereas it is 13.571 and 15.000 in case of smoker exposed group. MNC and

Table 1
General characteristics of study group and controls matched for age and sex

Sr. no.	Variables	Total	Exposed	Control
1	<i>n</i>	109	85	24
2	Average age (years)	23.82	24.19	22.71
3	Sex			
	Males	49(45%)	42 (49.4%)	7 (29.2%)
	Females	60(55%)	43 (50.6%)	17 (70.8%)
4	Smoking habits			
	Non-smokers	101 (92.7%)	78 (91.8%)	23 (95.8%)
	Smokers	8 (7.3%)	7 (8.2%)	1 (4.2%)
5	Drinking habits			
	Non-alcoholics	97(89%)	74 (87.1%)	23 (95.8%)
	Alcoholics	12 (11%)	11 (12.9%)	1 (4.2%)
6	Dietary habits			
	Vegetarian	79 (71.6%)	60 (70.6%)	19 (79.2%)
	Non-vegetarian	30 (28.4%)	25 (29.4%)	5 (20.8%)
7	Average duration of exposure (years)	–	2.35	0
8	Average duration of exposure daily (min)	–	61.26	0
9	Subjects proximity to base station (500 m)	40 (36.7%)	38 (45.2%)	2 (8.3%)

TMN in case of non-smoker controls is 3.478 and 3.739, respectively, whereas it is 10.000 (both in MNC and TMN) in a single smoker control. The MNC and TMN frequency in case of control male is 5.714 and 6.571, respectively, whereas it is 10.000 and 11.047 in exposed male group. On the other hand, MNC and TMN in case of female control is 3.058 and 3.176 whereas in female exposed it is 9.674 and 10.395, respectively.

Cells with MN count more than one were predominant in the exposed population apparently as a result of the clastogenic effect of electromagnetic radiations emitted from mobile phone antennas, on the other hand in control cells two or more MN per cell were not found. Some of the exposed subjects have as high as 57 cells carrying MN as compared to controls having maximum 12 cells containing MN/1000 cells. The mean of KL in controls was 13.17 ± 2.750 and in exposed subjects was 13.06 ± 1.793 . The value of means of KH in exposed subjects (1.84 ± 0.432)

slightly higher than in controls (1.42 ± 0.737). Mean broken egg was found to be more in exposed subjects (0.65 ± 0.276) as compared to controls (0.50 ± 0.217). Frequency of presence of more than one nucleus in a cell (binucleated) was also higher in exposed (2.72 ± 0.374) in comparison to controls (0.67 ± 0.231). Although there was a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference was not found statistically significant.

Cytological observations in relation to duration of exposure (years) to mobile phone radiation of exposed subjects are shown in Table 3.

Pearson correlation and Spearman's correlation (non-parametric correlation) was found to be positively correlated at 0.01 level. It is of interest that a positive correlation was found between duration of mobile phone use and MNC and TMN frequency showed gradual increase, although the correlation was

Table 2
Cytological observations for exposed (*n* = 85) and controls (*n* = 24)

Sr. no.	Cytological observations	Subjects	Mean no. of cells with nuclear anomalies $\pm$ S.E.	S.D.($\pm$)	Range
1	MNC	Controls	$3.75 \pm 0.774^*$	3.791	0–12
		Exposed	$9.84 \pm 0.745^*$	6.886	0–46
2	TMN	Controls	$4.00 \pm 0.808^*$	3.956	0–12
		Exposed	$10.72 \pm 0.889^*$	8.194	0–57
3	KL	Controls	13.17 ± 2.750	13.474	0–60
		Exposed	13.06 ± 1.793	16.532	0–104
4	KH	Controls	1.42 ± 0.737	3.611	0–16
		Exposed	1.84 ± 0.432	3.979	0–26
5	BE	Controls	0.50 ± 0.217	1.063	0–4
		Exposed	0.65 ± 0.276	2.548	0–22
6	BN	Controls	0.67 ± 0.231	1.129	0–4
		Exposed	2.72 ± 0.374	3.452	0–14

MNC: micronucleated cell, TMN: total micronuclei, KL: karyolysis, KH: karyorrhexis, BE: broken egg, BN: binucleated cells.

* Significant at 0.01 level (Student's *t*-test).

Table 3

Cytological observations in relation to duration of exposure (years) to mobile phone radiation of exposed subjects

Sr. no.	Duration of exposure (years)	Subjects (<i>n</i>)	Observations	Mean no. of cells with nuclear anomalies $\pm$ S.E.	S.D.($\pm$)	Range
1	0–1	5	Duration	0.464 $\pm$ 0.119	0.267	0.08–0.8
			MNC	8.800 $\pm$ 2.244	5.019	2–16
			TMN	9.200 $\pm$ 2.870*	6.418	0–18
2	1–2	21	Duration	1.085 $\pm$ 0.048	0.222	1–108
			MNC	10.095 $\pm$ 1.253	5.743	0–20
			TMN	10.857 $\pm$ 1.464*	6.710	0–26
3	2–3	24	Duration	2.010 $\pm$ 0.012	0.061	2–2.3
			MNC	9.291 $\pm$ 2.007	9.835	0–46
			TMN	10.583 $\pm$ 2.450*	12.010	0–57
4	3–4	21	Duration	3.000	0	0
			MNC	11.047 $\pm$ 1.126	5.162	4–22
			TMN	11.714 $\pm$ 1.244*	5.702	4.22
5	>4	14	Duration	4.711 $\pm$ 0.260	0.940	4–7
			MNC	9.357 $\pm$ 1.357	5.077	3–20
			TMN	10.214 $\pm$ 1.658	6.200	3–24

* Positively correlated (Pearson correlation and Spearman's correlation).

not found to be statistically significant. During the initial years (1–3) of mobile phone use, MNC and TMN frequency showed a gradual increase. Pearson correlation between 0–1, 1–2, 2–3 and 3–4 years of exposure has been calculated and found to be positively correlated for TMN, surprisingly more than 4 years exposure showed a slight decrease in MNC and TMN frequency.

4. Discussion

In spite of extensive increase in the mobile telephones with in last few years, very little is known about the effect of long-term exposure that is experienced by people using mobile phone or living near mobile phone base stations [20]. In the past several investigations have been conducted in order to evaluate the possible biological effects resulting from human exposure to mobile phone radiations [21].

Earlier researcher and engineers believed that electromagnetic field of low frequency could not cause alterations in human cells, based on the hypothesis that low electromagnetic field could not generate a sufficient amount of heat to increase the tissue temperature, not causing damage at the DNA level [22]. Recently, International Agency for Research on Cancer (IARC) has classified low frequency electromagnetic field as possible carcinogen—a categorization that necessarily implies that low electromagnetic field may promote DNA damage and hence may be genotoxic [23].

We have chosen exfoliated cell micronucleus assay because it is well documented that when the target tissue is epithelial tissue, the exfoliated cell micronucleus assay has advantage over more widely used micronucleus test in lymphocytes [24], lymphocyte must be stimulated to undergo mitosis, epithelial cells do not need to be stimulated, micronuclei in exfoliated cells reflect genotoxic events that occurred in the dividing basal layer 1–3 weeks earlier [25]. Micronuclei result from chromosome breakage or interference with the mitotic appa-

ratus and such events thought to be related to carcinogenesis [26,27].

The biological effects from genotoxic agents such as ionizing, UV radiations and extremely low frequency electromagnetic radiation are often postulated to be linearly linked to dose, i.e. not showing a threshold even at the lowest exposure level [28]. The inability to observe a biological effect does not necessarily imply that low radiations are harmless. The latter may exert biological effects by indirect mechanisms (non-stochastic).

There have been few studies regarding health effects of electromagnetic radiations caused by cellular phones, Melt et al. [29] studied the interaction of 850 MHz with DNA repair and DNA synthesis and found no effect. In contrast to these, Lai and Singh [5], using comet assay or single cell gel electrophoresis, have reported DNA strands break in rat brain cells irradiated *in vivo* with 2450 MHz radiation at SAR of 0.6 and 1.2 W/kg. Radio frequency exposure of cells *in vitro* has been linked to changes in transcription and cell proliferation assayed by the incorporation of an RNA-precursor and a DNA-precursor, respectively. RF exposure has also been linked to change in cell cycle [30].

Most *in vitro* studies reporting DNA or chromosomal damage were conducted at exposure levels that resulted in thermal effects. In contrast, several *in vivo* studies in rodents indicate a direct effect of RF fields on DNA [5]. Increased incidence of cancer in controlled experiment is equivocal and could be linked to thermal effects [7].

In previously published studies with cultured human lymphocytes no induction of chromosome aberrations, micronuclei or sister chromatid exchange was measured after exposure to a 900 MHz GSM signal (SAR between 0.2 and 10 W/kg) [30,31]. Evidence from *in vitro* studies to date is consistent with those epidemiological results, which state that there is no statistically significant difference between cancer and exposure to cell phone field [32]. Similar experiments on rats exposed to one of the mobile signal (FDMA or CDMA) were performed but no statistically significant difference was found between the exposed

animals for any tumor in any organ [33]. A few studies reported that exposure of cultured cells to mobile phone RF energy caused DNA damage [15].

Some of the findings reported that use of mobile phone was not associated with an increased risk of acoustic neuromas [34] and there was no statistically significant association of mobile phone use with overall incidence of brain cancer or the incidence of salivary gland cancer [35]. Regular use, or long-term (greater than 10 years) use, or heavy (greater than 500 h) use of mobile phones (analog or digital) was not associated with an increase in the incidences of malignant brain tumor [36]. A recent *in vitro* study reports no effect on the frequency of neoplastic transformation after exposure to cell phone RF-field (835 and 848 MHz) at 0.6 W/kg for 7 days in cell culture [37]. Some studies have postulated a connection between prolonged use of cellular phones with both brain cancer and leukemia [38,39].

Significantly, our results obtained from the investigation of micronucleus in exfoliated cells indicate that exposed individuals have a significant increased number of MNC and TMN (Table 2). Similar results were found in other reports, which showed an increase in MN frequency, following *in vitro* exposure of human lymphocytes to 1800 MHz microwave radiation was observed at a power density of 5, 10 and 20 MW/cm² [40]. Our results are in substantial agreement with the previous reports on the induction of micronuclei formation and chromosome damage by non-ionization radiation. A significant increase in MN frequency was reported by Carborari et al. [22]. However, some of the investigator found no effects on DNA damage, chromosomal aberrations or micronuclei in human peripheral blood lymphocytes or in rat peripheral blood or bone marrow cells exposed to two mobile phone signals (CDMA at 847.74 MHz, FDMA at 835.62 MHz) or to 2.45 GHz microwaves [41,42].

The latest interphone study found no risk of brain cancer among the North European population in relation to cumulative hours of mobile phone use or cumulative number of calls but more than 10 years of mobile phone use reported on the side of the head where tumor was located, a statistically increased risk of glioma was found [43]. Another recent study also found no association with non-Hodgkin tumors, testicular cancer, although an association with non-Hodgkin lymphoma (NHL) of T-cell type could not be ruled out [44].

A slight increase in the mean frequency of KH, BE and BN was also observed in the present study. Binucleation dose not likely involves direct interaction with DNA but rather involves interference with event occurring late in cell division and consequences of binucleation are unknown. BE is very abnormal nuclear appearance of unknown origin and significance [45]. KH and KL are evident in cells undergoing necrosis, a form of cell death that occurs following injuries by agents that cause gross perturbation of cellular environment. KH also accompany early stages of another type of cell death, apoptosis [46]. Although pycnosis and condensed chromatin are a part of normal epithelial cell differentiation and maturation, these phenomena may occur at elevated levels in response to cellular injury [47].

An important finding from our study is that, the duration of exposure and MN and TMN frequency shows a positive correlation in initial years of exposure, i.e. 0–1, 1–2, 2–3, 3–4 years

but slight decrease in the frequency of MNC and TMN was observed for subjects exposed to more than 4 years. It may be possible that some DNA repair mechanism gets activated, when it is exposed to prolonged use of low frequency EMR *in vivo* conditions, which worked to protect DNA.

It is conjectured that some clandestine mechanism helps DNA to develop some sort of adaptability however, when it is in continuous exposure of week genotoxic agents such as low frequency EMR, it is a matter of further investigation.

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