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Cytogenetic Damage in Mobile Phone Users: Preliminary Data

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ABSTRACT Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concerns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or *in vitro* studies. The aim of the present study hence was to detect any cytogenetic damage in mobile phone users by analysing short term peripheral lymphocytes cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

INTRODUCTION

The natural terrestrial electromagnetic environment does not significantly comprise radiofrequency (30 kHz-300MHz) or microwave fields (300-3000 MHz) but the artificial radiofrequency (RF)/ microwave fields emanating from wireless communication technology now have average intensities around 1 ìW/cm^2(4V/m) in general suburban environments (Adey 2003). In fact mobile phones radiate an average power of 0.2-0.6 W, 40 per cent of which is absorbed in the hand and the head (Kuster et al. 1997) and so a mobile phone may be regarded as a quite powerful radio transmitter. Its emission at the head surface is typically 10, 000 times stronger than that reaching the head of a user standing within 30m of the base of a mobile phone relay transponder mounted on a tower 30m above ground. Digital modulation in mobile phone systems include the North American Digital Cellular (NADC) standard used in North America and Japan employing Time Division Multiple Access (TDMA) modulation with speech encoding at 50 pulses/sec and the Global System for Mobile Communication (GSM) system employed throughout Europe and in most of the rest of the world encoding at 217 pulses/sec.

RF energy at cell phone frequency range is non-ionizing with biological effects resulting mostly from heating while other mechanisms are not well understood since effects produced by them occur only at very high exposure levels. The recommended exposure standards presently are only associated with excessive tissue heating. The FCC (Federal Communications Commission, USA) limits peak exposure to 1.6 W/kg of tissue averaged over any single gram of tissue (or 1.6 mW/g) though the European limits are less restrictive, specifying 1.6 W/kg averaged over 10 grams. Most modern phones' output is adaptively controlled by the base station and the handset constantly adjusts its power to provide the minimum signal needed to communicate reliably with the base station (Foster 2000).

The ever-growing incidence of mobile phone users globally (2 billion, www. more mobile.co.uk/ mobilephonenews) and nationally (40.6 million, www.ciol.com/content/news/2004), has been accompanied by an upsurge in public and media concern about the possible hazards of this technology and necessitates a comprehensive evaluation of mobile phone users. Mobile telephone handsets operate at low power levels (but the antenna, radiating ~ 600 mW for an analog mobile phone and 125 mW for a digital unit) is placed very close to the head, which can push exposure levels close to the regulatory limits. A complicating factor is that the exposure depends greatly on the exact position of the handset with respect to the head and on the exact shape and electrical characteristics of the head which are all variable quantities (Foster 2000). Though not clear what parameters of the field

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gives biological effect yet an-often used measure is the absorbed radiated energy into body tissues known as specific absorption rate (SAR), expressed as watts per kilogram (W/kg) or milliwatts per kilogram (mW/kg). It is probably not a true measure of the biological hazard from the phone but may be used as an indication of the energy being absorbed into the body.

Various studies on microwaves have been reported to cause chromosome aberrations (Maes et al. 1993, 1997, 2000; Lai and Singh 1995, 1997; Fritze et al. 1997; Malyapa et al. 1997; Svedenstal et al. 1999; Garaj-Vrhovac 1999; Yaguchi et al.1999; Zotti-Martelli et al. 2000; D'Ambrosio et al. 2002; Cho and Chung 2003; Mashevich et al. 2003). However, no direct cytogenetic investigations on mobile phone users have come to light. Therefore the present study was conducted in this direction to undertake cytogenetic analysis in different tissues of mobile phone users: to score any chromosomal aberrations in peripheral blood lymphocyte (PBL) cultures and micronuclei (MN) in buccal smear cells. Lymphocytes are distributed all over the body and move in all the tissues and so can be used to monitor exposures to any body part and the buccal mucosal cells appear to be the cells of choice in mobile phone users since they are in the direct route of exposure. The micronucleus test (MNT) in these cells has been used for biomonitoring of exposed populations as the cells can be easily and rapidly collected by brushing the buccal mucosa and because they are epithelial cell types in which 92% of human cancers arise (Salama et al. 1999).

MATERIALS AND METHODS

Interviews were conducted of mobile phone users and each individual was explained the reason for the present study and sample collection was carried out only after voluntary agreement and written consent. For assessing damage to the genetic material, chromosomal analyses of short term peripheral blood lymphocyte cultures (n=15) and of MN in buccal smear preparations (n=25; of these, 15 same for PBL cultures) were carried out. The cultured peripheral blood lymphocytes were screened for numerical and structural chromosomal aberrations while the MN test scores for both aneugenic and clastogenic effects. Samples from normal healthy controls (n=25) were similarly analyzed for background frequencies.

The short term peripheral blood lymphocyte cultures were set up as per the protocol given by Rooney and Czepulkowski (1986). Blood (5 ml) was drawn intravenously by venous puncture in a heparinized syringe, brought to the laboratory in an ice-box with minimum shaking and was kept in a refrigerator at 4°C for the plasma to separate. In a sterilized culture vial, 8 ml of RPMI-1640 medium was taken to which were added 0.2 ml of PHA-M and 2ml of the supernatant plasma containing the lymphocytes from the buffy coat along with a few drops of RBCs. The culture was kept at 37°C ±1 for 72 hours and was gently shaken once daily. The culture was harvested and 2-3slides/sample made. Well-spread G-banded metaphases of coded preparations were scored (120/sample) at 100x (oil immersion) for chromosomal aberrations.

The procedure of Nair et al. (1991) for buccal MN test was followed. The first scrapings were discarded, subsequent buccal samples were prepared on glass slides (2 each for the left and right cheeks), transported to the laboratory on ice, fixed in 3 methanol: 1 acetic acid (15'), hydrolyzed in 1N HCl (60°C, 8'), stained in Aceto-orcein (20' at 40°C) and counter-stained with 0.1% Fast Green solution(10'). The smear preparations were coded and scored blind. Micronucleated (MNd) cells were confirmed under oil immersion (100x) and also randomly by another observer. The criteria of Tolbert et al. (1992) were adhered to for scanning cells for MN and identifying MN in buccal mucosal cells.

RESULTS

The general details of the mobile phone users are given in Table 1. The age-range of these males was 17-51 years; eight of them were from upper class whereas others belonged to middle class (n=17). The sample group in varied occupations comprised pure vegetarians (n=4), non-vegetarians (n=21), alcohol drinkers (n=14) and non-alcoholics (n=11), smokers (n=2) and non-smokers (n=23). Healthy male volunteers (Table 2) comprised the control group matched for age, sex, socio-economic-status and habits like alcohol, smoking, dietary patterns and who had never used mobile phones at any time; they did not exercise nor had any occupational exposures. At the time of sampling, neither had they taken any medication nor had had any infections.

Table1: Cytogenetic damage in mobile phone users

Subject Code	*Brand & Model No.*	*SAR (W/kg)*	*Duration of use (yr.)*	*Daily freq. of calls (No.)*	*Duration of calls (min.)*		*Daily Exposure (hr.)*		*Kept on "On" mode (hr)*	*Phone attendance (ear)*[a]	*Micro-nucleated cells (%)*	*Aberrant metaphases (%)*
					IN	*OUT*	*IN*	*OUT*				
MM 1	Ericssion S-828	0.77	4.5	20	23	22	14	12.70	13	B	1.10	35.83
MM 2	Panasonic 9210	1.75	4.5	19	15	23	13	10.53	18	L	1.05	30.83
MM 3	Siemens C-35	1.33	5.0	13	17	12	23	9.11	18	R	0.91	31.67
MM 4	Nokia 3310	1.24	5.0	11	6	13	2	2.58	15	R	0.91	43.33
MM 5	Samsung 620	1.38	5.0	14	15	15	13	6.75	17	R	0.77	52.50
MM 6	Nokia 3310	1.24	5.0	28	20	25	13	16.00	12	R	0.91	40.00
MM 7	Nokia 5110	1.45	5.0	14	16	16	31	12.00	14	R	0.80	28.33
MM 8	Nokia 3310	1.24	5.0	11	3	12	16	2.16	13	R	0.74	29.17
MM 9	Nokia 3315	1.47	3.5	35	25	14	9	11.92	13	B	0.81	26.67
MM 10	Nokia 3315	1.47	3.0	15	13	13	4	4.12	18	R	0.72	26.67
MM 11	Nokia 3310	1.24	3.0	11	3	13	11	2.93	12	L	0.60	28.33
MM 12	Nokia 3310	1.24	3.0	13	14	13	11	5.38	14	B	0.85	20.00
MM 13	Samsung N-500	1.21	4.0	13	13	8	23	6.72	13	R	0.90	27.50
MM 14	Panasonic 9215	1.92	3.5	13	8	12	12	4.20	13	L	0.85	21.67
MM 15	Nokia 3315	1.47	3.5	25	13	15	13	9.15	17	R	0.84	26.67
MM 16	Samsung AR-220	1.56	3.5	35	25	10	12	10.83	16	R	0.77	
MM 17	Samsung AR-220	1.56	3.0	16	18	8	9	4.83	15	R	0.66	
MM 18	Nokia 3315	1.47	4.0	15	22	8	20	9.33	14	R	0.59	
MM 19	SonyCMB-1200	1.39	3.0	14	15	11	13	5.82	13	R	0.73	
MM 20	Siemens C-35	1.33	4.0	17	13	5	9	3.37	13	L	0.90	
MM 21	Nokia 3315	1.47	4.5	25	20	24	14	14.67	18	R	0.85	
MM 22	Nokia 3315	1.47	3.0	18	30	26	16	15.80	15	R	0.77	
MM 23	Motorola Startac-130	0.1	3.0	14	13	7	18	5.53	12	R	0.68	
MM 24	Samsung AR-220	1.56	3.5	13	4	13	9	3.41	18	R	0.88	
MM 25	Samsung AR-220	1.56	3.0	23	18	4	9	4.23	18	R	0.83	
TOTAL (n=25 for MNT; n=15 for PBL)										0.82**±0.094	31.28**±10.29	
Control (n=25 for MNT; n=15 for PBL)										0.06±.0003	10.66±4.59	

[a] L-left ear, R-right ear, B-both ears

** - Highly significant when compared to total control group (P<0.05 and P<0.01; Student's 't' test)

Table 2: General information of and cytogenetic damage in normal healthy individuals -control group

Subject Code	*Age (yr.)*	*Socio-economic status*	*Alcohol Intake ml/wk*	*Smoking (Cig/day)*	*Non-Veg gm/day*	*Micro-nucleated cells (%)*	*Aberrant meta-phases (%)*
MC 1	20	Middle	500	-	250	0.06	14.00
MC 2	27	Middle	500	-	250	0.06	10.00
MC 3	48	High	-	-	-	0.06	9.47
MC 4	21	Middle	750	-	250	0.06	8.42
MC 5	24	Middle	400	-	-	0.12	11.00
MC 6	26	Middle	400	-	250	0.06	6.00
MC 7	28	Middle	-	-	-	0.06	15.00
MC 8	45	Middle	-	-	400	0.11	9.00
MC 9	18	Middle	400	-	400	0.06	11.58
MC 10	32	Middle	-	-	400	0.06	8.00
MC 11	25	Middle	-	-	-	0.05	15.00
MC 12	23	Middle	400	-	250	0.06	9.00
MC 13	29	High	400	-	-	0.11	8.42
MC 14	45	Middle	-	-	-	0.06	12.00
MC 15	35	High	750	-	250	0.05	13.00
MC 16	45	Middle	-	-	250	0.00	
MC 17	45	High	750	2-3	250	0.11	
MC 18	21	Middle	500	-	250	0.00	
MC 19	45	Middle	-	4-7	-	0.06	
MC 20	32	Middle	750	-	500	0.06	
MC 21	30	Middle	750	-	-	0.05	
MC 22	28	Middle	-	-	250	0.00	
MC 23	50	Middle	750	-	250	0.10	
MC 24	25	Middle	-	-	250	0.05	
MC 25	40	Middle	750	-	250	0.06	
TOTAL (n=25 for MNT; n=15 for PBL)						0.06±.0003	10.66± 4.59

Of the various brands of cell phones available, Nokia was preferred by 12 (Table1), followed by those using Samsung (6). Other brands were comparatively preferred less (Siemens-n=2, Panasonic-n=2, Motorola-n=1, Sony-n=1, Ericssion-n=1). The SAR value is the specific absorption rate of radiation of the cell phone set and can be considered as a parameter of exposure. The highest number of individuals (n=17) used sets with SAR values in different models ranging from 1.02-1.47 W/kg, followed by those (n=6) with SAR value of 1.48-1.92 W/kg, and there was one each with models having SAR values of 0.77 W/kg and 0.10 W/kg. There were 16 individuals who had been using mobile phones for three to four years and nine individuals using mobile phones for more than four to five years. The daily frequency of incoming (11-35) calls and outgoing (4-30) calls and duration of incoming (5'-25') and outgoing (2'-23') calls showed extensive variations. There were more individuals below 30 years who were using mobile phones (n=15). The daily use of mobile phones varied from 2.16 to 16 hours and the set was kept on "ON" mode for 12-18 hours in a day. Most persons (n=16) attended the phone from their right ears, five individuals attended the phone from their left ears while the remaining four attended the phones from both the ears.

The placement patterns of mobile phones were also noted when an individual is on the move, in office, or at home. On the move all the 25 individuals kept the sets much closer to a part of the body, i.e. in shirt pockets (n=16), trouser pockets (n=2), waist pouches (n=2) or in the hands (n=5). In the office, 21 individuals kept the sets away from their bodies while some individuals kept the phones close to their bodies (in shirt pocket-n= 4). At home only four individuals kept the sets in their pockets while 21 individuals kept them away from their bodies. Headaches, heating sensation, and memory loss were some effects observed in mobile phone users. No aberrant reproductive performance was observed in the pedigree records of the married individuals (n=14). There were also no genetic disorders or major illnesses such as diabetes, cancer, etc.

DISCUSSION

In the present study, the buccal mucosa and the peripheral blood lymphocytes of mobile phone users were investigated for micronuclei and chromosomal aberrations, respectively. For the micronucleus test (MNT), depending on the buccal samples available, 1750 to 2000 cells per individual were scored. The percentage of micronucleated (MNd) cells varied from 0.59 to 1.10 (average 0.82±0.094) while in control individuals percentage of micronucleated cells varied from none to 0.12 (0.06±.0003). Low frequency of MN in oral mucosal cells in healthy individuals has also been reported in literature: 2.59±0.37 in an urban Mumbai population (Nair et al., 1991) and 0.6/1000 in Italy (Sarto et al., 1990). In earlier studies on the buccal MNT in our laboratory (Gandhi and Sharma 1991; Gandhi and Kaur 2000), there was complete absence of MN in healthy controls. Good dietary habits including green and leafy vegetables and fresh fruits as well as eating meat and fish products have been reported to lower MN frequency (Stich and Rosin 1984; Nair et al. 1991). The low frequency of MN in the control group of the present study also reflects on their dietary patterns. On the other hand, increased MN frequency in mobile phone users in some manner is associated with chromosome damage. Similar results are shown in peripheral blood lymphocyte cultures which showed that percentage of aberrant metaphases (31.28±10.29) in the risk group was significantly raised from those observed in the control group (10.66±4.59).

The highest percentage of cells with (1.10%) MN was observed in MM1 who is a male of 19 years, with middle socio-economic status who takes alcohol, does not smoke, is non- vegetarian and uses Ericssion-S-828 mobile phone model with SAR value 0.77 W/kg. He had been using mobile phone for 4.5 years with daily exposure of 12.7 hours and keeps it on "ON" mode for 13 hours and attends the phones from both ears. The individual was a student.

An average 31.28% of aberrant metaphases were found in the total 1800 metaphases scored while the percentage of aberrant metaphases in control data was 10.66 indicating higher significant damage ($p<0.05$, $p<0.01$) in peripheral blood lymphocyte cultures of mobile phone users. There was a preponderance of numerical chromosomal aberrations, especially triploid cells as well as cytological abnormalities like metaphases with acrocentric associations and centromere separation. The highest percentage of aberrant metaphases was observed in MM5 who is a laboratory technician, 24 years of age, belonging to upper socio-economic status who takes alcohol, does not smoke, is non- vegetarian and uses Samsung-620 with SAR value of 1.38 W/kg. His peripheral blood lymphocyte cultures showed 52.50% aberrant metaphases. His mobile phone usage history revealed mobile phone use for the past 5 years with daily exposure of 6.75 hours, keeping the phone on "ON" mode for 17 hours and attending the phone from his right ear.

The results of the present study find some similarity in the literature also. In human subjects occupationally exposed to electromagnetic frequencies (30-300 GHz; 10-50W/cm²SAR), increased chromosomal aberrations but no MN were reported (Garaj-Vrhovac et al. 1990); also increased chromosomal aberrations in lymphocytes of workers occupationally exposed to radar systems (Garaj-Vrhovac and Fucic 1993). However, in male workers (21-55years, average age 40.6 years) employed (10-19years, average age 13.3 years) on radar equipment and antenna system service (1250 MHz-1350MHz radiation) there was an increase in frequency of MN and disturbances in the distribution of cells over various mitotic divisions (Garaj -Vrhovac 1999). Though people working on TV towers had no chromosomal damage (Garson et al. 1991) and though no damage in telecommunication employees was observed, yet an incidence rate ratio for childhood leukaemia was reported as 1.56 and for all ages as 1.23 in a study conducted by Hocking et al. (1996) in populations exposed to RFR [100 kW video amplitude modulated (AM) and 10 kW audio frequency modulated (FM), frequency range from 63 to 215 MHz] from TV towers.

The absence of genotoxicity from exposure of RFR in mobile-phone range in terms of SCEs, chromosomal abnormalities, MN and DNA damage has also been documented. No effects on cell cycle progression or on sister chromatid exchange frequencies in human PBL exposed to 380, 900, and 1800MHz EMF were reported (Antopoules et al. 1997) as well as no chromosomal aberrations in human lymphocytes exposed to RFR-2450 MHz (Vijayalaxmi et al. 1997). Also no increase in MN or DNA damage in human lymphocytes exposed to 1.9 continuous wave

(CW) or pulsed-wave 50Hz RF for 2 hr at SAR of 0.0, 0.1, 0.26, 0.92, 2.4, and 10 W/kg was reported by McNamee et al. (2002). The present study is however a direct study on mobile phone users and elucidates the cytogenetic damage ensuing from mobile phone use. The increases in MNd cell frequencies and cytological abnormalities imply long-term detrimental effects since chromosomal damage is a mechanism relevant to the causation of birth defects and cancer and observations from the present results indicate the potential risk to the buccal mucosa and on the lymphatic system after exposure to microwave radiation through prolonged period of mobile phone use. On the other hand, it has to be recalled that one does not have to be a cell-phone user to become exposed to these radiations; exposure is there from simply living near a base-station which beams the radio waves or being a passenger on a crowded train full of mobile phone users.

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