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Research Article

MICRONUCLEI AND CYTOLOGICAL ABNORMALITIES IN INDIVIDUALS USING CELL PHONES

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ABSTRACT
Mobile phone systems operate within the radio frequency section (30KHz - 300GHz) of the electromagnetic spectrum, the microwaves. Some adverse effects reported from exposure to microwaves include physiological, neurological and cognitive changes, carcinomas, and other biological damage through heating effects. Although equivocal reports on their genotoxic/ carcinogenic potential exist, yet none have been reported in mobile phone users. This study for the first time reports cytogenetic damage in tissues of some individuals using cell phones over a period of time. Male volunteers (15-55y) using different phone models with specific absorption rates (SAR) between 0.10-1.92 W/kg, usage period of 3-5 years, and daily use from 12 to 18 h, were requested to provide user information and blood (n=15) and buccal smear (n=25) samples after informed consent. Significantly elevated micronucleated buccal cells (0.82±0.094 % frequency) and cytogenetically aberrant cells (31.28±10.29%) in short-term PBL cultures as compared to respective values in age- and sex-matched normal, healthy individuals were observed. The use period of mobile phone (daily and years of usage) and SAR values contributed strongly to the cytogenetic damage. Chromosomal damage in buccal cells correlates with exposure in the oral cavity in comparison to whole body exposure (PBL). These results imply caution for the mobile phone users in terms of the dire outcome of genetic damage.

INTRODUCTION
Considerable public concern exists about cellular telephone systems (cell/mobile phones) with regards to potential risks from absorption of radio frequency radiation (RFR), both to users of cell phones and from population exposure to cell phone transmitters. The absorbed radiated energy into body tissues –the specific absorption rate (SAR- expressed as W/kg or as mW/kg) is not a true measure of the biological hazard from the phone but has been used as an indication of the energy being absorbed into the body. Different organizations and governmental agencies for public health and safety with WHO have been monitoring developments and identifying research needs related to RF biological effects and have developed standards for exposure to RFR. The FCC limits peak exposure to 1.6 W/kg (or 1.6 mW/g) of tissue averaged over any single gram of tissue. European limits are less restrictive, specifying 1.6 W/kg averaged over 10 g. Such low electromagnetic frequencies may induce a classic stress response in cells (de Pomerai *et al.*,, 2000) involving the induction of heat-shock genes but no clear molecular mechanism to explain the observed effects have been understood.
India's mobile phone market has also grown rapidly in the last few years with spiraling income levels and falling phone tariffs and handset prices. This widespread prevalence of mobile telecommunications (RF fields 900 MHz -2000 MHz) has generated controversies regarding health outcomes. Published data have largely concentrated on a small number of outcomes, especially brain tumor and leukemia (Ahlbom *et al.*, 2004). Equivocal information has been obtained from animal and cell culture studies for genotoxicity and carcinogenesis. In fact, there are several possible sources of artifacts in such studies and there is often no clear molecular mechanism to explain the effects observed, which may occur only within the narrow windows of frequency and/or power level and/or pulse length/interval/and/or total exposure time (Goodman *et al.*, 1995). A four-year research project funded by the
European Union measured a significant increase in single- and double-strand DNA breaks in human and animal cells after being exposed to electromagnetic fields that are typical for mobile phones. The laboratory study did not prove that mobile phones are a risk to health but concluded that more research is

needed to see if effects can also be found outside the laboratory (The REFLEX project, 2004). Genotoxic effects and chromosomal damage are important starting points for many possible health effects. The present work reports the cytogenetic damage in different tissues of mobile phone users and appears to be the first of its kind. The objectives of this study were to score for any chromosomal aberrations in short term peripheral blood lymphocyte cultures, and for the presence of micronuclei in buccal smear cells of mobile phone users. Preliminary data from the laboratory have however been published for both chromosomal and DNA damage (Gandhi and Anita, 2005; Gandhi and Singh, 2005).

MATERIALS AND METHODS

Cytogenetic studies in peripheral blood lymphocyte cultures (n=15) and in buccal smear preparations (n=25; for 15 of these cultures were also set-up) of mobile phone users were carried out. The study was cleared by the institutional ethical review committee. Information from mobile phone users was recorded by the interview method on a pre-designed questionnaire and pertinent records were maintained for queries on type of cell phone set, daily frequency of calls (incoming and outgoing), duration of calls, use period in 24 hours and in years, specific absorption rate (SAR) of the model (obtained from the model's website) and the brand in use. Each individual was explained the reason for the present study and sample collection was carried out only after voluntary agreement and written consent. Individuals not exposed to hazardous agents at workplace and/or accidentally formed the sample group.

Peripheral blood lymphocyte (PBL) cultures of mobile phone users were set up according to standard procedures (Gosden et al.,1992) with the chemicals obtained locally. Briefly, intravenous blood was cultured in RPMI 1640 with PHA-M for 70-72 hours at 37 $\pm 1^{o}$C followed by addition of colchicine for termination of the cultures. The pellet was suspended in pre-warmed hypotonic solution and processed for slide preparation after fixation in chilled 3methanol:1 acetic acid mixture. The slides (2-3/individual) were coded and air dried .Well-spread metaphases were observed at 100x (oil immersion) and scored (120/individual) for both structural and numerical aberrations as well as for any cytological abnormalities.

The micronucleus (MN) test has been adopted by various laboratories for peripheral blood lymphocytes, epithelial cells, erythrocytes and fibroblasts. The presence of micronuclei is taken as an evidence of the appearance of chromatid/chromosome fragments or lagging chromosomes or of effects on the mitotic spindle and the appearance of numerical chromosome aberrations (Fenech *et al.*,,1999). Among the epithelial cells, the buccal smear cells cause little or no inconvenience to the individual and the sampling is fast and highly economical. The MN test in buccal cells has also been a method of choice for survey of large population groups, especially as a preliminary indicator of pre-cancerous lesions (Stich and Rosin, 1984) and hence was thought ideal for assessing chromosomal damage. The procedure of Nair *et al.*, (1991) for buccal MN test was followed. The first scrapings were discarded and the subsequent buccal smear samples on glass slides (separately for left and right cheeks) were transported to the laboratory on ice. Within 3-4 h of sampling, buccal mucosal cells were fixed in 3 methanol: 1 acetic acid for 15 min. The smears were hydrolyzed in 1N HCl at 60°C for 8 min followed by a rinse in tap water. Staining in Aceto-Orcein (2% aqueous) for 20 min at 40°C was followed by washing in ethanol and distilled water. Counterstaining of the slides was done in 0.1% Fast Green aqueous solution for 10 min followed by final rinses in ethanol and in distilled water. The slides (4/individual) were coded and scored blind for MN (2000 cells / individual). 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.

Information on a normal healthy control population who had never used mobile phones (n=25) was also collected and their samples were simultaneously analyzed along with those of mobile phone users for background frequencies. The Chi square (χ^2) test was performed to check for any differences for age, sex and dietary habits between the mobile phone users and control individuals. Statistical analysis of the data obtained for MNd cells and chromosomal damage in both the groups was performed with software package SPSS version 7.0 to check for any significance in genetic damage between the mobile phone

users and control individuals. The effect of confounding factors on genetic damage was assessed using multiple regression analysis and ANOVA while the Student's t-test was carried out to find whether the genetic damage in phone users was significant from that in controls.

RESULTS

In the present investigation, cytogenetic analysis of short-term peripheral blood lymphocyte cultures and of buccal mucosal cells of mobile phone users was carried out. A control group was similarly investigated. The Chi-square test (χ^2) results revealed that control individuals were matched with the sample group (Table 1) since differences in age, socio-economic status, diet, smoking and drinking habits were statistically non-significant. The occupational sub-groups revealed no other hazardous exposures except for the use of mobile phones by the sample group. The peripheral blood cultures lymphocytes did not reveal structural chromosomal abnormalities but numerical chromosomal aberrations including metaphases with acrocentric associations (AAs), triploidy, tetraploidy, diplochromosomes and centromeric separations, were observed. An average of 31.28% of aberrant metaphases was 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 (t_{cal}.-8.80, t_{tab}.-2.14, d.f.-14). The percentage frequency of metaphases with acrocentric associations (between chromosome groups D/G, D/D, DD/G, GG/D, G/G) in mobile phone users (16.89±3.79) was significantly higher (t_{cal}.-4.28, t_{tab}.-2.14. d.f.-14, $p<0.05$, $p<0.01$) as compared to the control value (8.38±2.23). Triploidy was more frequent (5.28% vs.1.01% in control) followed by cells with ploidy (tetraploidy : >n=98, >n=100) conditions (5.06% vs. 1.01% in control) as were cells showing centromere separation in all chromosomes (4.06% vs. 0.27% in controls). Diplochromosomes (endoreduplications) were also observed.

Micronuclei were observed in the buccal preparations of all the individuals and the percentage of micronucleated (MNd) cells was higher (0.82 ±0.094; range 0.59 -1.10) than in the control individuals (0.06±.0003). There were no differences for MN induction between the two cheeks and so their data were pooled.

Regression analysis (Table 2) was performed to determine whether confounders like age, socio-economic status and other life-style habits showed any correlation with genetic damage observed. In the control group, the analysis yielded non-significant results. Positive correlations for both, percentage of aberrant metaphases and cells with micronuclei were found for SAR values, duration of use of mobile phone and phone-use per day. These are all vital parameters of mobile phone use. The observation for ANOVA revealed significant P-values only for SAR values and exposure per day for both, the percent micronucleated cells and percent aberrant metaphases (Table 3). The data from the MNT and PBL cultures were further studied (Student's t-test) for observing whether induced genetic damage showed any correlations with age, period of use of mobile phone, daily use of mobile phone and the SAR values of the mobile phones.

In Table 4 is presented the data for the percentage of MNd cells in buccal cells and aberrant metaphases in PBL of mobile phone users in different age groups. The mean percentage frequency of MNd cells varied from 0.75±.011 to 0.85±.059 and the percentage of aberrant metaphases varied from 26.67±1.95 to34.44 ±3.95 in different age-groups though there was no significant increase with age. The age-range (17-55 years) includes both young and older mobile phone users but no significant effects for MN and chromosomal aberrations (CAs) were seen among age-groups. However, there were marked differences from the control group data.

The data from MNT and PBL cultures in mobile phone users using phones over different number of years are given in Table 5. There was observed a gradual increase in frequency of MNd cells among different groups for duration of use. The percent MN frequency was 0.78 for 3-4 years of use and this increased to 0.89% for a use of 4-5 years. There were 25.36% (±6.80) aberrant metaphases for a use of 3-4 years and

Table 1. Characteristics of mobile phone users and of control individuals

Characteristics			No. of mobile phone users	No. of controls	χ^2 (P<0.05)
Age-range (yr)			17-51	17-51	1.85
SES		Upper	8	4	1.76
		Middle	17	21	
Diet		Veg	4	8	1.76
		Non- Veg	21	17	
Smoking		Yes	2	5	1.49
		No	23	20	
Alcohol Consumption		Drinkers	14	15	0.08
		Non-Drinkers	11	10	
Married		Yes	14	-	-
		No	11	-	-
Duration of mobile phone use(yr)		>4-5	9	-	-
		3-4	16	-	-
Calls/day(frequency)		Incoming	11-35	-	-
		Outgoing	4-30	-	-
Daily use of mobile phone			2.16-16.00hrs	-	-
Mobile phone kept on "ON" mode/day			12-18 hrs	-	-
Attendance of phone(ears)		Left	5	-	-
		Right	16	-	-
		Both	4		-
Placement pattern of phone when :	On the move	Shirt Pocket	16	-	-
		Trouser Pocket	2	-	-
		Waist pouch	2	-	-
		In Hand	5	-	-
	In the Office	Shirt Pocket	4	-	-
		Table	21	-	-
	At home	Shirt Pocket	4	-	-
		Table	12	-	-
		Shelf	9	-	-
Occupation mobile phone		Chemists	6	-	-
		Medical representative	5	-	-
		Student	4	-	-
		Building contractor	2	-	-
		Doctor	1	-	-
		Others	7	-	-
Health effects		Headache	6	-	-
		Hearing sensation	3	-	
		Memory Loss	2	-	-

36.46% (±7.25) for a use of 4-5 years. Statistically significant differences were however observed only from total control values and none within the groups for both MNT and the PBL culture data.

Table 2. Regression coefficient analysis of various factors with percentage of MNd cells and of aberrant metaphases in mobile phone users and control individuals

	Age	SES	Alcohol	Veg/Non-Veg	Smoking	SAR	Duration of use	Exposure/day
Mobile phone users								
MNT								
r^2	0.031`	0.001	0.021	0.024	0.004	0.002	0.209	0.081
p	0.396	0.934	0.488	0.464	0.752	0.030	0.020	0.016
PBL Cultures								
r^2	0.109	0.201	0.131	0.069	.142	0.095	0.460	0.025
p	0.230	0.617	0.185	0.346	0.192	0.050	0.005	0.028
Control Group								
MNT								
r^2	0.046	0.094	0.059	0.051	0.056	-	-	-
p	0.305	0.137	0.243	0.278	0.253	-	-	-
PBL Cultures								
r^2	0.014	0.005	0.055	0.129	0.067	-	-	-
p	0.680	0.807	0.401	0.188	0.520	-	-	-

r- Regression coefficient, if (p) = 0.05, test is significant.

Table 3. Multifactorial Analysis of Variance of various factors with percentage of MNd cells and of aberrant metaphases in mobile phone users.

Source of Variation	**Sum of Squares**	**d.f.**	**Mean Squares**	**F-ratio**	**Significance (p-value)**
The Micronucleus Test					
AGE	2622.960	24	152.19	.602	.815
SES	5.440	24	.286	.708	.736
ALCOHOL	6.160	24	.357	.603	.814
SMOKING	1.840	24	9.524	.725	.724
VEG./NV.	3.360	24	.190	.626	.797
SAR	2.739	24	3.830	3.796	.040
DURATION OF USE	15.640	24	.476	1.520	.296
EXPOSURE/DAY	446.479	24	28.934	.496	.050
Peripheral blood lymphocyte cultures					
AGE	1294.933	14	68.000	**1.458**	.421
SES	3.600	14	.222	1.200	.497
ALCOHOL	3.600	14	.222	1.200	.497
SMOKING	1.940	14	8.257	1.281	.485
VEG./NV.	2.933	14	.167	1.327	.457
SAR	.954	14	7.350	11.527	.034
DURATION OF USE	9.833	14	.722	.965	.586
USE/DAY	254.221	14	24.135	.685	.470

If (p) = 0.05, test is significant.

Table 4. Effect of age on chromosomal damage in mobile phone users

The MN Test													
Age (yr)		**No.**	**Duration of use (Yr.)**		**SAR value (W/kg)**		**Daily usage (hr.)**		**Attending the phone (ears)**			**Micronucleated cells/cells scored (%)**	**Mean Percent Frequency of MNd cells±S.E.M**
Range	Mean		Range	Mean	Range	Mean	Range	Mean	L	R	B		
15-25	21.1	09	3-5	3.89	0.77-1.47	1.29	2.58-15.8	7.86	2	4	3	141/17280 (0.82)	0.81**±.017
26-35	29.2	09	3-5	4.00	0.10-1.75	1.30	3.41-16.0	9.13	1	7	1	142/16700 (0.85)	0.85**±.059
36-45	44.2	04	3-5	3.63	1.24-1.56	1.48	2.16-10.8	5.51	1	3	-	54/7150 (0.76)	0.75**±.011
46-55	48.0	03	3-5	3.83	1.33-1.92	1.55	4.20-9.11	6.37	1	2	-	47/5650 (0.83)	0.83**±.033
TOTAL (n=25) Control (n=25)		25							5	16	4	384/46780 (0.82) 27/45150 (0.06)	0.82**±.094 (0.06±.0003)

Peripheral blood lymphocyte cultures													
Age (yr)		**No.**	**Duration of use (yr)**		**SAR value (W/kg)**		**Daily usage (hr.)**		**Cells showing AA**	**Triploids**	**Cells Other than Triploids**	**Cells showing Centromere Separation**	**TAM/TMS (% ± S.E.M)**
Range	Mean		Range	Mean	Range	Mean	Range	Mean					
15-25	21.5	06	3-5	4	0.77-1.47	1.22	2.58-12.7	7.04	133	40	37	38	248/720 (34.44**±3.95)
26-35	33.33	06	3-5	4.17	1.21-1.75	1.43	4.12-16.0	9.75	119	36	34	27	216/720 (30.00**±3.47)

36-45	45.00	01	5	5	1.24	1.24	2.16	2.16	20	7	5	3	35/120 (29.17**±1.19)
46-55	46.50	02	3.5-5	4.25	1.33-1.92	1.63	4.2-9.11	6.67	32	12	15	5	64/240 (26.67**±1.95)
TOTAL (n=15) Control (n=15)		15 15							304 124	95 15	91 15	73 4	563/1800 (31.28**±10.29) 158/1480 (10.66± 4.59)

***- Highly significant when compared to total control group (P<0.05 and P<0.01; Student's 't' test).*
TMS- Total Metaphases Scanned, TAM- Total Aberrant Metaphases.
AA- Acrocentric Associations;L- Left Ear, R- Right Ear, B- Both Ears.

Table 5. Chromosomal damage as a function of period of use of mobile phones.

The MN Test													
Duration of use (yr)		**Age (yr)**		**No. of individuals**	**SAR value (W/kg)**		**Daily use (hr)**		**Attending the phone (ears)**			**Micronucleated cells/cells scored (%)**	**Mean Per cent Frequency of MNd cells ±S.E.M**
Range	Mean	Range	Mean		Range	Mean	Range	Mean	L	R	B		
3-4	3.34	17-51	31.5	16	0.10-1.92	1.38	2.93-11.92	6.72	4	9	3	235/30200 (0.78)	0.77**±.062
>4-5	4.83	19-46	30.0	09	0.77-1.75	1.32	2.58-16.00	9.61	1	7	1	149/16580 (0.89)	0.99**±.058
TOTAL (n=25) Control (n=25)				25					5	16	4	384/46780 (0.82) 27/45150 (0.06)	0.82**±.094 0.06±.0003
Peripheral blood lymphocyte cultures													

Duration of use (yr)		**Age (yr)**		**No. of individuals**	**SAR value (W/kg)**		**Daily use (hr)**		**Cells with AA**	**Triploid cells**	**Cells Other than Triploids**	**Cells showing centromere separation**	**TAM/TMS (% ± S.E.M)**
Range	Mean	Range	Mean		Range	Mean	Range	Mean					
3-4	3.36	17-47	29.71	07	1.21-1.92	1.43	2.93-11.92	6.35	95	44	48	26	213/840 (25.36**±6.80)
>4-5	4.88	19-46	30.13	08	0.77-1.75	1.30	2.16-16.00	8.98	209	51	43	47	350/960 (36.46**±7.25)
TOTAL (n=15) Control (n=15)				15 15					304 124	95 15	91 15	73 4	563/1800 (31.28**±10.29) 158/1480 (10.66± 4.59)

**- Highly significant when compared to total control group (P<0.05 and P<0.01; Student's 't' test).
TMS- Total Metaphases Scanned, TAM- Total Aberrant Metaphases.;AA- Acrocentric Association; L- Left Ear, R- Right Ear, B- Both Ears.

Table 6. Chromosomal damage as a function of daily use of mobile phones

The MN Test													
Daily usage (hr)		**No. of individuals**	**Age (yr)**		**Duration of use (yr)**		**SAR value (W/kg)**		**Attending the phone (ears)**			**Micronucleated cells/cells scored (%)**	**Mean Percent Frequency of MNd cells± S.E.M**
Range	Mean		Range	Mean	Range	Mean	Range	Mean	L	R	B		
1-4	2.89	05	21-45	27.6	3-5	4.1	1.24-1.56	1.32	2	2	1	76/9400 (0.81)	0.81**±.013
>4-8	5.29	09	23-51	35.44	3-5	3.39	0.10-1.92	1.31	2	6	1	134/17130 (0.78)	0.78**±.016
>8-12	10.41	07	17-46	31.86	3.5-5	4.14	1.33-1.75	1.50	1	5	1	105/12700 (0.83)	0.82**±.015
>12-16	14.79	04	19-29	23.5	3-5	4.25	0.77-1.47	1.23	-	3	1	69/7550 (0.91)	0.91**±.011
TOTAL (n=25) Control (n=25)		25							5	16	4	384/46780 (0.82) 27/45150(0.06)	0.82**±.094 0.06±.0003
Peripheral blood lymphocyte cultures													

Daily usage (hr)		**No. of individuals**	**Age (yr)**		**Duration of use (yr)**		**SAR value (W/kg)**		**Cells Showing AA**	**Triploid cells**	**Cells Other than Triploids**	**Cells showing centromere separation**	**TAM/TMS (% ± S.E.M)**
Range	Mean		Range	Mean	Range	Mean	Range	Mean					
1-4	2.56	03	21-45	30.3	3-5	4.33	1.24-1.56	1.32	61	23	15	22	121/360 (33.61**±3.45)
>4-8	5.43	05	23-47	31	3-5	3.7	1.21-1.92	1.44	84	35	37	22	178/600 (29.67**±4.47)
>8-12	10.90	06	17-46	29.33	3-5	4.33	0.77-1.75	1.37	129	32	33	22	216/720 (30.00**±5.86)
>12-16	16.00	01[a]	27	27	5	5	1.24	1.24	30	5	6	7	48/120 (40.00±1.85)
TOTAL (n=15)		15							304	95	91	73	563/1800 (31.28**±10.29)
Control (n=15)		15							124	15	15	4	158/1480 (10.66± 4.59)

***- Highly significant when compared to total control group (P<0.05 and P<0.01; Student's 't' test).; a not compared*
TMS- Total Metaphases Scanned, TAM- Total Aberrant Metaphases.; AA- Acrocentric Associations.; L- Left Ear, R- Right Ear, B- Both Ears.

Table 7. Chromosomal damage as a function of Specific Absorption Rate (SAR) of mobile phones

The MN Test

SAR value (W/kg)		**No.**	**Age (yr)**		**Duration of use (yr)**		**Daily usage (hr)**		**Attending the phone (ears)**			**Micronucleated cells/cells scored (%)**	**Mean Percent Frequency of MNd cells±S.E.M**
Range	Mean		Range	Mean	Range	Mean	Range	Mean	L	R	B		
0.10-0.56	0.10	01	26	26	3	3	5.53	5.53	-	1	-	13/1900 (0.68)	0.68**±.002
0.57-1.01	0.77	01	19	19	4.5	4.5	12.7	12.7	-	-	1	22/2000 (1.10)	1.10**±.007
1.02-1.47	1.35	17	17-51	29.18	3-5	4.03	2.16-17.16	8.10	2	13	2	256/31880 (0.80)	0.80**±.073
1.48-1.92	1.65	06	26-47	38.83	3-4.5	3.5	3.41-10.83	6.34	3	2	1	93/11000 (0.85)	0.84**±.027
TOTAL (n=25) Control (n=25)		25							5	16	4	384/46780 (0.82) 27/45150 (0.059)	0.82**±.094 0.06±.0003

Peripheral blood lymphocyte cultures

SAR value (W/kg)		**No.**	**Age (yr)**		**Duration of use (yr)**		**Daily usage (hr)**		**Cells showing AA**	**Triploids**	**Cells Other than Triploids**	**Cells showing centromere separation**	**TAM/TMS (% ± S.E.M)**
Range	Mean		Range	Mean	Range	Mean	Range	Mean					
0.77-1.24	1.17	07	19-45	27.00	3-5	4.21	2.16-16.00	6.92	146	45	42	36	269/840 (32.08**±6.86)
1.25-1.72	1.43	06	17-46	30.83	3.5-5	4.17	6.75-12.00	8.84	124	41	36	30	231/720 (10.50**±5.85)
1.73-2.20	1.84	02	28-47	37.50	3.5-4.5	4.00	4.20-10.53	7.37	34	9	13	7	63/240 (26.25**±2.40)
		15							304	95	91	73	563/1800 (31.28**±10.29)
		15							124	15	15	4	158/1480 (10.66±4.59)

*- Highly significant when compared to total control group (P<0.05 and P<0.01; Student's 't' test).
TMS- Total Metaphases Scanned, TAM- Total Aberrant Metaphases.
AA- Acrocentric Associations.
L- Left Ear, R- Right Ear, B- Both Ears.

Daily use was calculated by adding the duration of incoming and outgoing calls averaged per day (Table 6). The greatest percentage of MNd cells was obtained in the >12-16 hours' user group. The damage appeared to increase (though not significantly) proportionally with daily usage except in the second group (>4-8 hours'use). This could be related to less years of use and a different SAR value. The greatest percentage of aberrant metaphases was also obtained in the >12-16 hours' exposure group though this was not in any way significantly higher from values in other groups. The relation between SAR value and percentage of cells with MN and aberrant metaphases is given in Table 7. The greater SAR value groups (third and fourth) have less exposure in hours than the other two groups in which the number of individuals is significantly lower (only one each against 17 and 6 in the latter two). In latter two groups, percentage of cells with MN increased with increased SAR values in spite of decreased daily and years of usage. On the other hand, the maximum percentage of aberrant metaphases was 32.08 in the SAR range of 0.77-1.24 W/kg with lower percentage (10.50) in the group exposed to 1.25-1.72 W/kg. This may be in relation to the fact that factors like use-period and daily use are also interacting with SAR value to bring about the induced genetic damage. Higher MN frequencies were observed with increased SAR values though no concurrent increase of aberrant metaphases has been observed. However at all levels, the genetic damage was significantly elevated as compared to that in control individuals.

DISCUSSION

In the present study increases in MN frequency and cytological abnormalities in mobile phone users have been observed. These can imply long-term detrimental effects since chromosomal damage is a mechanism relevant to the causation of birth defects and cancer. Observations from the present results indicate the potential risk on the lymphatic system and the buccal mucosa after an exposure to microwave radiations over a period of mobile phone use.

Acrocentric association may be considered a preamble to non- disjunction and/or structural rearrangements (Ferguson-Smith and Handmaker, 1961) as are MN (Stich and Rosin, 1984; Fenech *et al.*,1999).Acrocentric associations have also been implicated in patients of Down Syndrome (Babu and Verma, 1985), in increasing frequency of spontaneous abortions and in the predisposition to Turner Syndrome, malignancy and genetic defects (Pathak, 1986; Jackson-Cook and Brown, 1987; Murthy *et al.*,1989; Sengupta *et al.*, 1994). In the present study such associations between and among satellited chromosomes also indicate a probable predisposition to genetic defects and malignancy depending on the nature of tissue exposed.

Premature centromere division/separation/condensation has been reported to lead to aneuploidy via non-disjunction (Fitzgerald, 1975) and to be associated with chronic myeligenous leukemia (Vig, 1990), Fanconi's anemia, ataxia telangiectasia, in chromosomal loss during ageing (Stone and Standberg, 1995) and in recurrent spontaneous abortions (Fitzgerald *et al.*,, 1986). The presence of centromere separation in PBL cultured cells also imply an impact of non-disjunction as in age-related changes, chromosomal instability syndromes and carcinogenesis.

The presence of both conventional chromosomes (monochromosomes) and diplochromosomes in the cell preparations indicates out-of-phase DNA synthesis, which has been also demonstrated cytogenetically as premature chromosome condensation (PCC) in endoreduplicated Chinese hamster ovary (CHO) cells induced by colchicine or vincristine (Sumner, 1988). Endoreduplication as defined by Levan and Hauschka (1953) consists of a microscopically non-visible duplication of the chromosomes within the cell nucleus leading to the formation of so-called 'diplochromosomes' in the following mitosis. Diplochromosomes therefore consisting of four chromatids lying side-by-side, instead of the normal two, are produced when cells go through two rounds of DNA replication without separation of chromatids. They are thus an indication of the failure of the normal chromosome separation mechanism and endoreduplication may allow the growth of cells beyond the limit defined by the nuclear/cytoplasmic ratio that normally restricts the size of diploid cells, and would thus allow considerable growth in post-mitotic cells (MacAuley *et al.*, 1988).

The technology of cell phones prevalent in the present study group included GSM, TDMA, and CDMA, which are innovations for good subjective speech quality, low terminal and service costs, support for international roaming, ability to support handheld terminals, support for range of new

services and facilities, and spectral efficiency. The maximum 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) having models with SAR values 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. It was also found that individuals below 30 years were extensive users of 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 attended from their left ears while four attended from both the ears.

In the literature perused, cytogenetic consequences of RFR exposure, especially for chromosome damage and MN formation, indicate both positive and negative results under different experimental conditions for studies conducted *in vitro* and *in vivo*. No genotoxicity studies on mobile phone users exist and to the best of our knowledge , the present study is the first of its kind. Hence only some investigations in human and rodent cells at comparable

SAR and RFR of mobile phones as used by the study group, are discussed.

Increased induction of DNA repair, DNA synthesis and inhibition of UV-induced DNA repair synthesis was reported (Meltz *et al.,* 1987) in human MRC-5 fibroblasts cells exposed to RFR frequencies [350MHz, 850MHz, 1200MHz, continuous wave(CW) and pulse wave(PW)] and there was also increased cytogenetic damage in human blood lymphocytes exposed to RFR 1250MHz1350MHz (Fucic *et al.,*1992) and to 954 MHz at a distance of 5 cm from a car phone antenna (Maes *et al.,*1997). Occupational exposure in microwave oven repairmen led to an increase in MN frequency in their lymphocytes (Garaj-Vrhovac ,1999). Significant induction of micronuclei in human peripheral blood lymphocytes exposed to microwave frequency used for mobile communication was also reported (d' Ambrosio *et al.,* 2002). However, no increases in DNA damage as observed by alkaline comet assay, and in micronuclei frequency in blood cells exposed to both, CW and PW of 1.9GHz RF field at SAR 0 to 10W/kg for 24h, have been reported (McNamee *et al.,* 2002a,b) but there was a linear increase in chromosome 17 aneuploidy in human peripheral blood lymphocytes exposed to continuous 830 MHz EMF (SAR 1.6-8.8 W/kg) for 72 hr (Mashevich *et al.,*2003). Primary DNA damage after *in vitro* exposure of Molt 4-lymphoblastoid cells to RFR 813.56 MHz (iDEN) and 836.55 MHz (TDMA) (Phillips *et al.,*1998) and both primary DNA damage and elevated MN frequency in human blood lymphocytes exposed to different frequencies, 837 MHz (TDMA), 837 MHz (CDMA) and 1900 MHz (PCS) (Vasquez *et al.,* 1999) have been reported besides neoplastic transformation (Roti Roti *et al.,* 2001) after exposure to RF radiation in the cellular-phone communication range (835.62 MHz FDMA; 847.74 MHz CDMA) at SAR 0.6W/kg in C3H 10T (1/2). Alternation in cell morphology and increased expression of mitogenic signal transduction genes and genes controlling apoptosis in human skin fibroblasts exposed to GSM radiofrequency for one hour have also been observed (Pacini *et al.,* 2002). Paulraj and Behari (2006) reported statistically significant ($p<0.001$) increase in DNA single strand breaks in brain cells of rat exposed to microwave radiation (2.45 and16.5 GHz, SAR 1.0 and 2.01 W/kg, respectively).

The absence of genotoxicity from exposure of RFR in mobile phone range in terms of sister chromatid exchanges (SCE), chromosomal abnormalities, MN and DNA damage also has been documented. No effects on cell cycle progression or SCE frequencies in human PBL exposed to 380, 900, and 1800MHz EMF were reported (Antonopoulos *et al.,* 1997) as well as no chromosomal aberrations in human lymphocytes exposed to RFR-2450 MHz (Vijayalaxmi *et al.,* 1997). No primary DNA damage was reported in human glioblastoma and C3H10T½ cells to RFR 835.62MHz (FDMA) and 847.74MHz (CDMA) for various periods of time up to 24 h (SAR 0.6 W/Kg) (Malyapa *et al.,*1997). No mutagenic/co-mutagenic/synergistic effects of 900 MHz radiation exposure on human lymphocytes, either GSM microwave exposure alone or after combination with X-rays or mitomycin C, were reported (Maes *et al.,*2000). Human blood leukocytes and lymphocytes after *in vitro* exposure to high power microwave pulses also did not induce DNA strand breaks, alkali-labile sites, and incomplete excision repair sites, which could be detected by the alkaline comet assay (Chemeris *et al.,* 2006).

Studies in rodents in different cells, both *in vivo* and *in vitro* have also documented equivocal results. Cell proliferation and changes in DNA synthesis rate in C6 glioma rat cells in vitro exposed to 836.55MHz (TDMA) RF radiation were reported (Stagg *et al.,* 1997) while potential mutagenic effects at DNA sequence level in mouse testes exposed to 2450MHz CW at an SAR of 1.18w/kg for 2h/day over a period of 120-200days was documented (Sarkar *et al.*,1994). Increase in number of leucocytes and micronucleated erythrocytes in peripheral blood in rats exposed to 50Hz magnetic fields was also reported (Svedenstal *et al.,* 1998). Increased single strand DNA breaks were reported (Lai and Singh,1995) in brain cells of rats exposed to 2450 MHz microwaves for 2 hrs at SAR 1.2 W/kg and in rats exposed to low intensity 60 HZ magnetic field (Lai and Singh,1997).

Negative reports include lack of SCE in mouse bone marrow cells exposed to 800 MHz and to 900 MHz RFR at SAR of 4W/kg for 8 h (Brown and Marshall, 1982) and no increase in CA/SCE in bone marrow cells of mice exposed in vivo to 2450 MHz microwaves radiation (Banerjee *et al.*,1983). No significant reductions in pregnancy rate, pre-implantation or post-implantation survival rates were observed (Saunders *et al.*,1988) in male mice

exposed to chronic low-level microwave radiation (100Wm-² of 2.45GHz CW for 6hrs/day for a total of 120 h over 8 weeks). There was also no damage in cerebral cortex or the hippocampus of rats exposed to 2450 MHz EMF for 2 hrs at SAR 1.2 W/kg (Malyapa *et al.,* 1998). Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay *in vitro*, Baohong *et al.*(2005) observed no DNA damage effects but DNA damage effects induced by MMC and 4NQO were enhanced. Peripheral blood samples collected from healthy human volunteers were exposed *in vitro* to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. The levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different (Vijayalaxmi., 2006). Stronati *et al.* (2006) used standard tests for chromosomal and DNA damage in Go human lymphocytes exposed *in vitro* to a combination of X-rays and RFR. They comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg was genotoxic *per se* or whether, it could influence the genotoxicity of X-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed. The combined genotoxic effects of radiofrequency electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen,3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone was examined in female rats for 2-years . There was no evidence for enhanced genotoxicity(alkaline comet assay, micronuclei) in rats exposed to RF radiation (Verschaeve et al, 2006).

The results of the present study in terms of elevated MN frequency in buccal cells and significant increase in aberrant metaphases in PBL cultures along with those of another study (Gandhi and Anita,2005) for DNA damage and MN induction in PBL imply the genotoxic nature of microwave radiations used in mobile phones (900-2000 MHz) in the absence of any other detrimental exposure. The effect of mobile phone usage has a direct bearing on the clastogenic and aneugenic potential of these microwaves. The greatly increased MN frequency in the cells of the buccal mucosa and the increase in aberrant metaphases showing increased ploidy levels, diplochromosomes, acrocentric associations and premature centromere separation in cultured PBL (whole body exposure to circulating cells) compliment each other since MN can arise from clastogenic and/or aneugenic action and acrocentric associations and centromere separation lead to nondisjunction. Another fact is that the induced MN frequency seen in direct buccal smear preparations is a result of genetic

damage ensuing in the basal epithelium which has manifested after cell division in the buccal epithelium cells. The cytological abnormalities observed in cultured PBL rather depict the damage in circulating blood cells, which have been induced to divide and may have partially repaired the damage.

Chromosomal lagging and non-disjunction are the main mechanisms of chromosomal malsegregation at mitosis and these two events contribute in the genesis of spontaneous or induced aneuploidy. The abnormal behaviour of centromeres may predispose the individual to cell division errors, the

consequence of which may be lead to malignancy. Bajnóczky and Méhes (1988) observed that the centromere separation sequence in lymphocyte mitoses of the parents of four infants with trisomy 18, five patients with trisomy 21, and five children with normal karyotype was evidence for the correlation between alteration of the parental centromere separation sequence and aneuploidy of the offspring.

'Premature chromatid separation (PCS)' is sometimes incorrectly referred to as 'premature centromere division (PCD).' PCD is a distinct entity; Premature chromatid separation consists of separate and splayed chromatids with discernible centromeres and involves all or most chromosomes of a metaphase. It is found in up to 2% of metaphases in cultured lymphocytes from approximately 40% of normal individuals. When PCS is present in 5% or more of cells, it is known as the 'heterozygous PCS trait' and may decrease fertility (Gabarron *et al*., 1986). Its inheritance is autosomal codominant (Kajii and Ikeuchi, 2004).The sample group of the present study show this condition with a possible cancer predisposition.

Mehes *et al.* (2002) reported a 22-month-old girl with nonsyndromic Wilms tumor who was found to have a normal karyotype but premature chromatid separation in 21% of her lymphocyte mitoses. This phenomenon was not found in her parents. PCS was noted in less than 4% of lymphocyte mitoses from 5 other children with Wilms tumor, similar to results obtained from 12 healthy controls.

Kajii *et al.* (2001) reported 5 infants (2 girls and 3 boys) with mosaic variegated aneuploidy (MVA) and total PCS from 4 families. All demonstrated severe pre- and postnatal growth retardation, profound developmental delay, etc. and four developed Wilms tumors, and one had cystic lesions in both kidneys. Cytogenetic analysis of 2 infants showed 48.5% and 83.2% lymphocytes in total PCS; their parents had 3.5 to 41.7% of their lymphocytes in total PCS.

Plaja *et al.* (2001) studied 3 patients with MVA related to PCS, showed that the phenomenon is expressed in vivo, and found that PCS is a cancer-prone disorder. One of their patients was the first in whom this condition was recognized Kawame *et al.* (1999) described a Japanese male infant with multiple congenital anomalies and mosaic variegated aneuploidy; later he developed bilateral Wilms tumors. Multiple cytogenetic analysis revealed various multiple numerical aneuploidies in blood lymphocytes, fibroblasts, and bone marrow cells, together with premature chromatid separation. Peripheral blood chromosome analysis in his parents also showed PCS, but no aneuploid cells i.e. a consequence of the homozygous PCS trait inherited from his parents.

A high frequency of acrocentric chromosome associations was also
observed in mothers of children of Down syndrome, this may have predisposed them to an enhanced risk for non-disjunction (Jyothy *et al*., 2000). Chromosome studies were carried out in long-term (142 and 184 d) human lymphocyte *in vitro* cultures in order to investigate the cytogenetic status of aging lymphocytes. and structural abnormalities, telomere fusions were detected in all donor cells, and associations of acrocentric chromosomes were found in six persons in the three age-groups. Investigated (Busson-Le Coniat et al., 2002).

Since endoreduplication disrupts the alternation of DNA synthesis and mitosis that maintains euploid DNA content during proliferation, with premature centromere separation, and acrocentric associations a preamble to non disjunction and aneuploidy, and micronuclei to clastogenicity too, ,besides the polyploidy observed in the PBL cultures of individuals using cell phones, there is an urgent need to exercise caution in their usage as the parameters observed all predispose towards malignancy.

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