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Research Article

DNA AND CHROMOSOMAL DAMAGE IN INDIVIDUALS USING MOBILE PHONES

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ABSTRACT
DNA and chromosomal damage in peripheral blood lymphocytes of individuals using mobile phones over the past few years were assessed using the single cell gel electrophoresis and the capillary blood *in vivo* micronucleus assays. The mean comet tail length (26.76±0.054 μm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group (8.11±0.028μm; 10.40% of cells damaged). The mean frequency of micronucleated cells was 0.25 in sample group being highly significant from that in the control group (0.05). The degree of manifested DNA and chromosomal damage in the same blood sample can be explained since the comet assay detects direct exposure, albeit repairable damage while micronuclei represent residual damage since they require a cell division for their manifestation. Statistical analysis revealed that duration of phone use, daily use and SAR influenced the genetic damage. Comets and micronucleated cells showed an increase with duration of use while comet tail lengths and comets also increased with daily phone use. Hence this correlation between mobile phone use (exposure to radio frequency radiations) and DNA and chromosomal damage in lymphocytes of individuals using mobile phones may augur for detrimental long-term consequences in terms of neoplasia and/or age-related changes.

Key Words: *Comet Assay, Micronucleus Test, Microwaves, Peripheral Blood Lymphocytes*

INTRODUCTION
There has been phenomenal development and deployment of wireless communication systems over the past decade and the use of the digital communication system that transmits Radio frequency radiations (RFR; 30 KHz to 30,000 MHz) at higher frequencies in this range has increased dramatically. Portable hand-held mobile phone use has and is increasing by popular demand and there are about 2 billion global users (Carlo, 2006). The Indian mobile industry in fact is growing rapidly with controversial 35.9 or 46 million CDMA and GSM customers as compared to 44.31 million land lines (Khetal, 2004). The government of India has plans for cellular coverage of 85% of the country's geographical area in this year and to increase the number of mobile connections to 500 million by 2010. In fact 2007 is being marked as the broadband year (Anonymous, 2006). However mobile phone safety, in terms of the effects of radio waves on blood pressure and cognitive functions, such as the abilities to concentrate, remember and learn, the connections between mobile phone use and brain cancer, acoustic neuroma, salivary gland cancer, leukaemia, and their long-term effects , has been controversial. Of special concern have been the effects on DNA. An evaluation of studies conducted in a frequency range from 800 to 3,000 MHz on direct toxicological effects of RFR and on the basic biological responses to RFR at the cellular and molecular levels, suggested that RFR is not directly mutagenic; adverse effects from exposure to high frequencies and high power intensities of RFR are the result of hyperthermia and probably some subtle indirect effects on the replication and/or transcription of genes under relatively restricted exposure conditions (Brusick et al., 1998). The mutagenic potential of RFR is hence far from being evident. Some *in vitro* and *in vivo* studies on RFR/microwaves have reported them to be positive in peripheral blood lymphocytes of humans and rats (Verschaeve et al., 1994), in brain cells of rats (Lai and Singh, 1996) or in synergism with Mitomycin C in human whole blood cells (Maes et al., 1997), while others have documented no increases in genotoxicity from exposure of these radiations in bone marrow cells of CD1 mice (McRee and MacNicholas, 1981), in Fischer 344 rats and their off springs (Vijayalaxmi et al., 2003), *in vitro* in

human MRC-5 fibroblasts (Meltz et al., 1987, 1990a) and in C3H 10 T½ mouse fibroblasts (Malyapa et al., 1997). Rather, the literature provides equivocal evidence for mutagenicity of RF and/ or microwaves. There seem to be no reports on any genotoxicity induced in mobile phone users. The present investigation was hence planned to estimate genetic damage by using the Single Cell Gel Electrophoresis assay and the capillary *in vivo* micronucleus test in the peripheral blood lymphocytes of individuals using mobile phones.

One of the techniques which permits the sensitive detection of DNA damage for studies of environmental toxicology, carcinogenesis, aging, genetic damage, etc., is the single cell gel electrophoresis assay. The alkaline single cell gel electrophoresis (SCGE) or comet assay has found routine use as it is a simple, rapid, visual and sensitive technique (Singh et al., 1988). It has many advantages over other DNA damage assays: it allows collection of data at the individual cell level and can be performed on small cell samples (Tice et al., 1992); it does not require proliferating cell populations, is suitable for any eukaryotic cell population for which a healthy cell suspension can be obtained and allows the scoring of DNA damage both in *in vivo* and in *in vitro* studies (Green et al., 1992).

Micronuclei, on the other hand, provide a measure of both, chromosome breakage and chromosome loss, and the micronucleus test (MNT) is as sensitive an indicator of chromosome damage as classical metaphase chromosome analysis (Fenech and Morley, 1985).The use of micronuclei as a measure of chromosome damage in peripheral blood lymphocytes (PBL) was first proposed by Countryman and Heddle (1976) and was subsequently improved with the development of the cytokinesis-block micronucleus method (Fenech and Morley, 1986). However this requires culturing of lymphocytes and so is a laborious and intensive method preventing its use in monitoring large populations. In order to circumvent these difficulties, the *in vivo* micronucleus test (MNT) in lymphocytes of human capillary blood was developed by Xue et al. (1984, 1988). It is a simpler, convenient and informative *in vivo* cytogenetic technique and (Lee et al., 1997) though differences in mean frequencies of MN were observed with capillary and venous blood using cytokinesis-block-MNT, favouring the latter (Tian et al., 2003). In the present study the micronucleus protocol given by Xue et al. (1992) was followed as it requires a small amount of blood with no need for cells to be cultured and it is simple and rapid.

MATERIALS AND METHODS

Some individuals using mobile phones over a time period were contacted and after voluntary written informed consent, were requested to blood samples. Their records were maintained for the time since they were using the phone, model of phone, Specific Absorption rate (SAR) value of the model, daily frequency and duration of calls (incoming and outgoing), duration of phone use in 24 hr, any health effects after using phone,etc. Finger-prick blood samples were collected in heparinized eppendorf tubes, transported on ice to the laboratory and processed for SCGE assay and the MNT within 3-4 hr of collection. Age- and sex-matched healthy individuals who had never used mobile phones formed the control group.

A modified protocol (Ahuja and Saran, 1999) of the original SCGE assay of Singh et al. (1988) was followed except for increase in lysis time from 1 hr in the original study to 2-3hr in the present study. The method in brief involved layering of blood (25µl)in phosphate buffered saline(PBS) mixed with low melting point agarose (75µl, 0.5%LMPA) over a plain slide pre-coated with normal melting point agarose (150 µl of hot 1% NMPA in PBS dried at 37°C overnight).This was followed by layering again with LMPA (0.5%; 75µl). The cells on the slides were lysed (pH=10, 4°C for 2-3 hr), electrophorosed in an alkaline buffer (pH≥13 for 20 min at 25V and 300 mA), flooded with neutralization buffer and stained with silver nitrate as per the method of Cerda et al. (1997). Cells (100/ sample) were scanned under a standard transmission binocular microscope. Damaged cells (comets) were scored avoiding overlapping and apoptotic-like figures. DNA migration lengths were measured under 40X using an ocular micrometer calibrated with the help of a stage micrometer. The tail length was measured from the trailing edge of the nucleus to the leading edge of the tail.

For the MN test (Xue et al., 1992), to blood samples (0.06-1.0ml) obtained through finger puncture, methyl cellulose (0.3%) was added in a v/v ratio of 1: 3. The sample was mixed carefully with a fine glass rod and kept in a water-bath maintained at 37°C for 40-60 min. The lymphocytes' suspension was decanted into a micro-centrifugation tube and centrifuged at 1000 rpm for 6 min. The supernatant was discarded and the pellet in 43µl of the supernatant was mixed together using a small vortex stirrer. The suspension was poured onto a slide and its smears prepared. These were allowed to air dry, fixed in 100% methanol for 1 min. and again air dried. Staining was carried out in buffered Giemsa (pH 6.4, 1:10) for 20 min. Cells ($2x10^3$/sample) were scored for the presence of MN under the low power (40 x) of a binocular microscope. The main nucleus and MN showed dark blue against the light blue cytoplasm. MN were detected as small, spherical, separated chromatin masses in small (T) lymphocytes (Xue et al., 1992). They were confirmed at 100x under oil immersion and randomly by another observer.

The data obtained for DNA and chromosomal damage were statistically analyzed using Chi-square test, regression analysis, analysis of variance and Student's t-test. Chi square was calculated manually whereas a software package SPSS version 7.0 was used for regression analysis, ANOVA and for the Student's t-test.

RESULTS

A total of 21 males and 3 females belonging to upper middle strata (n=6) and middle socioeconomic (SES) class (n=20) were investigated. All those evaluated for the MN test (n=20) were also investigated for DNA damage and so are included among those (n=24) for which the SCGE assay was performed. An age-,sex- and SES- matched control group (n=11) was similarly investigated. Chi-square (χ^2) analysis revealed that the control sample was similar to the subject group with respect to age, sex, SES, alcohol consumption and smoking habits ($p<0.01$) except for the vegetarian and non-vegetarian status since there were no non-vegetarians in the control sample (Table 1). Data for DNA and chromosomal damage of female subjects was clubbed with that of male subjects as there were no differences in the values.

The SCGE assay results demonstrated DNA damage with ~ 40% (39.75) cell damage in mobile phone users and a mean tail length (comet tail length also known as DNA migration length) of 26.76±0.054 µm versus 8.11±0.028 µm in control individuals in whom only 10.40% of cell damage was observed. Chromosomal damage scored as micronuleated cells was also significantly elevated in mobile phone users (mean per cent frequency of 0.25 MNd cells) from that in the controls (mean per cent frequency of 0.05 MNd cells). To find out whether confounding factors (age, smoking, dietary habits and alcohol consumption) influenced genetic damage, multiple regression analysis was performed (Tables 2). The analysis revealed no correlation between these factors and DNA and chromosomal damage. On the other hand, the exposure period in 24 hr (as can be presumed from daily usage of mobile phone) and specific absorption rate (SAR) affected the number of cells damaged; tail length and MN frequency were influenced by SAR values only. To look for the significance of regression lines, analysis of variance (ANOVA) was also carried out (Table 3). There were no effects from age, smoking, alcohol consumption, diet, exposure in 24 hr, time since using phone and SAR values for tail lengths. Rather SAR values influenced both percentage of damaged cells and MNd cells frequency; the non-vegetarian dietary pattern also influenced the MN frequency. The Student's t-test was performed on the results obtained from SCGE assay and MN test to analyse whether daily usage of mobile phone, duration of its use and its SAR values exhibited differential genetic damage.

The use of mobile phone varied from 1-5 yr among the selected individuals (Table 4).Statistical analysis revealed that the percentage of damaged cells in 1-2 yr users significantly increased from that in users with ≤1 yr of use ($t_{cal} = 3.487$, $t_{tab} = 2.880$, df = 18, $p<0.01$). A significant difference in tail lengths was observed between the users with 3-4 yr of use and with 4-5 yr of use ($t_{cal} = 7.215$, $t_{tab} = 4.300$, df = 2, $p<0.05$). On the other hand, the difference in MNd cell frequency was not statistically significant among the groups though these and the differences in tail lengths, percentage of damaged cells and MN frequency were highly significant from the total control values.

Table 1. Characteristics of Individuals Using Mobile Phones and Control Individuals.

Characteristics			No. of mobile phone users	No. of controls	χ^2 **(P<0.05)**
Age-range (yr)			15-35	26-35	-
Sex	Male		23	8	2.42
	Female		3	3	
SES	Upper		6	2	0.12
	Middle		20	9	
Diet	Veg		10	10	8.55
	Non- Veg		16	1	
Smoking	Yes		2	0	0.88
	No		24	11	
Alcohol Consumption	Drinkers		2	0	0.08
	Non-Drinkers		24	11	
Married	Yes		19	-	-
	No		7	-	-
Duration of mobile phone use(yr)	1-2		7	-	-
	>2-3		9		
	3		9	-	-
	>4-5		1		
Calls/day(frequency)	Incoming		7-9	-	-
	Outgoing		5-7	-	-
Daily use of mobile phone			1-16.00hrs	-	-
Attendance of phone(ears)		Left	5	-	-
		Right	21	-	-
		Both	-		-
Placement pattern of phone when :	On the move	Shirt Pocket	13	-	-
		Trouser Pocket	7	-	-
		Waist pouch	1	-	-
		Bag	4	-	-
	In the Office	Pocket	8	-	-
		Table/Drawer	9	-	-
	At home	Pocket	6	-	-
		Table	12	-	-
		Shelf	1	-	-
Occupation mobile phone		Businessman	7	-	-
		Student	6	-	-
		Others	13	-	-
Health effects		Heart pain	2	-	-
		Sleeplessness	1	-	-
		Memory Loss	2	-	-

Table 2. Correlation Between DNA (SCGE Assay) And Chromosomal (MNT) Damage And Confounding Variables Among Individuals Using Mobile Phones And Controls.

Group	Type of damage		Age	SES	Alcohol consumption	Smoking	Veg/Non. Veg.	Use in 24 hr.	SAR value	Duration of use
Mobile Phone Users	Percentage of damaged cells	r	0.293	0.167	0.071	0.286	0.063	0.197	0.237	0.118
		p-value	NS	NS	NS	NS	NS	p<0.05	p<0.05	NS
	Mean comet length	r	0.063	0.126	0.032	0.321	0.387	0.377	0.095	0.000
		p-value	NS	NS	NS	NS	NS	NS	p<0.05	NS
	Frequency of MNd cells	r	0.045	0.318	0.032	0.032	0.089	0.032	0.338	0.212
		p-value	NS	NS	NS	NS	NS	NS	p<0.05	NS
Controls	Percentage of damaged cells	r	0.251	0.077	-	-	0.118			
		p-value	NS	NS	-	-	NS			
	Mean comet length	r	0.000	0.176	-	-	0.179			
		p-value	NS	NS	-	-	NS			
	Frequency of MNd cells	r	0.481	0.219	-	-	-			
		p-value	NS	NS	-	-	-			

r= regression coefficient; If p≤0.05, test is significant ; SES -Socio-economic Status; p-probability value; NS -Non significant; MNd cells - Micronucleated cells;SAR-Specific Absorption Rate

Table 3. Multivariate Analysis Of Variance (ANVOA) In Individuals Using Mobile Phones And Conrols.

Group	Type of damage	Source of variation	Sum of squares	df	Mean Square	f-ratio	p-value
Mobile Phone Users	Percentage of damaged cells	Age	269.33	23	1.500	8.828	NS
		SES	3.33	23	0.167	0.850	NS
		Alcohol	1.83	23	0.167	0.400	NS
		Smoking	1.83	23	0.167	0.400	NS
		Veg./Non-Veg.	5.83	23	0.333	0.725	NS
		SAR value	1.83	23	8.483	0.926	<0.05
		Use in 24 hr.	466.00	23	7.333	3.027	NS
		Duration of use	15.50	23	1.375	0.414	NS
	Frequency of MNd cells	Age	178.95	19	11.561	0.560	NS
		SES	3.75	19	0.220	0.759	NS
		Alcohol	2.55	19	0.182	0.378	NS
		Smoking	2.55	19	0.159	0.629	NS
		Veg./Non-Veg.	4.55	19	0.114	3.630	<0.05
		SAR value	2.05	19	0.109	0.979	<0.05
		Use in 24 hr.	418.00	19	23.720	0.828	NS
		Duration of use	16.20	19	1.129	0.419	NS
Controls	Percentage of damaged cells	Age	146.90	9	43.000	0.202	NS
		SES	1.60	9	0.333	0.400	NS
		Alcohol	0.00	9	0.000	-	NS
		Smoking	0.00	9	0.000	-	NS
		Veg./Non-Veg.	0.90	9	0.333	0.100	NS
	Frequency of MNd cells	Age	150.909	10	15.422	0.893	NS
		SES	1.636	10	0.188	0.364	NS
		Alcohol	0.000	10	0.000	-	NS
		Smoking	0.000	10	0.000	-	NS
		Veg./Non-Veg.	0.909	10	0.109	0.156	NS

If p≤0.05, test is significant *df - degree of freedom*
SES - Socio Economic Status *p - probability value*
NS - Non significant
MNd cells - Micronucleated cells
SAR - Specific Absorption Rate

Table 4. DNA and Chromosomal Damage As Functions Of Period Of Use Of Mobile Phones.

Comet Assay												
S. No.	Duration of use (y)		No. of individuals	Age (y)		Daily Exposure (hr)		SAR value (W/kg)		No. of cells scored	No. of damaged cells (%)	Mean[$] comet tail length (µm) ± SEM
	Range	Mean		Range	Mean	Range	Mean	Range	Mean			
1.	1-2	1.83	6	21.28	24.00	1.15-11.00	6h 33 min	0.59-1.47	1.10	600	308 (51.33)***,!	27.14***±0.062
2.	>2-3	2.75	14	21.35	26.71	1.15-11.00	4 h 29 min	0.87-1.66	1.19	1400	448 (32.00)***,#	26.02***±0.107
3.	>3-4	3.83	3	20.28	23.67	1.30-1.45	2h 10 min	1.27-1.45	1.36	300	154 (51.33)***,+	25.33***,a±0.351
4.	>4-5	5.00	1	27	27.00	7.30	7 h 30 min	7.30	1.51	100	44 (44.00)	29.09[b]±0.000
	Total									2400	954 (39.75)***	26.76***±0.054
	Total Control									1000	104 (10.40)	8.11±0.028
MN Test												
										No. of cells scored	No. of MNd cells (%)	Mean† (%frequency of MNd cells) ± SEM
1.	1-2	1.85	7	19-28	23.43	1h 15 min-11h	6h 05 min	0.59-1.47	1.07	14000	32 (0.23)	0.23***±0.0012
2.	>2-3	2.72	9	21-35	27.33	1h 15 min- 11h	3h 22 min	0.87-1.66	1.23	18000	43 (0.24)	0.24***±0.0007
3.	>3-4	3.83	3	20-28	23.63	1h 30min-3h 30 min	2h 02 min	1.27-1.45	1.36	6000	18 (0.30)	0.30***±0.0017
4.	>4-5	5.00	1	-	27.00	-	7h 30 min	-	1.51	2000	7 (0.35)	0.35***±0.0000
	Total									40000	100 (0.25)	0.25***±0.0006
	Total Control									16000	8 (0.05)	0.05±0.0006

$: Calculated as an average of individual DNA migration lengths in that group; †calculated as an average of individual frequencies of micronucleated cells in that group.
****: Highly significant from total control (p≤0.01; 0.001; Student's 't' test) but not within the group except for a,!,#*
a : Significant from b ! : Significant from # # : Significant from +
SAR : Specific Absorption Rate

Table 5. DNA And Chromosomal Damage As Functions Of Daily Use Of Mobile Phones.

Comet Assay												
S. No.	Daily exposure (hr)		No. of individuals	Age (years)		Duration of use (years)		SAR value (w/kg)		No. of cells scored	No. of damaged cells (%)	Mean$ comet length tail (μm) ± SEM
	Range	Mean		Range	Mean	Range	Mean	Range	Mean			
1.	1-4	2h 11min	15	20-35	27.73	2-4	2.83	0.59-1.66	1.31	1500	586 (39.07)***	27.00***[a]±0.074
2.	<4-8	7h 30min	3	21-27	25.00	1-5	3.00	1.24-1.51	1.41	300	107 (35.67)**[c]	27.30***[b]±0.153
3.	>8-12	9h 58min	5	23-28	24.60	2-3	2.40	0.59-1.27	1.07	500	233 (46.60)***$_d$	25.37***±0.217
4.	>12-16	15h 30min	1	30	30.00	2.5	2.50	1.24	1.24	100	28 (28)	28.50±0.000
	Total									2400	954 (39.75)***	26.76***±0.054
	Total Control									1000	104 (10.40)	8.11±0.028
MN test												
										No. of cells scored	No. of damaged cells (%)	Mean† (%frequency of MNd cells) ± SEM
1.	1-4	2h 32 min	13	20-35	26.23	2.4	2.83	0.59-1.66	1.29	26000	59 (0.23)	0.23***±0.0006
2.	<4-8	6h 58 min	4	19-27	22.75	1-5	2.50	0.59-1.51	1.28	8000	17 (0.21)	0.21***±0.0011
3.	>8-12	10h 1min	3	24-28	25.33	2-3	2.40	0.59-1.24	0.94	6000	24 (0.40)	0.40***±0.0011
	Total									40000	100 (0.25)	0.25***±0.0006
	Total Control									16000	8 (0.05)	0.05±0.0006

$: Calculated as an average of individual DNA migration lengths in that group
†calculated as an average of individual frequencies of micronucleated cells in that group.
*** : *Highly significant from total control ($p \leq 0.01$; 0.001; Student's 't' test) but not within the group except for a and c*
a : significant from b c : significant from d
SAR : Specific Absorption Rate

Table 6. DNA And Chromosomal Damage As Functions Of Specific Absorption Rate (SAR) Value Of Mobile Phones.

Comet Assay												
S. No.	SAR Value (W/kg)		No. of individuals	Age (y)		Duration of use (y)		Daily Exposure (hr)		No. of cells scored	No. of damaged cells (%)	Mean$ comet length tail (µm) ± SEM
	Range	Mean		Range	Mean	Range	Mean	Range	Mean			
1.	0.57-1.01	0.84	6	21-35	26.67	2-3	2.58	1h 15 min-11h	4h 31 min	600	256*** (42.67)	25.42***±0.230
2.	1.02-1.47	1.34	15	20-30	25.53	1-4	2.50	1h 30min-15h 30min	5h 6min	1500	607*** (40.47)	26.64***±0.070
3.	1.48-1.92	1.58	3	23-27	24.30	2.5-5	3022	1h 45min-7h 30 min	3h 17 min	300	91* (30.33)	30.01***±0.016
	Total									2400	954*** (39.75)	26.76***±0.054
MN test												
										No. of cells scored	No. of MNd cells (%)	Mean†(% frequency of MNd cells) ± SEM
1.	0.57-1.01	0.80	7	19-35	25.57	1½-3	2.43	1h 15 min-11h	5h 29 min	14000	32(0.23)	0.23***±0.0008
2.	1.02-1.47	1.39	10	20-29	25.50	1-4	2.70	1h 30min-8h 30min	3h 42 min	20000	52(0.26)	0.26***±0.0010
3.	1.48-1.92	1.57	3	23-27	24.67	2½-5	3.33	1h 15min-7h 30 min	3h 30 min	6000	16(0.26)	0.27***±0.0013
	Total									40000	100(0.25)	0.25***±0.0006
	Control Total									16000	8(0.05)	0.05±0.0006

$: Calculated as an average of individual DNA migration lengths in that group; †calculated as an average of individual frequencies of micronucleated cells in that group.

**** : Highly significant from total control (p≤0.01; 0.001; Student's 't' test)*
** : Significant from total control (p≤0.05; Students 't' test)*
SAR : Specific Absorption Rate

Table 7. Categorization Of Comets According To Degree Of Damage As A Function Of SAR Value

SAR value		No. of individuals	No. of cells scored	No. of undamaged cells grade-0 (%)	Grade-I damage (%)	Grade-II damage (%)	Grade-III damage (%)	Total no. of damage cells (%)
Range	Mean							
0.57-1.01	0.84	6	600	344 (57.33)	186 (31.00)	55 (9.17)	15 (2.50)	256 (42.67)
1.02-1.47	1.34	15	1500	893 (59.53)	412 (27.47)	144 (9.60)	55 (3.67)	607 (40.47)
1.48-1.92	1.58	3	300	209 (69.67)	59 (19.67)	22 (7.33)	11 (3.67)	91 (30.33)
Total		24	2400	1446 (60.25)	657 (27.38)	221 (9.21)	81 (3.38)	954 (39.75)

Daily use of mobile phone varied from one to 16 hr calculated on the basis of frequency and duration of calls attended and made. Statistically significant differences in tail lengths of PBL of individuals using the phone for 4-8 hr from that of 1-4 hr (t_{cal} = 4.113, t_{tab} = 4.020, df = 16, $p<0.001$) were observed (Table 5). The mean tail lengths were also highly significant from total control value for all the groups analysed (1-4 hr vs. total control- t_{cal} = 14.590, t_{tab} = 3.770, df = 23, $p<0.001$; 4-8 hr vs. total control -t_{cal} = 8.351, t_{tab} = 4.440, df = 11, $p<0.001$; 8-12 hr vs. total control - t_{cal} = 9.675, t_{tab} = 4.220, df = 13, $p<0.001$).

A similar significance was also observed in percentage of damaged cells between the 4-8 hr and 8-12 hr of daily use (t_{cal} = 4.044, t_{tab} = 2.780, df = 4, $p<0.05$) while increase in percentage of damaged cells was highly significant from total control values for all the sample groups (1-4 hr vs. total control- t_{cal} = 5.803, t_{tab} = 3.770, df = 23, $p<0.001$; 4-8 hr vs. total control - t_{cal} = 3.660, t_{tab} = 3.110, df = 11, $p<0.01$; 8-12 hr vs. total control - t_{cal} = 9.450, t_{tab} = 4.220, df = 13, $p<0.001$). The differences in MN frequency between the groups was not found to be statistically significant, though seemingly an almost two fold increase was observed in the individuals between the 4-8 hr (0.21% MN frequency) and 8-12 hr (0.40% MN frequency) of daily mobile phone use. However, the overall chromosomal damage significantly increased from that of the total control group at all intervals of daily phone use.

Though SAR value is not an exact or only parameter useful in estimating radiation emittance and so probably inducing biological effects, yet it is the only one available. The SAR values of mobile phones being used either fall within the SAR limit of 1.6 W/kg set by FCC or the higher limit of 4 W/kg given by NCRP, IEEE, ICNIRP. The SAR value was shown to influence both DNA and chromosomal damage (Table 6). All sample groups exhibited high significance from total control (0.57-1.01 W/kg vs. total control - t_{cal} = 5.699, t_{tab} = 4.140, df = 14, $p<0.001$; 1.02-1.47 W/kg vs. total control - t_{cal} = 8.509, t_{tab} = 3.770, df = 23, $p<0.001$; 1.48-1.92 W/kg vs. total control - t_{cal} = 2.435$_{tab}$ = 2.200, df = 11, $p<0.05$). Comet tail lengths also increased with increasing SAR but these were non-significant among the groups though highly significant from total control values (0.57-1.01 W/kg vs. total control - t_{cal} = 8.194, t_{tab} = 4.140, df = 14, $p<0.001$, 1.02-1.47 W/kg vs. total control - t_{cal} = 16.750, t_{tab} = 3.770, df = 23, $p<0.001$; 1.48-1.92 W/kg vs. total control - t_{cal} = 32.195, t_{tab} = 4.440, df = 11, $p<0.001$).The mean frequency of MNd cells also showed a linear increase within the samples groups but the increase was statistically non-significant albeit significant from the control group value.

The cells were categorized in to four classes according to the degree of damage to the cells and therefore on the appearance of the comet. Collins et al. (1997) and Collins (2004) also formed 5 classes, from 0 (no tail) to 4 (almost all DNA in tail). There were no cells with the entire DNA in the tail in the sample under study, hence the fifth class is lacking (Table 7). The comets in control group fell mainly into classes 0 and I. From the 100 comets scored per sample, each comet was assigned a value of 0 to 4 according to its class, so that the total score was between 0 to 400 arbitrary units (Collins, 2004). The number of damaged cells at all grade levels (I, II, III) as well as the total damaged cells decreased but non-significantly with increasing SAR values. However, the differences in the percentage of damaged cells with different grades of damage were found to be statistically significant for all the groups.

DISCUSSION

The present study revealed a high degree of DNA damage (percentage of damaged cells and mean tail length) but not such a high degree of chromosomal damage (per cent frequency MNd cells) in peripheral blood lymphocytes of mobile phone users. This can be explained on the basis of the fact that the MN test detects injuries that survive at least one mitotic cycle, while the comet assay identifies repairable injuries and/or alkali-labile sites also (Van Goethem et al., 1997). In this study the *in vivo* capillary blood lymphocytes were directly used to assess the MN frequency without the need to culture them. Hence it reflects that the damage manifesting as MN in T (small) lymphocytes probably occurred before the cell division which led to their formation.

A number of mobile telecommunication networks have been available to the local users from the period starting in 1998, viz., Spice telecommunications (900 MHz), Reliance India Ltd. (CDMA, 837.37 MHz), Bhartiya Sanchar Nigam Ltd. (GSM), Airtel (1800 MHz) and Connect (CDMA, 837.37 MHz). The quality of service, availability of network and offer of any special deals by these networking companies were the decision factors for the preference of the users for a particular telecommunication company. A perusal of literature for investigations on the genotoxic potential of RF radiation as emitted by mobile phones (800 – 2400 MHz) has revealed both positive and negative effects in varied *in vitro* and *in vivo* studies. However, no reports on the direct effects of mobile phone usage have come to attention.

Some of the documentations on mobile phone RF and/or comparable SAR values in which positive results were observed are discussed first. Dominant lethal mutations were induced in Swiss mice exposed to 1700 MHz EMF (SAR 50 W/kg) for 30 min and changes in melting temperature, base composition and optical density as indicators of mutagenicity and strand separation were reported in Swiss mice exposed to 985 and 1700 MHz RFR for 80 min at power density 10 W/cm^2 and to 1700 MHz RFR at power density 50 mW/cm^2 for 30 min (Verma and Traboulay, 1976). In another study (Verma and Traboulay, 1977) exposure of male Swiss mice to 1.7 GHz at 500 W/m^2 for 30 min or 100 W/m^2 for 40 min over a 2wk period or 500 W/m^2 for 30 min (SAR 50 W/kg) in one day also resulted in dominant lethality, increase in DNA damage and changes in testicular DNA. Cytogenetic damage in blood lymphocytes of occupationally exposed humans to X-rays, vinyl chloride and 1250 MHz – 1350 MHz RFR for 15 yr (power density 10$\mu W/cm^2$ 20 $\mu W/cm^2$) was reported in the form of clastogenic effects of microwaves. The frequencies of size-distribution of micronuclei in the lymphocytes were compared and it was concluded that in contrast to X-rays, microwaves exhibited a dual action, partly as radiations and partly acting as a chemical clastogenic agents (Fucic et al., 1992). Human blood samples from 29 male and 3 female healthy donors exposed *in vitro* to 954 MHz for 1-2 hr revealed an increase in mean comet tail lengths in 30 out of 32 subjects. An increase in SCE frequency and a slightly longer tail length on comet assay were observed in blood samples from 22 "mobile telephone maintenance personnel" exposed to different(450 MHz, 900 MHz and others) frequencies (Verschaeve et al.,1994).An increase in chromosome aberration was reported in human lymphocytes exposed *in vitro* to 954 MHz RFR at a distance of 5 cm from a GSM antenna for 2h at SAR 1.5 W/kg. The authors (Maes et al.,1995) however suggested that since the control samples were kept inside a metallic can, they were not sufficiently shielded from electromagnetic fields and the data could be explained as a direct effect of the magnetic component of EMF or as a secondary effect caused by metallic can, which behaves like an antenna. On exposure of human whole blood cells to continuous waves (CW; 935.2 MHz,SAR 0.3-0.4 W/kg) followed by mitomycin-C , a synergistic effect was reported in the form of an increase in SCE (Maes et al.,1997). *In vitro* exposure to Molt-4 lymphoblastoid cells to 813.56 MHz (iDEN) for 2 to 21 hr (SAR 24-26 mW/kg) resulted in primary DNA damage (Phillips et al.,1998).An increase in MN frequency was observed in human PBL exposed for 3 and 24hr to voice- modulated 837 MHz produced by analog signal generator, 837 MHz TDMA, 837 MHz CDMA and voice- modulated 1909.8 MHz generated by PCS cell phone at SAR 1.0–10.0 W/kg, and under extended exposure conditions, induction of chromosomal damage in human lymphocytes was seen at SAR 5.0 W/kg (Tice et al. , 2002). Human PBL cultures of 16 volunteers exposed to 1.748 GHz phase modulated wave RFR (SAR 45W/kg) resulted in a statistically significant MN effect suggesting the genotoxic power of the phase modulated waves(d'Ambrosio et al. , 2002).*In vitro* exposure of human peripheral blood lymphocytes to continuous 830 MH Electromagnetic frequency (EMF) EMF (SAR 1.65-8.8 W/kg) for 72 hr caused losses and gains of chromosomes. A linear increase in Chr # 17 aneuploidy was observed as a function of SAR value at 34.5-37.5°C indicating that the genotoxic effect of the EMF is elicited via a non-thermal pathway (Mashevich et al., 2003).

Negative studies for chromosomal aberrations include no increases in sister chromatid exchanges in bone marrow cells of mice exposed to 900 MHz and 800 MHz RFR at SAR 4 W/kg for 8 hr (Brown and Marshall, 1982). Both, SCE frequency and chromosome aberrations were also not observed (Meltz et al., 1990b) in Chinese Hamster Ovary cells exposed to 200 MHz (SAR 24.33 W/kg) and to 850 MHz (SAR

14.4 W/kg).Blood lymphocytes of six workers responsible for the maintenance of transmission antennas linked to the mobile telephone network, who were occupationally exposed to RFR of 450 MHz, 900 MHz and other frequencies showed no increase in chromosome aberrations (Maes et al.,1995). In another study (Maes et al.,1997) it was observed that neither direct chromosomal damage (chromosome aberrations and SCEs) nor tail moment and tail lengths increased in comet assay when human whole blood cells were exposed to continuous 935.2 MHz (SAR 0.3-0.4 W/kg). An exposure of human lymphocytes to 455.7 MHz microwaves from a car antenna (5 cm away, SAR 6.5 W/kg) and in lymphocytes subsequently exposed to X-rays or MMC, no synergistic effect with EMFs was revealed for chromosome aberrations or SCE (Maes et al.,2000). In another study no evidence of mutagenic and/co-mutagenic/synergistic effect was found in human lymphocytes exposed to 900 MHz (power output 0, 2, 8, 15, 25, 50 W) alone or in lymphocytes subsequently exposed to X-rays or MMC (Maes et al.,2001).Human PBL incubated in the presence of 380, 900 and 1800 MHz RFR showed no effect on cell cycle progression or on SCE frequency (Antonopoulos et al. ,1997). There was no induction of neoplastic transformation following exposure of C3H10T (½) cells to 835.62 MHz (FDMA) and 847.74 MHz (CDMA) at SAR 0.6 W/kg as observed in the cell transformation assay system by Roti Roti et al. (2001). No evidence was found for the induction of chromosome aberrations and MN in human PBL after an *in vitro* 24 h exposure to 835.62 MHz RFR FDMA at SAR 4.4. or 5.0 W/kg (Vijayalaxmi et al.,2001a) and to 847.74 MHz CDMA for 24h at SAR 4.9 or 5.5. W/kg (Vijayalaxmi et al.,2001b). Human PBL cultures of 16 volunteers exposed to 1.748 GHz CW and/or PW RFR (SAR 45W/kg) also did not induce significant effect on MN incidence and cell proliferation (d'Ambrosio et al. ,2002). PBL cultures of 20 healthy donors exposed to (1) CW intermittent exposure (SAR 1.6 W/kg) for 6 min. followed by 3hr pause (14 on/off cycles) (2) GSM signal (3) GSM signal exposure before 24h phytohemaglutinin-stimulation (4) GSM exposure at SAR 0.2 W/Kg for 1 h/day for 3 days, did not reveal an increase in MN frequency or any significant changes in cell cycle kinetics in the cytokinesis – block MN assay (Zeni et al. ,2003).Fischer 344 rats and their offsprings were exposed to far-field 1.6 GHz Iridium wireless communication signals for 2h/day, 7 days/wk (SAR 0.036-0.077 W/kg). For the offsprings, another head-only exposure to a near field of 1.6 GHz signals for 2h/day, 5 days/wk was given from 38 day to 2 yrs of age. No evidence of excess genotoxicity was found in bone marrow smears assessed for increased MN frequency as compared to sham-exposed and caged controls (Vijayalaxmi et al.,2003).

Despite the number of prominent, peer-reviewed studies indicating that cell phone radiation can cause genetic damage, brain and blood cell dysfunction and a host of health problems including cancer, a Danish study reports no danger at all (Carlo,2006). Rather no effects on DNA and on the process of DNA repair were reported in human MRC-5 fibroblasts exposed to 1200 MHz (CW or PW SAR 2.7 ± 1.6 W/kg) or to 850 MHz (SAR 4.5 ± 3.0 W/kg) and to 350 MHz (SAR 0.39 ± 0.15 W/kg) for 1-3 hr (Meltz et al.,1987). *In vivo* experiments conducted with rats caged for 1 to 5 wks in proximity to a 954 MHz antenna showed an increase in comet tail length but similar increases were also observed in lymphocytes of transported rats and it was concluded that this might be due to stress resulting from the transportation (Verschaeve et al. ,1994). Neither direct chromosomal damage (chromosome aberrations and SCE) nor tail moment and tail lengths increased in comet assay (Maes et al. ,1997) when human whole blood cells were exposed to continuous 935.2 MHz (SAR 0.3-0.4 W/kg). C6 glioma rat cells showed no cell proliferation or changes in DNA synthesis rate after a 24h exposure to 836.55 MHz (TDMA) RFR at SAR 0.15 and 59 mW/kg (Stagg et al., 1997).No primary DNA damage was detected in U87MG human glioblastoma and C3H 10 T½ mouse fibroblast cells exposed to 835.62 MHz (FDMA) and 847.74 MHz (CDMA) RFR, respectively at SAR 0.6 W/kg (Malyapa et al. ,1997a,b). *In vitro* exposure to Molt-4 lymphoblastoid cells to 836.55 MHz (TDMA) for 2 to 21 h (SAR 24-26 mW/kg) resulted in no primary DNA damage in cells exposed to TDMA. A decrease in DNA migration was also observed in cells exposed to both iDEN(813.56 MHz) and TDMA signals at SAR 2.4-2.6 mW/kg (Phillips et al. ,1998). Equal number of DNA breaks in rat lymphocytes were reported in both controls and animals exposed to 945 MHz RFR for 1-5 wks (Verschaeve and Maes,1998).Human blood lymphocytes exposed to 837 MHz

(TDMA), 837 MHz (CDMA) and 1900 MHz (PCS) showed no increase in primary DNA damage or of micronucleated binucleated human blood lymphocytes (Vasquez et al.,1999). Exposure of 847.74 MHz CDMA and 835.62 MHz FDMA frequencies (SAR 3.2 – 5.1 W/kg) to cultured C3H10T (½) murine fibreblast cells at 37°C did not result in DNA damage using the alkaline comet assay (Li et al., 2001). No DNA damage (SCG assay) was observed in human PBL exposed for 3h and 24h to voice modulated 837 MHz produced by analog signal generator, 837 MHz TDMA, 837 MHz CDMA and voice-modulated 1909.8 MHz generated by PCS cell phone at SAR 1.0–10.0 W/kg (Tice et al., 2002).No DNA damage as assayed by the SCGE assay and no increase in MN frequency as assayed by the cytokinesis block MN assay were observed in human blood lymphocytes exposed to 1.9 GHz CW and PW radiofrequency for 2h at SAR 0.0, 0.1, 0.26, 0.92 and 10 W/kg (McNamee et al.,2002a,b). Molt-4T lymphoblastoid cells exposed to 847.74 MHz CDMA (SAR 3.2 W/kg); 835.62 MHz FDMA (SAR 3.2 W/kg); 813.56 MHz iDEN (R) (iDEN) (SAR 2.4 or 24 mW/kg) and 836.55 MHz TDMA (SAR 2.6 or 26 mW/kg) for 24h at 37°C±0.3°C revealed no statistically significant differences in the level of DNA damage or apoptosis by SCGE assay and annexin V affinity assay, respectively between sham-treated and RF- exposed cells (Hook et al., 2004). No evidence for an association between tumor risk and cellular telephone use among either short-term or long-term users (Shuz et al.,2006). The authors further stated that the narrow confidence intervals provide evidence that any large association of risk of cancer and cellular telephone use can be excluded. However, Carlo (2006) has stated that the study, funded by the telecommunications industry, was clearly created in order to produce a positive, low-risk finding and that this is a ruse based upon a programme initiated by the telecommunications industry more than a decade ago to control the global scientific research agenda concerning cell phones and health effects. In this regard, the evidence for genetic damage in the present study can in no manner be overlooked, either at the DNA or at the chromosomal levels, thereby implying a probable carcinogenic outcome in the wake of genetic damage.

In conclusion and despite *in vitro* and *in vivo* reports to the contrary, the results of the present investigation reveal that mobile phone use (after ruling out any other exposures), causes significant genetic damage in PBL, both with respect to number of damaged cells and DNA migration lengths in the SCGE assay, and also to some extent, chromosomal breaks and/or aneugenicity in the MNT, probably because of exposure to RFR (800-2000 MHz). In an earlier study (Gandhi and Singh,2005) PBL cultures showed a higher number of aberrant metaphases (with satellite associations and centromere separation) and MNd cells in the buccal mucosal cells of mobile phone users, thereby also implicating a high probability of non-disjunction - a preamble to carcinogenesis/genetic defects. The underlying threat to mobile phone users is hence apparent since genotoxicity may be indicative of carcinogenicity and/or precocious age- related distress.

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