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Mutagenic Potential of Radio Frequency Electromagnetic Fields

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Abstract: The present study was conducted to determin the potential genetic damage of occupational exposure to EM field. The studied subjects are engineers and air traffic controllers exposed to radio frequency emitted from different instruments. Lymphocytes of exposed and control individuals were analyzed for structural and numerical chromosomal aberrations, sister chromatid exchanges, mitotic activity and cell kinetics. Cells with structural chromosomal aberrations were significantly increased in both engineers and air traffic controllers ($P < 0.001$). Also, The number of aberrant cells with total numerical aberrations increased significantly in both exposed groups ($P < 0.001$). Numerical aberrations were mainly hypodiploidy. The frequencies of SCEs in engineers and air traffic controllers were slightly increased over the control but this increase was not statistically significant. A decrease in mitotic activity was reported in EM field exposed engineers and air traffic controllers at statistically significant levels of $P < 0.01$ and $P < 0.001$, respectively. Exposure to EM field did not affect the cell kinetics in engineers and air traffic controllers.

Key words: Electromagnetic field, radio frequency, lymphocytes, mutagenic potential

Introduction

People are continuously exposed to increasing levels of electromagnetic (EM) fields emitted from various electrical installations and telecommunication systems. These EM fields are waves with a broad range of frequencies including radio frequency (RF, ranging from 100 KHz to 300 GHz) and extremely low frequency (ELF, below 300 Hz) (Juutilainen and Lang, 1997).

Several epidemiological studies suggest a possible relationship between exposure to EM fields, in residential or occupational environments, and the incidence of certain types of cancer. They include leukemia, brain tumors and breast cancer (Wertheimer and Leeper, 1979; Thomas *et al.*, 1989; Savitz *et al.*, 1988; Feychting and Ahlbom, 1995; Ahlbom, 1996; Verkasalo, 1996).

However, some scientists doubt that this apparent association between EM field exposure and cancer is real, because it is difficult to explain biologically and because the research results are inconsistent. Most agree that more information is needed to resolve the issue about whether or not EM fields affect human health.

A number of investigations have been carried out to test the potential genotoxicity of electric and magnetic fields. Several reviews on the genotoxic and carcinogenic potential of EM fields studies have appeared (Michaelson, 1987; Scarfi *et al.*, 1989; McCannn *et al.*, 1993; McCannn *et al.*, 1998).Because initiation of carcinogenesis is believed to involve DNA damage, assays for genotoxicity are considered to supply evidence relevant to carcinogenic potential (Dennis *et al.*, 1991).

In vivo studies showed the genome damages including the increases of chromosome abnormalities and the frequencies of micronuclei formation, in workers occupationally exposed to radio frequency (Fucic *et al.*, 1992; Garaj-Vrhovac, 1999; Othman *et al.*, 2001) and low frequency EM field (Nordenson *et al.*, 1984; Nordenson *et al.*, 1988). Also, the results of many *in vitro* studies showed the mutagenic potential of these radio frequency (Maes *et al.*, 1993; Maes *et al.*, 1995; D'Ambrosio *et al.*, 1995; Garaj-Vrhovac *et al.*, 1996) and low frequency EM fields (Nordenson *et al.*, 1994; Galt *et al.*, 1995; Tofani *et al.*, 1995).

Other *in vivo* studies were performed on experimental animals to investigate the mutagenic effect of exposure to electric and EM field. Exposure to low frequency electric fields caused a significant increase in SCEs, chromosomal aberrations and in the number of micronucleated polychromatic erythrocytes in bone marrow of mice (El Nahas and Anis, 1986; El Nahas and Oraby, 1989; El Nahas *et al.*, 1998; Timchenko and Ianchevskaia, 1995). Also, exposure to radio frequency caused an increase in the frequency of chromosome exchanges in spermatocytes and in number of translocations (Manikowska *et al.*, 1979). It also caused an alteration in the length of a DNA microsatellite sequence in cells from brain and testis of mice (Sarkar *et al.*, 1994) and an increase in DNA damage in exposed male rats (Lai and Singh, 1995; Lai and Singh, 1996). Some *in vitro* studies also showed positive mutagenic effect of EM field (Yao, 1976; 1982; Garaj-Vrhovac *et al.*, 1991).

In contrast to the above results, negative effect of exposure *in vivo* and *in vitro* to low frequency EM field (Skyberg *et al.*, 1993; Valjus *et al.*, 1993; Zwingelberg *et al.*, 1993; Antonopoulos *et al.*, 1995; Paile *et al.*, 1995; Jacobson-Kram *et al.*, 1998) and to radio frequency (Garson *et al.*, 1991; Antonopoulo *et al.*, 1997; Eberle *et al.*, 1996; Maes *et al.*, 1996 ; Maes *et al.*, 1997 ; Vijayalaxmi *et al.*, 1997) were reported.

In view of conflicting results, this project was undertaken to further evaluate the possible genetic effects of occupational exposure to electromagnetic field. Stimulated human lymphocytes of individuals exposed to radio frequency EM field were analyzed. Chromosomal aberrations, sister chromatid exchanges, mitotic activity and cell kinetics were investigated.

Materials and Methods

In this study, the cytogenetic effect of electromagnetic field on lymphocytes from occupationally exposed individuals was evaluated. Induction of chromosomal aberrations and sister chromatid exchange (SCEs) were the two cytogenetic parameters analyzed. The effects on mitotic activity and replicative index were also studied.

Fifty male workers, 26 air traffic controllers and 24 engineers, exposed for 8-27 years to radio frequency radiation EM fields were chosen as a random sample. From a questionnaire filled by the workers, non of them were exposed to any mutagenic agents for the last six months.

The amount of radiation to whom the workers were exposed ranged from 60.43 to 105.7% of ANSI standard (American National Standard Institute) (ANSI/IEEE, 1991) as measured at different locations by Atomic Energy Authority (AEA), Cairo, Egypt. Ten males, none of them had been occupationally exposed to EMF, were used as a control group.

Culture conditions of lymphocytes: Fresh heparinized peripheral blood (0.5 ml), from each EMF exposed and non-exposed individuals, was cultured at 37°C for 72 hours in 5 ml RPMI 1640 medium (Gibco) supplemented with 20% fetal calf serum (Gibco), 0.1% garamycin (Schering), 1% L-glutamine (Gibco) and 4% phytohaemagglutinin (Wellcome). The blood cultures for SCE analysis and cell cycle kinetics were treated with bromodeoxyuridine (BrdU) at a final concentration of 10 μg/ml, 24 hours from culture initiation.

Chromosome preparation and analysis: Two hours before harvesting, colchicine was added to all cultures at a final concentration of 20 μg/ml. At harvest, the cells were treated with a hypotonic solution (0.075M KCl) and incubated at 37°C for 20 minutes, then the cells were fixed three times in fixative (3 methanol: 1 acetic acid). Finally, the cells were spread onto cold slides dipped in 70% ethyl alcohol. The slides were air dried and stained using the fluorescence plus Giemsa technique (Goto *et al.*, 1978).

Scoring and statistical analysis: For chromosomal aberrations analysis, 50 cells from each individual were analyzed. Structural and numerical aberrations were recorded. For sister chromatid exchange (SCE) study, the frequency of SCE was recorded for each individual in at least 30 second division cells. Mitotic activity was studied by analyzing 2000 cells in each individual and calculating the mitotic index (number of dividing cells/1000 cell). For cell cycle kinetics, 100 metaphase cells from each individual were analyzed and the number of first (MI), second (M2) and third (M3) divisions were recorded and the replicative index (R.I.) was calculated according to Schneider and Lewis (1981).

Statistical analysis: For chromosomal aberrations analysis the Chi-Square test was used, whereas the Student t-test was used for sister chromatid exchange, mitotic index and replicative index data analysis.

Results

Chromosome aberrations: The structural chromosomal aberrations reported in this investigation were mainly in the form of breaks and gaps. Whereas the numerical aberrations were mainly hypodiploid.

The number of cells with structural chromosomal abnormalities are significantly higher in exposed workers of both groups as compared with the control (Table 1).

Chromosomal breakage constitutes about 56 and 78% of the total structural aberrations in engineers and air traffic controllers, respectively. The increase in chromosomal breakage in both exposed groups were statistically significant ($P<0.01$). The number of cells with gaps in exposed groups also increased as compared to the control group. However only in engineer group that the increase was significant ($P<0.01$).

In order to study the effect of duration of exposure, the engineers and air traffic controller were further subdivided into two subgroups according to their average exposure times (20 years for engineers and 16 years for air traffic controllers) (Table 1). The percentage of total aberrant cells was 4.86% in engineers exposed for $\geq$ 20 years and 5.33% in engineers exposed for $<$20 years. These numbers were significantly ($P<0.001$) higher than the control in the two subgroups. The difference in total structural aberrations between these two subgroups was not statistically significant. In air traffic controllers, the percentage of total aberrant cells was 3.38% in individuals exposed for $\geq$16 years and 2.73% in individuals exposed for $<$16 years compared with 0.8 in the control group. The increases were significant at a P levels of 0.01 and 0.05, respectively. As in engineers the difference in total structural aberrations between the two subgroups was not significant.

Concerning the numerical aberrations, the percentages of cells with total numerical aberrations were 9.62, 9.17 and 3.20% in engineers, air traffic controllers and control individuals, respectively. The increase in cells with numerical aberrations in both exposed groups was statistically significant at $P<0.001$. Hypodiploid cells constituted 88 and 91% of total cells with numerical aberrations in engineers and air traffic controllers, respectively. Table 1 also showed the effect of exposure-duration on numerical aberrations. The percentage of cell with total numerical aberrations were higher (9.86%) in engineers exposed for $\geq$ 20 years than in engineers exposed for $<$20 years (9.33%), with no significant difference between the two subgroups. Also in air traffic controllers, the percentages of total numerical aberrations in individuals exposed for $\geq$ 16 years (9.38%) were higher than those exposed for $<$16 years (8.91%) with no significant differences between the two subgroups.

Mitotic index: The MI was decreased in exposed engineers (19.56 $\pm$ 5.92), and in air traffic controllers (18.58 $\pm$ 5.28) compared to the control group (30.20 $\pm$ 13.09) (Table 2). This decrease was statistically significant at $P<0.01$ and at $P<0.001$, respectively. Table 2 presents the mitotic activity in exposed workers in relation to duration of exposure. The mitotic indices were 17.18 $\pm$ 4.49 and 22.30 $\pm$ 6.35 in engineers exposed for $\geq$ 20 and $<$ 20 years, respectively. A decrease in the mitotic activity was only significant ($P<0.01$) in engineers exposed for $\geq$ 20 when compared with the control group (30.20 $\pm$ 13.09). Also a significant difference ($P<0.05$) as a result of exposure- duration occurs between the two subgroups. In air traffic controllers the mitotic indices were 18.42 $\pm$ 3.11 and 18.77 $\pm$ 7.24 in individuals exposed for $\geq$ 16 years and those exposed for $<$ 16 years, respectively. The mitotic activities of both subgroups were significantly decreased at $P<0.01$ and $P<0.05$, respectively. However, no significant differences were found between the two subgroups.

Sister chromatid exchange analysis: The frequency of sister chromatid exchanges (SCEs) in engineers, air traffic controllers and control group are presented in Table 3. Cells from 38 exposed individuals (19 engineers and 19 air traffic controllers) and 10 control individuals were analyzed. The frequencies of SCEs in control group, engineers and air traffic controllers were 4.5 $\pm$ 0.94, 5.00 $\pm$ 1.20 and 4.80 $\pm$ 1.25 SCEs/cell, respectively. Although there was a slight increase in exposed groups over the control, however such increase was not statistically significant. No significant differences were found between individuals exposed for different times.

Cell kinetics: RI values for control group, engineers and air traffic controllers. They were 1.78 $\pm$ 0.180, 1.79 $\pm$ 0.18 and 1.80 $\pm$ 0.21 for the studied groups respectively (Table 3). Statistical analysis showed that exposure to EMF did not affect the RI in both exposed groups when compared with the control group. Also no significant differences were found between subgroups exposed for different durations.

Discussion

Several epidemiological studies have correlated exposure of human to electromagnetic fields with a high incidence of cancer (Coleman *et al.*, 1983; McDowall, 1983; Pearce *et al.*, 1985; Speers *et al.*, 1988). In addition to cancer induction, other biological effects have been reported. The relationship between spontaneous abortion and exposure to electromagnetic fields has been considered in several studies (Schnorr *et al.*, 1991; Lindbohm *et al.*, 1992; Belanger *et al.*, 1998). The association between occupational exposure and Alzheimer's disease was considered in other studies (Sobel *et al.*, 1995). Generally, change in DNA or chromosome structure of somatic cells are considered to be very important, as these changes could be associated with cell death and possibly with the development of cancer. Such change in male or female germ cells are important, as surviving mutations might be passed on to the next generation. Relatively few studies have addressed the questions of whether EMF causes genetic mutations changes after RF exposure (Verschaeve and Maes, 1998) and ELF exposure (McCann *et al.*, 1998).

The aim of this study was to evaluate the genetic changes in air traffic controllers and engineers occupationally exposed to RF electromagnetic fields. In this study, the frequency of structural chromosomal aberrations in both EMF-exposed worker groups increased significantly. The percentage of chromosomal aberrations was higher in the engineers group than in the air traffic controllers group. Individuals with longer duration of

Table 1: Chromosomal aberrations in electromagnetic fields-exposed workers and control groups

				Cells with structural aberrations						Cells with numerical aberrations					
				Breaks		Gaps		Total		Hypodiploid		Hyperdiploid		Total	
Exposed Individuals	Duration of exposure (years)	No. of cases	No. of cells examined	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	-	10	500	2	0.40	2	0.40	4	0.80	16	3.20	-	-	16	3.20
Engineers	≥ 20	14	700	23***	3.29	11	1.57	34***	4.86	***60	8.57	*9	1.29	69***	9.86
	< 20	12	600	14**	2.33	18**	3.00	32***	5.33	***50	8.33	*6	1.00	56***	9.33
	Total	26	1300	37**	2.85	29**	2.23	66***	5.08	***110	8.46	*15	1.15	125***	9.62
Air traffic	≥ 16	13	650	18**	2.77	4	0.62	22**	3.38	***55	8.46	*6	0.92	61***	9.38
Controllers	<16	11	550	11*	2.00	4	0.73	15*	2.73	***45	8.18	4	0.73	49**	8.91
	Total	24	1200	29**	2.42	8	0.67	37***	3.08	***100	8.33	*10	0.83	110***	9.17

* P<0.05, ** P<0.01, *** P<0.001

Table 2: Mitotic activity in electromagnetic fields-exposed workers and control groups

Exposed Individuals	Duration of exposure (years)	No. of cases	No. of cells examined	No. of dividing cells	Mitotic index Mean±SD
Control	-	10	2000	604	30.20±13.09
Engineers	20	14	28000	481	**17.18±4.49
	<20	12	24000	536	22.30±6.35
	Totals	26	52000	1017	**19.56±5.92
Air traffic controllers	16	13	26000	479	**18.42±3.11
	<16	11	22000	413	*18.77±7.24
	Totals	24	48000	892	***18.58±5.28

* P<0.05, ** P<0.01, *** P<0.001

Table 3: Frequencies of sister-chromatid exchanges and cell cycle kinetics in electromagnetic fields-exposed workers and control groups

			SCEs/cells			Replicative index (RI)	
Exposed Individuals	No. of cases	No. of cells examined	Range	Mean±SD	No. of Examined	cellsRange	Mean±SD
Control	10	480	0-8	4.50±0.94	1000	1.62-2.24	1.78±0.18
Engineers	19	810	0-17	5.00±1.20	1900	1.49-2.13	1.79±0.18
Air traffic controllers	19	700	0-18	4.80±1.25	1900	1.40-2.22	1.80±0.21

exposure showed higher number of chromosomal aberrations than the shorter duration of exposure in both exposed groups. Although few studies have been performed on the mutagenic potential of exposure to radio frequency, positive effects have been reported. *In vivo* exposure to RFR. In human, an increased incidence in micronucleated white blood cells from professionally exposed subjects was reported by (Fucic *et al.*, 1992; Garaj-Vrhovac, 1999). In animals, an increase in the frequency of chromosome exchanges in spermatocytes and increase in number of translocations has been reported in mice exposed to 9.4 GHz (Manikowska *et al.*, 1979). Sarkar *et al.* (1994) found evidence of an alteration in the length of a DNA microsatellite sequence in cells from brain and testis of mice exposed to 2.45-GHz fields. In another series of experiments, (Lai and Singh, 1995; Lai and Singh, 1996), using the same frequency, demonstrated that acute exposure to low-intensity radio frequency radiation increased DNA strand breaks in brain cells of the rat. Lai (1992) suggested that radio frequency radiation activated endogenous opioids in the brain which in turn cause biological effects. Also *in vitro* studies showed positive effect. Garaj-Vrhovac *et al.* (1990) exposed human lymphocytes to 7.7 GHz and reported an increase in chromosome aberrations and micronuclei. An increase in micronuclei frequency was also noted when human lymphocytes were exposed to 415 MHZ Garaj-Vrhovac *et al.* (1996). Studies on exposure of animal cells to 2.45 GHz revealed an increase in chromosome aberration frequency in exposed rat Kangaroo cells (Yao, 1976, 1982).

However other authors have reported that radio frequency EM field does not cause chromosomal aberrations. Garson *et al.* (1991) reported no increase in chromosome damage in lymphocytes of radiolinemen who work with radiofrequencies ranging from 400 KHz to 20 GHz. No significant effect was reported on frequency of micronuclei in mice exposed to 2.45 GHz compared to sham-exposed animals (Vijayalaxmi, 1997). Also, no significant evidence of germ cell mutagenesis or alteration in reproductive efficiency was reported by Berman *et al.* (1980) when male rats were exposed to 2.45 GHz. Negative effects of *in vitro* exposure were also reported. No effect on chromosomal aberrations and sister chromatid exchanges was reported in a study by Lloyd *et al.* (1984, 1986) where human lymphocytes were exposed to 2.45 GHz. Also, no effect on chromosomal aberrations, micronuclei and HGPRT-mutations were noted when human lymphocytes were exposed to 440,900 and 1800 MHZ (Eberle *et al.*, 1996).

The significant increase in hypodiploid cells reported in this studies agrees with the results obtained in a previous study on air traffic controllers and engineers by Othman *et al.* (2001). They reported a significant increase in monosomy of chromosomes 7 and 17 and loss of Y chromosome in both EMF-exposed groups as compared to the control. This explains the increase in hypodiploid reported in this study.

No significant increase in frequency of SCE was found in this study in both air traffic controllers and engineers groups. Although there was a slight increase in exposed groups over the control, such increase was not statistically significant and does not reach the level to be accepted as a positive response according to the UKEMS guide lines on mutagenecity testing (UKEMS, 1983) where at least a doubling in SCE frequency should occur. The results agree with the few studies dealing with effect of radio frequency on frequency of sister chromatid exchanges was reported in study by Lloyed *et al.* (1984, 1986); Eberle *et al.* (1996) where no increase in SCE were reported.

Investigating the mitotic activity and cell kinetics revealed that the mitotic activity (MI) was significantly decreased in engineers and air traffic controllers exposed to radio frequency at P level of 0.01 and 0.001, respectively. Whereas the cell cycle kinetics in engineers and air traffic controllers groups were not affected when compared with control. A decrease in mitotic index was reported in human peripheral lymphocytes exposed to EMF *in vitro* (Khalil and Qassem, 1991). On the other hand, Cossarizza *et al.* (1989); Scarfi *et al.* (1994) and Antonopoulos *et al.* (1995) reported that mitotic indices were elevated in human peripheral lymphocytes exposed *In vitro* to EMF when compared to controls. Also a significant increase in mitotic activity was reported in mice exposed to low frequency electric field (El Nahas *et al.*, 1998). Zwingelberg *et al.* (1993) reported that the magnetic field did not influence the proliferation characteristics of peripheral lymphocytes. Contrary to our results , Garaj- Vrhovac (1999), reported disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls in subjects occupationally exposed to microwave radiation.

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