Aneuploidy in Workers Occupationally Exposed to Electromagnetic Field Detected by FISH

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Summary Exposure to power frequency electromagnetic fields (EMF) may cause adverse health effects in humans. Some epidemiological studies support an association between exposure to EMF and the incidence of different types of cancer, which may be associated with the presence of chromosomal aneuploidy. In this study, fluorescence *in situ* hybridization (FISH) was used to detect numerical chromosomal aberrations induced by occupational exposure to EMF. The labeled probes used in this study were repetitive α -satellite probes for chromosomes 7, 12, 17 and heterochromatic region of the Y chromosome. This study was conducted using 23 male individuals, 18 of which are occupationally exposed to EMF whereas the other 5 individuals are used as controls. Our study shows monosomy of chromosomes 7 and 17 in 4 and 3 exposed individuals, respectively. The mean percentages of nuclei with monosomy of chromosomes 7 and 17 in these affected individuals are 18.3% and 16.1%, respectively. The loss of Y chromosome was detected in 6 exposed individuals and the mean percentage of nuclei showing this aberration is 15.8%. In control individuals, the mean percentages of nuclei with monosomy of chromosomes 7, 17 and loss of the Y chromosome were 3.2%, 3.7% and 4.5%, respectively. The increase in monosomy of chromosomes 7 and 17 and the loss of Y chromosome compared to the control is statistically significant ($P < 0.001$). No significant increase in numerical aberrations of chromosome 12 was observed in exposed individuals.

Key words EMF exposure, Aneuploidy, FISH.

There is growing concern that environmental and household exposure to power frequency electric and/or magnetic fields may cause adverse health effects in humans. Several epidemiological studies indicate an association between exposure to magnetic fields, in residential or occupational environments, and the incidence of cancer (Lin *et al.* 1985, Savitz and Calle 1987, Thomas *et al.* 1987, Savitz *et al.* 1988, Dennis *et al.* 1991, London *et al.* 1991, Theriault 1991). The cancers most frequently identified are acute leukemia and brain cancers.

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 EMF studies have appeared (Michaelson 1987, Scarfi *et al.* 1989, McCann *et al.* 1993, 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).

Controversy still exists on the genetic effect of electromagnetic fields. Several studies reported the mutagenic effect of EMF, including increase in chromosome and chromatid abnormalities, sister chromatid exchanges and also micronuclei formation in both *in vivo* and *in vitro* studies on human peripheral lymphocytes and other mammalian cells (Nordenson *et al.* 1984, 1988, 1994, Butler *et al.* 1987, Rosenthal and Obe 1989, El Nahas and Oraby 1989, Khalil and Qassem 1991, Tofani *et al.* 1995, Miyakoshi *et al.* 1996, 1997).

However in other studies, these same end points were negative (Bauchinger *et al.* 1981, Cohen

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et al. 1986, Livingston *et al.* 1991, Takahashi *et al.* 1987, Rosenthal and Obe 1989, Garcia-Sagredo *et al.* 1990, Saalman *et al.* 1991, Scarfi *et al.* 1991, Hintenlang 1993, Tabrah *et al.* 1994, Antonopoulos *et al.* 1995, Galt *et al.* 1995, Paile *et al.* 1995). From the carcinogenic effect point of view, these negative results suggest that EMF may not have potential as an initiator of carcinogenesis (Livingston *et al.* 1991). Most recent research on the question of a causal role of EMF in the development of cancer has focused on the later stages of carcinogenesis and cancer development, namely promotion and progression (McCann *et al.* 1993).

The objective of this study is to assess whether occupational electromagnetic field exposure is a risk factor for the induction of aneuploidy damage using fluorescence *in situ* hybridization technique to interphase nuclei in stimulated blood lymphocyte cultures.

Materials and methods

The present study includes 18 male air traffic controllers and engineers who have been exposed for 10–27 years to electromagnetic fields emitted from radar screens, antennae, satellite installations and closed circuit televisions (CCTV). All worked in shifts of 6 h period and they rotated clockwise changing their shift every day with a night shift of 12 h which was followed by 2 days off. Also, 5 comparable males randomly chosen were used as a control group, matched for age, smoking habits and socio-economic status, none of them had been occupationally exposed to EMF. None of the workers under investigation nor the control group had been exposed to known chromosome-damaging agents, including drugs, nor they received ionizing radiation for medical purposes except for occasional chest roentgenograms.

Cell cultures

Peripheral blood lymphocytes from EMF exposed and non exposed individuals were cultured at 37°C for 72 h in 5 ml of RPMI 1640 medium (Gibco) containing 20% foetal calf serum (Gibco), 0.1% garamycin (Schering), 1% L-glutamine (Flow) and 4% phytohaem-agglutinin (Wellcome). Slides were prepared for the analysis of interphase nuclei according to standard cytogenetic protocols including hypotonic swelling with 0.075 M KCl and 3 times fixation with methanol : acetic acid (3 : 1).

Probe labeling and fluorescence *in situ* hybridization

The 3 human α -satellite DNA specific for the peri-centromeric regions of chromosomes 7, 12, 17 and the heterochromatic region of the Y chromosome [which detect the 2.1-Kb repeat of the heterochromatic region of the Y_q arm (pHY2.1) (Cooke *et al.* 1982)] were used. The probes were labeled by nick-translation using biotin-11-dUTP according to BRL protocol.

The labeled DNA was ethanol-precipitated with salmon sperm DNA. The precipitate was dissolved in 60% formamide/2× SSC (1× SSC=0.15 M NaCl, 0.015 M sodium citrate) to a probe concentration of 2.5 ng/ μ l.

FISH was performed according to the protocol of Kievits *et al.* (1990) with some modifications. Briefly, the slides were treated with RNase A for 1 h at 37°C (10 μ g in 100 μ l of 2× SSC under 24×50 mm² coverslip) followed by pepsin treatment for 10 min at 37°C (0.01% pepsin {Serva} in 0.01 M HCl), postfixed for 10 min in 1% acid-free formaldehyde in PBS, and then dehydrated in an ethanol series at room temperature. The probe and the target DNA were denatured simultaneously for 10 min at 75°C.

Hybridization was performed overnight at 37°C in a humid chamber. Posthybridization washings were carried out at 45°C in 50% formamide, 2× SSC (pH 7.0) 3 times for 2 min each, followed by 2× SSC (pH 7.0) once at room temperature for 2 min. The slides were immersed in blocking buffer and were never allowed to dry after this point.

Signal detection

The slides were incubated at room temperature with FITC conjugated avidin (5 $\mu\text{g/ml}$ in $4\times$ SSC, 5% nonfat dry milk). The intensity of biotin-linked fluorescence was amplified by adding a layer of biotinylated goat anti-avidin antibody (5 $\mu\text{g/ml}$ in $4\times$ SSC, 5% nonfat dry milk), followed, after washing, by another application of FITC conjugated avidin. Slides were dehydrated and mounted in antifade medium (9 parts glycerol containing 2.3% DABCO {Sigma}: 1 part 0.2 M Tris-HCl, 0.02% NaN_3 , pH 7.5) containing DAPI of 0.5 $\mu\text{g/ml}$ as chromosomal counterstain.

The evaluation of FISH results with the centromeric probes used was done according to criteria described previously (van Dekken *et al.* 1990a). One thousand intact and non-overlapping interphase nuclei were analyzed. The number of bright fluorescing spots per nucleus was scored for each probe with the signals having more or less the same homogenous fluorescence intensity. Minor hybridization spots were not counted. Spots in a paired arrangement (split spots) were counted as one signal.

Statistical analysis

For each probe, the significant differences between the signals/nucleus distributions obtained in both exposed and control groups, was determined using the chi-square test whereas significant differences between the personal characteristics were determined using the *t*-test.

Results

Statistical analysis was conducted to determine if there are significant differences in personal characteristic habits between EMF exposed and the control groups (Table 1). No statistically significant difference was found regarding their age and smoking habits. The median age of the exposed

Table 1. Personal characteristic of the exposed and control individuals

Subject	Case No.	Age (years)	Marital status	Smoking habit	Duration of exposure to EMF (years)
Exposed individuals	1	51	Married	Non-smoker	25
	2	45	Married	Non-smoker	17
	3	52	Married	smoker	14
	4	52	Married	Non-smoker	17
	5	52	Married	Non-smoker	23
	6	44	Married	smoker	20
	7	36	Single	Non-smoker	10
	8	42	Married	smoker	16
	9	44	Married	smoker	20
	10	52	Married	Non-smoker	23
	11	51	Married	Non-smoker	27
	12	49	Married	Non-smoker	15
	13	44	Married	Non-smoker	17
	14	45	Married	smoker	16
	15	45	Married	Non-smoker	20
	16	44	Married	smoker	19
	17	43	Married	Non-smoker	15
	18	42	Single	Non-smoker	15
Controls	1	44	Married	Non-smoker	-----
	2	49	Married	Non-smoker	-----
	3	40	Married	smoker	-----
	4	39	Single	smoker	-----
	5	51	Single	Non-smoker	-----

Table 2 Evaluation of aneuploidy detected in lymphocyte cultures of exposed and control individuals after FISH with four biotinylated probes on interphase nuclei

Subject	Case No.	No. of examined cells	No. and (%) of nuclei with monosomy of chromosome 7	No. and (%) of nuclei with monosomy of chromosome 12	No. and (%) of nuclei with monosomy of chromosome 17	No. and (%) of nuclei with loss of Y chromosome
Exposed individuals	1	1000	29 (2.9)	40 (4.0)	40 (4.0)	45 (4.5)
	2	1000	194 (19.4)	45 (4.5)	38 (3.8)	196 (19.6)
	3	1000	31 (3.1)	37 (3.7)	46 (4.6)	52 (5.2)
	4	1000	38 (3.8)	33 (3.3)	160 (16.0)	149 (14.9)
	5	1000	182 (18.2)	39 (3.9)	40 (4.0)	145 (14.5)
	6	1000	39 (3.9)	40 (4.0)	33 (3.3)	49 (4.9)
	7	1000	26 (2.6)	36 (3.6)	48 (4.8)	39 (3.9)
	8	1000	28 (2.8)	46 (4.6)	179 (17.9)	150 (15.0)
	9	1000	190 (19.0)	44 (4.4)	42 (4.2)	47 (4.7)
	10	1000	32 (3.2)	35 (3.5)	45 (4.5)	50 (5.0)
	11	1000	30 (3.0)	33 (3.3)	39 (3.9)	46 (4.6)
	12	1000	31 (3.1)	39 (3.9)	43 (4.3)	47 (4.7)
	13	1000	27 (2.7)	37 (3.7)	145 (14.5)	163 (16.3)
	14	1000	32 (3.2)	40 (4.0)	40 (4.0)	50 (5.0)
	15	1000	165 (16.5)	35 (3.5)	37 (3.7)	45 (4.5)
	16	1000	36 (3.6)	46 (4.6)	35 (3.5)	46 (4.6)
	17	1000	38 (3.8)	39 (3.9)	39 (3.9)	43 (4.3)
	18	1000	43 (4.3)	32 (3.2)	47 (4.7)	147 (14.7)
Controls	1	1000	38 (3.8)	38 (3.8)	40 (4.0)	47 (4.7)
	2	1000	35 (3.5)	36 (3.6)	39 (3.9)	52 (5.2)
	3	1000	32 (3.2)	39 (3.9)	34 (3.4)	46 (4.6)
	4	1000	25 (2.5)	47 (4.7)	30 (3.0)	39 (3.9)
	5	1000	30 (3.0)	45 (4.5)	43 (4.3)	43 (4.3)

workers was 46.3 years (range 36–52 years) and the median age of control individuals was 44.6 years (range 39–51).

After establishing the proper conditions for signal analysis, the same conditions and probe concentrations were used for hybridization of the 4 probes to interphase nuclei of both exposed and control individuals. FISH was successful in all cases studied. Table 2 presents the number and percentages of signals for the 4 probes in exposed and control individuals. Only monosomy of chromosome 7 and 17 and loss of the Y chromosome was detected. It is known that specimen was considered monosomic if the percentage of nuclei exceeds 10%. This same percentage applies for loss of chromosome Y (van Dekken *et al.* 1990b). Ten out of the 18 exposed cases had normal diploid chromosome count. Four cases (22.2% of exposed individuals) demonstrated a loss of chromosome 7 (Fig. 1) and the percentages of nuclei showing monosomy 7 ranged from 16.5–19.4% compared to 3.2% in control (mean value). In 2 cases (nos. 2, 5), monosomy of chromosome 7 was accompanied by loss of Y chromosome. Loss of chromosome 17 was detected in 3 cases (16.7%) (Fig. 2). The percentages of nuclei showed monosomy 17 ranged from 14.5–17.9% with a mean of 16.1% compared to 3.7% in the control. Monosomy of chromosome 17 was accompanied by loss of Y chromosome in the 3 cases.

In 6 cases (33.3%), a loss of Y chromosome was observed. The percentages of nuclei, which showed the loss of Y chromosome ranged from 14.5–19.6% with a mean of 15.8%, compared to 4.5% in the control. As mentioned earlier, the loss of Y chromosome in 5 cases were accompanied by a loss of 7 or 17 chromosomes.

The increase in monosomy of chromosomes 7 and 17 and the loss of Y chromosome compared to the control were statistically significant at $P < 0.001$. No significant numerical aberrations of

Figs. 1–2. 1) Fluorescence *in situ* hybridization with chromosome 7-specific alpha satellite DNA probe. Nuclei in the photomicrographs exhibit 1 and 2 centromeric spots. Omega double filter was used for the visualization of FITC only. 2) Fluorescence *in situ* hybridization with chromosome 17-specific alpha satellite DNA probe. Nuclei in the photomicrographs exhibit 1 and 2 centromeric spots. The used filter was specific for the visualization of FITC and DAPI.

chromosome 12 were observed in all exposed individuals tested. The mean percentage of nuclei with monosomy 12 in exposed individuals was 3.9% compared to 4.1% in the control.

Neither age of workers and smoking habits nor the duration of exposure to EMF for more than 10 years had a statistically significant correlation with the presence or absence of any of the numerical chromosomal aberrations observed in this study.

Discussion

The controversy still exists on the genetic effect of electromagnetic fields. Although some reports concerning DNA damage and mutagenic effects of EMF show negative results (Zwingelberg *et al.* 1993, Murphy *et al.* 1993, McCann *et al.* 1993, 1998, Scarfi *et al.* 1994), others are reporting positive effects (Miyakoshi *et al.* 1996, 1997, El Nahas and Oraby 1989, Nordenson *et al.* 1994).

Several epidemiological studies have correlated exposure of human to electromagnetic fields with a high incidence of cancer (Coleman *et al.* 1983, McDowall 1983, Milham 1985, Pearce *et al.* 1985, Speers *et al.* 1988, Loscher *et al.* 1993, Loscher and Mevissen 1994).

Hardell *et al.* (1995) and McCann *et al.* (1997) suggest that experimental evidence seems to exclude the possibility of an initiating carcinogenic effect of EMF but promoting effects are possible, one of these could be stimulation of the cell cycle. Extensive efforts are now underway to assess the carcinogenic potential of EMF in animal model systems. Specific numerical chromosomal aberrations play an important and possibly essential role in neoplastic development.

The present study shows monosomy of chromosomes 7 in 22.2% of exposed individuals and monosomy of chromosome 17 in 16.7% of exposed individuals. Six exposed individuals (33.3%) showed loss of Y chromosome. The percentage of signals in individual with monosomy 7, 17 and loss of Y chromosome ranged from 14.5–19.6%. It is known that specimen was considered monosomic if the percentage of nuclei exceeds 10%. This same percentage applies for loss of chromosome Y (van Dekken *et al.* 1990b). The increase in monosomy of chromosomes 7 and 17 and the loss of Y chromosome compared to the control were statistically significant at $p < 0.001$. No numerical aberrations of chromosome 12 were observed in all exposed individuals tested. Loss of chromosome Y was accompanied by monosomy 7 in 2 cases and by monosomy 17 in 3 cases. Loss of Y chromosome has been reported to be accompanied by other karyotypic abnormalities (van Dekken and Alers 1993, Aly *et al.* 1993).

The monosomy of chromosomes 7 and 17 and the loss of Y chromosome observed in the present study, may shed some light on the relation between the exposure to EMF and the incidence of cancer.

Chromosomal loss is the most frequent karyotypic abnormality in the myelodysplastic syndromes (Boultonwood and Fidler 1995). Recent attention has focused on the loss of genetic material as a result of chromosomal monosomy or deletion in the myelodysplastic syndromes. The most frequently reported deletions in these myeloid syndromes are of chromosome 7 (Wyandt *et al.* 1998). It is probable that the deleted chromosomal bands involve the location for the unidentified myeloid specific tumor suppressor loci (Boultonwood and Fidler 1995). Loss of a chromosome 7 is also associated with a poor prognosis in acute myeloid leukemia (Kadam *et al.* 1993, Fischer *et al.* 1996, Pedersen *et al.* 1997).

As for chromosome 17, the gene for p53, neu (c-erbB-2) and nm23 are all located on this chromosome. Abnormality of chromosome 17, and in particular the loss of parts of the short arm, had previously been related as characteristic in disorders such as blast crisis of chronic myeloid leukemia (Borgstrom *et al.* 1982) and acute myeloblastic leukemia de-novo (Fourth International Workshop on Chromosomes in Leukemia 1984). Numerical aberrations of chromosome 17 have been frequently observed also in many other tumor types and often as a secondary change such as multiple myeloma (Drach *et al.* 1995), colorectal carcinoma (Wang *et al.* 1997), breast carcinoma cells (Sauer *et al.* 1998), ovarian carcinomas (Diebold 1999), chromophobe renal cell carcinoma (Gunawan *et al.* 1999), and primary gastric cancers (Terada *et al.* 1999).

Loss of Y chromosome was found to be a normal aging phenomenon in bone marrow cells of elder men (Pierre and Hoagland 1971, 1972). However, Y chromosome loss may not be entirely related to aging and the possibility remains that, at least in an occasional cases, cells with a missing Y may be neoplastic (Aly *et al.* 1993). Loss of Y chromosome was reported as a sole anomaly in many malignant as well as benign tumors (Mitelman 1991).

From our results we can conclude that exposure to EMF lead to significant chromosomal aneuploidy in chromosomes 7 and 17 which are reported in cancer cases. Also, loss of Y chromosome reported here reflect an increase in the rate of aging at least at the cellular level in addition to its role in some cancers. In view of these findings and the contradictory reports in the literatures, it is important to carry out more investigations using various types of cells under different experimental conditions to resolve the controversy concerning the possible risks associated with EMF. It is therefore recommended that exposure to EMF should be minimized through increasing exposure distance between the workers and the functioning units and shielding through the use of electricity-proof materials. Regular monitoring of the work place for both electric and magnetic fields is mandatory.

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