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A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations

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A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05 , respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

Keywords: electric and magnetic field exposure; genotoxicity; CA; MN

Introduction

The general public has been deriving the benefits from the use of electricity, at home and in the workplace, for well over 100 years. It is almost impossible to imagine life without the use of electricity. Environmental exposure to the extremely low frequency electric (ELF) and magnetic fields (EMF) generated by power lines and by domestic and industrial equipment is quite ubiquitous, especially for populations living in the developed world. In the last few decades, the hazard to human health following exposure has become a subject of considerable interest by public health authorities and decision-making bodies. The concern is primarily focused on populations at risk, such as children. The cancer risk of electro-magnetic fields (ELFMFs) is currently being evaluated by the International Agency for Research on Cancer (IARC). Workers at the sites of electric production are chronically exposed to ELF and EMF produced by transmission through power lines and transformers. Several independent epidemiological studies based on job titles have shown an increased risk of leukemia, and others have found an elevated risk of brain and breast tumors among “electrical workers” (Savitz and Ahlbom 1994; Ahlbom 1997).

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Several in vivo and in vitro models have been investigated with the effort to determine a link, if any, between such fields and mutagenesis and to determine the possible mechanism of cancer risk. It was attempted to define whether 50/60 Hz ELF-EMF as generated by high-voltage power lines or electrical appliances could give rise in vivo to primary DNA damage (e.g. single- and double-strand breaks) and cytogenetic effects such as chromosomal aberrations, sister-chromatid exchanges, or micronuclei in humans (Skyberg et al. 1993; Vijayalaxmi and Obe 2005; Miyakoshi 2006).

DNA damage and chromosome aberrations (CA) in somatic cells are closely related to carcinogenesis. It is generally believed that magnetic fields are devoid of genotoxic potential (McCann et al. 1998; Repacholi and Greenebaum 1999). Their oncogenic activity, if any, can be better explained in terms of tumor promotion or co-promotion than in terms of mutagenic ability. Nevertheless, in some studies, genotoxic effects have been reported (Khalil and Qassem 1991; Nordenson et al. 1994; Tofani et al. 1995; Singh and Lai 1998). The issue however remains controversial.

Despite the large number of studies performed, the effect of ELF-EMF on genotoxicity is still unclear. However, these fields are strongly suspected to have detrimental biological effects. A number of independent scientific committees and working groups have reviewed the literature and concluded with various degrees of confidence that exposures to EMF, as encountered in residential and in most occupational settings, were not proven to impose adverse health effects on humans (Davis et al. 1992; National Radiological Protection Board [NRPB] 1993). Nonetheless, considering the scientific "weight of evidence", mostly from human epidemiological data, the IARC concluded that EMF exposures act as a "possible human carcinogen" in the IARC category of Class 2B (IARC 2002).

Turkish electrical employees in transformers and power lines are occupationally exposed to ELFMFs chronically, while exposure to other genotoxic agents is considered to be low or non-existent. To find out if ELFMF exposure has a genotoxic effect, we performed a study on 55 smoking–non-smoking electrical workers. The control population is formed by working or retired people from a different workforce, housewives and students, as termed "non-occupational exposure to ELFMFs". Therefore, we aimed to investigate the association between occupational exposure to ELFMF and the occurrence of genotoxic outcomes using short-term genotoxicity tests.

Materials and methods

Electric and magnetic field exposure and study population

The ELF-EMF frequency used in Turkey is 50 Hz. This study was performed on employees of 154–380 kV energy transmission lines in Bursa, Turkey. At the surrounding and inside of power generation and transmission systems, the electric and magnetic fields were measured by the equipment Holaday-HI3604 model. The electric field was found to be in the range from 130–8310 V/m and from 300–15,000 V/m, the magnetic field was also measured between 0.5 and 1.7 A/m and 0.25–17 A/m around and inside transformer buildings as termed "outside of transformer" and "inside transformer buildings", respectively.

The study population was composed of 55 workers (32 panelists, technicians, etc.) and 23 office workers (manager, doctor, chief, secretary and the other personnel

of the department, etc.); the workers were occupationally exposed to the ELFMMFs in power generation and transmission (e.g. power lines, transformers) systems over long periods. The groups were described as 'exposed groups'. The control groups included 17 referent subjects for both assays that were non-occupationally exposed to ELFMMFs and all of them live in Bursa. Individuals of all groups also completed a questionnaire including general information about age, gender, medical and occupational records, alcohol intake, and smoking habits. Informed consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and with the approval of the local ethics committee.

Lymphocyte cultures and cell harvesting

Heparinized peripheral blood used in all experiments, was obtained from occupationally exposed and control donors. Two cultures/person were set up by adding 0.3 ml of whole blood to RPMI 1640 medium (10X, Sigma) supplemented with 20% fetal calf serum (Biochrom AG), 100 IU/ml penicillin-100 µg/ml streptomycin (Biological Industries), 0.5 mg/ml L-glutamine and 6 µg/ml phytohemagglutinin (PHA-L, Biochrom AG). Lymphocytes were incubated at 37°C for 72 h. Metaphases were obtained by adding colcemid (0.2 µg/ml final concentration, Sigma) 2 h prior to harvest. Chromosomes were prepared using standard procedures (Benn and Perle 1992). For cytokinesis block micronucleus test, 4.5 µg/ml cytochalasin B was added lymphocyte cultures at 44 h for blocking of cell division. After that lymphocyte cultures were harvested after 72 h (Fenech 2000).

Microscopic evaluation

Chromosomal aberrations

The analysis of cells with CAs was performed in 50 metaphases for each culture/person. The total chromosome aberrations data determined in two categories as gaps and pulverizations were both included and excluded from total chromosome aberrations. CAs were classified according to the recommendation of EHC 51 (Environmental Health Criteria) for Short-term Tests for Mutagenic and Carcinogenic Chemicals (IPCS 1985).

Micronuclei (MN)

Two thousand binucleated cells for each experimental point were examined, following the scoring criteria adopted by the Human Micro Nucleus Project (Bonassi et al. 2001). We evaluated the binucleated micronucleated lymphocytes (BNMN) frequency as the number of binucleated lymphocytes containing one or more MN per 1000 binucleated cells.

Mitotic index (MI) and nuclear division index (NDI)

The mitotic index was calculated as the number of metaphases in 1000 cells analyzed in CA assays. Moreover, in micronuclei assay, 500 lymphocytes were scored to

evaluate the percentage of binucleated cells and the nuclear division index (NDI) was calculated according to the following formula:

$$\text{NDI} = [\text{MONO} + 2\text{BN} + 3\text{TRI} + 4\text{TETRA}]/500;$$

where MONO, BN, TRI and TETRA are mononuclear, binucleated, trinucleated and tetranucleated lymphocytes, respectively (Eastmond and Tucker 1989).

Statistical analysis

The significance levels of CAs and MN in exposed person's cultures and their controls were determined using the non-parametric statistics (Mann–Whitney U test). One sided tests were found appropriate, considering the hypothesis of the study. The statistical significance level was set at $p \leq 0.05$. Further analysis of the relative importance of different explaining variables was done using multiple linear regression analysis. These analyses were carried out with commercial software programs SPSS 11.5 and Statistica 6.0, respectively.

Results

The study group included 55 exposed and 17 control persons. The exposed group was divided into two subgroups; 'outside of transformer' and 'inside of transformer'. They filled in a questionnaire to record occupational and non-occupational exposures with possible influence on chromosome damage. Table 1 shows the characteristics of the exposed and control groups. Age, exposure time, current smoking habit, and alcohol intake were similar in the subgroups.

A summary of CA assay data is given by taking smoking habits of persons into consideration in Table 2. The results obtained by total chromosome and chromatid aberration and all parameters show statistically significant differences between control and both two exposed groups ($p < 0.05$ – 0.001). The differences were present among smokers and non-smokers compared with their controls for all documented parameters in Table 2. MI data are also shown in Table 2. The MI was slightly decreased when compared with their control group. Exposed groups who were

Table 1. Description of the study groups*.

	Number of persons	Age (y)	Smoking habit		Alcohol intake		Exposure time (y)
			Smokers	Non-smokers	Drinker	Non-drinkers	
Controls	17	38.76 ± 9.12	9	8	9	8	–
Out of transformer (office worker)	23	44.09 ± 6.35	10	13	7	16	19.41 ± 6.75
Inside of transformer (panelist)	32	42.31 ± 6.78	19	13	10	22	19.70 ± 6.78
Total	72	42.04 ± 7.44	38	34	26	46	19.58 ± 6.70

*Mean values $\pm$ SD.

Table 2. Types and frequencies of chromosome aberrations and mitotic index in occupationally exposed with ELFMFs and its statistical evaluations (mean values $\pm$ SD).

	<i>n</i>	Total	Smokers	Non-smokers	<i>p</i> -value ^a
Chromatid breaks ^{a*}					
Controls	17	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.01 $\pm$ 0.01	
Out of transformer (office worker)	23	0.04 $\pm$ 0.03*	0.04 $\pm$ 0.03	0.05 $\pm$ 0.03	0.019
Inside of transformer (panelist)	32	0.05 $\pm$ 0.03*	0.04 $\pm$ 0.03	0.05 $\pm$ 0.02	0.020
Chromosome breaks ^{a*}					
Controls	17	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	
Out of transformer (office worker)	23	0.01 $\pm$ 0.02	0.02 $\pm$ 0.03	0.01 $\pm$ 0.01	0.157
Inside of transformer (panelist)	32	0.01 $\pm$ 0.02*	0.00 $\pm$ 0.01	0.02 $\pm$ 0.02	0.042
Gaps ^{a*}					
Controls	17	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.03	
Out of transformer (office worker)	23	0.08 $\pm$ 0.05*	0.08 $\pm$ 0.04	0.09 $\pm$ 0.06	0.034
Inside of transformer (panelist)	32	0.07 $\pm$ 0.04*	0.08 $\pm$ 0.04	0.10 $\pm$ 0.05	0.002
Total aberrations inc. (G + P) ^{a*}					
Controls	17	0.07 $\pm$ 0.03	0.08 $\pm$ 0.03	0.07 $\pm$ 0.04	
Out of transformer (office worker)	23	0.18 $\pm$ 0.07*	0.16 $\pm$ 0.09	0.19 $\pm$ 0.06	0.001
Inside of transformer (panelist)	32	0.18 $\pm$ 0.07*	0.16 $\pm$ 0.07	0.20 $\pm$ 0.06	0.001
Total aberrations exc. (G + P) ^{a*}					
Controls	17	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	
Out of transformer (office worker)	23	0.09 $\pm$ 0.04*	0.08 $\pm$ 0.05	0.09 $\pm$ 0.03	0.001
Inside of transformer (panelist)	32	0.09 $\pm$ 0.04*	0.09 $\pm$ 0.04	0.09 $\pm$ 0.04	0.001
MI ^{b**}					
Controls	17	6.54 $\pm$ 4.80	4.29 $\pm$ 1.60	9.08 $\pm$ 6.00	
Out of transformer (office worker)	23	6.33 $\pm$ 2.73	6.78 $\pm$ 3.26	5.99 $\pm$ 2.33	0.556
Inside of transformer (panelist)	32	6.42 $\pm$ 2.68	6.65 $\pm$ 2.89	6.09 $\pm$ 2.41	0.298

MI, mitotic index; inc., including gaps and pulverizations; exc., excluding gaps and pulverizations; *: ^aparameters scored in 100 cells per person; **: ^bparameters scored in 1000 cells per person. ^{a*}Significant when used with Mann-Whitney U test.

smokers and non-smokers showed no significant increases in all cytogenetic parameters in cultured human lymphocytes than their controls ($p < 0.05$, not shown in Table 2).

The results obtained by the MN analyses and NDI data are summarized in Table 3. The MN frequency was significantly increased by the ELFMFs in the group of workers inside the transformer. The ELFMF exposure did not seem to influence cell proliferation as calculated by the NDI ($p > 0.05$).

Correlation and multiple linear regression analyses were carried out for the data of all cytogenetic parameters on the whole population. The dependent variables were: total aberrations including, and excluding gap and pulverizations, chromosome and chromatid breaks, gaps and MN. Independent variables attempted were exposure time and age. Results of multiple linear regressions are shown in Table 4.

Discussion

Many researchers have examined the prevalence of chromosomal aberrations and the frequency of sister chromatid exchange (SCE) while investigating the effects of exposure to ELFMs in occupationally exposed workers or exposed cultures. The

Table 3. Frequencies of Micronuclei (MN) and Nuclear Division Index (NDI) in occupationally exposed workers with ELFMFs and its statistical evaluations (Mean values $\pm$ SD).

	<i>n</i>	Total	Smokers	Non-smokers	<i>p</i> -value
MN^a					
Controls	17	1.29 $\pm$ 0.77	1.44 $\pm$ 0.73	1.13 $\pm$ 0.83	
Out of transformer (office worker)	23	1.65 $\pm$ 0.71	1.70 $\pm$ 0.67	1.62 $\pm$ 0.77	0.201
Inside of transformer (panelist)	32	1.81 $\pm$ 0.69*	1.74 $\pm$ 0.73	1.92 $\pm$ 0.64	0.026
NDI^b					
Controls	17	1.67 $\pm$ 0.11	1.61 $\pm$ 0.12	1.73 $\pm$ 0.08	
Out of transformer (office worker)	23	1.63 $\pm$ 0.09	1.64 $\pm$ 0.09	1.62 $\pm$ 0.09	0.221
Inside of transformer (panelist)	32	1.61 $\pm$ 0.11	1.59 $\pm$ 0.11	1.65 $\pm$ 0.10	0.196

^aParameters scored in 2000 cells per person; ^bparameters scored in 500 cells per person. *Significant when used with Mann–Whitney U test.

Table 4. Results of multiple linear regression analysis of cytogenetic parameters of the whole population.

Dependent variable	Independent variable	R ² sum	β	<i>p</i> -value
Gaps	Exposure time	0.190	0.436	0.0001
	Age	0.066	0.256	0.030
Chromatid breaks	Exposure time	0.196	0.443	0.0001
	Age	0.021	0.145	0.225
Chromosome breaks	Exposure time	0.072	0.268	0.023
	Age	0.013	0.113	0.343
Total aberrations (G + P)	Exposure time	0.393	0.627	0.0001
	Age	0.067	0.258	0.028
MN	Exposure time	0.060	0.245	0.038
	Age	0.003	0.054	0.651

MN, micronuclei frequency.

results of these studies are contradictory. The incidence of chromosomal aberrations or SCE frequency was not increased in these studies (Cohen et al. 1986a, 1986b; Takahashi et al. 1987; Yaguchi et al. 1999), with one exception (Khalil and Qassem 1991). Therefore, the likelihood that chromosomal aberrations are induced by exposure to ELF or EMF cannot be neglected. In this investigation, several types of chromosomal aberrations were observed and it was found that the most common aberrations were gaps, chromatid and chromosome breaks. As seen in Table 2, we found that the chromosomal aberration frequency significantly increased in exposed workers ($p < 0.001$). Genotoxicity biomarkers have received considerable interest as tools for detecting human genotoxic exposure and effects. MN test is increasingly utilized as a less tedious and expensive alternative to CA frequency in monitoring human population for possible environmental or occupational exposure to chemical (Thomson and Perry 1988; Surrallés et al. 1995) and physical (Fenech and Morley 1986; Silva et al. 1994) genotoxic agents. In this regard, data retrieved in the literature are not consistent. Positive results (genotoxic effects of EMF) have been reported by several authors, including in vitro induction of MN (Khalil and Qassem 1991; Simko et al. 1998), chromosomal aberration (D'Ambrosio et al. 1988; Nordenson et al. 1994), and sister chromatid exchanges (Rosenthal and Obe 1989).

Moreover, it was also reported that acute exposure of rats to EMF results in an increase in the DNA single- and double-strand breaks (Lai and Singh 1997), as well as to the formation of DNA-protein and DNA-DNA cross-links (Singh and Lai 1998) in brain cells, probably via an increased activity of free radicals. However, several studies reported negative results suggesting that ELFMF is not genotoxic (Livingston et al. 1991; Antonopoulos et al. 1995; Cantoni et al. 1995). In the study by Valjus et al. (1993) which examined occupational exposure to EMF, the incidence of chromatid breaks was increased 2-fold when compared to controls in exposed individuals; while no difference was observed in MN and SCE frequencies. Nordenson et al. (1988) reported a statistically significant increase in the rate of chromosomal aberrations (CA) and cells with micronuclei (MN) in lymphocytes of 400 kV-substation workers. Fatigoni et al. (2005) found that the exposure of *Tradescantias* to the ELFMF at a flux density of 1 mT for 1, 6 and 24 h had a time-dependent increase in the frequency of MN formation and these results suggest that 50 Hz MF of 1 mT field strength is genotoxic. Winkler et al. (2005) found that the intermittent (5 min field-on/10 min field-off, for 2–24 h) exposure to a 50 Hz sinusoidal 1 mT magnetic field caused a time-dependent increase in frequency of MN in cultured human fibroblasts. Simko et al. (1998) showed that 50 Hz 1 mT continuous exposure to horizontal magnetic fields in different duration of exposure induced formation of MN in human amnion cells and in human squamous cell carcinoma cells. Our results indicated that, MN and CA frequency was significantly higher in the workers inside of the transformer than in controls (Tables 2 and 3). This finding is in agreement with other studies reporting an increase in MN and CA frequency in those exposed to ELMF, as mentioned above (Simko et al. 1998; Fatigoni et al. 2005; Winkler et al. 2005).

In the literature, several studies demonstrated that CA, SCE and MN frequencies generally increased in workers occupationally exposed to environmental contaminants. Some of these investigations revealed that there is a relationship between age, smoking and increase in abnormalities (Maki-Paakkanen et al. 1980; Topaktas et al. 2002). However, some researchers reported that there was no relationship between age, smoking and increasing abnormalities (Khalil et al. 1994; Surrales et al. 1997). In this study, it also exhibited that there are no relationships between smoking, age and increased chromosome abnormalities and MN, and slightly decreasing MI and NDI (Table 4). However, the incidence of CA was shown to be positively correlated with duration of exposure time: a clear dose-response relationship was evident with years of working ($r = 0.33$, $p < 0.01$, Table 4). We think that the chromosomal damage appears to be cumulative for continuous exposure to ELMF where people chronically exposed to ELMF seem to be more susceptible to chromosomal damage.

A large number of epidemiological studies investigated the possible association between residential or occupational exposure to ELFMF and cancer. Several reviews of these studies are focused on the potential role of ELFMF in the etiology of cancer (Kheifets et al. 1997; Wartenberg 1998; Caplan et al. 2000). Several epidemiological reports suggest a possible association between exposure to ELFMF and an increased incidence of acute childhood leukemia, cancer of the nervous system and lymphomas. It is generally accepted that ELFMF are unable to transfer energy to cells in sufficient amounts to directly damage DNA. However, it is possible that certain cellular processes altered by exposure to ELFMF, such as free radicals production and/or activity (Brockleburst and McLauchlan 1996; Ahlbom et al. 2000), might indirectly affect the structure of DNA. Also, we comprehended from

questionnaire forms that some workers have cancer patients in their family, especially their children. It can be explained that the increase of childhood cancer incidence may result from living in apartments provided for the employee by their employer at the nearest transformer buildings for a long time.

In conclusion, our findings suggest that ELFMF might possess genotoxic capabilities, as measured by CA and MN assays; and that CA analysis was more sensitive to environmental contaminants than other cytogenetic end-points. It can be concluded from this study that chronic occupational exposure to ELFMF may lead to an increased risk of genetic damage among electrical workers. The genotoxic and potential carcinogenic risks ELMF should be taken into account, and precautions in the transformers and electric distribution lines may be increased.

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