RESEARCH COMMUNICATION

Evaluation of Chromosomal Alteration in Electrical Workers Occupationally Exposed to Low Frequency of Electro Magnetic Field (EMFs) in Coimbatore Population, India

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Abstract

Extremely low frequency electro magnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of non ionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure ($P<0.05$). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

Keywords: Electromagnetic field exposure - chromosome aberration - micronucleus - occupational hazard

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Introduction

Humans are exposed either environmentally or occupationally to a large number of genotoxic agents that can cause a variety of health hazards including cancer and genetic diseases. The 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. Electric utility workers may be exposure to any combination of Electromagnetic fields (EMFs), nuisance shocks (from spark discharges and continuous currents), imperceptible contact currents, and electrical injuries. Collectively these exposures referred to as EMF Factors. Worker at the sites of electric production are chronically exposed to EMFs produced by transmission through power lines and transformers. Extremely low frequencies of EMFs have classified as a possible human carcinogen (Class 2B) by the International Agency for Research on Cancer (IARC, 2002). During the last decade, several studies have focused on the adverse effects on health caused by exposure to EMFs. Several independent epidemiological studies based on job titles have shown an increased risk of cancer such as leukemia, and others have found an elevated risk of brain tumors among electrical workers (Murphy et al., 1993; Valjus et al., 1993; Savitz and Ahlbom, 1994; Blank, 1995; Savitz and Loomis, 1995; Salvatore et al., 1996; Ahlbom, 1997). Human data concerning the cytogenetic effects of EMFs exposure demonstrate conflicting evidence from a series of *in vitro* and *in vivo* studies (Nordstrom, 1979: 1981; Nordstrom et al., 1981: 1983; Nordenson et al., 1984).

According to Nordenson (1988) and Serap Celikler (2009) found out the chromosomal aberrations (CA) in peripheral blood lymphocytes from workers occupationally

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exposed to EMFs at 50-60Hz and extremely low frequency EMF of 50/60 Hz by high-voltage power lines or electrical appliances could give rise *in vivo* to primary DNA damage and cytogenetic effects such as CA, SCE, or MN in humans (Skyberg et al., 1993; Vijayalaxmi and Obe, 2005; Miyakoshi, 2006). Studies of the peripheral lymphocytes of humans have suggested that exposure to EMFs have no specific effect on the induction of the sister chromatid exchange (SCE) frequencies, but it can result in an increase in chromatid-type breaks and other chromosomal damage (Garcia-Sagredo et al., 1980; Nordenson et al., 1984; Khalil and Qassem, 1991; Yamazaki et al., 1993). Nevertheless, in some earlier studies, Bauchinger et al. (1981) showed no CA in lymphocytes from electrical workers. Furthermore Nordenson et al. (1984), Cohen et al. (1986), and Rosenthal and Obe, (1989) have observed no statistically significant differences regarding cell cycle progression between human lymphoid cells exposed to low-level 60-Hz, 50-Hz EMFs. The issues however remain controversial.

Hence, there was a need to identify the low level of non ionizing radiation accumulation EMFs in electricity workers and to analyze the genotoxic parameters based on their work duration. Therefore, the focal aim of the present study was to find out the possible Cytogenetic effects of exposed to EMFs exposed Electricity workers in Coimbatore District and surrounding region, Tamil Nadu, South India. Direct exposure (DE) EMFs includes electrical employees in transformers and power lines and the indirect exposure (IE) include office workers in place adjacent to electric supply substations. High voltage transmission lines and distribution network lines are among the field sources considered in this study.

Materials and Methods

Electric and magnetic field exposure and study population

The ELF-EMF frequency used in Coimbatore is 50-60 Hz. This study was performed on employees of 180-420 kV energy transmission lines in Coimbatore city, Tamilnadu, South India. At the surrounding and inside of power generation and transmission systems, 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.

Measurement of Electric and Magnetic Fields

Exposure to the electric and magnetic fields was measured using a personal device designed for worker sampling, BE-log dosimeter. The load of the line was checked at each exposure condition. The linesmen wore the dosimeter during the control condition and thus both the extra weight and the practical difficulties with the dosimeter during inspection of the insulators were identical in both conditions. The BE-log dosimeter has been developed within the framework of a prospective epidemiological investigation of workers occupied in the production and distribution of electricity in progress at present.

Specification of the BE-log dosimeter: Frequency, 50 Hz, Magnetic field, x, y, z direction 0-2-200 1T; Electric field, 0-30 kV/m unperturbed field, Linearity 12%, Weight of equipment, 2-8 kg, A check of the field strength Bx, By, Bz, and E is stored every 15 sec, Resolution, a change of 1 LSD equals 2-8% of reading

Measurements made by the BE-log dosimeter show values about 10% higher than measurements made by handheld instruments.

Study Population

The study population includes 70 samples, which includes 50 exposed workers and 20 controls. The exposed workers were categorized as DE and IE which includes 28 electric field workers and 22 office worker (manager, chief, secretary and the other personnel of the department, etc.) respectively. The workers were occupationally exposed to the EMFs in power generation and transmission (power lines, transformers) systems over long periods. DE worked in extra high voltage (EHV) substations in operating and maintaining the EHV electricity transmission network (210 and 440 KV). Their work involves installing couplings between EHV lines as well as voltage transformers. The electric lines arriving in substations further increase the ambient magnetic field levels there. The control population shows no previous occupational exposure to EMFs. They were subjected only to the normal electromagnetic fields of our daily environment as termed ''non-occupational exposure to EMFs''. Moreover, both the subjects were categorized as follows based on their age: Group I ≤ 40 years [*n*=37 (DE=16; IE=10; CS=11)] and Group II > 40 [*n*=33 (DE=12; IE=12; CS=9)] years. A questionnaire including general information about age, all the subjects recorded medical and occupational records and smoking habits. Informed written consent obtained from all participants and the study performed in accordance with the Declaration of Helsinki and with the approval of the local ethics committee.

Chromosomal Aberration (CA) Assay

Cultures of leucocytes obtained from peripheral blood were set-up in our laboratory as described in the standard protocol of Hoyos et al. (1996). The chromosomal preparations obtained and stained with Giemsa. All authors in the research team made critical observations and recorded the results. For the CA analysis, 100 well spread complete metaphase cells in first cell cycle were evaluated per subject under a microscope at 100x magnification to identify numerical and structural CA. CA was identified according to the recommendation of (ISCN, 1995) norms under the oil-immersion microscope. Chromatid-type CAs: (chromatid gaps; chromatid breaks) Chromosome-type CAs: (break; gap; exchange) were observed. The collected data registered on master tables and later transferred to a computer file.

Micronucleus (MN) assay

The MN assay was performed by using the cytochalasin B technique (Fenech and Morley, 1985). Lymphocytes were cultured in the same manner as described above. Cytochalasin B (6 μg/ml) was added at 44 h of incubation. After 72 h of culture, the cells were harvested. Slides

Table 1. Description of the Study Groups

S.No	Particulars	No. of Subjects (*n*)	Age (Y)	Groups (Age)		Exposure Time (Y)	Smoking habit	
				I	II		Smokers	Nonsmokers
1	Controls	20	41.45±6.53	11	9	NA	12	8
Exposed subjects:								
2	Direct Exposures (Transformer and power line workers)							
		28	41.78±5.93	16	12	20.03±4.70	15	13
3	Indirect exposures (EB office worker)							
		22	32±7.71	10	12	23±6.06	13	9
4	Total	70	43.22±7.01	37	33	21.68±5.60	40	30

* Mean value ±SD, EB - Electricity Board office workers; n - Number of subjects; Y - Years; NA - Not Applicable; Group (group's were categorized based on age wise manner) Group I ≤ 40 years and Group II > 40.

were coded and scored by light microscopy at 400x magnification. For each experiment, 1000 binucleated lymphocytes with well-preserved cytoplasm were scored following the scoring criteria adopted by the Human Micro Nucleus Project (Fenech et al., 2003). MN frequency was determined on coded slides in at least 1000 lymphocytes.

Statistical analysis

All statistical analysis were performed using software SPSS for Windows, version 13 to assess the group statistics for exposed workers and controls such as mean±SD for the age, smokers and duration of the exposure of the subjects and CA. The p values were calculated at the 0.05 level by ANOVA.

Results

The study group includes 50 exposed and 20 control persons. The exposed group was divided into two subgroups; 'DE (*n*=28) and IE (*n*=22)'. The characteristics of the exposed and control groups showed in Table 1. Age, exposure time, and current smoking habit were similar in the subgroups. Furthermore the exposed subjects and controls were Categorized based on their age into two groups, Group I≤40 years (*n*=37) and Group II >40 (*n*=33) years.

A summary of CA assay data and MN analyses were given by occupationally exposed EMFs and controls by taking smoking habits of persons into consideration in Table 2. The frequency of CA was higher in DE (4.89±3.28), compared to IE (3.59±1.59) and Controls (1.55±0.75). The results obtained by total chromosome and chromatid aberration and also MN show statistically significant differences between controls and both two exposed groups (p <0.05). The MN frequency was significantly increased by the EMFs in DE (1.32±1.12) workers compared to IE (1.18±0.73) and controls (0.45±0.60). The differences were observed among smokers and non-smokers compared to their controls for all documented parameters in Table 2. Exposed smokers and non-smokers showed significant increases in cytogenetic parameters in cultured human lymphocytes. Moreover this study reveals that increase in age increases CA and MN in both the exposed subjects and controls with a significantly increase in exposed subjects as compared to controls Table 3. The frequency of total CA (8.16±2.08; p<0.05) and MN (2.55±1.05; p<0.05) in direct exposures

Table 2. Frequency of Chromosome Alterations and Micronucleus in Occupationally Exposed EMFs and Controls (mean ± SD)

	n	Total	*n* Smokers	*n* Non-Smokers	
Chromatid aberration					
Controls	20	1.00±0.64	12 1.00±0.75	8	1.00±0.60
Direct exposure	28	**3.75±2.42**	15 **4.20±2.65**	13	**3.23±2.12**
Indirect exposure	22	2.68±1.24	13 2.84±1.34	9	2.44±1.13
Chromosome aberration					
Controls	20	0.55±0.75	12 0.66±0.49	8	0.37±0.51
Direct exposure	28	**1.14±0.97**	15 **1.27±1.08**	13	**1.07±0.86**
Indirect exposure	22	0.90±0.52	13 1.00±0.57	9	0.77±0.44
Total CA					
Controls	20	1.55±0.75	12 1.66±0.65	8	1.37±0.91
Direct exposure	28	**4.89±3.28**	15 **5.40±3.60**	13	**4.30±2.89**
Indirect exposure	22	3.59±1.59	13 3.84±1.81	9	3.22±1.20
MN/1000 cells					
Controls	20	0.45±0.60	12 0.33±0.49	8	0.62±0.74
Direct exposure	28	**1.32±1.12**	15 **1.66±1.29**	13	1.00±0.81
Indirect exposure	22	1.18±0.73	13 1.38±0.76	9	0.88±0.60

n- Number of Subjects; CA, Chromosomal aberrations; MN, Micronucleus; numbers in bold, significant at p < 0.05 level by ANOVA

Table 3. Comparative Analyses of Chromosome Alterations and Micronuclei Based on their Age in the Group I and II DE, IE and Controls (mean ± SD)

S.No	Particulars:	Cases	Group I ≤ 40 yrs	Group II > 40 yrs
1	Age		37.8±2.17	50.3±4.28
2	Chromatid type aberration:			
	Exp	Direct Exposures	1.93±0.68	6.2±1.64
		Indirect Exposures	1.72±0.94	3.5±0.79
	Cont		0.72±0.64	1.33±0.5
3	Chromosomal Type aberration:			
	Exp	Direct Exposures	0.5±0.63	2.0±0.60
		Indirect Exposures	0.7±0.48	1.08±0.51
	Cont		0.54±0.52	0.55±0.52
4	Total CA:			
	Exp	Direct Exposures	2.5±1.09	**8.16±2.08**
		Indirect Exposures	2.3±1.13	4.58±1.16
	Cont		1.27±0.64	1.88±0.78
5	MN / 1000 cells:			
	Exp	Direct Exposures	0.68±0.60	**2.55±1.05**
		Indirect Exposures	0.7±0.67	1.58±0.51
	Cont		0.18±0.40	0.77±0.66

*Exp - Exposed subjects; Cont - Controls; Group (group's were categorized based on age wise manner) Group I ≤ 40 years and Group II > 40; bold, p < 0.05 by ANOVA

(DE) in Group II was higher, when compared to IE in Group II, Controls and Group I DE, IE. The controls of group I (1.27±0.64), (0.18±0.40) and group II (1.88±0.78), (0.77±0.66) shows lesser frequency of CA and MN compared to exposed groups.

Discussion

Many researchers have examined the prevalence of CA and MN while investigating the effects of exposure to EMFs in occupationally exposed workers. In the present study, increased frequencies of CA and MN in cultured human lymphocytes exposed to a 50-Hz EMF were observed. From the results obtained, it was in good agreement with previous reports indicating that *in vivo* and *in vitro* exposure to human (Nordenson et al., 1988; Khalil and Qassem, 1991; Simko et al., 1998; Fatigoni et al., 2005; Winkler et al., 2005; Celikler et al., 2009). Nordenson and his co-workers (1988) reported a statistically significant increase in the rate of CA and MN in lymphocytes of 400 kV-substation workers. Fatigoni et al. (2005) found that the exposure of Tradescantias to the EMFs at a flux density of 1mT for 1, 6 and 24 h had a time dependent increase in the frequency of MN formation and these results suggest that 50 Hz Magnetic field strength was 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 1mT magnetic field caused a time-dependent increase in frequency of MN in cultured human fibroblasts. Simko and his colleague (1998) showed that 50 Hz 1mT continuous exposures to horizontal magnetic fields in different duration of exposure induce the formation of MN in human amnion cells and in human squamous cell carcinoma cells. D'Ambrosio et al. (1985) have reported significantly increased CA in cultures of bovine lymphocytes was exposed to a 50Hz electric field.

The factors influencing genetic damage such as smoking habit, age and duration of exposure were analyzed in our study. The present study clearly exhibits that there was a significant relationship between smoking and increased chromosome abnormalities and MN. Some of these investigations revealed that there was a relationship between age, smoking and increase in abnormalities (Maki et al., 1980; Topaktas et al., 2002). However, some researchers reported that there was no significant relation between age, smoking and increasing abnormalities (Khalil et al., 1994; Surrales et al., 1997). In contrast, our investigation reveals increase in age increases CA and MN in exposed subjects compared to controls. Moreover, the results of this study also indicate a role for age in chromosome alterations observed in peripheral blood lymphocytes as noted in the controls (Nowinski et al., 1990; Balachandar et al., 2008). However, the frequency of CA was higher among the exposed employees than to controls. A large number of epidemiological studies investigated the possible association between residential or occupational exposure to EMFs and cancer (Wertheimer and Leeper, 1979; Milham, 1982; Tomenius et al., 1982; Wright et al., 1982; Coleman et al., 1983; Mc-Dowall, 1983; Pearce et al., 1985; Speers et al., 1988), the incidence of CA shown to be positively correlated with duration of exposure time: a clear dose-response relationship was evident with years of working, that the chromosomal damage appears to be cumulative for continuous exposure to EMFs where people chronically exposed to EMFs seem to be more susceptible to chromosomal damage. Several reviews of these studies focused on the potential role of EMF 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 EMF and an increased incidence of acute childhood leukemia, cancer of the nervous system and lymphomas. It is generally accepted that EMFs 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 EMFs, such as free radicals production and/or activity (Brockleburst and McLauchlan, 1996; Ahlbom et al., 2000), might indirectly affect the structure of DNA. To the best of our knowledge, this study constitutes the first scientific evidence of cytogenetic effects of electrical employees exposed on EMFs in Tamil Nadu population.

In conclusion, our findings suggest that EMFs possess genotoxic capabilities, as measured by CA and MN assays; in which CA analysis was more sensitive to environmental contaminants than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers. The genotoxic and potential carcinogenic risks among EMFs workers should be taken into account, and precautions in the transformers and electric distribution lines may be increased. However, the best remedy for occupational exposure is prevention. Workers in many occupational settings were exposed to certain genotoxic agents and they have no awareness about the genotoxic agents, the type and the amount of agent to which they have exposed. Therefore, there is a need to educate those who work at EMFs exposure area, about the potential hazard of occupational exposure and the importance of using protective measures.

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