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Cytogenetic Studies of Individuals with Occupational Exposures to Extremely Low Frequency Electromagnetic Fields (ELF–EMF)

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ABSTRACT

The present cytogenetic study was conducted on subjects occupationally exposed to extremely low frequency electromagnetic fields (ELF–EMF), whereby Chromosomal Aberrations (CAs) and Sister Chromatid Exchange (SCE) frequencies were premeditated. These subjects were electric substation attendants, electric arc welders and individuals working in the vicinity of electric distribution point (DP) transformers.

The results of the present study revealed that occupational exposure to ELF–EMF alone cannot exert genotoxic effects in the exposed individuals. However, it was found that ELF–EMF exposure along with Mitomycin–C (MMC) treatments did influence the levels of Chromosomal Aberrations (CAs), indicating the possibility of synergestic/co–mutagenic effects.

Key Words: Occupational Exposures, Extremely Low Frequency Electromagnetic Fields (ELF–EMF), Cytogenetic Studies, Mitomycin C, Synergestic/co–mutagenic effects

INTRODUCTION

Electromagnetic waves are known to directly interact with the biological systems such as cells, plants, animals and human beings as well. Almost none of the electric fields penetrate the human body; while magnetic fields easily penetrate the human body without any significant attenuation [1].

A plethora of studies involving classical cytogenetic end points such as chromosomal aberrations (CAs); Micronuclei (MN) formation and Sister Chromatid Exchange (SCE) frequencies to measure the damage caused by the electromagnetic field exposure has been reported. The results of these studies involving 'whole body exposure' of animals [2-4] showed no significant increase in either SCE frequencies or micronuclei formation. In addition, proliferation rate characteristic of cultured peripheral lymphocytes also remained unaltered. The lack of realistic mechanisms to couple exposure to electromagnetic fields and biological events has resulted in much unfocused research, inconsistent observations and interpretations. Till date, there is no such accepted mechanism by which power line electromagnetic fields has consistently produced a disease or a pre–disease condition.

From the ongoing discussion and the above mentioned facts it appears that further investigations are necessary in order to clarify and fill in the gaps in the existing literature. Observing these facts the present cytogenetic study was conducted whereby CAs and SCE frequencies were studied from the individuals occupationally exposed to extremely low frequency electromagnetic fields (ELF–EMF).

The present study comprised of a total of 60 male subjects. These subjects were classified into the following four different groups each consisting of 15 individuals.

Group A: Unexposed Donors	–	Controls
Group B: Exposed Donors	–	Electricity Substation Attendants
Group C: Exposed Donors	–	Electric Arc Welders
Group D: Exposed Donors	–	Donors working in vicinity of Electricity Distribution Point (DP) Transformers

Although the voltage varied from 11,000 Volts to 66,000 Volts, each individual was exposed to the same frequency of 50 Hz magnetic fields. The mean years of employment of each of the donor in the present task ranged from 14 years to 27 years. To the best of knowledge, there are no reports dealing with the cytogenetic analysis of electricity substation attendants, in India. Although there are cytogenetic reports on electric arc welders, many researchers have considered welding fumes as the genotoxic agents. Individuals working in the vicinity of electricity distribution point (DP) transformers are also exposed to similar magnetic fields. Till date, there are no cytogenetic reports on these occupationally exposed individuals.

The aim of the present study as a whole was to analyze CAs and SCE frequencies among the individuals occupationally exposed to ELF–EMF.

MATERIALS AND METHODS

Peripheral venous blood was collected in sodium heparinized vaccuttainers for the cytogenetic study. The study group comprised of 45 individuals who were occupationally exposed to ELF–EMF. 15 more individuals who were not exposed to ELF–EMF and had same age and socio–economic conditions were chosen as controls. Electric field strength in terms of voltage was known, whereas the magnetic field strength at the work environment was measured using magnetometers.

Routine peripheral blood lymphocyte cultures were setup for each individual, following the standard protocols with some modifications. Three separate culture vials were setup per individual. One culture vial was not treated with any chemical mutagen while the second culture vial received a treatment of 6 ng/ml of MMC, in order to find out the probability of synergistic effects of ELF–EMF along with chemical exposure. The third culture vial was used for SCE studies. Prior to blood collection, each individual was well informed about the nature of the study in a language well understood by them and an informed consent was taken.

For counting chromosomal aberrations one hundred well spread metaphase plates, each containing not less than 44 chromosomes were considered. About 30 well spread second division (M_2) metaphase plates were scored for calculating SCE frequencies. The percentages of the first (M_1) metaphases as well as third (M_3) metaphases were also counted so as to calculate the Replicative Index (RI).

Statistics

Two tailed Student's 't' Test was employed in order to compare two means, whereas ANOVA analysis was used to find out the variations in the mean CAs as well as SCE frequencies among the various groups. Replicative Index (RI) was calculated by applying the following formula

$$RI = \frac{1 \text{ X } \% M_1 + 2 \text{ X } \% M_2 + 3 \text{ X } \% M_3}{100}$$

RESULTS AND DISCUSSION

To analyze possible effects of ELF–EMF among the occupationally exposed individuals, the present study used CAs as well as SCE frequencies as cytogenetic end points. The parameters of the exposed individuals were compared with the non–exposed controls. **Table–I** indicates the particulars of each study group.

The chromosomal aberration frequencies observed in the lymphocytes of the individuals occupationally exposed to ELF–EMF as well as the control groups have been represented in **Table–II**; while **Table–III** represents the chromosomal aberration frequencies among the same control, and ELF–EMF exposed groups after *in vitro* treatment of their lymphocytes with 6 ng/mL of MMC.

Table–II indicates that the total chromosomal aberrations, either including chromatid gaps or excluding chromatid gaps, do not differ significantly among the control, or any of the exposed groups. Whereas, **Table–III** reveals a different picture, there were statistically significant differences in the total CAs including chromatid gaps among the Electric substation attendants ($p<0.02$); Electric arc welders ($p<0.01$) and individuals working in the vicinity of electricity DP transformers ($p<0.02$) when compared with the control individuals group. Further, when the total CAs excluding chromatid gaps were considered, it was seen that the CAs of Group E (Electric arc welders) were significantly different from the control ($p<0.001$). This indicated that electric arc welders group was the only group with highest risk factor, after MMC treatment.

The results on 'ANOVA analysis' carried out in order to know the levels of significance for the observed variations in the frequency of the total CAs including as well as excluding chromatid gaps among the control, and the exposed groups, with and without MMC treatments, are represented in **Table–IV**. There were significant differences in the mean chromosomal aberrations including chromatid gaps ($p<0.05$) as well as mean CAs excluding chromatid gaps ($p<0.01$) among the control, as well as exposed group individuals after MMC treatment.

Table–V indicates mean SCE frequencies in the lymphocytes of the control, and the exposed groups. There was no statistically significant difference in the mean SCE frequencies of the individuals among any of the groups. The results are consistent with the findings of the CA frequencies without MMC treatments. Further, the cell cycle replicative index also remained unaltered in all the individuals in any of the groups.

The results of the present study revealed that occupational exposure to ELF–EMF alone cannot exert genotoxic effects in the exposed individuals. However, it was found that ELF–EMF exposure along with MMC treatments did influence the levels of CAs, indicating the possibility of synergestic/co–mutagenic effects. It also suggested no significant effect of EMF exposure on CAs or SCE frequencies in human peripheral lymphocytes. Further EMF exposures had no obvious effects on the cell cycle Replicative Index.

However, lymphocytes of all exposed subjects when treated with MMC revealed significantly higher CAs when compared to the MMC treated lymphocytes of the control group subjects; thereby suggesting synergistic effects.

It is generally suggested that ELF–EMF alone does not produce enough energy to induce DNA or chromosomal damage [5]. The present study considered synergestic effects of EMF exposure on CAs frequency with a known mutagen MMC on human lymphocytes. The results suggested that EMF exposure enhances the effect of MMC.

In a similar study [6] the possible carcinogenic or co–carcinogenic potency of 60 Hz ELF–EMF, (flux density of 0.8 mT) on human lymphocytes along with a genotoxic agent Benzo–a–pyrene was analyzed using frequencies of MN and SCE as cytogenetic end points. Their results showed that, ELF–EMF interacted with the cellular systems by an indirect mechanism, probably as an enhancer of initiation or as a co–carcinogen, leading to an increased MN and SCE formations *in vitro*.

The present study included a group of individuals, working with the high tension power lines were linemen, or handled 66 KV electric transformers and switch yard operators. The lymphocytes of these individuals showed no significant increase, in either CAs or SCE frequencies when compared to controls. Upon MMC treatment CAs increased significantly again indicating co–mutagenic effects. The results are in much agreement with the early findings [7, 8].

To the best of knowledge, so far in India there are no published cytogenetic studies on electric substation attendants. However, there is an Indian report [9] on the chromosomal damages in the lymphocytes of stainless steel welders. The authors mainly investigated the genotoxic effects of welding fumes generated by manual metal arc, metal inert gas and oxyacetylene welding. They concluded that welders in their occupational settings were prone to high genetic risk, since they observed high frequencies of Mitotic Index (MI), CAs and SCE as well as satellite associations among welders as compared to controls. They further noted that the duration of exposure and hence age, alcohol consumption and smoking habits added to the damage.

As with the other study groups (Substation workers and DP transformer vicinity workers) total CAs both including chromatid gaps as well as excluding chromatid gaps were significantly higher than the control. Further, this group of individuals showed highest (but not significant) CAs after MMC treatment, as well as SCE rates. This could be due to the fact that the subjects had either close body contact with the source of ELF–EMF or the exposure of electric arc to other chemical or physical agents released during welding.

Early studies [10, 11] have reported elevated levels of CAs and SCE in welders respectively. Still there are contrary reports where no increase in the CAs [12, 13] and SCE frequencies [14, 15] from the lymphocytes of welders when compared to controls.

Another group of individuals included in the present study were subjects working in the vicinity (within five meters) of electricity DP transformers. Occupationally either they were tea stall holders, shopkeepers or pan/gutkha sellers. They typically spent 12 to 14 hours in the ELF–EMF generated by the electricity DP transformers. Again CAs and SCE of these individuals were not significantly

higher than either control or referents. After *in vitro* treatment of MMC, CAs including chromatid gaps as well as excluding chromatid gaps were significantly higher than the CAs of the MMC fed cultures from the controls. Till now, no published cytogenetic reports on the individuals with this type of exposure have been reported, and therefore the study is unique of its own kind.

As discussed no guidance has emerged from experimental studies to identify characteristics of magnetic field exposure (if any) which would translate into a biologically effective dose that should be targeted for exposure assessment. Should we be concerned with transients or harmonics? What level of field intensity is important? What duration of exposure is necessary? Are there acute or chronic threshold effects of magnetic fields that need to be crossed before damage can occur?

None of these answers are known, and until they are, the current EMF exposure assessment techniques such as wire codes, ambient levels in the home, personal dosimetry, calculated household exposure from historical information, job classifications, or work site measurements can only be considered crude measures. It is inevitable that exposure assessment will be inaccurate if we do not know what to measure, and the validity of the methods used for both residential and occupational exposure assessment have long been of concern. As with many areas of environmental and occupational epidemiology, this is the principle challenge facing future EMF research in human populations.

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TABLE I: The table below indicates the details of the four different study groups.

Groups	Types	Mean Age in years	Occupation	Average Duration (years) of Exposure to ELF – EMF	Strength of ELF-EMF in the Working Environment	Number of Subjects per Group
A	Control (No Exposure)	40.1	Research Students, Clerks, Officers, etc	-	-	15
B	Exposure 1	48.7	Electricity Substation Workers	26 – 27 Years	66,000 Volts and 50 Hz	15
C	Exposure 2	42.3	Electric Arc Welders	14 – 15 Years	12,000 Volts and 50 Hz	15
D	Exposure 3	41.6	Shop keepers, Pan and Gutkha sellers, Tea Stall Workers working in the vicinity of Electricity DP Transformers	14 – 15 Years	11,000 Volts and 50 Hz	15

TABLE II: Chromosomal Aberrations in the lymphocytes of individuals occupationally exposed to ELF – EMF

SUBJECTS	NUMBER OF SAMPLES	NUMBER OF CELLS SCORED	ABERRATIONS PER 100 CELLS											
			CHROMOSOME TYPE ABERRATIONS					CHROMATID TYPE ABERRATIONS			OTHER ABERRATIONS		TOTAL ABERRATIONS	
			G	B	D	AF	R	G	B	CI	PSC	ER	INCLUDING CHROMATID GAPS	EXCLUDIMG CHROMATID GAPS
GROUP A	15	1500	0.80	0.40	0.20	0.13	0.00	1.67	1.20	0.00	0.87	0.33	5.60	3.90
GROUP B	15	1500	1.00	0.53	0.20	0.13	0.07	2.60	1.33	0.07	1.33	0.80	8.06	5.47
GROUP C	15	1500	0.87	0.73	0.13	0.13	0.07	2.33	1.20	0.00	1.07	0.87	7.40	5.07
GROUP D	15	1500	0.73	0.47	0.13	0.33	0.27	2.00	1.07	0.07	1.07	1.00	7.20	5.13

G = Gap, B = Break, D = Dicentric, R = Ring, AF = Acentric Fragment, CI = Chromatid Interchange,
PSC = Premature Separation of Centromere and ER = Endoreduplication

TABLE III: Chromosomal Aberrations in the lymphocytes of individuals occupationally exposed to ELF – EMF and also treated with Mitomycin-C (6ng/ml)

SUBJECTS	NUMBER OF SAMPLES	NUMBER OF CELLS SCORED	ABERRATIONS PER 100 CELLS											
			CHROMOSOME TYPE ABERRATIONS					CHROMATID TYPE ABERRATIONS			OTHER ABERRATIONS		TOTAL ABERRATIONS	
			G	B	D	AF	R	G	B	CI	PSC	ER	INCLUDING CHROMATID GAPS	EXCLUDIMG CHROMATID GAPS
GROUP A	15	1500	1.06	0.80	0.20	0.33	0.00	1.80	1.20	0.00	0.93	0.46	6.80	5.00
GROUP B	15	1500	1.47	1.00	0.27	0.27	0.00	2.00	1.67	0.00	1.80	0.93	9.40 **	7.40
GROUP C	15	1500	1.40	1.00	0.27	0.33	0.00	1.67	1.33	0.00	1.73	1.47	9.20 *	7.53 **
GROUP D	15	1500	1.33	0.73	0.33	0.53	0.00	2.27	1.40	0.13	1.60	1.07	9.40 **	7.20

***** Significantly greater than Control at $p < 0.05$
****** Significantly greater than Control at $p < 0.02$
\# Significantly greater than Control at $p < 0.001$
G = Gap, B = Break, D = Diacentric, R = Ring, AF = Acentric Fragment, CI = Chromatid Interchange,
PSC = Premature Separation of Chromatids and ER = Endoreduplication

TABLE IV ANOVA Analysis

CATEGORY	GROUP A	GROUP B	GROUP C	GROUP D	STATISTICAL VALUE 'F'	TABLE VALUE	P VALUE
I	5.60	8.06	7.40	7.10	2.36	2.37	
II	3.90	5.47	5.07	5.13	1.47	2.37	
III	6.80	9.40	9.20	9.40	2.60	2.37	< 0.05
IV	5.00	7.40	7.53	7.20	3.08	2.37	< 0.01

I = Mean Chromosomal Aberrations including Chromatid Gaps
II = Mean Chromosomal Aberrations excluding Chromatid Gaps
III = Mean Chromosomal Aberrations including Chromatid Gaps after Mitomycin C treatment
IV = Mean Chromosomal Aberrations excluding Chromatid Gaps after Mitomycin C treatment

TABLE V: Sister Chromatid Exchange (SCE) frequencies in the lymphocytes of various grouped individuals exposed to ELF – EMF

SUBJECTS	NUMBER OF SAMPLES	MEAN AGE (YEARS)	NUMBER OF CELLS SCORED	PERCENTAGE OF CELLS			REPLICATIVE INDEX RI	NUMBER OF M_2 PLATES SCORED	MEAN SCE/CELL X ± SEM
				M_1	M_2	M_3			
GROUP A	15	39.6	1500	42	30	28	1.86	450	3.23 ± 0.24
GROUP B	15	48.66	1500	38	30	32	1.94	450	3.92 ± 0.46
GROUP C	15	37.53	1500	42	30	28	1.86	450	4.31 ± 0.41
GROUP D	15	38.33	1500	41	30	29	1.88	450	3.33 ± 0.17

M_1 = First Division Metaphase, M_2 = Second Division Metaphase, M_3 = Third Division Metaphase