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# Cytogenetic Studies on Railway Engine Drivers Exposed to Extremely Low Frequency Electromagnetic Fields (ELF-EMF)

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**KEYWORDS** ELF-EMF Railway Engine Drivers. Cytogenetics. Chromosomal Aberrations. Sister Chromatid Exchanges

**ABSTRACT** Electric train engine drivers are occupationally exposed to relatively high magnetic field flux densities, while exposure to the other genotoxic agents is considered to be low or non-existent. The present study aimed to analyze the Chromosomal Aberrations (CAs) and Sister Chromatid Exchange (SCE) frequencies among the railway engine drivers occupationally exposed to ELF–EMF. Additionally, to know the synergestic/co–mutagenic effects, the blood samples of these individuals were exposed *in vitro* to 6ng/ml Mitomycin–C (MMC) and Chromosomal Aberrations (CAs) were studied. The results of the present study do not give any support to the hypothesis that occupational exposure to ELF–EMF can exert a genotoxic effect in these exposed individuals. In addition, it seems that ELF–EMF exposure along with Mitomycin–C (MMC) treatments does not influence the levels of Chromosomal Aberrations (CAs), indicating no possibility of synergestic/co–mutagenic effects.

## INTRODUCTION

The study of the interaction between electromagnetic energy and living things involves aspects of both physical and biological science that are less than perfectly understood. Electromagnetic energy, one of the four basic forces of the universe, is neither quite particulate nor quite wave-like in nature but displays properties of both simultaneously. Biological effects produced by the electromagnetic waves may sometimes, but not always lead to adverse health effects. A biological effect occurs when exposure to the electromagnetic waves causes some noticeable or detectable physiological changes in a biological system. An adverse health effect occurs when the biological effect exceeds the normal range for the body to compensate and thus leads to some detrimental health conditions.

Whether or not electromagnetic fields have genotoxic potential is a controversial issue. It is generally suggested that neither electromagnetic fields nor static electric or magnetic fields have a clearly demonstrated potential to cause genotoxic effects due to their low energy (Repacholi and Greenbaum 1999). Till date almost four comprehensive reviews (McCann et al. 1993; Murphy et al. 1993; McCann et al. 1998 and Moulder 1998) related to the genotoxic potentials of electromagnetic fields have been published in the literature. All these reviews pointed to the unconfirmed and/or inconclusive positive results and suggest additional research (Vijayalaxmi and Obe 2005).

Electric train engine drivers are occupationally exposed to relatively high magnetic field flux densities, while exposure to the other genotoxic agents is considered to be low or non-existent. To find out if magnetic field exposure has any genotoxic potentials, Nordenson et al .(2001) first performed a pilot study on 18 non–smoking Swedish male engine drivers. Seven dispatchers constituted the reference group. Since there was significant difference in the number of cells with chromosome-type aberrations between the two groups, the authors extended the follow–up study on 30 engine drivers and 30 referents (policemen). Again, as consistent to the pilot study, results of the follow–up study also showed an increase in the frequency of cells with chromosome type aberrations (gaps excluded) among the 30 engine drivers as compared to the 30 referent policemen. The authors concluded that exposure to the

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magnetic field at mean intensities of 2–15 ìT can induce chromosomal damages.

To investigate the relationship between extremely low frequency magnetic field (ELF-MF) exposure and mortality from leukaemia and brain tumor in a cohort of Swiss railway workers, recently, Röösli et al .(2007) carried out a study on 20141 Swiss railway employees with 464129 person-years of follow-up between 1972 and 2002. Mortality rates for leukemia and brain tumor of highly exposed train drivers (21 mT average annual exposure) were compared with medium and low exposed occupational groups (i.e. station masters with an average exposure of 1 mT). In addition, individual cumulative exposure was calculated from on-site measurements and modeling of past exposures. The authors concluded that "Some evidence of an exposure–response association was found for myeloid leukemia and Hodgkin's disease, but not for other haematopoietic and lymphatic malignancies and brain tumors".

In the recent years, there has been a steady increase in the investigations examining the genetic and epigenetic effects of electromagnetic field exposure in prokaryotic as well as eukaryotic cells. Researchers have used the recently developed experimental techniques as well as classical cytogenetic methods in their studies. As far as we are aware, there are no such reports on railway engine drivers (Loco pilots) from India, in particular. The working schedule of these drivers include an average of eight hours a day, thereby constantly being under a high tension wire carrying 25000 volts and exposed to extremely low frequency electromagnetic field so generated under the roof of engine. An attempt in this study was to know whether such 'long time-low dose' exposure does cause any health problem, in particular, chromosomal damage to these individuals. As referents the train guards were chosen since they share the same working schedules, have almost the same socio economic status and share many common features with the engine drivers, except that they are not exposed to the electromagnetic fields.

Therefore, the present cytogenetic study was conducted on railway engine drivers occupationally exposed to extremely low frequency electromagnetic fields (ELF – EMF) whereby, Chromosomal Aberrations (CAs) and Sister Chromatid Exchange (SCE) frequencies were studied. The study comprised of a total of 15 railway engine drivers and 15 guards as controls.

## MATERIALS AND METHODS

Routine peripheral whole blood lymphocyte cultures were set up following the standard protocols of Hungerford (1965) with some modifications (Gadhia et al. 2004). The culture vials were incubated at 37°C with 5 % $CO_2$ level. For CAs study incubation duration was of 50 hours and for SCE study, 72 hours. Colcemid treatment was given during the last two hours of incubation so as to arrest mitotic division. Air - dried slides were prepared with routine protocols of hypotonic treatment and fixation in Carnoy's fixative. All the slides were immediately blind coded.

Air- dried slides were stained in 2 % Giemsa staining solution prepared in Sorenson's buffer. Optimum staining time varied between slides and the stain batches, however, was generally of the order of about 5–7 minutes. For counting chromosomal aberrations one hundred well-spread metaphase plates, each containing not less than 44 chromosomes were considered. Both chromosomes as well as chromatid type of aberrations were considered.

The method of Perry and Wolff (1974) with some modifications (Gadhia et al.2005) was followed for studying Sister Chromatid Exchanges (SCE). After 24 hours of initiation of cultures, 5-Bromodeoxyuridine (5-BUdR) was added in a final concentration of 10 µgm per ml of culture. About 30 well spread second division ($M_2$) metaphase plates were scored for calculating Sister Chromatid Exchange (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).

Two tailed Student's 't' Test was employed in order to compare two means of Chromosomal Aberrations (CAs) as well as Sister Chromatid Exchange (SCE) frequencies among Drivers and Guards.

Replicative Index (RI) was calculated by applying the following formula

$$RI = 1 \times \% M_1 + 2 \times \% M_2 + 3 \times \% M_3 / 100$$

### Working Protocol

Peripheral venous blood was collected in sodium heparinised vacutainers for the cytogenetic study. The study group comprised of 30 individuals of which 15 Electric Train Engine Drivers were occupationally exposed to extremely

low frequency electromagnetic fields (ELF–EMF) while rest 15 individuals were train guards who were chosen as occupationally related controls. The criterion of same age and socio economic conditions were kept in mind while selecting the controls. Electric field strength in terms of voltage was 25000, whereas the magnetic field strength at the work environment was measured using magnetometers.

Routine peripheral blood lymphocyte cultures were setup for each individual. 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 Mitomycin-C (MMC), in order to find out the probability of synergistic effects of extremely low frequency electromagnetic fields (ELF-EMF) along with chemical exposure. The third culture vial was used for Sister Chromatid Exchange (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.

## RESULTS

In the present study Chromosomal aberrations (CAs) as well as Sister Chromatid Exchange (SCE) frequencies were used as

**Table 1: Particulars of the study group individuals**

| *S. No.* | *Particulars* | *Guards* | *Drivers* |
|---|---|---|---|
| 1 | Number of individuals studied | 15 | 15 |
| 2 | Mean Age (years) | 43.6 | 42.4 |
| 3 | Smoking Habits | 2 | 2 |
| 4 | Tobacco Chewing Habits | 5 | 2 |
| 5 | Mean Years of Employment in the present Task | 19.30 | 21.06 |
| 6 | Mean Hours of Exposure per day | 0 | 9.20 |
| 7 | Strength of ELF-EMF in the Working Environment | | 25, 000 Volts and 50 Hz |
| 8 | Number of Individuals consuming medications for diabetes, high or low blood pressure, headache, allergies, etc. frequently | 8 | 6 |
| 9 | Number of Individuals with High Blood Pressure | 6 | 2 |
| 10 | Number of Individuals with Low Blood Pressure | 0 | 2 |
| 11 | Number of Individuals taking any Protective measures against ELF-EMF exposure | 0 | 0 |

**Table 2: Chromosomal aberrations in the lymphocytes of drivers and guards without and with treatments of Mitomycin-C**

| *Subjects* | *Treatments* | *Number of samples* | *Number of cells scored* | *Aberrations per 100 cells* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Chromosome type Aberrations* | | | | | *Chromatid type aberrations* | | | *Total aberrations* | | *Other aberrations* | |
| | | | | *G* | *B* | *D* | *AF* | *R* | *G* | *B* | *CI* | *PSC* | *ER* | *Including chromatid gaps* | *Excluding chromatid Gaps* |
| Guards | | 15 | 1500 | 0.67 | 0.47 | 0.20 | 0.13 | 0.00 | 2.00 | 1.33 | 0.00 | 1.00 | 0.40 | 6.20 | 4.20 |
| Drivers | | 15 | 1500 | 0.73 | 0.87 | 0.67 | 0.40 | 0.00 | 2.40 | 1.20 | 0.27 | 0.80 | 0.40 | 7.93 | 5.40 |
| Guards | Mitomycin-C6 ng/mL | 15 | 1500 | 0.93 | 0.67 | 0.13 | 0.27 | 0.07 | 1.73 | 1.07 | 0.13 | 1.27 | 0.73 | 7.00 | 5.27 |
| Drivers | Mitomycin-C6 ng/mL | 15 | 1500 | 0.80 | 0.80 | 0.86 | 0.53 | 0.07 | 2.47 | 1.33 | 0.40 | 1.07 | 0.66 | 9.00 | 6.53 |

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

**Table 3: 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/ CellX ± SEM* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $M_1$ | $M_2$ | $M_3$ | | | |
| Group B | 15 | 43.6 | 1500 | 40 | 30 | 30 | 1.90 | 450 | 3.16 ± 0.15 |
| Group C | 15 | 42.4 | 1500 | 39 | 30 | 31 | 1.92 | 450 | 3.35 ± 0.17 |

$M_1$-First division metaphase, $M_2$ – Second division metaphase, $M_3$- Third division metaphase

cytogenetic end points. The parameters of the exposed railway drivers were compared with the non exposed occupationally related controls - the railway guards. Table 1 indicates the particulars of each study group. Both chromosome as well as chromatid type of aberrations were observed. Chromosome type of aberrations included chromosome gap, chromosome break, dicentric and ring configurations as well as acentric fragments; while chromatid type of aberrations included chromatid gap, chromatid break and chromatid interchanges. Occasionally, chromosomes with endoreduplicated configurations and prematurely separated centromeres were seen.

The chromosomal aberration frequencies observed in the lymphocytes of drivers and guards without and with *in vitro* treatments of their lymphocytes with 6 ng/mL of Mitomycin–C (MMC) are represented in table 2. It is clear from the table that the total chromosomal aberrations, either including chromatid gaps or excluding chromatid gaps, do not differ significantly among the control and the exposed drivers, under both conditions of without and with treatments of MMC.

Table 3 indicates mean Sister Chromatid Exchange (SCE) frequencies in the lymphocytes of the control guards and the exposed drivers. There was no statistically significant difference in the mean Sister Chromatid Exchange (SCE) frequencies of the drivers as compared to guards. The results are consistent with the findings of the Chromosomal Aberration (CA) frequencies. Further, the cell cycle Replicative index also remained unaltered.

## DISCUSSION

The present study aimed to analyze the Chromosomal Aberrations (CAs) and Sister Chromatid Exchange (SCE) frequencies among the railway engine drivers occupationally exposed to ELF–EMF. Additionally, to know the synergestic/co–mutagenic effects, the blood samples of these individuals were exposed *in vitro* to 6ng/ml Mitomycin–C (MMC) and Chromosomal Aberrations (CAs) were studied.

The results of the present study do not give any support to the hypothesis that occupational exposure to ELF–EMF can exert a genotoxic effect in the exposed individuals. In addition it was seen that ELF–EMF exposure along with Mitomycin–C (MMC) treatments did not influence the levels of Chromosomal Aberrations (CAs), indicating no possibility of synergestic/ co–mutagenic effects.

The present results are in lieu to the findings of Bauchinger et al. (1981), Husgafvel-Pursianen Kalliomaki and Sorsa (1982), Elias et al. (1989), Nagaya et al. (1989), Popp et al. (1991), Ciccone et al. (1993), Skyberg et al. (1993), (2001), Gobba et al.(2003), Stronati et al. (2004). However, there are reports with contrary results as well, *viz.* Bigliev et al. (1977), Sarto et al. (1982), Valjus et al. (1993), and Nordenson et al. (1984, 1988, 2001)

From the data currently available on various human cell types, studied under a variety of experimental conditions and assessing different biological parameters, no apparent cytogenetic effects of electromagnetic field (EMF) are discernible. The present study results also suggest no significant effect of electromagnetic field (EMF) exposure on Chromosomal Aberrations (CAs) or Sister Chromatid Exchange (SCE) frequencies in peripheral lymphocytes of railway drivers. Further, the electromagnetic field (EMF) exposures had no obvious effects on the traverse through the cell cycle, since the distribution of the first ($M_1$); second ($M_2$) and third ($M_3$) divisions was similar in exposed drivers and the referent guards.

It is generally suggested that extremely low frequency electromagnetic fields (ELF–EMF) alone does not produce enough energy to induce DNA or chromosomal damage (Lagroye and Poncy 1997). Any relationship between increased incidences of cancer and extremely low frequency

electromagnetic fields (ELF–EMF) should therefore be explained by a promoting effect on cellular transformation together with the effect of known carcinogens/mutagens (Loescher and Mevissen 1994; Baum et al. 1995; McLaen et al. 1997). The present study therefore, considered synergestic/co–mutagenic effects of electromagnetic fields (EMF) exposure on Chromosomal Aberrations (CAs) frequency with a known mutagen Mitomycin–C (MMC). The results suggested that electromagnetic fields (EMF) exposure do not enhance the effect of Mitomycin–C (MMC) at least for the studied exposure.

However, Cho and Chung (2003) in a similar study analyzed the possible carcinogenic or co–carcinogenic potency of a 60 Hz extremely low frequency electromagnetic fields (ELF–EMF), (flux density of 0.8 mT) on human lymphocytes along with a genotoxic agent Benzo–a–pyrene using frequencies of Micronuclei (MN) and Sister Chromatic Exchange (SCE) as cytogenetic end points. Their results showed that, cells co–exposed to Benzo–a–pyrene and 0.8 mT extremely low frequency electromagnetic fields (ELF–EMF) for 24 hours, followed by Benzo–a–pyrene exposure for 48 hours enhanced the frequency of Micronuclei (MN) and Sister Chromatic Exchange (SCE) compared to Benzo–a–pyrene treatment alone. This suggested that extremely low frequency electromagnetic fields (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 Micronuclei (MN) and Sister Chromatic Exchange (SCE) formations *in vitro*.

So far there is only one published cytogenetic study (Nordenson et al. 2001) on railway employees of Sweden. Both the pilot as well as the follow–up study of these authors showed a significant increase in the number of cells with Chromosomal Aberrations (CAs) among the engine drivers in comparison with the respective reference groups. The pilot study had only seven concurrently sampled non–exposed referents (dispatchers), which made the interpretations uncertain. The follow–up study however supported the hypothesis that exposure to electromagnetic fields (EMF) may induce chromosomal damage. Based on their results the authors concluded that it is possible that train engine drivers and other train attendants experience a risk of future tumor development.

The results of the present study are however contrary to the results of Nordenson et al. (2001). In the present study the Chromosomal Aberrations (CAs) and Sister Chromatid Exchange (SCE) frequencies of 15 loco pilots were compared with the occupationally related controls (train guards) who matched the age/habit/socio–economic conditions of the former. In the either case ,no statistically significant differences were observed. Even, *in vitro* treatment of lymphocytes from train drivers with a known mutagen Mitomycin–C (MMC) did not increase the frequency of chromosomal aberrations. The results suggest no increased sensitivity of lymphocytes of railway train engine drivers to the mutagen than the controls.

To the best of our knowledge, so far in India there are no published cytogenetic studies on railway employees. Several *in vitro* studies (Khalil et al.1991; Livingston et al. 1991; Nordenson et al.1994; Scarfi et al. 1994; Paile et al.1995; Galt et al.1995; Scarfi et al. 1997a, b, c; Simko et al. 1998; Zeni et al. 2002; and Ivancsits et al. 2002) have addressed a possible association between exposure to 50 Hz–60 Hz magnetic fields and induction of genetic damage. Accumulating evidences suggest that this type of exposure is not able to induce mutations and initiate malignant transformation. Moreover, most studies (McCann et al. 1993; Murphy et al. 1993; McCann et al. 1998; Loberg et al.2000) that reported the induction of genotoxic effects have not been independently confirmed or have failed to replicate positive results. Nevertheless, the sporadic positive results could not be overlooked, for they stimulate further in depth laboratory investigations.

The results of the present study do not give any support to the hypothesis that occupational exposure to ELF–EMF can exert a genotoxic effect in these exposed individuals. In addition it seems that ELF–EMF exposure along with Mitomycin–C (MMC) treatments does not influence the levels of Chromosomal Aberrations (CAs), indicating no possibility of synergestic/co–mutagenic effects.

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