Risk Assessment of Human Subjects occupationally exposed to Extremely Low Frequency Electromagnetic Fields (ELF-EMFs) and Light at Night (LAN) with particular reference to Melatonin Hypothesis

Surender Vemula[1], Varak Kavitha[1], Potti Ravindra Babu[2] and Tiwari R.[3]*
1. University Grants Commission (UGC) Research Unit, Bhavan's New Science College, Hyderabad, Telangana State, INDIA
2. Department of Biotechnology, Sreenidhi Institute of Science and Technology, Hyderabad, Telangana State, INDIA
3. Global Institute of Engineering and Technology, Moinabad, Hyderabad, Telangana State, INDIA
*rravindra_tiwari@yahoo.com

Abstract

Modern society makes pervasive use of electric power producing electromagnetic Fields (EMFs) and light at night (LAN). This has resulted in the indiscriminate use of electrical and electronic gadgets which release electromagnetic radiations which are supposed to cause the biological effects by some scientists. However, ELF- EMFs and LAN are hypothesized to be responsible for the changes in hormonal configurations leading to development of cancer in women particularly nurses who are occupationally exposed to ELF- EMFs and LAN.

The blood samples of 342 exposed subjects and 150 non exposed individuals were analyzed. Plasma melatonin was measured by radioimmunoassay (RIA). DNA damage was studied by alkaline comet assay along with micronucleus test and RT-PCR. Our results suggest that the plasma melatonin levels were significantly suppressed in the occupationally exposed subjects ($p < 0.05$) and DNA damage ranged between 8µm to 10µm. Group-C exposed subjects showed more DNA damage. The occupationally exposed subjects were found to be vulnerable for electromagnetic stress with decreased melatonin concentrations and increased DNA damage.

Keywords: ELF-EMFs, Melatonin, Light at Night, Comet Assay, DNA damage, MNT and RT-PCR.

Introduction

The generation, distribution and use of electric power are hallmarks of modern life. Most of us are depending on the wireless communications like cell phones, internet, satellite devices, Wi-Fi, personal systems etc. Due to this, humans as well as other living beings are continuously exposed to the EMFs day and night in an extreme way[7,35]. The extensive use of technology generates electro-magnetic fields (EMFs). EMFs are supposed to increase temperature of live tissue and have thermal effect on bio-organisms. These effects can change membrane electric potential and ion distribution and finally can disrupt biochemical reactions in cells.

However, a few experiments have shown a thermal cellular effects of EMFs[19]. These EMFs are supposed to have effect on endocrine system, particularly pineal hormone-melatonin. Several studies have also reported an association between exposure to ELF-EMFs and neurodegenerative disorders[6,30]. Electric power resulting in exposure to light at night (LAN) and anthropogenic electromagnetic fields is likely to decrease the melatonin levels. The role of melatonin in circadian regulation and prevention of cancer is well established. There is mounting evidence of an association between night shift work and breast cancer risk which is of increasing concern[5,24]. Some studies have evaluated the evidence linking women's occupation and workplace exposures to breast cancer. Overall, the data do not suggest that occupational exposures to EMF increase the risk of breast cancer[14].

Melatonin is a neuroendocrine hormone, an indoleamine (N-acetyl-5 methoxytryptamine) secreted by the pinealocytes of the pineal gland situated in the hypothalamic region of the brain. It transduces the body's circadian rhythms and controls the sleep-wake cycle, an internal 24 hour time keeping system (biological clock). Melatonin's hypothermic, antioxidant and free radical scavenging properties attribute it to an immune modulator and an oncostatic agent. According to the 'melatonin hypothesis' of cancer, the exposure to light at night (LAN) and anthropogenic electric and magnetic fields (EMFs) are related to the increased incidence of breast cancer due to melatonin disruption. It is important to understand that night shift workers are more likely to be obese and to have unhealthy life style which may also contribute to risk of breast cancer[41].

Controversial reports addressing health effects like neurodegenerative disorders and brain tumors due to RF electromagnetic field exposure have been mounting[9]. At low frequency waves, EMFs have the effect on the nervous system due to their intensive sensitivity. Not only the nervous system but they also affect the psychological conditions. Due to the heat released by them, the temperature of the body also rises[40,43].

The aim of our study is to understand the association between melatonin levels and the increased incidences of breast cancer in urbanized cities. It is reported that working at night shifts could be responsible for the depletion of melatonin and disruption of circadian rhythm. Such sleep

disturbances are supposed to suppress immune system by suppressing melatonin production. Breast cancer is the leading cause of cancer death in women in industrialized countries.[10,59]

Light and Melatonin: The effect of light on pineal function in humans has been extensively studied[62]. The effect is qualitatively similar to the effect in other mammals where the intensity of nocturnal illumination suppresses melatonin production to daytime levels[33,34]. The normal melatonin rhythm in humans has characteristics that may be relevant to breast cancer risk[37]. In animals, very brief light exposure (minutes or even seconds) at night can suppress melatonin production[46]. The exposure to light at night (LAN) and anthropogenic electric and magnetic fields is related to the increased incidence of breast cancer due to melatonin disruption[21]. In recent years, a large number of studies are held on this topic but the results are controversial and largely unanswered.

However, ELF-EMFs and LAN are hypothesized to be responsible for the changes in hormonal configurations leading to development of cancer in women, particularly night shift workers[52]. The mechanism by which ELF-EMFs and LAN exposure could increase cancer risk, lies in the possible oncostatic property of melatonin and its circulating levels[38].

Electric and Magnetic Fields and Melatonin: The first reports that the pineal body might respond to an artificial EMF appeared in the early 1980s. The electrical activity of pineal cells in anesthetized male guinea pigs was studied[49]. The exposure of rats to a 60-Hz electric field suppressed the normal nocturnal rise in pineal melatonin production in male. Sprague-Dawley-derived rats were reported[63]. In humans, an association between use of wireless phones and brain tumors was also indicated[16].

We propose the role of melatonin as and antioxidant to explain the biological effects of EMFs. The rationale behind our study is that the increased free radical production in response to magnetic field exposure might result in suppressed circulating melatonin levels. The suppression of melatonin with extended free radical lifetime may also enhance DNA damage[17,45]. In female workers exposed to magnetic fields, Nocturnal 6-hydroxy melatonin sulfate levels were suppressed[28]. This hypothesis could have an important implication for the possible health effects associated with exposure to ELF magnetic fields in the public and occupational environments. Based on the epidemiological association between residential exposure to ELF-EMFs and childhood leukemia, the International Agency for Research on Cancer (IARC) classified ELF-EMFs as a "possible human carcinogen"[57].

However, the literature survey indicates a controversy on whether the ELF-EMFs can induce adverse biological effects particularly cancer. Several meta-analyses showed a statistical association between childhood leukemia and a range of exposure 0.1–2.36 µT MF intensity[50]. Hence, to rule out the controversy, the subjects exposed to EMFs from occupational environments i.e. light at night are included in this study. Although it is generally accepted that EMFs can exert biological effects, in general, epidemiological studies show a weak and sometimes inconsistent association between exposure to power frequency fields (PFF) and cancer. In most cases, the studies fail to show a dose-response relationship[11,29].

Material and Methods

The women workers (n=342) working in the night shifts for about 5 years, from different hospitals and BPO centers situated in various locations in Hyderabad, India, were considered for the study. Age, diet and recent infection if any were used as criteria for the selection of both exposed and control population (n=150) who are not night shift workers [Table 1]. Detailed questionnaire on subjective symptoms related to EMFs exposure included self-assessment of non-specific symptoms such as headache, dizziness, tinnitus, visual impairments and sleep disturbances.

Table 1
General characteristics of the study group and controls

S.N.	Variables	Exposed	Controls
1.	Number(*n*)	342	150
2.	Age (years)	19-45	20-45

Sampling: After receiving informed consent, 5 ml peripheral blood was collected at the late night times between 12 am to 4 am from each volunteer by venepuncture into a sterilized disposable syringe. 1ml of whole blood was allowed to clot to collect the serum. The remaining 4 ml of blood was heparinized and both the samples were placed in ice to prevent exogenous damage. The samples were processed in the laboratory for estimating Melatonin by RIA, DNA damage by comet assay and Micronucleus Test and RT-PCR.

Alkaline Comet Assay: Alkaline comet assay or Single Cell Gel Electrophoresis (SCGE) was used to study DNA damage[4,36,53].

Chemicals: The source of chemicals were as follows: Agarose [low melting (20°C) and normal melting point (35°C)], sodium lauryl sarcosinate, titron X-100, silver nitrate, catalase from Sigma-USA; tungstostilic acid from Koch-light Laboratories, England; sodium chloride, sodium hydroxide, potassium chloride, tris, EDTA, potassium dihydrogen phosphate and sodium phosphate dibasic from Glaxo, Mumbai, India; zinc sulphate and ammonium nitrate from Fischer, Madras, India; thiobarbituaric acid, butylated hydroxyl toluene, sulphosalicyclic acid and N-1-napthyl ethylene diamine dihydrochloride from SD Fine, Mumbai.

Preparation of slides: On a clean, dry plain slide 100 µl of 0.75% normal melting agarose (NMA) prepared in

phosphate buffered saline (PBS) was first layered. These precoated slides were dried at 37°C. On top of this layer, 30 µl of peripheral blood leukocytes (PBL) suspended in PBS, mixed with 110 µl of 0.5% low melting agarose (LMA) prepared in PBS was layered second. The third layer consisted of 100µl of LMA. The slides were incubated in cold lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris;1% sodium lauryl sarcosinate;1% Triton X-100 and DMSO added fresh) at 4°C overnight.

Alkali treatment and electrophoresis: The slides were removed from the lysing solution and placed side by side in a horizontal electrophoresis unit. The slides were completely immersed in freshly prepared alkaline electrophoretic buffer (1 mM Na_2EDTA and 30 mM NaOH, pH 13) for 30 min to facilitate the DNA unwinding and expression of alkali labile sites.

After alkali treatment, the electrophoresis was carried out for 30 min at 300 mA and 0.67V/cm. The slides were carefully lifted from the buffer and gently washed with neutralizing buffer (0.4M Tris buffer, pH 7.5). The slides were then washed with distilled water and air dried.

Silver staining: The air dried slides were immersed in the fixing solution (15% w/v trochloroacetic acid, 5% w/v zinc sulphate and 5% w/v glycerol) for 10 min and washed gently with double distilled water several times. For staining, 32 ml of staining solution A (5% w/v Na_2CO_3) was mixed with 68ml of staining solution B (0.02% w/v NH_4NO_3, 0.02% w/v $AgNO_3$, 0.1% w/v tungtosilic acid and 0.05% v/v formaldehyde) and poured over the slides so as to cover the slides uniformly.

The slides were kept in small boxes covered with aluminium foil until a light grey colour was obtained. To stop the staining process, the slides were immersed in stopping solution (1% acetic acid) for 5 min, washed with double distilled water and air dried.

Evaluation of DNA damage: For visualization of DNA damage, a bright field, transmission light microscope (Oympus Research Microscope CH20i (Binocular vision), India) was used at 400X magnification. Comet tail length was measured, using an ocular micrometer fitted in the eyepiece, in 200 cells per treatment. Mean comet tail length, which is an estimate of DNA damage, was calculated for each sample.

Melatonin Assay (Direct Radio Immuno Assay): Plasma melatonin was quantitatively measured by Direct Radio Immuno Assay (RIA) described by Fraser et al.[18] The amount of ^{125}I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. Standard melatonin from the Melatonin Direct RIA Kit (BA R 3300, LDN Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany) used in this assay, represents the principle where during equilibrium, the antibody bound radioactivity gets precipitated with a second antibody in the presence of polyethylene glycol. The precipitate was counted in a gamma counter from Automatic Gamma Counter (Model No: 1480-11 Wizard 3), Perkin Elmer, USA. Quantification of unknown samples was achieved by comparing their activity with a reference curve prepared with known calibrators.

Micronucleus Test (MNT) in Buccal Epithelial Cells (BECs): The study of exfoliated buccal epithelial cells was performed by the standard technique[13,58], with slight variations made for the requirements of this investigation. BECs were analysed for micronuclei, which are indicators of genetic damage. The slides were air dried, fixed in Carnoy's fixative for 10 minutes and stained in 2% Giemsa for 10 minutes. Under a bright field transmission light microscope (Oympus Research Microscope CH20i (Binocular vision), India) at 400X magnification, at least 1000 cells from each individual were examined. The number of cells with nuclear anomalies was scored. The criterion was followed for scanning cells for micronuclei and other nuclear anomalies.

Gene expression by real-time PCR (RT-PCR): Protocol of Schmittgen and Livak was followed for RT-PCR analysis. According to this protocol, the RNA was first isolated from the sample. Then the RNA was exposed with DNAse I, later cDNA was synthesized. In the next step, RT-PCR from Eppendorf (Mastercycler gradient) was performed, where triplicates PCRs per gene, per cDNA sample were made.

To the triplicates, master mix containing SYBER green reagent was added as per the protocol. PCR was performed using real time instrument. 40 cycles of 15s at 95°C were followed for SYBER green detection. Further PCR efficiency of gene of interest and control genes was determined.

Statistical Analysis: Mean + SE of each parameter for each type of exposure is calculated. Student t-test and ANOVA were carried out for comparison. Statistical analysis was performed using International Business Machines (IBM) Corporation Statistical Package for the Social Sciences (SPSS) Statistics for Windows (version 20.0. Armonk, New York: IBM Corporation).

Results and Discussion

The general characteristics of the exposed group and controls are shown in table 1. The duration of occupationally exposed groups based on age is shown in table 2.

Effect of ELF-EMFs on DNA by Comet Assay: The comet tail was coinciding with 100 divisions of the Ocular ruler. Tail length observed was 4 divisions of Oculometer (OM). One Oculometer division (OD) for 40X objective lens was 2.5 µm. So the tail length of comet is 4 OD x 2.5 µm = 10 µm.

The results of basal DNA damage assessed by alkaline comet assay in terms of Mean ± SD comet tail length are summarized [Table 2]. Independent t test showed significant difference in the mean comet tail length values [Figure 3 and 4] of exposed and control groups [Table 3].

Effect of ELF-EMFs on Melatonin Concentration: The results of melatonin concentration levels gradually decreased when the duration of exposure increased as shown in table 2 and also between exposure and control groups as shown in table 3 and figures 5 and 6.

Effect of ELF-EMFs on Buccal Epithelial Cells (BECs): The results of micronuclei test, the percentage of micronucleated cells (%MNC) of the exposed groups [Figure 7] based on duration found difference in values and the decrease of mean RT-PCR value from short duration to long duration exposure groups was observed [Table 2].

Table 2
Occupationally Exposure characteristics based on duration

Duration Group	Age	Subjects (n)	Parameter	Mean ± SD
A (1-6 days)	19-45	121	Comet Tail length (CTL)	10.21 ± 1.28
		121	Melatonin (MEL)	38.1 ± 2.5
		121	Micronuclei Test (MNT)	1.41 ± 0.65
		121	RT-PCR	8.04 ± 6.62
B (1 to 4 weeks)	19-45	114	Comet Tail length (CTL)	8.5 ± 1.1
		114	Melatonin (MEL)	37.5 ± 2.3
		114	Micronuclei Test (MNT)	1.38 ± 0.62
		114	RT-PCR	7.84 ± 6.61
C (1 to 6 Months)	19-45	107	Comet Tail length (CTL)	8.8 ± 1.3
		107	Melatonin (MEL)	37.8 ± 2.5
		107	Micronuclei Test (MNT)	1.42 ± 0.71
		107	RT-PCR	7.06 ± 6.53

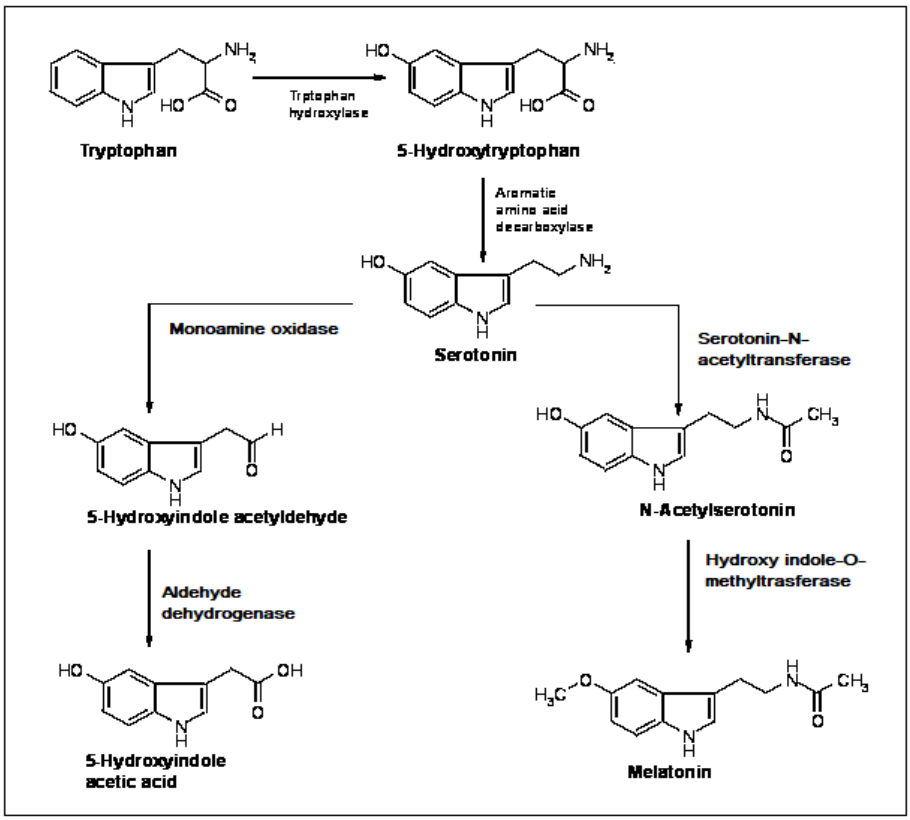


Figure 1: Synthesis of Melatonin

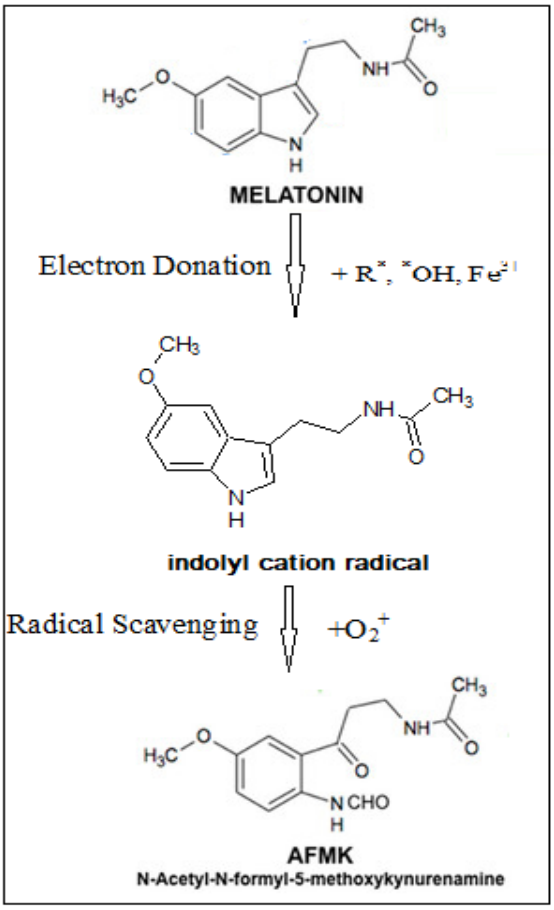


Figure 2: Free-radical scavenging activity of melatonin

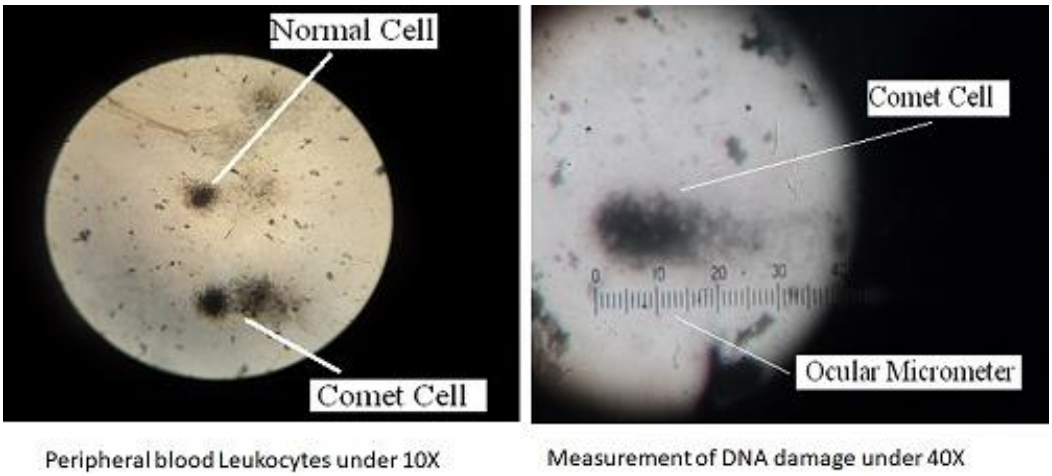


Figure 3: Single Cell Gel Electrophoresis Comet cell images

Table 3
Results of Mean Concentrations of DNA damage, Melatonin, Micronucleus Test and RT-PCR levels between the Exposure and Control groups

Parameters	EXPOSED		CONTROLS		t-value	p-value
	N	Mean ± SD	N	Mean ± SD		
DNA damage	342	10.21 ± 1.28	150	4.21 ± 1.13	49.556	<0.0001
Melatonin(pg/ml)	342	37.92 ± 2.72	150	98.00 ± 140.59	7.909	<0.0001
MNT (%)	342	1.40 ± 0.66	150	1.24 ± 0.67	2.464	<0.0141
RT-PCR (pg/ml)	342	7.65 ± 6.59	150	1.24 ± 1.13	11.930	<0.0001

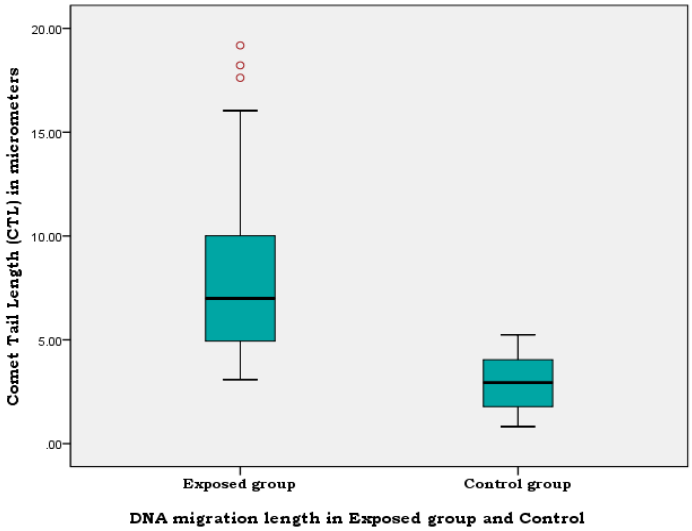


Figure 4: Box plot of DNA Damage in presence of comet tail length

Figure 5: Melatonin Assay by RIA counted in a gamma counter from Automatic Gamma Counter (1480-11 Wizard 3), Perkin Elmer, USA.

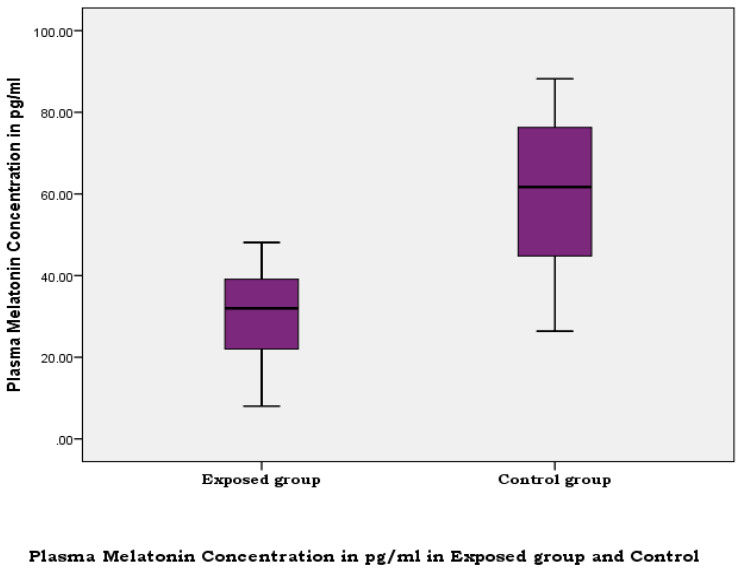
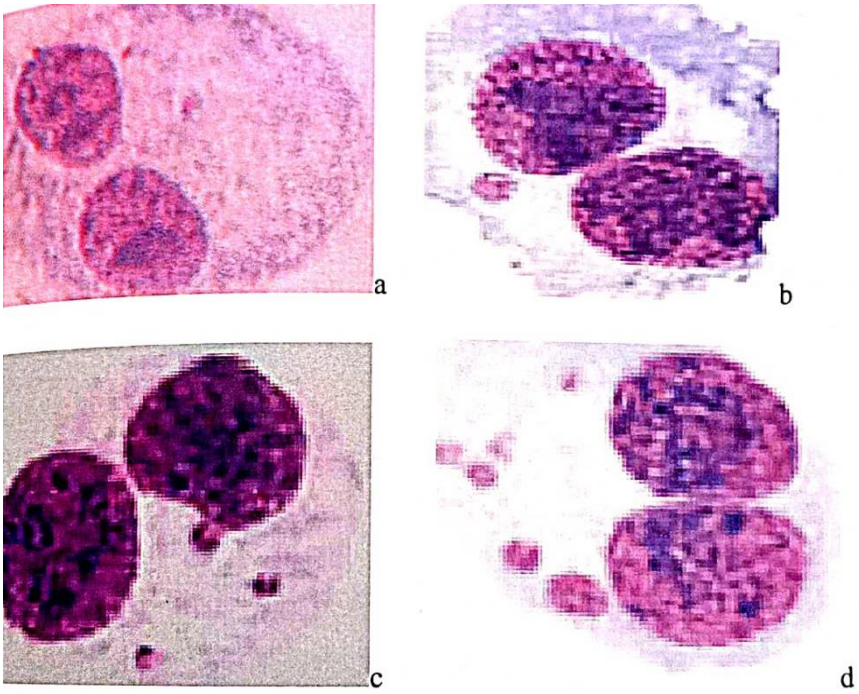


Figure 6: Box plot of Melatonin concentration levels



a. Binucleated cell, b. Single micronucleated cell, c and d. Binucleated cells with more than one micronuclei
Figure 7: Micronuclei in binucleated lymphocytes

The results observed from one-way ANOVA revealed significant differences in DNA damage, melatonin concentrations, incidence of micronuclei and also RT-PCR among subgroups based on duration of occupationally exposed subjects [Table 2].

Overall, the observation was carried out between different parameters to assess the extent of Mean ± SD relationship between the endpoints of comet assay [8.51 ± 1.28], melatonin concentrations [37.92 ± 2.72], micronuclei percentage [1.40 ± 0.66] and RT-PCR analysis [7.65 ± 6.59] showing a significant difference in the exposed as well the control subjects [Table 3].

The research into the potential health effects of exposure to EMFs has been underway for several decades. A number of independent scientific committees and working groups have reviewed the literature and concluded with various degrees of confidence that exposures to EMF are encountered in residential and in most occupational settings, to impose adverse health effects in humans[1,2,8,23,61]. Nonetheless, considering the scientific ‘‘weight of evidence,’’ mostly from human epidemiological data, the International Agency for Research on Cancer (IARC) concluded that EMF exposures act as ‘‘possible human carcinogen’’ in the IARC category of Class 2B[56]. Despite the epidemiologic association of magnetic fields with health hazards, a cause-and-effect relationship cannot be inferred.

The present study has shown the possible bioeffects of ELF-EMFs on exposed subjects occupationally exposed to light at night. However, it is the general consensus of the majority of the scientific community that scientific studies, to date, have suggested that the existence of harmful effects from environmental levels of exposure has not been substantiated but remains a possibility.

Bioeffects on occupational exposure to ELF-EMFs: The advent of electricity has brought about far greater and increasing ELF-EMFs exposures over the last 120 years from the generation, transmission and use of electricity[27,55]. The hypothesis that chronic exposure to residential and/or occupational ELF-EMFs may cause adverse health hazards, has led to a great deal of research. Epidemiological reports particularly on increased risk of childhood leukaemia, breast cancer and neurological disorders had been a subject of national and international reviews[22,54].

However, very few epidemiological studies have directly evaluated the cause of such bioeffects. As studies on genotoxicity are highly relevant in evaluation of carcinogenicity, the present study focused on such an issue in occupational settings of ELF exposure. Moreover, in India, no studies are conducted in evaluating genotoxicity and stress effects of magnetic fields on such occupational exposure.

ELF Magnetic field effect on Biomarkers of Genetic Damage: An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the DNA and chromosomal level. The findings from alkaline comet assay carried out in peripheral blood lymphocytes showed induction of DNA damage in occupationally exposed when compared against controls. In the previous experiments carried out at our laboratory using comet assay, increase in DNA strand breaks was observed in cells exposed to low level and low frequency EMFs[3,61]. This observation was also similar to our

earlier reports on human peripheral blood leukocytes exposed to 5 flux densities in the range of 2 to 10 Mt[4].

Using comet assay, an increase in DNA damage in rat brain cells exposed to ELF-EMFs (0.25 mT and 0.5 mT) was also reported[32]. Similarly, the data from an *in vivo* study indicated the cytotoxic / genotoxic potential of long-term EMF exposure at 1mT[15]. A report also showed evident increase in all parameters consistent with damaged DNA after 1 hour exposure to power frequency magnetic fields[66]. Some other studies on cytogenetic effects of ELF-EMFs have also shown supporting evidences[25,31,64,65].

Similarly, a dose dependent increase in DNA strand breaks from microwave RF at low intensity levels was also evaluated, indicating a causal relationship between RF exposure and genetic damage[48]. Some other workers also have not observed an increased inducible DNA damage by exposure to radiofrequency signals[39]. In general, the energy of ELF-EMFs is considered to be insufficient to break chemical bonds in DNA. However, recent observations have shown that DNA can transfer electrons within its base pairs. These studies suggest that EMF may initiate transcription by generating repulsive forces causing chain separation at specific DNA sequences.

As in the state of transcription, the DNA presents itself as a vulnerable target to genotoxic influences, ELF-EMF exposure may lead to DNA disruption, causing DNA strand breaks[26].

Indeed, epigenetic changes, including modifications of histones and microRNA expression and DNA methylation, can be associated to ELF MF exposure[12,20]. Nonetheless, it has been suggested that EMFs may induce cell specific activation processes which in turn activate signal transduction pathways causing excessive reactive oxygen species (ROS) formation/ cell cycle disturbances[47]. These disturbances have the potential to disrupt DNA integrity.

Relevance of Electromagnetic field Exposure Indices: The exposure indices such as magnetic field strength, duration on night shift in nights, number of hours exposure at night, age intensity and diet were considered. The inclusion of duration and intensity of exposure in evaluating personal exposure level can be of prime importance.

For the duration estimates, we systematically used the number of nights of exposure which are expected to provide better estimates of the duration of long-term exposure. When occupationally exposed, nurses were categorized based on duration in nights. Evaluation of melatonin showed a significant decrease.

Magnetic field Effect on Neuroendocrine Hormones: Few studies were carried out on melatonin secretion in occupationally ELF-EMFs exposed subjects. The effect of ELF-EMFs occupational exposure on production of pineal gland hormone, melatonin was evaluated in our study. The mean melatonin concentration [Table 2] of the exposed was significantly different from those of healthy individuals. The exposed night shift workers have suppressed melatonin levels against the control group. Another occupational study of a group of female garment industry workers of night shifts showed reduced 6-OHMS[46].

We propose the role of melatonin to explain the biological effects of EMFs. The rationale behind our study is that the increased DNA damage in response to magnetic field exposure, resulting in suppressed circulating melatonin is due to the scavenging property of melatonin. The suppression of melatonin synthesis with extended lifetime, may also enhance DNA damage[45,51].

Conclusion

Although the effect of EMF on melatonin release was extensively studied, the site and mechanism of action of magnetic field on the pineal gland that leads to changes in melatonin synthesis, are unclear. The effect of magnetic field on melatonin synthesis can result from changes in neural input. Magnetic fields are perceived and interpreted by photoreceptors in eye as 'light', resulting in the suppression of melatonin. This hypothesis could have an important implication for the possible health effects associated with exposure to ELF magnetic fields in the public and occupational environments particularly at night.

Our results show a significant effect associated with longer duration of intense night shifts and relatively lesser effect for few night shifts. Our results warrant more epidemiological studies considering the confounding factors.

Acknowledgement

The authors would like to thanks University Grants Commission (UGC), New Delhi for funding this major research project. We would like to express our deep gratitude to Late Prof. Y.R. Ahuja, Director, Genetics Unit, Vasavi Medical and Research Centre, Hyderabad and also project Co-Principal Investigator, for their patient guidance and enthusiastic encouragement for the research work. We also acknowledge the management of Bharatiya Vidya Bhavan's New Science College for their support and cooperation.

References

1. AGNIR-Advisory Group on Non-Ionising Radiation, Power Frequency Electromagnetic Fields, Melatonin and the Risk of Breast Cancer, Chilton, Health Protection Agency 2006, (Documents of the Health Protection Agency, Series B: Radiation, Chemical and Environmental Hazards, RCE-1) **(2006)**

2. Ahlbom A., Bridges J., De Seze R., Hillert J.L., Juutilainen J., Mattsson M.O., Neubauer G., Schüz J., Simko M. and Bromen K., Possible effects of electromagnetic fields (EMF) on human health--opinion of the scientific committee on emerging and newly identified health risks (SCENIHR), *Toxicology*, **246(2-3)**, 248-250 **(2008)**

3. Ahuja Y.R., Bhargava S.C., Safia C., Rizwani W., Lima S. and Devdas A.H., Comet assay to evaluate DNA damage caused by magnetic fields, Proceedings of International Conference in Electromagnetic Interference, 273-276 **(1997)**

4. Ahuja Y.R. and Saran R., Alkaline single cell gel electrophoresis assay/comet assay protocol, *Journal of Cytology and Genetics*, **34**, 57–62 **(1999)**

5. Åkerstedt T., Knutsson A., Narusyte J., Svedberg P., Kecklund G. and Alexanderson K., Night work and breast cancer in women: a Swedish cohort study, *BMJ Open*, **5(4)**, e008127 **(2015)**

6. Andel R. et al, Work-related exposure to extremely low-frequency magnetic fields and dementia: results from the population-based study of dementia in Swedish twins, *J Gerontol A BiolSci Med Sci*, **65(11)**, 1220-1227 **(2010)**

7. Bodewein L. et al, Systematic review on the biological effects of electric, magnetic and electromagnetic fields in the intermediate frequency range (300 Hz to 1 MHz), *Environment Research*, **171**, 247-259 **(2019)**

8. Celikler S., Aydemir N., Vatan O., Kurtuldu S. and Bilaloglu R., A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations, *International Journal of Environmental Health Research*, **19(6)**, 421–430 **(2009)**

9. Choi K.H. et al, Multiple assessment methods of prenatal exposure to radio frequency radiation from telecommunication in the Mothers and Children's Environmental Health (MOCEH) study, *Int J Occup Med Environ Health*, **29(6)**, 959-972 **(2016)**

10. Davis D.L., Hoel D., Fox J. and Lopez A., International trends in cancer mortality in France, West Germany, Italy, Japan, England and Wales and the USA, *Lancet*, **336(8713)**, 474-481 **(1990)**

11. Deutsch S. and Wilkening G.M., Electromagnetic field cancer scares, *Health Phys*, **73**, 301-309 **(1997)**

12. Diab K.A., The impact of the low frequency of the electromagnetic field on human, In Cell Biology and Translational Medicine, Volume 7, Stem Cells and Therapy: Emerging Approaches; Turksen K., Eds., Springer: Berlin/Heidelberg, Germany, **1237**, 135-149 **(2020)**

13. El-Setouhy M. et al, Genotoxic effects of waterpipe smoking on the buccal mucosa cells, *Mutat Res*, **655(1-2)**, 36–40 **(2008)**

14. Engel C.L., Rasanayagam M.S., Gray J.M. and Rizzo J., Work and female breast cancer: The state of the evidence, 2002–2017, *New Solut*, **28**, 55-78 **(2018)**

15. Erdal N., Gurgul S. and Celik A., Cytogenetic Effects of extremely low frequency magnetic field on Wister rat bone marrow, *Mutation Research*, **630(1-2)**, 69-77 **(2007)**

16. Falcioni L. et al, Report of final results regarding brain and heart tumors in Sprague-Dawley rats exposed from prenatal life until natural death to mobile phone radiofrequency field representative of a 1.8 GHz GSM base station environmental emission, *Environ Res*, **165**, 496-503 **(2018)**

17. Floderus B., Persson T. and Stenlund C., Magnetic-field exposures in the workplace, Reference distribution and exposures in occupational groups, *Int J. Occup Environ Health*, **2(3)**, 226-238 **(1996)**

18. Fraser S., Cowen P., Franklin M., Franey C. and Arendt J., Direct radioimmunoasssay for melatonin in plasma, *Clin Chem*, **29**, 396-397 **(1983)**

19. Gaestel M., Biological monitoring of non-thermal effects of mobile phone radiation: recent approaches and challenges, *Biol Rev CambPhilos Soc*, **85(3)**, 489-500 **(2010)**

20. Giorgi G. and Del Re B., Epigenetic dysregulation in various types of cells exposed to extremely low-frequency magnetic fields, *Cell Tissue Res*, **386**, 1-15 **(2021)**

21. Halgamuge M.N., Pineal melatonin level disruption in humans due to electromagnetic fields and ICNIRP limits, *Radiat Prot Dosimetry*, **154(4)**, 405-416 **(2013)**

22. Hardell L. and Sage C., Biological effects from electromagnetic field exposure and public exposure standards, *Biomedicine and Pharmacotherapy*, **62(2)**, 104-109 **(2008)**

23. IEGEMF, (current report), Recent Research on EMF and Health Risks, Fourth annual eport from SSI's Independent Expert Group on Electromagnetic Fields, Stockholm: Statens Stralskyddsinstitut **(2006)**

24. Ijaz S. et al, Nightshift work and breast cancer-a systematic review and meta-analysis, *Scandinavian Journal of Work, Environment and Health*, **39**, 431-447 **(2013)**

25. Ivancsits S., Diem E., Pilger A., Rudiger H.W. and Jahn O., Induction of DNA strand breaks by intermittent exposure to extremely low frequency electromagnetic fields in human diploid fibroblasts, *Mutat Res*, **519(1-3)**, 1-13 **(2002)**

26. Ivancsits S., Pilger A., Diem E., Jahn O. and Rudiger H.W., Cell type specific genotoxic effects of intermittent extremely low frequency electromagnetic fields, *Mutation Res/Genetic Toxicology and Environmental Mutagenesis*, **583(2)**, 184-188 **(2005)**

27. Jackson J.D., Are the stray 60-Hz electromagnetic fields associated with the distribution and use of electric power a significant cause of cancer?, *Proc Natl Acad Sci*, **89(8)**, 3508-3510 **(1992)**

28. Juutilainen J. et al, Noctural 6-hydroxymelatonin sulfate excretion in female workers exposed to magnetic fields, *J. Pineal Res*, **28(2),** 97-104 **(2000)**

29. Karimi A., Moghaddam F.G. and Valipour M., Insights in the biology of extremely low-frequency magnetic fields exposure on human health, *Mol Biol Rep*, **47**, 5621-5633 **(2020)**

30. Kheifets L. et al, A pooled analysis of extremely low-frequency magnetic fields and childhood brain tumors, *Am J Epidemiol*, **172(7)**, 752-761 **(2010)**

31. Kindzelskii A.L. and Petty H.R., Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phase matched with metabolic oscillators, *Bioche Biophys Acta*, **1495(1)**, 90-111 **(2000)**

32. Lai H. and Singh N.P., Acute exposure to 60Hz magnetic field increases strand breaks in rat brain cells, *Bioelectromagnetics*, **18(2)**, 156-165 **(1997)**

33. Lewy A.J., Wehr T.A. and Goodwin F.K., Light suppresses melatonin secretion in humans, *Science*, **210(4475)**, 1267-1269 **(1980)**

34. Lynch H.J., Deng M.H. and Wurtman R.J., Light intensities required to suppress nocturnal melatonin secretion in albino and pigmented rats, *Life Sci*, **35(8)**, 841-847 **(1985)**

35. Markov M. and Grigoriev Y., Protect children from EMF, *Electromagn Biol Med*, **34(3)**, 251-256 **(2015)**

36. McGlynn A.P., Wasson G.R., O'Connor J., McKerr G., McKelvey-Martin V.J. and Downes C.S., The Bromodeoxyuridine Comet Assay Detection of Maturation of Recently Replicated DNA in Individual Cells, *Cancer Res*, **59**, 5912–5916 **(1999)**

37. Morris M., Lack L. and Barrett J., The effect of sleep/wake state on nocturnal melatonin excretion, *J Pineal Res*, **9(2)**, 133-138 **(1990)**

38. Ohayon M.M., Stolc V., Freund F.T., Milesi C. and Sullivan S., The potential for impact of man made super low and extremely low frequency electromagnetic fields on sleep, *Sleep Medicine Reviews*, **47**, 28-38 **(2019)**

39. Pall M.L., Wi-Fi is an important threat to human health, *Environmental Research*, **164**, 405-416 **(2018)**

40. Pedersen C. et al, Occupational exposure to extremely low-frequency magnetic fields and risk for central nervous system disease: an update of a Danish cohort study among utility workers, *International Archives of Occupational and Environmental Health*, **90(7)**, 619-628 **(2017)**

41. Pepłońska B. et al, Night shift work and modifiable lifestyle factors, *International Journal of Occupational Medicine and Environmental Health*, **27(5)**, 693-706 **(2014)**

42. Position Statement on EMF Radiation Concerns, https://ehs.unc.edu/radiation/emf/ **(2018)**

43. Pritchard C., Silk A. and Hansen L., Are rises in Electro-Magnetic Field in the human environment, interacting with multiple environmental pollutions, the tripping point for increases in neurological deaths in the Western World?, *Medical Hypotheses*, **127**, 76-83 **(2019)**

44. Radiofrequency (RF) Evaluation Report, URS, Omaha Nebraska **(2013)**

45. Ravindra T., Lakshmi N.K. and Ahuja Y.R., Melatonin in pathogenesis and therapy of Cancer, *Indian Journal of Medical Sciences*, **60(12)**, 523-535 **(2006)**

46. Reiter R., Action spectra, dose-response relationships and temporal aspects of light's effects on the pineal gland, *Ann NY Acad Sci*, **453**, 215-230 **(1985)**

47. Reiter R.J., Melatonin aspects of exposure to low frequency electric and magnetic fields, *In: Advances in Electromagnetic Fields in Living Systems*, **50(2)**, 1-27 **(1997)**

48. Rubtsova N., Paltsev Y., Perov S. and Bogacheva E., Intensity-time dependence dosing criterion in the EMF exposure guidelines in Russia, *Electromagn Biol Med*, **37(1)**, 43-49 **(2018)**

49. Semm P., Schneider T. and Vollrath L., Effects of an earth-strength magnetic field on electrical activity of pineal cells, *Nature*, **288(5791)**, 607-608 **(1980)**

50. Seomun G., Lee J. and Park J., Exposure to extremely low-frequency magnetic fields and childhood cancer: A systematic review and meta-analysis, *PLoS One*, **16**, e0251628 **(2021)**

51. Simco M. and Mattson M.O., Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro:possible immune cell activation, *J Cell Biochem*, **93(1)**, 83-92 **(2004)**

52. Singh H., Kumar C. and Bagai U., Effect of Electromagnetic Field on Red Blood Cells of Adult Male swiss albino Mice, *International Journal of Theoretical and Applied Sciences*, **5(1)**, 175-182 **(2013)**

53. Singh N.P., McCoy M.T., Tice R.R. and Schneider E.L., A simple technique for quantitation of low levels of DNA damage in individual cells, *Experimental Cell Research*, **17**, 184-191 **(1988)**

54. SSI, Swedish Radiation Protection Authority, Recent Research on EMF and Health Risks, Fifth Annual Report from SSI's Independent Expert Group on Electromagnetic Fields, 2007, SSI Report, 12 **(2008)**

55. Swanson J., Long-term variations in the exposure of the population of England and Wales to power frequency magnetic fields, *J Radiol Prot*, **16(4)**, 287-301 **(1996)**

56. The International Agency for Research on Cancer (IARC), *Non-ionizing Radiation, Part 2: Radiofrequency Electromagnetic Fields*, **102**, 462 **(2013)**

57. The International Agency for Research on Cancer (IARC), RF EMF Class 2B, *Lancet Oncol*, **12(7)**, 624-626 **(2011)**

58. Tolbert P.E., Shy C.M. and Allen J.W., Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users, *Am J Epidemiol*, **134(8)**, 840–850 **(1991)**

59. Ursin G., Bernstein L. and Pike M.C., Breast Cancer, *Cancer Surv*, **19/20**, 241-264 **(1994)**

60. Vijayalaxmi Obe G., Controversial cytogenetic observations in mammalian somatic cells exposed to radiofrequency radiation, *Radiation Research*, **162(5)**, 481-496 **(2004)**

61. Vijayalaxmi Obe G., Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: a review and future research recommendations, *Bioelectromagnetics*, **26(5)**, 412-430 **(2005)**

62. Wetterberg L., Light and Biological Rhythms in Man, Wenner-Gren International Series, Oxford: Pergamon Press **(1993)**

63. Wilson B.W. anderson L.E., Hilton D.I. and Phillips R.D., Chronic exposure to 60-Hz electric fields: effects on pineal function in the rat, *Bioelectromagnetics*, **2(4),** 371-380 **(1981)**

64. Winker R., Ivancsits S., Pilger A., Adlkofer F. and Rudiger H.W., Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low frequency electromagnetic fields, *Mutation Res/genetic Toxicology and Environmental Mutagenesis*, **585(1-2)**, 43-49 **(2005)**

65. Wolf F.I. et al, 50 Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage; possible involvement of a redox mechanism, *Biochemica et Biophysica Acta (BBA)-Molecular Cell Research*, **1743(1-2)**, 120-129 **(2005)**

66. Zmyslony M., Politanski P., Rajkowska E. and Szymczak W., Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions, *Bioelectromagnetics*, **25(5)**, 324-328 **(2004)**.

(Received 30th September 2022, accepted 01st December 2022)