

Occupationally Exposure to Extremely Low Frequency Electromagnetic Fields and Light at Night on Melatonin & related Oxidative Stress in Human Subjects

Vemula Surender¹, Ravindra Tiwari*², Ravindra Babu Potti³

¹University Grants Commission (UGC) Research Unit, Bhavan's New Science College, Hyderabad, Telangana State, India

*²Dean, Global Institute of Engineering & Technology, Moinabad, Hyderabad, Telangana State, India

³Department of Biotechnology, Sreenidhi Institute of Science and Technology, Hyderabad, Telangana State, India

Corresponding Author: Dr. (Mrs).Ravindra Tiwari, Principal Investigator, UGC Research Unit, Bhavan's New Science College, Narayanguda, Hyderabad-500029, Telangana State, India, Email: rrvindra_tiwari@yahoo.com

Short running title: Occupationally Exposure to ELF-EMFs and LAN on Melatonin & Oxidative Stress

ABSTRACT

During one decade, an increasing percentage of world population has to be attributed to an array of environmental, health and lifestyle factors. The potential effect of extremely low frequency electromagnetic fields (ELF-EMFs) emitted from electrical and various electrical devices in the occupational environment and light at night (LAN) to elicit biological response are a major concern for the public and scientists. Controversial genetic and oxidative stress effects have been reported by these polluted environments. Hence this study was undertaken with a purpose to evaluate melatonin hormone and oxidative stress markers in exposure workers along with cumulative effects of smoking and drinking. The study subjects (n=342) included night shift workers for more than 2 years with age, gender, socioeconomic status matched non exposure controls (n=150). Self reported symptoms were sought using questionnaire. Plasma Melatonin was evaluated using radioimmuno assay. Oxidative stress was estimated by measuring plasma malondialdehyde (MDA), serum nitric oxide (NO) and erythrocyte catalase levels. When the data of exposed subjects was analyzed, a significant decrease in melatonin levels was observed. Plasma MDA levels demonstrated a significant increase in one sub-group. The serum NO levels and erythrocyte catalase levels shows a significant increase. The cumulative effect of a chemical agent or environmental pollutant could be a possible contributor in enhancing the adverse effect of extremely low frequency electromagnetic fields. Certain caution is necessary.

Keywords: ELF-EMFs, Melatonin, Light at Night, Oxidative Stress, MDA and NO

INTRODUCTION

The generation, distribution, and use of electric power is a hallmark 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 [1,2]. 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 thermal cellular effects of EMFs [3].

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 [4,5]. 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 [6,7]. 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 increases the risk of breast cancer [8].

Melatonin is a neuroendocrine hormone (Figure 1 & 2), 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) is 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 [9]. Controversial reports addressing health effects like neurodegenerative disorders and brain tumors due to RF electromagnetic field exposure has been mounting [10].

At low frequency waves, EMFs have the effect on the nervous system due to their intensive sensitivity. Not only the nervous system they also affect the psychological conditions. Due to the heat released by them the temperature of the body also raises [11,12].

The aim of our study is to understand the association between melatonin levels and the increase 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 [13,14].

Light Effects on Melatonin

The effect of light on pineal function in humans has been extensively studied [15]. The effect is qualitatively similar to the effect in other mammals where the intensity of nocturnal illumination suppresses melatonin production to daytime levels [16,17]. The normal melatonin rhythm in humans has characteristics that may be relevant to breast cancer risk [18]. In animals, very brief light exposure (minutes or even seconds) at night can suppress melatonin production [19]. 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 [20]. 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 [21]. The mechanism where ELF-EMFs and LAN exposure could increase cancer risk lie in the possible oncostatic property of melatonin and its circulating levels [22].

Effects of Electric and Magnetic Fields on 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 [23]. 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 was reported [24]. In humans an association between use of wireless phones and brain tumors was also indicated [25].

We propose the role of melatonin as an 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 [26,27]. 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" [29].

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 μ T MF intensity [30,31]. 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 [32,33].

METHODOLOGY

The women workers (n=342) working in the night shifts 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. 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.

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 placed in ice to prevent exogenous damage. The samples were processed in the laboratory for estimating Melatonin by RIA and oxidative stress by LPO.

Melatonin Assay (Direct Radio Immuno Assay)

Plasma Melatonin was quantitatively measured by Direct Radio Immuno Assay (RIA) described by Fraser et al.[34]. The amount of ¹²⁵I-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.

Lipid Peroxidation - Thiobarbituric Acid Reactive Substances (TBARS) Assay

Malondialdehyde (MDA) is the stable end product of lipid peroxidation and an efficient parameter for lipid oxidative damage [35,36]. Formation of MDA is assessed by quantification of thiobarbituric acid reactive substrate (TBARS).

Thiobarbituric acid reactive Substances (TBARS), Malondialdehyde (MDA), an end product of lipid peroxidation, reacts with TBA to form a 1:2 adduct with thiobarbituric acid which can be measured by spectrophotometry.

In the process, one ml of protein free plasma obtained after centrifugation was treated with thiobarbituric acid (0.33%) in boiling water bath for 30 min to form an 1:2 adduct which is measured at 535 nm with spectrophotometer (Schimadzu UV-160A, Cat No:204-04550-01, Made in Japan). Mean and \pm SD or SE was calculated for the samples. The concentration of MDA equivalents in nmol/ml in samples were interpolated from the standard curve.

Estimation of Serum Nitric Oxide

As NO is an unstable compound, it is rapidly converted to nitrates and nitrites in the body; hence their concentration is parallel to NO levels. Total production of Nitric Oxide (NO) was determined by assaying for nitrite and nitrate [37]. Nitrate and nitrite concentrations were then estimated by the Griess method. In this method nitrate is first reduced to nitrite which is treated with sulfanilamide and N1-naphthyl-ethylene diamine. The coloured compound is formed after which its characteristic absorption spectrum was determined on spectrophotometry.

500 μ l of sulfosalicylic acid was added to 500 μ l of serum in a centrifuge tube. The reaction was allowed for 5 minutes with mild stirring carried out for every 30 sec followed by centrifugation at 3000 rpm for 20 min. After centrifugation, to 200 μ l of supernatant, 30 μ l of 10% NaOH and 300 μ l of Tris HCl buffer (pH 9.0) were added, 500 μ l of freshly prepared Griess reagent was added to the above mentioned reaction mixture. The reaction was allowed in dark for 10 min and later read at 540 nm against double distilled water as blank. The lower limit detection of the assay was 5 μ moles/L. The optical densities of the samples were then obtained on a spectrophotometer (Schimadzu UV-160A, Cat No: 204-04550-01, Made in Japan) at 540 nm.

Enzymatic Determination of Catalase

The catalase assay [38] was performed in Red cell concentrate samples of 500 μ l, added onto 2 ml of cold distilled water (1:5 v/v), vortexed and left to stand at 4 °C for 15 min for hypotonic lysis. A further 1:1000 dilution was prepared in 5fold diluted stock hemolysate using 50 mM-phosphate buffer. A portion of the diluted lysate (1 ml) was transferred to a quartz cuvette and allowed to react with either 0.5 ml phosphate buffer (reference) or 0.5 ml freshly prepared 30 mM-H₂O₂ as the test. The decomposition of the substrate (H₂O₂) was recorded spectrophotometrically (Schimadzu UV340) at 240 nm for 60s at 30s time interval. Catalase activity was expressed as K/g hemoglobin.

STATISTICAL ANALYSIS

For statistical evaluation, observations on each parameter for each group were pooled and mean \pm SD (Standard Deviation) was calculated. Student's t-test (paired and unpaired comparisons) and one way ANOVA were performed to evaluate various differences. One-way ANOVA was used to compare the sub-groups based on exposure and non-exposure subjects. Multiple regression analysis was done to study the effects of confounding factors, and correlation analysis was carried out to adjudge the sensitivity of parameters used. Chi-square test was used to study the relationship between prevalence of self reported symptoms and mobile phone use. Statistical software SPSS 19 (IBM, Bangalore, India) was used to carry out statistical analysis.

RESULTS AND DISCUSSION

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 [39,40]. 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 [41,42]. 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. Some occupational epidemiological studies have demonstrated an increased incidence of breast cancer among mainly male electrical workers [43]. Moreover, in India no studies are conducted in evaluating genotoxicity and stress effects of magnetic fields on such occupational exposure.

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 subjects were categorized based on duration in nights, evaluation of melatonin and oxidative stress levels showed a significant report.

Results of 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 & Figure 3) of the exposed was significantly different from those of healthy individuals. The exposed night shift workers have suppressed melatonin levels against the control group. In some studies occupational study of a group of female garment industry workers of night shifts, showed no significance in melatonin with reduced 6-OHMS [44].

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 [45,46].

Results of Oxidative Stress

The MDA levels in samples of ELF-EMFs occupationally exposed subjects suggest a statistical significance in LPO activity (Figure 5) owing to the great number of biological actions of free radicals, the LPO activity could have an important implication in the comprehension of the biological stress response to ELF-EMFs. Oxidative DNA damage and LPO activity in exposure to ELF-EMFs was reported in certain in vivo experimentations and animal models. A time-dependent significant higher TBARS levels ($p < 0.001$) and induced DNA damage ($p < 0.05$) in the exposure group (50Hz, 0.97 mT) was observed [47]. EMF is known to impact on biological systems by increasing reactive oxygen species, which causes oxidative stress by altering or increasing the catalase activity levels of tissues [48].

It is also observed that nitric oxide synthase is stimulated by the EMF exposure [49,50]. This compound can be involved in the stimulation of lipid peroxidation indirectly. This hypothesis could also explain the increased TBARS production in our exposed group as well. Similarly, the influence of ELF-EMFs on oxidative anti-oxidative balance in cells was demonstrated [51]. In recent studies observed that chronic EMF exposure is an environmental factor affecting blood serum parameters and could impair oxidant-antioxidant function, increase lipid peroxidation and oxidative stress depending on the continuity of ELF-MF exposure [52].

The result of TBARS assay showing plasma MDA as end product of lipid peroxidation are shown in Figure-5, the

mean MDA concentration (nmoles/ml) of the universal analysis of variance showed significant p value between groups (Table 2). the ELF-EMFs showed significant increase in MDA levels in exposed group when compared with controls. While significant increase in MDA level was demonstrated in ELF-EMFs exposed groups.

In both control and study groups, the serum nitric oxide levels are demonstrates as nitrites or nitrates. in this complete data disclose an increased levels of serum NO in the exposed groups (Figure 6).

The anti-oxidative enzyme erythrocyte catalase activity was analyzed in controls and exposed groups. Statistical analysis showed there is a significant alteration between the exposed groups.

The overall findings of oxidative stress parameters in ELF-EMFs exposed had significant difference with non-exposed groups. However, the sub groups based on duration of exposure showed higher significance in the mean concentration of MDA, NO and erythrocyte catalase levels. The effect of duration of work in the exposed night shift workers were significantly high in MDA, NO and catalase levels among the sub groups (Table 3).

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 is 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.

ACKNOWLEDGMENT

The authors thank University Grants Commission (UGC) for funding this Major Research project. The authors are grateful to Mr. M. Sheshacharyulu, Senior Technician and Mr. K. Bhaskar, Senior Statistician, National Institute of Nutrition for their help revising manuscript. The team of authors express their profuse thanks to the management of Bharatiya Vidya Bhavans New Science College for their support and cooperation.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- [1]. Markov M, Grigoriev Y. 2015. Protect children from EMF. *Electromagnetic Biology and Medicine*. 34(3): 251-256.
- [2]. Bodewein L, Schmiedchen K, Dechent D, Stunder D, Graefrath D, Winter L, *et al.* 2019. 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.
- [3]. Gaestel M. 2010. Biological monitoring of non-thermal effects of mobile phone radiation: recent approaches and challenges. *Biological reviews of the Cambridge Philosophical Society*. 85(3):489-500.
- [4]. Andel R, Crowe M, Feychting M, Pedersen NL, Fratiglioni L, Johansson B, *et al.* 2010. Work-related exposure to extremely low-frequency magnetic fields and dementia: results from the population-based study of dementia in Swedish twins. *The journals of gerontology. Series A, Biological Sciences and Medical Sciences*. 65 (11):1220-1227.
- [5]. Kheifets L, Ahlbom A, Crespi CM, Feychting M, Johansen C, Monroe J, *et al.* 2010. A pooled analysis of extremely low-frequency magnetic fields and childhood brain tumors. *American Journal of Epidemiology*. 172(7):752-61.
- [6]. Ijaz S, Verbeek J, Seidler A, Lindbohm ML, Ojajärvi A, Orsini N, *et al.* 2013. Nightshift work and breast cancer—a systematic review and meta-analysis. *Scandinavian Journal of Work, Environment & Health*. 39: 431-447.
- [7]. Åkerstedt T, Knutsson A, Narusyte J, Svedberg P, Kecklund G, Alexanderson K. 2015. Night work and breast cancer in women: a Swedish cohort study. *BMJ open*. 5(4): e008127.
- [8]. Engel CL, Rasanayagam MS, Gray JM, Rizzo J. 2018. Work and female breast cancer: The state of the evidence, 2002–2017. *New Solutions* 28: 55–78.

- [9]. Peplowska B, Burdelak W, Krysicka J, Bukowska A, Marcinkiewicz A, Sobala W, *et al.* 2014. Night shift work and modifiable lifestyle factors. *International Journal of Occupational Medicine and Environmental Health.* 27(5): 693-706.
- [10]. Choi KH, Ha M, Burm E, Ha EH, Park H, Kim Y, *et al.* 2016. Multiple assessment methods of prenatal exposure to radio frequency radiation from telecommunication in the Mothers and Children's Environmental Health (MOCEH) study. *International Journal of Occupational Medicine and Environmental Health.* 29(6):959-972.
- [11]. Pedersen C, Poulsen AH, Rod NH, Frei P, Hansen J, Grell K, *et al.* 2017. 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.
- [12]. Pritchard C, Silk A, Hansen L. 2019. 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.
- [13]. Ursin G, Bernstein L, Pike MC. 1994. Breast Cancer. *Cancer Surveys.* 19/20: 241-264
- [14]. Davis DL, Hoel D, Fox J, Lopez A. 1990. International trends in cancer mortality in France, West Germany, Italy, Japan, England and Wales, and the USA. *Lancet.* 336(8713): 474-481.
- [15]. *Light and Biological Rhythms in Man.* Edited by Wetterberg L. Wenner-Gren International Series. Oxford: Pergamon Press.1993.
- [16]. Lynch HJ, Deng MH, Wurtman RJ. 1984. Light intensities required to suppress nocturnal melatonin secretion in albino and pigmented rats. *Life Sci.* 35(8): 841-847.
- [17]. Lewy AJ., Wehr TA, Goodwin FK. 1980. Light suppresses melatonin secretion in humans. *Science.* 210 (4475): 1267-1269.
- [18]. Morris M, Lack L, Barrett J. 1990. The effect of sleep/wake state on nocturnal melatonin excretion. *Journal of Pineal Research.* 9(2): 133-138.
- [19]. Reiter R. 1985. Action spectra, dose-response relationships, and temporal aspects of light's effects on the pineal gland. *Annals of the New York Academy of Sciences.* 453: 215-230.
- [20]. Halgamuge MN. 2013. Pineal melatonin level disruption in humans due to electromagnetic fields and ICNIRP limits. *Radiation Protection Dosimetry.* 154(4):405-416.
- [21]. Singh H, Kumar C, Bagai U. 2013. Effect of Electromagnetic Field on Red Blood Cells of Adult Male swiss albino Mice. *International Journal of Theoretical & Applied Sciences.* 5(1): 175-182.
- [22]. Ohayon MM, Stolc V, Freund FT, Milesi C, Sullivan S. 2019. The potential for impact of man made super low and extremely low frequency electromagnetic fields on sleep. *Sleep medicine reviews.* 47:28-38
- [23]. Semm P, Schneider T, Vollrath L. 1980. Effects of an earth-strength magnetic field on electrical activity of pineal cells. *Nature.* 288 (5791): 607-608.
- [24]. Wilson BW, Anderson LE, Hilton DI, Phillips RD. 1981. Chronic exposure to 60-Hz electric fields: effects on pineal function in the rat. *Bioelectromagnetics.* 2(4): 371-380.
- [25]. Falcioni L, Bua Tibaldi E, Lauriola M, De Angelis L, Gnudi F, Mandrioli D, *et al.* 2018. 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. *Environmental Research.* 165:496-503.
- [26]. Ravindra T, Lakshmi NK, Ahuja YR. 2006. Melatonin in pathogenesis and therapy of Cancer. *Indian Journal of Medical Sciences.* 60(12): 523-535.
- [27]. Floderus B, Persson T, Stenlund C. 1996. Magnetic-field exposures in the workplace, Reference distribution and exposures in occupational groups. *International Journal of Occupational Medicine and Environmental Health.* 2(3): 226-238.
- [28]. Juutilainen J, Stevens RG, Anderson LE, Hansen NH, Kilpeläinen M, Kumlin T. 2000. Nocturnal 6-hydroxymelatonin sulfate excretion in female workers exposed to magnetic fields. *Journal of Pineal Research.* 28(2): 97-104.
- [29]. The International Agency for Research on Cancer (IARC). 2011. Volume 102, RF EMF Class 2B. *The Lancet Oncology.* 12 (7): 624-626.
- [30]. Seomun G, Lee J, Park J. 2021. Exposure to extremely low-frequency magnetic fields and childhood cancer: A systematic review and meta-analysis. *PLoS ONE.* 16, e0251628.
- [31]. Zaki AM, Abd Rahim MA, Zaidun Z, Ramdzan AR, Isa ZM. 2020. Exposure to non-ionizing radiation and childhood cancer: A meta-analysis. *Middle East Journal of Cancer.* 11: 1–11.
- [32]. Karimi A, Moghaddam FG, Valipour M. 2020. Insights in the biology of extremely low-frequency magnetic fields exposure on human health. *Molecular Biology Reports.* 47:5621-5633.
- [33]. Deutsch S, Wilkening GM. 1997. Electromagnetic field cancer scares. *Health Physics.* 73:301-309.
- [34]. Melatonin. In *Methods of Hormone Radioimmunoassay*, (Jaffe BM, Behrman BM.) Edited by Arendt J, Wilkingson M. New York: Academic Press;1979:101-119.
- [35]. Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology.* 186: 407-421.

- [36]. 36.Gutteridge JM, Halliwell B. 1990.The measurement and mechanism of lipid peroxidation in biological systems. Trends in Biochemical Sciences. 15:129-135.
- [37]. 37.Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. 1982. Analysis of nitrate, nitrite, and (¹⁵N) nitrate in biological fluids. Analytical Biochemistry. 126: 131-138.
- [38]. 38. Aebi H. 1984. Catalase in vitro. Methods in Enzymology. (Colowick SP, Kaplan NO, Ed) Academic Press; Florida. 105:114-121.
- [39]. 39. Jackson JD. 1992. Are the stray 60-Hz electromagnetic fields associated with the distribution and use of electric power a significant cause of cancer ? Proceedings of the National Academy of Sciences. 89(8): 3508-3510.
- [40]. 40.Swanson J. 1996. Long-term variations in the exposure of the population of England and Wales to power frequency magnetic fields. Journal of Radiological Protection. 16(4): 287-301.
- [41]. 41.SSI. Swedish Radiation Protection Authority 2007. Recent Research on EMF and Health Risks. Fifth Annual Report from SSI's Independent Expert Group on Electromagnetic Fields. SSI Report: 2008:12.
- [42]. 42.Hardell L, Sage C. 2008. Biological effects from electromagnetic field exposure and public exposure standards. Biomedicine and Pharmacotherapy. 62(2): 104-109.
- [43]. 43.Caplan LS, Schoenfeld ER, O'Leary ES, Leske MC. 2000. Breast cancer and electromagnetic fields a review. Annals of Epidemiology, 10(1):31-44.
- [44]. 44.Reiter R. 1985. Action spectra, dose-response relationships, and temporal aspects of light's effects on the pineal gland. Annals of the New York Academy of Sciences. 453: 215-230.
- [45]. 45.Simco M, Mattson MO. 2004. Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. Journal of Cellular Biochemistry. 93(1): 83-92.
- [46]. 46.Ravindra T, Lakshmi NK, Ahuja YR. 2006. Melatonin in pathogenesis and therapy of Cancer. Indian Journal of Medical Sciences. 60 (12): 523-535.
- [47]. 47.Sandstrom M, Wilen J, Oftedal G, Hansson Mild K. 2001. Mobile phone use and subjective symptoms. Comparison of symptoms experienced by users of analogue and digital mobile phones. Occupational Medicine. 51:25-35.
- [48]. 48. Elfide Gizem Kivrak, Kıymet Kübra Yurt, Arife Ahsen Kaplan, Isinsu Alkan, Gamze Altun. 2017. Effects of electromagnetic fields exposure on the antioxidant defense system. Journal of Microscopy and Ultrastructure. 5:167-176
- [49]. 49.Al-Khlaiwi T, Meo SA. 2004. Association of mobile phone radiation with fatigue, headache, dizziness, tension and sleep disturbance in Saudi population. Saudi Medical Journal. 25:732-736.
- [50]. 50.Johansson O. 2006. Electrohypersensitivity: state-of-the-art of a functional impairment. Medicine. 25:245-258.
- [51]. 51.Mortazavi SMJ, Ahmadi J, Shariati M. 2007. Prevalence of subjective poor health symptoms associated with exposure to electromagnetic fields among university students. Bioelectromagnetics. 28:326-330.
- [52]. 52. Zozan Guleken, Devrim Sarıbal, Çağlar Uyulan, Aybike Keles, Joanna Depciuch. 2022. Investigating Bio-interface Effects of Chronic ELF-MF Exposure before and after Neonatal Life on Rat Offspring Using Spectroscopy and Biochemical Assays. Biointerface Research in Applied Chemistry. 12(1): 795-808.

Table 1: General characteristics of the study group and controls

Serial Number	Variables	Controls	Exposed subjects	Category of Exposed Subjects	
				Symptomatic	Non-symptomatic
1	Number(<i>n</i>)	150	342	100	242
2	Age (Range) (Mean ± SD)	19-45 22.9 ± 4.5	19-45 25.1 ± 4.1	19-45 26.7 ± 4.2	19-45 24.2 ± 3.7
3	Dietary Habits Vegetarian Non-vegetarian	50 (33.33%) 100 (66.66%)	100 (29.23%) 242 (70.8%)	42 (42%) 58 (58%)	142 (58.67%) 100 (41.32%)

Table 2: Mean ± SD values of melatonin and oxidative stress levels in exposed and non-exposed subjects

Parameters	Exposed subjects		Control		T value	p value
	N	Mean ± SD	N	Mean ± SD		
Melatonin (pg/ml)	342	37.92 ± 2.72	150	98.00 ± 140.59	1.326	< 0.0001
MDA (nm/ml)	342	2.58 ± 0.82	150	1.87 ± 1.47	1.055	< 0.0001

Nitric oxide (nmoles/ml)	342	2.58 ± 0.09	150	1.98 ± 0.07	2.151	< 0.776
Catalase (K/Hb)	342	135.7 ± 26.8	150	140.4 ± 28.6	1.223	0.222

Table 3: Oxidative stress in relation to period of exposure (years) to extremely low frequency lectromagnetic fields and light at night of exposed subjects

* Different literals indicate statistically significant difference between mean values at p=0.05

Group	Period (years)	Subjects (n)	Parameter	Mean ± SD	95% CI for mean
A	2-4	121	MEL	38.1 ± 2.5	36.2 – 2.7
		121	MDA	2.34 ± 0.14	2.2 – 2.9
		121	NO	2.34 ± 0.21	2.0 – 2.7
		121	Catalase	137.60 ± 7.1	127.0 – 148.2
B	5-7	114	MEL	36.4 ± 2.1	34.2 – 2.9
		114	MDA	2.30 ± 0.10	2.0 – 2.5
		114	NO	2.32 ± 0.21	2.0 – 3.0
		114	Catalase	140.22 ± 6.15	129.4 – 151.1
C	8-10	107	MEL	37.92 ± 2.72	36.2 – 2.9
		107	MDA	2.40 ± 0.20	2.0 – 2.8
		107	NO	2.57 ± 0.43	1.9 – 3.3
		107	Catalase	129.04 ± 7.9	116.1 – 142.0

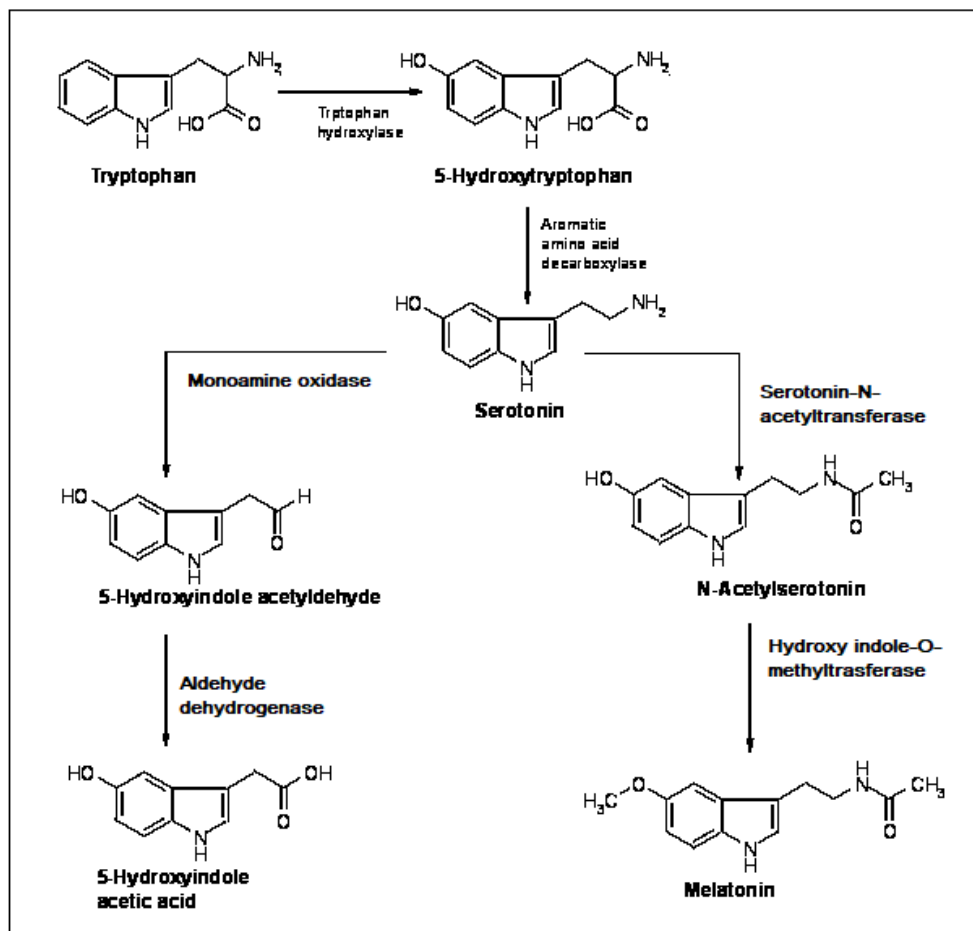


Figure 1: Synthesis of Melatonin

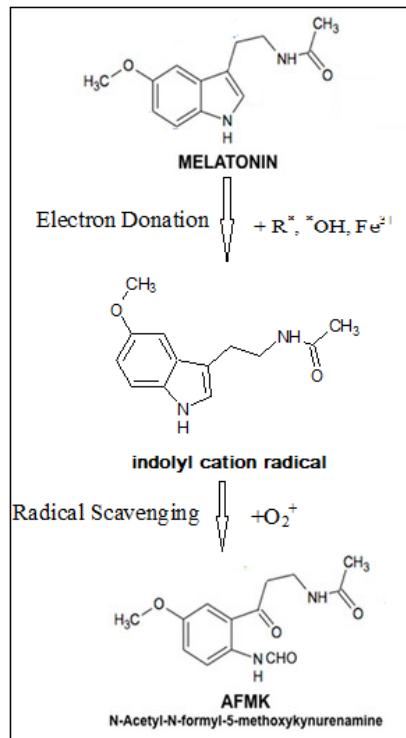
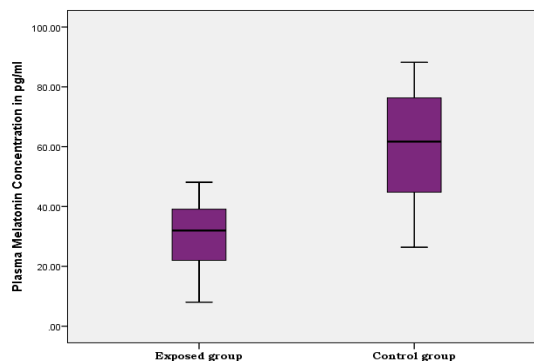


Figure 2: Free-radical scavenging activity of melatonin



Plasma Melatonin Concentration in pg/ml in Exposed group and Control

Figure 3. Box plot of Melatonin concentration levels



Figure 4. Melatonin Assay by RIA counted in a gamma counter from Automatic Gamma Counter (Perkin Elmer=Tri-Carb 2910 TR), Perkin Elmer, USA.

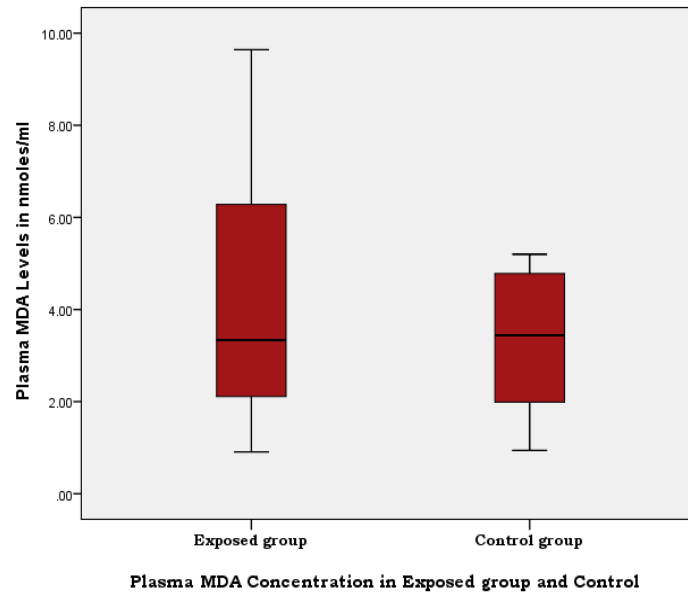


Figure 5. Box plot of MDA concentration levels

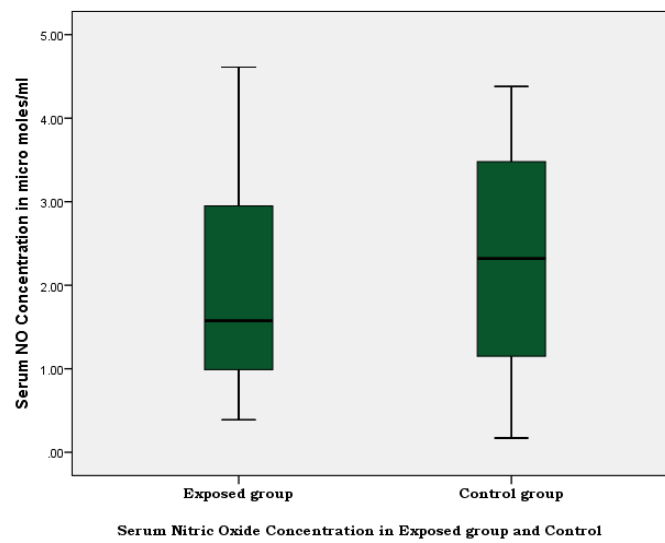


Figure 6. Box plot of serum nitric oxide concentration levels