Continuous exposure of three successive generations of mice to electromagnetic fields: implication on double minute frequency

Puji Sari,[1] Asmarinah,[1] Oentoeng Soeradi,[1] Raden Susworo[2]

[1] *Department of Biological Sciences, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*
[2] *Department of Radiotherapy, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*

Abstrak

Latar belakang: *Penelitian epidemiologi pada pekerja kelistrikan yang terpajan medan elektromagnet (EMF) memperlihatkan terjadi peningkatan risiko leukemia, limfoma dan tumor otak. Hasil penelitian terdahulu dengan pajanan medan elektromagnet (1-5 kV) hingga 4 generasi mencit menimbulkan kelainan morfologi dan tumor. Penelitian ini bertujuan untuk mengetahui apakah pajanan dengan medan elektromagnet terus menerus dengan tegangan 3, 4 dan 5 kV pada mencit berimplikasi terhadap rusaknya kromosom yang dideteksi sebagai pembentukan kromosom double minute.*

Metode: *Empat pasang mencit galur Swiss Webster umur 3-4 bulan, dipajankan terhadap EMF 3, 4, dan 5 kV, dan satu pasang diambil sebagai kontrol tanpa pajanan. Pembentukan double minute diperiksa pada semua turunan, kecuali satu pasang untuk dipajan seperti di atas untuk mendapatkan generasi F2, dan F3 . Dua puluh metafase kromosom diperiksa, dan frekuensi double minute dihitung pada tiga generasi semua kelompok.*

Hasil: *Frekuensi double minute pada mencit F1, F2, dan F3 yang dipajan EMF 3 kV adalah berturut-turut (0,78±0,08; 0,83±0,09; dan 0,80±0,05). Pada pajanan 4 KV (0,083±0,11; 0,73±0,03; dan 0,96±0,15), dan 5 kV (0,96±0,25; 0,75±0,05; dan 0.99±0.33), sedangkan pada kelompok kontrol tidak ditemukan. Frekuensi double minute pada mencit yang dipajan dengan EMF lebih tinggi secara bermakna dibandingkan kontrolnya.*

Kesimpulan: *Pajanan medan elektromagnet terus menerus dengan tegangan 3 kV, 4 kV, dan 5 kV selama tiga generasi menyebabkan peningkatan perubahan pada kromosom yang menghasilkan double minute.* ***(Med J Indones 2011; 20:109-13)***

Abstract

Background: Epidemiological studies indicate increased risk of leukemia, lymphoma, and brain tumor among electrical workers exposed to electromagnetic field (EMF). Other investigator reported that continuous exposure of four successive generations of mice to EMF in doses of 1 kV to 5 kV caused tumor formation in offspring. The objective of this study was to evaluate the effect of continuous exposure of three successive generations of mice (Mus musculus L) to EMF of 3 kV, 4 kV, and 5 kV and its implication of chromosomal breakage, as detected by double minute formation.

Methods: Four couples of mice of Swiss Webster strain, 3-4 months of age, and 7-40 gram of body weight were exposed to EMF at the doses of 3 kV, 4 kV, and 5 kV, and one couple served as control. Double minute formation was examined in all offspring, except one couple of each group to be exposed with the same doses of EMF to get the F2 generation, and so forth until F3 generation. Twenty metaphases of chromosomes were examined and frequencies of double minute were calculated in the three generations of all group.

Results: Frequencies of double minute in F1, F2, and F3 of mice exposed to EMF of 3 kV were respectively 0.78±0.08; 0.83±0.09; and 0.80±0.05. In the 4 kV group were 0.083±0.11; 0.73±0.03; and 0.96±0.15, and in the 5 kV group were 0.96±0.25; 0.75±0.05; and 0.99±0.33, whereas no double minute chromosomes were noted in control group. Frequencies of the double minute in mice exposed to EMF were significantly higher than control group.

Conclusions: Continuous exposure of mice during three successive generations to EMF at doses of 3 kV, 4 kV, and 5 kV causes increased chromosomal breakage as detected as double minute chromosome formation. ***(Med J Indones 2011; 20:109-13)***

Key words: *chromosomal breakage, double minute, electromagnetic field, mice*

Modern civilization exposes humans, animals, and other organism to extremely complex surrounding electric and magnetic field. Studies during past decade suggest that both electric and magnetic fields, either alone or in "electromagnetic" combination, may induce biological effects in living organisms that could have untoward consequences, even though the fields are generally considered to be innocuous. Several household appliances like microwave ovens, television, cellular phone, etc make people aware of not to be intentionally exposed to certain frequencies of complicated electric fields. Electric and magnetic fields cannot be felt by human senses, because human physiology is not equipped with receptors and neural structures capable to detect or even measure such environmental fields, so that they will be exposed unintentionally.[1] Actually, humans, like other organisms, are continuously exposed to earth electromagnetic fields (EMF) since the beginning of existence of life and evolution. The strength of such EMF ranges from 100-500 V/m and the strength of magnetic fields from 0.004-0.007 μT.[2]

Correspondence email to: puji_sari@yahoo.com

Epidemiological evidence was obtained in the USA during 1950 until 1982 from mortality analysis in workers employed in occupations with intuitive exposure to high voltage EMF.[3] These workers showed a significant increase in proportionate mortality ratios (PMR) associated with leukemia and non-Hodgkin lymphomas. Moreover, leukemia incidence was 2-3 times higher than among people who lived faster away from the EMF power lines.[3] Exposure to extremely low frequency (ELF) and magnetic fields of 5-500 μT for 2 hours per day for two years was found to cause skin cancer.[4] Two decades ago, Brain and Pool reported increasing risk of leukemia, lymphomas, and brain tumors among children who live close to power lines or among men whose jobs expose them to unusually high voltage.[5, 6]

A Canadian study found that occupational exposure of pregnant woman to magnetic fields may be related with leukemia of their children in two pooled retrospective studies. Increased risk of childhood leukemia was found associated with exposure to magnetic fields above 0.3 or 0.4 μT, respectively.[7-9] These findings support the hypothesis that electric and magnetic fields are carcinogenic. Epidemiological studies show links between exposure to EMF and cancer, especially leukemia's, lymphomas, and brain tumors. According to the International Agency for Research on Cancer electromagnetic fields probably play a role as a carcinogenic factor in humans.[10] The biological mechanism by which human cancer could be induced by EMF is unknown. Nevertheless, experimental studies in animals suggest causal relationship.[11]

Congenital anomalies in the offspring were found after exposing the testes of adult male rats to electrostatic fields of 6 and 7 kV for one hour daily during one month,[12] while other investigation reported that electrostatic fields increased proliferation and chromosomal aberration of lymphocytes.[13]

Continuous exposure of four successive mouse generations to EMF showed increased mortality rates and congenital anomalies in their offspring. Usually, life expectancy of offspring suffering anomalies or tumors was only 5 – 6 month as compared to 2 – 2.5 years of normal mice.[14] Further investigation has been done by Lai and Singh on the brain cells of rats exposed to EMF in which DNA stand breaks were significantly increased probably caused by the generation of free radicals.[15, 16]

Double minute chromosomes are small a centric fragment as cellular proto-oncogene amplification, which become progressively oncogenic.[17] In connection with the formation of tumors the question was raised whether continuous exposure to EMF will affect chromosomal alterations in mice. The purpose of the present study is to evaluate the effects of continuous exposure to EMF of 3, 4 and 5 kV respectively, on chromosomal breakage and its implication to double minute formation in three successive generations of mice.

METHODS

Four couples of mice (*Mus musculus* L) of Swiss Webster strain, 3-4 months of age, and 7-40 gram of body weight were kept in a controlled environment and fed a standard diet. Three couple were exposed to EMF at the doses of 3 kV, 4 kV, and 5 kV, and one couple served as control. Twelve plastic cages (26 x 20 x11 cm; length x width x height) with metal wire cages tops were used for one couple of mice, each. The cages of experimental mouse groups were then put on negative terminal plate (120 x 34 cm) of a pair of parallel aluminum plate electrodes perpendicular to positive electrode (120 x 34 cm) at a distance of 10 cm. Subsequently, the electrodes were connected to an alternating current power supply. All mice belonging to experimental groups were allowed to mate, gestate, and deliver the F1 generation. At maturity, the mice from F1 generation were similarly allowed to mate, gestate, and bear their offspring of the F2 generation while being continuously exposed to EMF. Couples from the F2 generation were then mated to produce F3 generation. A parallel procedure was followed for the controls, however without EMF exposure. Parent mice were placed in individual cages and remained with their offspring until weaning at about 4 weeks after birth.

In all offspring, except one couple to be exposed to EMF to obtain next F2, and F3 generation, bone marrow was aspirated from foot and thigh bones after the mice had been sacrificed. Haemopoetic bone marrow cells were cultured or a period of 72 hours at 37° C; mitosis was stopped two hours before harvest. Chromosomal analysis was carried out after fixation with Carnoy fixative solution and subsequent staining with Giemsa. Twenty metaphases of chromosomes were examined and frequencies of double minute calculated in three successive generations of mice in each group of EMF exposures. The frequency of double minutes was then analyzed using two-way ANOVA with Duncan test.

RESULTS

Data obtained in control group with score zero were transformed by using $X = \sqrt{Y + 0.5}$ to enable statistical analysis.[18] The frequency of double minute formation in F1, F2, F3, after exposure of EMF is shown in Table 1.

The higher frequency (compared to control) of double minute in mice exposed to EMF of 4 kV or 5 kV were

found in F1 and F3, but not in F2. Whereas in group exposed to 3 kV, the significant different from control group was found in F2, but not in F1 and F3.

From our experimental setting we obtained two sets of results, one on the variation of strength of EMF and another one on the three successive generations. Table 2 represents the average double minute frequency in groups exposed to 3 kV, 4 kV, and 5 kV of EMF by grouping all generation. Significant different were found between group of 4 kV and 5 kV compared to control group.

Table 3 represents the average double minute frequency F1, F2, and F3, when doses of exposure were grouped and averaged. Significant differences were found between generations F1 and F3 (but not F2) compared to control group.

Concerning the three successive generations, frequency of double minute appears generally lower in F2 than in F1 and F3 generation.

Table 1. Mean frequency of double minute in three successive generations of mice after continuous exposure to electromagnetic fields (values in control grouped were transformed to enable statistical analysis).

Treatment	Generation		
	F1	F2	F3
Control	0.71 ± 0.00	0.71 ± 0.00	0.71 ± 0.00
3 kV	0.78 ± 0.08	0.83 ± 0.09*	0.80 ± 0.05
4 kV	0.83 ± 0.11*	0.73 ± 0.03	0.96 ± 0.15*
5 kV	0.96 ± 0.25*	0.75 ± 0.05	0.99 ± 0.33*

Data from 5 replicates; * = p<0.05 vs. control group.

Table 2. Average frequency of double minute in all generations of mice after continuous exposure to electromagnetic fields.

Treatments			
Control	3 kV	4 kV	5 kV
0.71±0.00	0.80±0.07	0.84±0.14*	0.90±0.25*

* = p<0.05 vs. control group

Table 3. Average frequency of double minute in three successive generations of mice after continuous exposure to electromagnetic field. Frequencies were taken from mean values of exposure to 3 kV, 4 kV, and 5 kV exposure.

Generations		
F1	F2	F3
0.86±0.16*	0.77±0.07	0.92±0.24*

* = p<0.05 between groups

Figure 1. Demonstrates double minute formation (Fig. 1A) compared to normal mouse chromosomes (Fig. 1B).

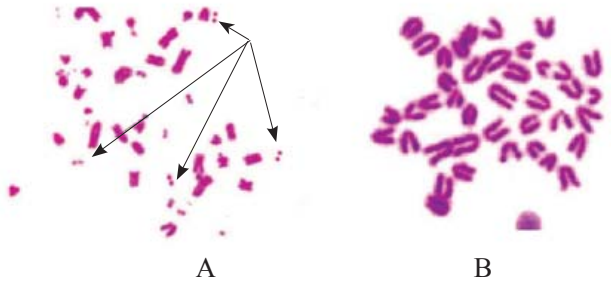

Figure 1. Double minute is shown in A, and normal mouse chromosomes in B

DISCUSSION

In this study the control group mice (with no exposure to electromagnetic fields) from generations F1 to F3 generations did not form double minute chromosome. This means that the double minute is not formed on normal cells without any treatment. This is different from the mice exposed to EMF at the doses of 3 kV, 4 kV, and 5 kV from generations F1 to F3 generation, in which double minute chromosomes can be formed, although not all exposure lead to large quantities of double-minute formation. Although not all double minute formation between EMF doses group show significant difference, it is clear that EMF exposure in Swiss Webster strain mice was associated with significantly higher double minute formation as compared with the control group.

It might be possible that exposure to EMF induces strong recovery mechanisms in F2 which transiently protect this generation from chromosomal alterations. Further exposure to EMF, especially at 4 or 5 kV over rules these protective mechanisms and induces extensive double minute formation.

Usually, double minute can be found in cancer cells associated with the amplification of oncogenes. Gene amplification is common in many tumors including neuroblastoma, squamous cell carcinoma of the head and neck, and malignant glioblastoma of the brain.[17] Although double minute is rarely found in leukemia, several studies reported the frequency vary from 1% to 12.5% and double minute frequency found in myelodysplastic syndrome was around 7 or more per 100 metaphases.[19, 20] In general, existence of double minute in leukemia is caused by amplification of oncogene c-MYC.

The results of a study by Obe and Vijayalaksmi conducted in animals, cell culture, and human lymphocytes exposed to extremely low frequency of electromagnetic fields (ELF), 46% of the researchers

said there was no increase in DNA damage such as chromosome aberrations, micronucleus, and sister chromatic exchange, double minute, while another 32% stated an increase in DNA damage.[21] Further studies using cell culture exposure mice with high frequency electromagnetic field 2.45 GHz for 2 hours did not induce chromosome aberrations.[22]

The result from Magnussen study, concluded from various studies on the effects of electromagnetic fields, that electromagnetic fields can trigger changes in membrane signal that involves changes in gene expression and in turn there is a change in DNA repair. Accumulation of DNA damage repair caused chromosome damage, thus increasing the risk of cell death such as diseases that are directly related to Alzheimer's and cancer. If damage occurs on chromosome fragile sites will be promoting gene amplification, which is then referred to as double minute.[23] Chromosome double minute chromosomes are structures that do not have a centromere and telomere. It is known that if there is a fault on the segment of chromosome arms (chromosome breakage), then the normal chromosome in the cell will make the settings again (rearrangement) which can be either stable or not. In the rearrangement process, centromere and telomere take roles.[24] Telomere are nucleoprotein structures that contain DNA sequences, which among other functions to protect chromosome ends, protect chromosomes from fusion, as well as maintain the integrity of chromosomes during division cell.[16,25] Centromere is part of the DNA in chromosomes associated with several specific proteins to form kinetokor. Kinetokor functions, among others, to attach chromosomes to microtubules and regulate chromosome movement during mitosis.[16, 26] In this case, the double minute chromosomes are structures that do not have a centromere and telomere. Thus, double minute can not maintain the integrity of chromosomes during mitosis. In the absence of the centromere, the double minute can not do the segregation at the time of gamete formation.[26]

Our data indicate that continuous exposure to EMF of 3 kV, 4 kV and 5 kV may not always evoke the formation of chromosomal alterations in each generation of mice, depending on the strength and frequency of exposure. However, it suggests that continuous exposure may cause double minute chromosomes of considerable clinical relevance to carcinogenicity and mutagenicity.

It is concluded that continuous exposure to electromagnetic field may cause chromosomal alterations, namely double minute, with the frequencies depends on the strength and the length of exposure.

Acknowledgments

We thank University of Indonesia for the Research Grant to the Doctoral Program that was delivered by Directorate of Research and Public Services and the Department of Biology at the Faculty of Medicine UI for providing laboratory facilities. Furthermore, we thank Prof. H.-J. Freisleben for his help in writing the manuscript.

REFERENCES

1. Lunquist, S. Biological effects of electromagnetic fields. Royal Swedish Academy of Engineering Sciences IV A-Meddelande 210. 1980, Stockholm. P.10
2. www.elektroindonesia.com/Pengukuran Medan listrik dan medan magnit di bawah SUTET. (Cited 10 September 2006).
3. Deventer, E.Environmental health criteria 238: Extremely low frequency fields. World Health Organization. 2007
4. Tynes T, Klaeboe L, Haldorsen T. Residental and occupational exposure to 50 Hz magnetic fields and malignant melanoma: a population based study. Br Med J. 2003;60:343-7.
5. Brain JD, Kavet R, McCormick DL, Poole C, Silverman LB, Smith TJ, et al. Childhood leukemia: electric and magnetic fields as possible risk factors. Environ Health Perspect. 2003;111:962-70.
6. Cherry N. Evidence that elektromagnetic radiation is genotoxic: The implications for the epidemiology of cancer and cardiac, neurological and reproductive effects. 2000, http://www.feb.se/EMFguru/EMF/genotoxi/ Genotoxic-EMR-paper.html. (Cited 27 November 2007).
7. Infante-Rivard C, Deadman JE. Maternal occupational exposure to extremely low frequency magnetic fields during pregnancy and childhood leukemia. Epidemiology. 2003; 14: 437-41.
8. Ahlbom A, Day N, Feychting M, Roman E, Skinner J, Dockerty J, et al. A pooled analysis of magnetic fields and childhood leukemia. Br J Cancer. 2000;83(5):692-8.
9. Greenland S, Sheppard AR, Kaune T, Poole C, Kelsh MA. A pooled analysis of magnetic fields, wire codes, and childhood leukemia. Childhood Leukemia-EMF Study Group. Epidemiology. 2000;11(6):624-34.
10. www.mindfully.org/Nucs/2002/Non-Ionizing-80-IARC7mar02htm. Static and extremely low-frequency (ELF) electric and magnetic fields (Cited 8 January 2005).
11. Lacy-Hulbert L, Metcalfe JC, Hesketh R. Biological responses to electromagnetic fields. FASEB J. 1998;12:395-420.
12. Soeradi O, Tadjudin MK. Congenital anomalies in the offspring rats after exposure of the testis to an electrostatic field. Int J Androl. 1986;9:152-60.
13. Sari P. Pemajanan medan elektrostatik pada mencit strain Swiss Webster dan pengaruhnya terhadap kromosom serta proliferasi limfosit. Magister Thesis at the Postgraduate program, University of Indonesia, Jakarta, 1998.
14. Soeradi O, Yurnadi, Sari P, Pujianto DA. The effect of continuous exposure to electromagnetic field on four successive generations of mice. Med J Indones. 2002;11:3-10.
15. Lai H, Singh N. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect. 2004;112:1-12.
16. Snustad DP, Simmons MJ. Principles of Genetics. 4th edition. John Wiley & Sons, Inc. 2010. p. 659.
17. Miller OJ, Therman E. Chromosomes and cancer: activation of oncogenes. In. Miller OJ, Therman E: Human chromosomes. 4th edition, 2001. Springer, Berlin. P.405.
18. Stell RGD, Torrie JH. Prinsip dan prosedur statistika. Suatu pendekatan biometrik. Gramedia Pustaka Utama. 2000. p.283.

19. Mathew, S. Double minute chromosomes and c-MYC amplification in a child with secondary myelodysplastic syndrome after treatment for acute lymphoblastic leukemia. Leukemia. 2000. 14 : 1314 – 1331.
20. Von Hoff, D.D., Forseth, B, Clare, C.N., Haneen, K.L., and Van Deventer, D. Double minute arise from circular extra chromosomal DNA intermediates which integrate into chromosomal sites in human HL-60 leukemia cells. J Clin Invest. 1990. 15 : 1887 – 1895.
21. Vijayalaxmi, Obe G. Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency elektromagnetic radiation: A review and future research recommendations. Bioelektromagnetics. 2005;26: 412-30.
22. Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, and Miyakoshi J. Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. Mutat Res. 2005; 587: 114-9.
23. Magnussen T. Key to understanding the EMF issue: Piecing together the EMF puzzle to view the total picture. EMX Corporation. 2006 :1-13.
24. Nussabaum RL, McInnes RR, Willard HF. Principle of clinical cytogenetics. In: Nussabaum RL,McInnes RR, Willard HF, editors. Thompson & Thompson. Genetics in medicine. 6th ed. 2001. WB. Saunders. Philadelphia.
25. Bailey SM, and Murnane JP. Telomeres, chromosomes instability, and cancer. Nucleic Acid Res. 2006; 34:2408-17.
26. Lo AWI, Sprung CN, Fouladi B, Pedram M, Sabatier L, Ricoul M, Reynolds GE, Murnane JP. Chromosome instability as a result of double-strand breaks near telomeres in mouse embryonic stem cells. Mol Cell Biol. 2002; 22: 4833-6.