Determination of the effects of extremely low frequency electromagnetic fields on the percentages of peripheral blood leukocytes and histology of lymphoid organs of the mouse

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ABSTRACT

الأهداف: من أجل تحديد آثار الحقول الكهربائية المغناطيسية للتيار المتردد المنخفض الشديد (٥٠ ميجاهرتز) والضعيف للغاية (إي إل إف– إي إم إف) على وزن الطحال النسبي والنسيج العضوي اللمفاوي وكريات الدم البيضاء المحيطية وألفا– نافثيل الموجب (أيه إن أيه إي – موجب) والنسب المئوية للخلايا اللمفاوية في الفئران.

الطريقة: أجريت الدراسة بمركز العلوم والتطبيق بجامعة سيلكوك بمدينة كونيا بتركيا في عام ٢٠٠٥م. تم تقسيم إجمالي عدد ١٢٠ فأراً من نوع ألبينو السويسري الى ستة مجموعات بحيث يكون في كل مجموعة ٢٠ حيوان. تم تعريض حيوانات التجربة الى ١و٢و٣و٤و٥ من الكثافة الصغرى وتدفق الكثافات للحقول الكهربائية المغناطيسية ذات التردد المنخفض عند ٥٠ ميجا هرتز لمدة أربعين يوماً.

النتائج: في المجموعات المتعرضة والتي تحتوي كل مجموعة على ٢٠ فأر، ازداد وزن الجسم تدريجياً في كثافات الحقل العالي ووصلت في الذروة إلى مستوى ٤ من الكثافة الصغرى ومن ثم تم تخفيضها تدريجياً. لم يتأثر وزن الطحال النسبي (٪ من وزن الجسم). لم تسبب معالجة آثار الحقول الكهربائية المغناطيسية للتيار المتردد المنخفض الشديد أي تغير ملحوظ في الخلايا اللمفاوية والكريات البيضاء الوحيدة و ألفا– نافثيل الموجب (أيه إن أيه إي–الموجب) للخلايا اللمفاوية، بينما تغيرت نسب الكريات المتعادلة والخلايا البيض متألفة الأساس. لم يتم مراقبة أي تغير في النسيج العضوي اللمفاوي المنسوب الى تأثير الحقل في المجموعات المعرضة للتيار.

خاتمة: لم يسبب تعرض آثار الحقول الكهربائية المغناطيسية للتيار المتردد المنخفض الشديد مع تدفق الكثافات بين ١–٥ من الكثافة الصغرى على مدى أربعين يوماً أي أثر على وزن الطحال النسبي والنسيج العضوي اللمفاوي وكريات الدم البيضاء ونسب الخلايا اللمفاوية موجبة ألفا– نافثيل (أيه إن أيه إي – موجب) للفأر تحت ظروف الدراسة الحالية.

Objectives: To determine the effects of very weak, extremely low frequency (50 Hz) electromagnetic field (ELF-EMF) on the relative spleen weight, lymphoid organ histology, peripheral blood leukocyte and alpha-naphthyl acetate esterase positive (ANAE-positive) lymphocyte percentages of the mouse.

Methods: The study was carried out in Scientific Research and Application Center of Selcuk University, Konya, Turkey in 2005. A total of 120 Swiss albino mice were divided into 6 groups (20 in each group). The experimental animals were exposed to 1, 2, 3, 4 and 5 μT flux intensities (rms) of EMF at 50 Hz for 40 days.

Results: In the exposure groups with 20 animals, the body weight (BW) increased gradually in higher field intensities and reached at peak level in the 4 μT, and then slightly decreased. The relative spleen weight (% of the BW) was not affected. The ELF-EMF treatment did not cause any significant change in lymphocyte, monocyte and ANAE-positive lymphocyte ratios, whereas percentages of neutrophils and basophiles changed non-linearly. Any change in the lymphoid organ histology, which is attributable to the field effect, was not observed in the exposure groups.

Conclusion: Extremely low frequency-EMF exposure with the flux intensities between 1-5 μT for 40 days did not cause any effect on the relative spleen weight, lymphoid organ histology, leukocyte and ANAE-positive lymphocyte percentages of the mouse.

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In the last 30 years, an increasing public concern has accumulated on the possible health effects of extremely low frequency (<300 Hertz) electromagnetic fields (ELF-EMFs) from a wide variety of sources.[1,2] The main ELF-EMF sources of everyday exposure are electrical power transmission and distribution lines (50/60 Hz), electrical substations, indoor medical and industrial appliances. In biological studies, a magnetic field (MF) was termed weak if it is of non-thermal intensity and B ≥0.1 mT (1 G), whereas if B is <0.1 mT it is considered a very weak field. Very weak ELF-EMF effects are referred to in the literature as a thermal, or alternatively, non-thermal field effects.[3] The immune system is mainly dependent on the lymphoid system, which is divided into central, and peripheral lymphoid organs.[4] Spleen is the largest peripheral lymphoid organ. Environmental stressors can induce changes in number of the cells and structure of the cell and tissues of the lymphatic system.[5] There is considerable evidence relating EMF exposure to reduce immune system competence. In the previous studies, non-thermal ELF-EMF intensities have been shown to modify peripheral blood leukocyte counts,[6,7] inflammatory responses,[8] peripheral blood natural killer (NK) cell activity[9] and numbers,[10] CD4 expression[11] and the appearance or weight of primary and secondary lymphoid organs.[12] The alpha-naphthyl acetate esterase (ANAE) activity in peripheral blood lymphocytes (PBL) is a good marker for T lymphocytes in both peripheral blood and lymphoid tissues of the mouse.[13] Because that the lack of the detailed information on the long term effects of the very weak ELF-EMF, this study was designed to examine the effects of 40 days exposure on the body weight and relative spleen weight, histology of the lymphoid organs, percentages of the peripheral blood leukocyte and ANAE-positive lymphocyte.

Methods. ***Animals and animal husbandry.*** A total of 120 (60 males and 60 females) 20-day-old Swiss albino mice were used in the experiments. After one-week habitation period, the animals were divided into 6 groups as controls (sham-exposed), 1, 2, 3, 4 and 5 µT exposure groups, (20 animals in each group) (10 males and 10 females) and maintained under a 12-hour light and 2-hour darkness schedule (light 06-18.00 hours) at 21-23ºC, and 50+5% relative humidity. Both controls and experimental groups were housed in a completely non-metallic environment. The number of animals appropriate to each cage (10 each) were fed with a commercial mouse food and watered ad libitum. The experimental groups exposed continuously to different flux intensities of ELF-EMF at 50 Hz, for 40 days. Control animals were maintained under the similar experimental conditions, except inducing MF. Scientific and Ethics Committee of the Experimental Medicine and Application Centre of Selcuk University, Turkey approved all manipulations performed in this study.

Magnetic field exposure system. Extremely low frequency-EMF densities were measured and monitored with a wide band, high sensitivity (one nT at 2 µT, 10 nT at 20 µT) AC magnetometer (Walker Scientific, BBM-3D Model, Walker Scientific Inc, Rockdale Street, Worcester, Massachusetts 01606, USA). A homogenous MF was created by using 6 solenoid coils similar to that reported by Mevissen et al.[14] The coils were consisted of a single 300 turns of cooper wire on a cylindrical plastic core with 32 cm diameter. A cylindrical plastic cage in 30 cm diameter was located into the centre of each coil. The coil systems were settled in the laboratory in which ambient EMF was less than 10 nT. Electric power for coil system was supplied with 50 Hz alternating current, which was taken from local 220 V power network via voltage regulating power transformer (SR Servo-Matik Transformatör ve Regulator Sanayii, İstanbul/Turkey). Coarse adjustment of EMF densities were carried out with a dimmer circuit connected to the primary of a step-down transformer, which was powered by the regulating power transformer; moreover, fine adjustment was carried out with a mechanical rheostat connected to the output of the step-down transformer. Magnetic field densities were measured in x, y, and z directions. Root mean square (rms) was calculated with the formula given below.[15]

$$B = \sqrt{B_x^2+B_y^2+B_z^2}$$

Local temperature variations inside the cages were periodically measured with a digital multi meter (Maxcom, MX 250TX) equipped with a high sensitivity positive temperature coefficient probe and no temperature variations occurred in the cages through the experiments.

Histological procedures. At the termination of the exposure period, the animals were weighed with a digital scale (Sartorius PT-100) and following euthanized by ketamine hydrochloride over dose (Ketanes®, Alke, Turkey). After gross morphological examination, cardiac blood and tissue samples were taken. From each animal, 2 mL of cardiac blood sampled into heparinized (10 IU Heparin mL-1 of blood) tubes for T-lymphocyte specific ANAE demonstration and May-Grünwald-Giemsa staining. Each spleen was excised in toto and weighed. Tissue samples from spleen, thymus and ileum with Peyer's patches were taken and divided into 2 pieces. One piece of each tissue sample was fixed in 0.1M phosphate buffered (pH 7.4) formal-saline for 24-hour, and following dehydrated in a graded series of ethyl alcohol and embedded in paraffin blocks for sectioning. The 6-µm thick sections were stained with Crossman's trichrome.[16] The other pieces of the samples

were fixed in formal-sucrose fixative for 24-hours and following, the pieces were kept in Holt's solution for additional 24-hours. Frozen sections were taken with cryostat (Slee, London) and ANAE was demonstrated in the sections. From the body and spleen weight data, relative spleen weight of each animal was calculated and expressed as a percentage of the animal's body weight (BW%).

Demonstration of ANAE in blood smears and frozen sections. From each blood sample, 4 smears were prepared and fixed in glutaraldehyde-acetone for 3 minutes at -10°C. Two of the smears were stained with May Grunwald-Giemsa. In the remaining blood smears, ANAE was demonstrated histochemically.[18] The ANAE incubation solution was freshly prepared by mixing 40 mL of 0.067 M phosphate buffer (pH 5.0) and 2.4 mL of hexazotized pararosaniline (Sigma, color index number 42500), following 10 mg of alpha-naphtyl acetate (Sigma, N-8505) and dissolved in 0.4 mL acetone. The final pH was adjusted to 5.8 with 2N NaOH. Both the blood smears and frozen sections were treated with the freshly prepared incubation solution. The smears were incubated for 4 hours; the sections were incubated with controlled periods at 37°C. Following the incubation, the slides were washed in distilled water and stained for nuclei with 1% methyl green (Merck, color index number 2585) in 0.1 M acetate buffer pH 4.2. The slides were dehydrated through the alcohol series, cleaned in xylene and mounted with a synthetic mounting medium. The lymphocytes in both smears and sections having 1-3 reddish-brown cytoplasmic granules were scored as positive for ANAE (ANAE-positive). The percentage of ANAE-positive lymphocytes was determined for each blood smear by counting at least 200 lymphocytes.

Statistical analyses. The data was expressed as mean±SD and analyzed using one-way Analysis of Variance. The significance of differences between control and exposed groups were further analyzed using Duncan's test. The difference between these groups yielded a significant p value of less than 0.05. In all analyses, Statistical Package for Social Sciences Version 10.0 for Windows software was used.[17]

Results. The animals of both genders regularly gained body weight through the experimental period without any health problem. Tumor formation was not observed in any of the internal organs at necropsy. Male animals had larger body weight than females. Mean body weight of the experimental animals was higher than that of the controls; it gradually increased with the increase of field intensity, gained a peak level at 4 µT exposure group and then slightly decreased (**Figure 1**). The females had larger spleens than the males and it was at the highest level in 4 µT exposure group. There was no significant difference between the relative spleen weights of the groups when both genders were taken into consideration. The value decreased in 1 µT group, whereas in 2, 3 and 4 µT field the levels increased and regressed in the 5 µT exposure group (**Figure 2**). In the control animals, lymphocyte ratio had the highest level (58.5%), and it was followed by neutrophils (29.8%), monocytes (10.3%), eosinophils (5.4%) and basophils (1.6%). The ELF-EMF treatment did not result any significant change in lymphocyte, monocyte and ANAE-positive lymphocyte ratios, whereas percentages of neutrophil and basophils changed nonlinearly with the increase of field intensity (**Figure 3**). The ANAE-positive peripheral blood lymphocyte

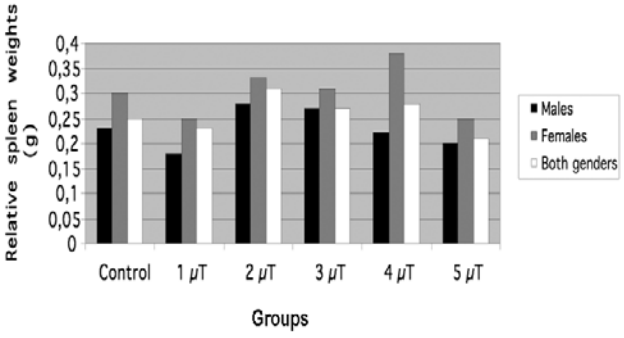

Figure 2 - Mean relative spleen weight of the groups.

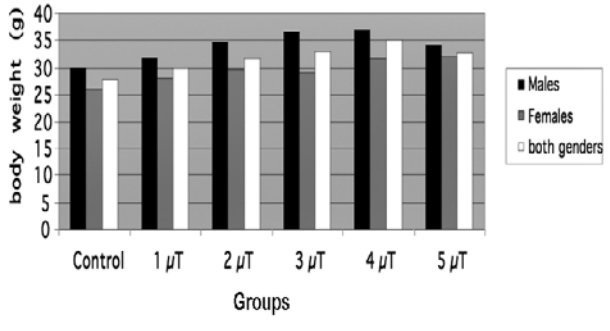

Figure 1 - Mean body weight of the groups.

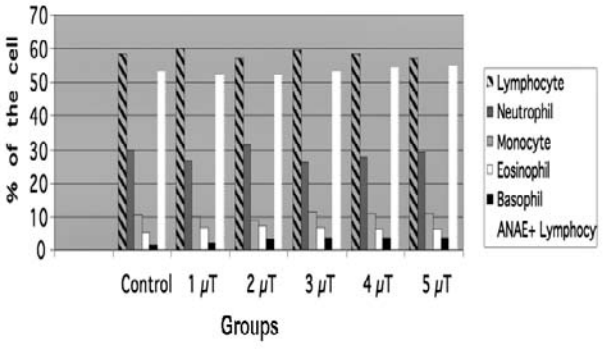

Figure 3 - Leukocyte and alpha-naphthyl acetate esterase-positive lymphocyte percentages of the groups.

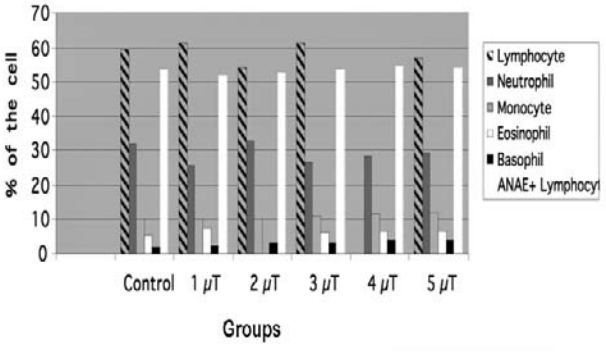

Figure 4 - Leukocyte and alpha-naphthyl acetate esterase-positive lymphocyte percentages of the males.

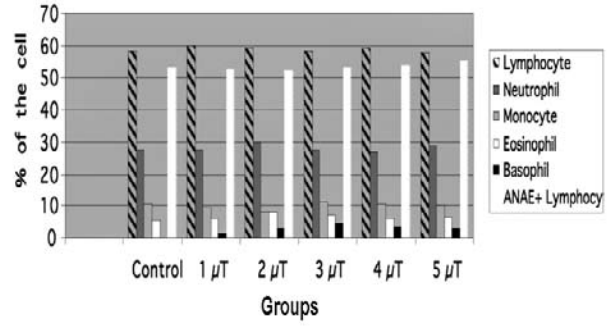

Figure 5 - Leukocyte and alpha-naphthyl acetate esterase-positive lymphocyte percentages of the females.

frequency (53.4%) of the control animals was superior to that of the ANAE-negative lymphocytes. Both males and females responded to the ELF-EMF in a similar manner, and gender differences were significant in the treatment groups (**Figures 4 & 5**). Light microscopically investigated lymphoid organs from exposed animals displayed quite normal histology similar to those of the control animals, and any change attributable to the field effect was not observed. Alpha-naphthyl acetate esterase reaction product in the ANAE-positive lymphocytes were observed as 1-3 reddish-brown granules located beneath the cell membrane, whereas monocytes displayed diffuse granular cytoplasmic staining. The ANAE-positive lymphocytes in the sections of thymus, spleen and ileal Peyer's patches displayed similar staining features to those of the peripheral blood lymphocytes. They mainly located in periarteriolar lymphoid sheets (PALS) and marginal zone of the splenic follicles (**Figure 6**), in the medullar region of the thymic lobes (**Figure 7**), and in the dome area and germinal centres of ileal Peyer's patches (**Figure 8**). Cells of the monocyte/macrophage series displayed similar localization to the ANAE-positive lymphocytes.

Discussion. The effects of experimentally induced 50 Hz ELF-EMFs fields were investigated on the relative

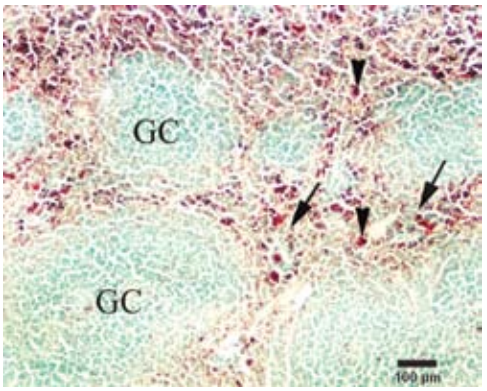

Figure 6 - A section from the spleen of a male animal 5 µT field-exposure group. Alpha-naphthyl acetate esterase (ANAE)-positive cells are mostly periarteriolar located (arrow) and inter follicular area, germinal centers (GC) are devoid ANAE-positive cells. Larger cells (arrowheads) are megakaryocytes. ANAE staining, magnification scale: 100 µm.

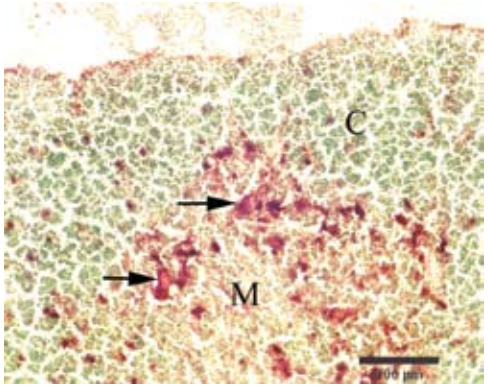

Figure 7 - A section from the thymus of a male animal from 5 µT field-exposure group. Alpha-naphthyl acetate esterase (ANAE)-positive cells are mainly located in the medulla (M), cortex (C) contains less number of the cells. Arrows show the thymic corpuscles. ANAE staining, magnification scale: 100 µm.

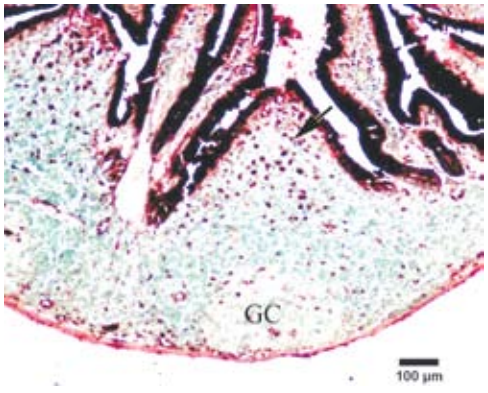

Figure 8 - A section from the ileal Peyer's patch of a female animal from 2 µT field-exposure group. Dome area (arrow) is highly populated with alpha-naphthyl acetate esterase (ANAE)-positive cells. Germinal center (GC) and diffuse lymphoid tissue are relatively lack of the positive cells. ANAE staining, magnification bar: 100 µm.

spleen weight, lymphoid organ histology, peripheral blood leukocyte and alpha-naphthyl acetate esterase positive (ANAE-positive) lymphocyte percentages of the mouse due to the very weak 50 Hz ELF-EMFs fields that we mostly encounter in daily life. Exposure period was 40 days, which is equal to approximately 1/18 of a 2-year-life span of the mice. Field intensities of 1, 2, 3, 4 and 5 μT (rms values) were chosen since public exposure levels to 50 Hz ELF-EMFs were arrange between 0.01-1000 μT. The levels around the home are generally in the range of 0.01-0.25 μT. For homes near power lines, these levels may be as high as 0.5-1 μT.[18] Nevertheless, immediately under the power line, MF intensities of 6-10 μT may be found.[19] Values of up to 12 μT may occur intermittently in rooms heated using electric oil heaters as well as peak levels of 1-30 μT at 30 cm distance from various appliances.[20] However, some (3.0 cm) appliances such as electric blankets, hair dryers, shavers and magnetic mains voltage stabilizers, the magnetic flux intensity can approach to levels of 100-1000 μT. The main problem with many household appliances is that they often run very close to the body.[21] The growing body of evidence suggests that EMFs affect a number of cellular structure and activities. Many reports were published during the 1980's, implying potential mutagenic, teratogenic and carcinogenic effects of EMFs, sometimes with contrasting results; no study has established unequivocally a causal relationship between EMFs and cancer to date.[22] Ornithine decarboxylase (ODC),[23] Na+/K+-pump,[24] quantity of RNA transcripts and protein synthesis,[25] cell surface,[26] melatonin-linked endocrine mechanisms, calcium influx,[27] have been reported as the more sensitive properties. Nevertheless, a satisfactory explanation for the influence of EMF on the cell cycle cannot be given.[28] Results of the present study have showed that exposure to 4 and 5 μT intensities of ELF-EMF increased the mean body weight of the groups. This might have arisen from an increase in the growth and division of the cells. Nevertheless, ELF-EMF exposure did not cause any significant change in lymphocyte, monocyte and ANAE-positive lymphocyte ratios, whereas neutrophil and basophil percentages of different exposure groups changed nonlinearly. Gender differences were not significant in response to the field effect. Any change in the histology of the lymphoid organs of the field-exposed animals was not observed in the present study. Nevertheless, Tremblay et al[29] reported that the numbers of T and B-cell sub-populations decreased after 42 days in rats exposed to 20-160 μT intensities of EMF. For unapparent reasons, however, the results of similar studies have often differed markedly from one another. It had generally been assumed, in the studies, that EMF effects would exhibit a dose-effect relationship, which is a basic property of linear systems. However, Marino et al[30] found nonlinear changes in their study with 60 Hz 500 μT field, and any effect due to the field was not found when the analysis was restricted to linear relationships. They assumed that only a nonlinear approach could explain their data. Prompted by recent developments in the theory on nonlinear systems, the researchers[30] hypothesized that there was a nonlinear relationship between EMFs and the effects they produced in the endocrine and immune systems. Consequently, Marino et al[30] have strongly stress that the existence of nonlinear physiological changes due to EMFs may necessitate reevaluation of assessments of potential public-health risks that were based on linear effects. Although MF intensities were not given, Shafey et al[4] showed that the embryonic spleen was the most sensitive lymphoid organ of birds to the exposure of 30 kV/m at 60 Hz electric fields. The researchers[4] have reported that the electrical field significantly increased relative spleen size. In a previous study, Mukewar and Baile[31] found significant increase in spleen weight due to the enlargement of leukocytes in the pulp of the spleen. However, in the present study, the field effect did not induce any change in the relative spleen weight of the exposure groups when both genders were taken into consideration. Although the results of the present study are far from giving evidence for possible action mechanism of ELF-EMF, the cell membrane might be one of the targets and specifically that process involving the receptor-ligand interaction and the ion channels.[32] When direct effects of EMFs are considered, the type of dosimetry needed, depends upon the nature of the field. One mechanism, which probably requires alternating magnetic flux intensities at least 100 μT involves free-radical reactions.[33] Two other mechanisms, however, are in principle applicable at lower ELF fields, down to 10 μT and possibly lower. One of them interacts via magnetic crystals found in the tissues;[34] the other model is that of ion parametric resonance.[35] Results of the present study showed that exposing for 40 days to 1, 2, 3, 4 and 5 μT intensities of ELF-EMFs at 50 Hz increased body weight gain, whereas the fields did not cause to any significant effect on the lymphoid organs and cells of the mouse, under the circumstances of the present study.

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