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Total Antioxidant Capacity, Total Oxidant Status and Oxidative Stress Index in Rats Exposed to Extremely Low Frequency Magnetic Field

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The aim of this study is to investigate the effects of a low frequency (50 Hz), low intensity (0.25 mT) magnetic field on oxidative stress of male rats. The effects of a 0.25 mT, 50 Hz magnetic field on the total antioxidant capacity, total oxidant status and oxidative stress index in male rats were investigated. In this study, 45 male rats were introduced and were divided into three groups as sham, control and experimental group. Experimental group was exposed to 50 Hz, 0.25 mT magnetic field for 14 days, 3h/d. Total antioxidant capacity, total oxidant status and oxidative stress index levels of each subject was determined. Total antioxidant capacity in exposed group was found to be decrease. But, this decrease was not statistically significant. Total oxidant status and oxidative strress index levels in the exposed group were found as increased. But, this increase was not statistically significant ($p > 0.05$). Control group and sham group; sham group and exposed groups were compared with each others. Statistical analysis was done by Mann-Whitney U test using SPSS 11.5 program in computer. The magnetic field generated in environmental magnetic field and by our home appliances and by industrial electrical apparatus and caused a few changes in oxidative system of rats. But, this change is not statistically significant.

Key Words: Weak magnetic field, Oxidative stress index, Total antioxidant capacity, Total oxidant status.

INTRODUCTION

In modern societies, humans are frequently exposed to magnetic fields, including extremely low frequency (< 300 Hz), low intensity (microtesla and militesla) and high intensity (Tesla) magnetic fields (MF). Low intensity magnetic fields are generally produced by power lines and many kinds of electrical appliances. High intensity magnetic fields are produced by the apparatus such as magnetic resonance imaging (MRI) equipment.

Several experimental and biological studies have found an association between low intensity magnetic field exposure and the increased incidence of various types of cancer, including childhood leukemia, lymphomas, brain tumours and breast cancers and some biological problems[1-3]. However, other studies have not found such associations and so any potential link remains the subject of debate[4-6].

Several *in vivo* and *in vitro* investigations claim that extremely low-frequency magnetic field produced a genotoxic effect, originating from types of free radicals[7-11]. The radical pair mechanism was suggested as a possible way[12]. For biological effects of free radicals, especially reactive oxygen species (ROS) may produce cellular and toxic effects such as lipid peroxidation in cell membrane, protein degradation, enzyme inactivation and damage to DNA[5].

Free radical species and their concentration in extremely low frequency magnetic fields have been investigated with different methods and approaches. One of the proposed mechanisms of free radical suggests that static magnetic fields may interact with the living organism through electronic interactions, that is the radical pair mechanism. Magnetic fields influence the kinetics of reactions with radical pair intermediates[13,14]. An external magnetic field can increase the concentration and diversion of free radicals in living cells[15,16], thereby causing cellular damage[17].

More recent findings have confirmed that 50 Hz magnetic fields at the intensity of 1 mT increase free radical formation in mouse bone marrow-derived promonocytes and macrophages[18]. Oxyradical-mediated effects of electromagnetic fields have been reported in rats exposed to 0.01 mT for 24 and 48 h[19]. Increased damage to DNA was prevented by pretreatment with free radical scavengers. It is suggested that magnetic fields can promote a Fenton-like reaction with formation of hydroxyl radicals, which damage lipids, proteins, DNA and calcium homeostasis[7]. Although extremely low frequency (ELF) magnetic fields have been widely studied under laboratory conditions, their biological effects have not been adequately explained under realistic conditions[1,2].

Regoli *et al.*[20], investigated TOSC (total oxyradical scavenging capacity) by exposing land snails to 2.5, 10 and 50 μT extremely low frequency magnetic field. They observed increased TOSC in the snails exposed to extremely low frequency magnetic field. Lai and Singh[18] reported that acute exposure to a 60 Hz magnetic field caused DNA strand breaks in animal brain cells. Lalo *et al.*[21] and Kubato *et al.*[22] suggested that a steady magnetic field could accelerate lipidperoxidation. Fiorani *et al.*[23] reported that a magnetic field (50 Hz, 0.5 mT) increased the damage in an oxidative stressed rabbit erytrocyte system. These reports suggested that magnetic fields affect biological systems by prolonging the life of free radicals in the systems. Excess oxygen free radicals induce lipid peroxidation, especially in the brain. Thus, the living organism employs defensive systems against free radicals, such as the production of antioxidant enzymes[24].

In spite of much previous investigation, the biophysical mechanism of magnetic field-induced effects is still not clear. Reactive oxygen species are generally non-specific. All cellular components *i.e.*, protein, phospholipids, carbohydrates and nucleic acids may be damaged by reactions with ROS. When these species react with non-radicals, new free radicals can be formed, which leads to chain reactions, *i.e.* lipid peroxidation[25].

Overproduction of oxygen free radicals can give rise to functional and morphological disturbances in the cell through oxidative stress[15]. The increase in production of ROS or a deficiency of the antioxidant defense mechanism may result in reversible or irreversible cell and tissue injury (*e.g.* DNA damage)[25]. Under physiological conditions, the production of ROS is strictly controlled by all antioxidants *viz.*, enzymatic and non-enzymatic. Thus antioxidants protect cells, tissues and organs from oxidative damage in macromolecules (DNA, protein, lipids)[15].

Malondialdehyde (MDA)[16], nitrogen oxide, superoxide[19], peroxidase, catalase and glutathione reductase[26] and glutathione (GSH)[27] had been studied previously in different studies. However, total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) parameters were not previously investigated. Therefore, the aim of this study is to determine TAC, TOS and OSI levels in male rats exposed to 0.25 mT magnetic fields for 14 days.

EXPERIMENTAL

The research was designed and implemented according to the principles of the Declaration of Helsinki. This experimental research was performed with the approval of the ethics committee of the Medicine Faculty of Harran University, Turkey. Forty five male rats were exposed to 0.25 mT sinusoidal magnetic field for 2 weeks.

Spraque Dawley rats were obtained from the Medical Science Application and Research Center of Dicle University. All animals were 2 months old at the beginning of the study, weighing 250-280 g and were fed with standard pellet food (Tavas Inc, Adana, Turkey). The rats were divided three groups of 15: control, sham and experimental groups. The experimental group was exposed to a 0.25 mT extremely low frequency magnetic field in metacrylate boxes (17 × 17 × 25 inches). The experimental group (n = 15) was exposed to EMF for 14 days, 3 h a day. The sham group was treated like the experimental group, except for extremely low frequency-magnetic field exposure. Treatment of the control group differed from the experimental and sham groups. Animals in the control group were kept in a 14/10 h light/dark environment at constant temperature of 22 ± 10 % humidity.

Magnetic field generation and exposure to magnetic field: The magnetic field was generated by a device designed by the researchers that had two pairs of Helmholtz coils of 25 cm diameter. The frequency of sinusoidal current was 50 Hz. This magnet was constructed by winding 300 turns of insulated soft copper wire with a diameter of 0.85 mm. Coils were placed vertically and horizontally, facing one another. The distance between coils was 25 cm. The average magnetic field intensity was measured as 0.25 mT ± 0.01 mT at 12 different points both transverse and axial within the metacarylate cage by using a digital hall effect Gaussmeter (Bell 5170, SYPRIS, USA). The measurements were made by an independent researcher who was not involved in the animal experiment. No temperature difference was observed between exposure and sham coils during the exposure. The animals were euthanized after the final exposure by anesthesia with ketalar (50 mg/kg, intramuscular) and the blood of the animals was withdrawn into ethylenediaminetetraacetic acid (EDTA) containing syringes by cardiac puncture. The blood samples were centrifuged for 8 min at a rate of 5,000 revolutions/min and plasma was separated. Centrifugation was made at a constant temperature of 24 ºC by a cooled centrifuge.

Total antioxidant capacity (TAC) measuring method: The plasma was analyzed in terms of TAC, thiol, ascorbic acid, uric acid, bilirubin, total protein and albumin. The TAC levels were determined by two different and novel automated methods developed by Erel[28]. In the first method, the hydroxyl radical is produced by the Fenton reaction and reacts with the colourless substrate *o*-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in colour. The assay results are expressed in milimole trolox equivalent units per liter and the precision of this assay is excellent-less than 3 %. Total peroxide concentrations of the plasma were determined by the FOX2 methods with minor modifications. The FOX2 test system is based on the oxidation of ferrous ion to ferric ion by various types of peroxides contained in the plasma samples in the presence of xylenol orange, which produces an orange-coloured ferric-xylenol complex whose absorbance can be measured. Aliquots (200 μL) of plasma were mixed with 1.8 mL of the FOX2 reagent.

After incubation at room temperature for 0.5 h, the vials were centrifuged at 12'000xg for 10 min. The absorbance of the supernatant was then fixed at 560 nm. The total peroxide contents of the plasma samples were determined as a function of the difference in absorbance between the test and blank samples with a solution of H_2O_2 used as a standard. The coefficient of variation for individual plasma samples was less than 5 %. Plasma total protein, albumin, uric acid and bilirubin levels were measured using commercial kits (Abbott Laboratories). Vitamin C concentration was measured by the FRASC method. The per cent ratio of the total peroxide to the TAC yields OSI, an indicator of the degree of oxidative stress.

Total oxidant status (TOC) measuring methods: Oxidant in the sample oxidizes the ferrous ion-*o*-dianisidine complex

to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion produces a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules. The results are expressed in terms of micromolar H_2O_2 equivalent per liter. The reading was taken at 450 nm and subsequently, the plate was incubated for 20 min in the dark. Then, the reaction was stopped with 50 µL of 2 N H_2SO_4 and the second absorbance reading was taken at 450 nm. The total peroxide levels of the samples were calculated as the differences between the absorbance readings related to the H_2O_2 standard curve[29].

Oxidative stress index (OSI): The per cent ratio of TOS to TAC was accepted as the OSI, an indicator of the degree of oxidative stress. To perform the calculation, we changed the resulting unit of TAC, millimoles of Troloxs, to micropoles per litre and the OSI value was calculated from the formula:

$$OSI = \frac{TOS\ \mu mol / L}{TAC\ \mu mol\ of\ Trolex} \times 100$$

Data was analyzed using the non-parametric Mann-Whitney U test. The following comparisons were made: control group: sham group and; sham group: experimental group (Exposed group). P values below 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

No statistically significant difference was observed in TAC, TOS and OSI values between the control group, sham group and exposed group ($p > 0.05$). The results are given in Figs. 1-3.

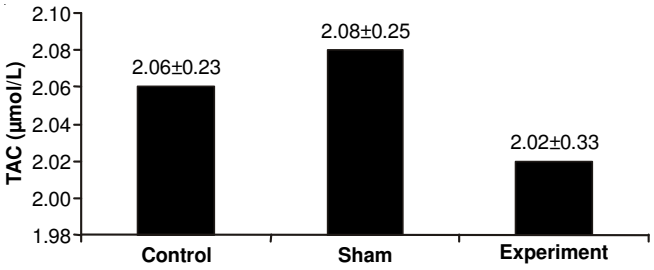

Fig. 1. Total antioxidant capacity of control, sham and experimental group exposed to 2.5 Gauss magnetic field

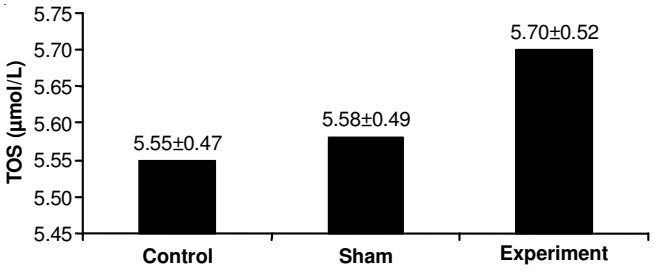

Fig. 2. Total oxidant status of control, sham and experimental group exposed to 2.5 Gauss magnetic field.

Extremely low-frequency (ELF) magnetic field have been the subject of public debate for some time, but whether exposure at low frequency (50 and 60 Hz), low intensity magnetic field

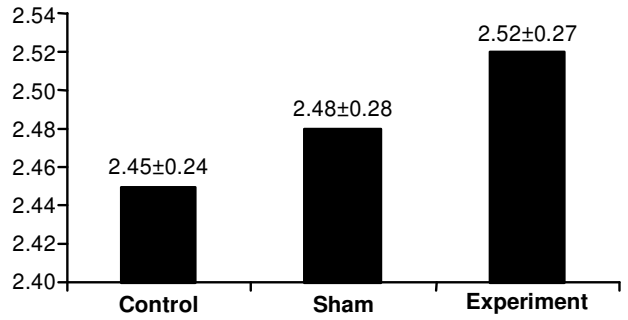

Fig. 3. Oxidative stress index of control, sham and experimental group exposed to 2.5 Gauss magnetic field.

represents a hazard to human health is still open to question. At present, no epidemiological evidence has supported the widely accepted association with cancer processes and the assessment of the biological impact of ELF magnetic field remains a complex issue[4-6]. In this regard, several investigations have been directed toward understanding the cellular-molecular mechanisms by which magnetic fields interact with biological systems, possibly facilitating the initiation of carcinogenesis and the onset of long-term effects[30].

Enhanced pro-oxidant conditions and free radical formation have been suggested in different biological models as important pathways of response induced by EMF, which modulate both the turnover of oxyradicals and cellular effects, including cell proliferation, induction of ROS-generating enzymes, signal transduction processes, modulation of proto-oncogenes and genotoxcicity[19,31].

Various mechanisms have been postulated for oxidative damage, but different pathways can overlap with both indirect and cascade effects and the same pro-oxidant stressor can interact with several targets and with different actions[20,32]. Those studies suggested that in µT and mT levels, extremely low frequency magnetic field adversely affect biological systems at the molecular level.

In cells, free radical concentration is determined by the balance between their rate of production and their rate of clearance, controlled by different enzymes and antioxidant compounds. High level production of free radicals in the organism has shown an increased potential for cellular damage of substances such as DNA, proteins and lipid-containing structures[33]. In contrast to molecules such as cytokines, molecules such as ROS could react with diverse cell compounds in a non-specific mechanism. Therefore, free radicals play a decisive role in cytotoxicity and also as cellular messengers to control non-cytotoxic physiological responses[19]. It is assumed that the activation of NADH-oxidase also plays a major role in phagocytic cells and is responsible for the magnetic field-induced free radical production.

In some studies, free radical species and changes of antioxidant with exposed to magnetic field (50-60 Hz, sinusoidal or constant) were investigated. Rolwitz *et al.*[19] investigated the effects of 50 Hz magnetic field on free radical production, in mouse bone marrow-derived (MBM) macrophages and promonocytes.

Chater *et al.*[27] investigated MDA, SOD, glutathione peroxidase (GSH-Px), Glutathione (GSH) and Catalase in female rats exposed to 128 mT magnetic field 1 h/day for 14 days.

They did not observe any change in the level of MDA, CAT, SOD or GSH-Px; an increase was observed in the level of total GSH.

Jajte *et al.*[16] investigated MDA and oxidative stress-induced necrotic and apoptotic cell deaths but found that the observed changes were not statistically significant.

Amara *et al.*[34] studied changes in the activities of MDA, GSH-Px CAT and SOD in rats exposed to 128 mT. They observed a reduction in GSH-Px, CAT and SOD and an increase in the MDA level.

Lee *et al.*[24] exposed Balb/c mice to 12G magnetic field for 3 h. They observed increased SOD activities in the brain. Piacentini *et al.*[26] reported unchanged peroxide (POD) activities in plant cell (*Cucumis sativus*) exposed to a 1 Gauss magnetic field (1 h/day for 14 days).

Exposure to magnetic fields at levels of Gauss, μT and mT is inevitable, as the tools that are used in our houses, environment and offices produce such a magnetic field. As seen, free radicals and enzymes changed in some studies, but didn't change in some other studies. Furthermore, in previous studies, MDA, GSH-Px, CAT and SOD activities in rats exposured to magnetic field have been investigated. But TAC and TOS have never been investigated. The present authors investigated the changes of TAC, TOS and OSI in male subjects who were exposed to an acute, high intensity static magnetic field[35]. In 1.5 T static magnetic fields, TAC was shown to increase significantly, while TOS and OSI decreased significantly. Another study by the present authors investigated the increase of nitric oxide (NO) concentration in male subjects who were exposed to a 1.5 Tesla static magnetic field[36]. In contrast to our earlier studies, which used a high intensity magnetic field, this present study used an extremely low intensity magnetic field.

In this study, we investigated TAC, TOS and OSI changes in rats which were exposed to 0.25 mT sinusoidal magnetic fields. In the animals which were exposed to this level of magnetic field for 2 weeks, we observed insignificant changes of TAC, TOS and OSI. These findings are important, as these factors indicate total antioxidant defense and oxidative stress. In practical terms, it may be concluded that the magnetic fields to which we are exposed in this level in daily life do a few increase free radical products. But, this increase is not statistically significant. In a magnetic field with intensity higher than 0.25 mT, oxidative stress may increase.

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REFERENCES

1. M. Feytching, U. Forssen and B. Floderus, *Epidemiology*, **8**, 384 (1997).
2. C.Y. Li, G. Theriault and R.S. Lin, *Epidemiology*, **8**, 25 (1997).
3. UK Childhood Cancer Study Investigators, *The Lancet*, **354**, 1925 (1999).
4. J. McCann, F. Dietrich and C. Rafferty, *Mutat. Res.*, **411**, 45 (1998).
5. I.C. Ahlbom, E. Cardis, A. Gren, M. Linet, D. Savitz and A. Swerdlow, *Environ. Health Perspect.*, **109**, 911 (2001).
6. M.J. Crumpton and A.R. Collins, *DNA Repair*, **3**, 1385 (2004).
7. S. Ivanesist, E. Diem, O. Jahn and H.W. Rüdiger, *Int. Arch. Occup. Environ. Health*, **76**, 431 (2003).
8. N. Singh and H. Lai, *Mutat. Res.*, **400**, 313 (1998).
9. N.K. Chemeris, A.B. Gapeyev, N.P. Sirota, O. Gudkova, I.V. Konovalov, M.E. Buzoverya, V.G. Suvorov and V.A. Logunov, *Mutat. Res.*, **558**, 27 (2004)
10. H. Lai and N. Singh, *Environ. Health Perspect.*, **112**, 687 (2004).
11. F.L. Wolf, A. Torsello, B. Tedesco, S. Fasanella, A.D. Boninsegna, M. Ascenzo, C. Grassi, G.B. Azzero and A. Cittadini, *Biochim. Biophys. Acta*, **743**, 120 (2005).
12. B. Brocklehurst and K.A. McLauchlan, *Int. J. Radiat. Biol.*, **69**, 3 (1996).
13. C.B. Grissom, *Chem. Rev.*, **95**, 3 (1995).
14. K.A. Mclauchlan and U.E. Steiner, *Mol. Phys.*, **73**, 241 (1991).
15. J. Jajte, M. Zymslony, J. Palus, E. Dziubaltowska and E. Rajkowska, *Mut. Res.*, **483**, 57 (2001).
16. J. Jajte, J. Grzegorczyk, M. Zmyslony and E. Rajkowska, *Bioelectrochemistry*, **57**, 107 (2002).
17. J.C. Scaino, N. Mohtat, F.L. Cozens, J. McClean and A. Thansandote, *Bioelectromagnetics*, **15**, 549 (1994).
18. J. Rollwitz, M. Lupke and M. Simko, *Biochim. Biophys. Acta*, **1674**, 231 (2004).
19. H. Lai and N.P. Singh, *Bioelectromagnetics*, **18**, 156 (1997).
20. F. Regoli, S. Gorbi, G. Frenzilli, M. Nigra, I. Corsi, S. Focardi and G.W. Winston, *Environ. Res.*, **54**, 419 (2002).
21. U.V. Lalo, Y.V. Pankratov and O.M. Mikhailik, *Redox Rep.*, **1**, 71 (1994).
22. H. Kubato, N. Yokoi, A. Ogawa, A. Mori and R.P. Liburdy, *Pathophysiology,* **7**, 283 (2001).
23. M. Fiorani, B. Biagiarelli, F. Vetrano, G. Guidi, M. Dacha and V. Stocehi, *Bioelectromagnetics*, **18**, 125 (1997).
24. B.C. Lee, H. Johng, T.J. Nam, J.H. Lee, J. Kim, U.D. Sohn, G. Yan, S. Shin and K.S. Soh, *J. Photochem. Photobiol. B: Biol.*, **73**, 43 (2004).
25. Y. Sun, *Free Rad. Biol. Med.*, **8**, 583 (1990).
26. M.P. Piacentin, D. Freternale, E. Piatti, D. Ricci, F. Vetran, M. Dacha and M. Accorsi, *Plant Sci.*, **161**, 45 (2001).
27. S. Chater, H. Abdelmelek, T. Douki, C. Garrel, A. Favier, M. Sakly and K.B. Rhouma, *Arch. Med. Res.*, **37**, 941 (2006).
28. O. Erel, *Clin. Biochem.*, **37**, 112 (2004).
29. O. Erel, *Clin. Biochim.*, **38**, 1103 (2004).
30. A. Lacy-Hulbert, J. Metacalfe and R. Hesketh, *FASEB J.*, **12**, 395 (1998).
31. K. Fernie and D. Bird, *Environ. Res.*, **86**, 198 (2001).
32. F. Regoli, S. Gorbi, N. Machella, S. Tedesco, M. Benedetti, R. Bochetti, A. Notti, P. Fattorini, R. Pivohetti and G. Principato, *Free Rad. Biol. Med.*, **39**, 1620 (2005).
33. J. Abe and B.C. Berk, *Trends Cardiovas. Med.*, **8**, 59 (1998).
34. S. Amara, H. Abdelmelek, C. Garrel, P. Guirand, T. Douki, J.L. Ravanat, A. Favier, M. Sakly and B.R. Rhouma, *J. Trace Elem. Med. Biol.*, **20**, 263 (2006).
35. Ö. Sirmatel, C. Sert, C. Tümer, Z. Ziylan and M. Bilgin, *Bioelectromagnetics*, **28**, 152 (2007).
36. Ö. Sirmatel, C. Sert, F. Sirmatel, S. Selek and B. Yokus, *Gen. Physiol. Biophys.*, **26**, 86 (2007).