

Extremely Low Frequency Electromagnetic Field (ELF-EMF) Reduces Oxidative Stress and Improves Functional and Psychological Status in Ischemic Stroke Patients

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As a result of ischaemia/reperfusion, massive generation of reactive oxygen species occurs, followed by decreased activity of antioxidant enzymes. Extremely low frequency electromagnetic fields (ELF-EMF) can modulate oxidative stress, but there are no clinical antioxidant studies in brain stroke patients. The aim of our study was to investigate the effect of ELF-EMF on clinical and antioxidant status in post-stroke patients. Fifty-seven patients were divided into two groups: ELF-EMF and non-ELF-EMF. Both groups underwent the same 4-week rehabilitation program. Additionally, the ELF-EMF group was exposed to an ELF-EMF field of 40 Hz, 7 mT for 15 min/day for 4 weeks (5 days a week). The activity of catalase and superoxide dismutase was measured in hemolysates, and total antioxidant status (TAS) determined in plasma. Functional status was assessed before and after the series of treatments using Activities of Daily Living (ADL), Mini-Mental State Examination (MMSE), and Geriatric Depression Scale (GDS). Applied ELF-EMF significantly increased enzymatic antioxidant activity; however, TAS levels did not change in either group. Results show that ELF-EMF induced a significant improvement in functional (ADL) and mental (MMSE, GDS) status. Clinical parameters had positive correlation with the level of enzymatic antioxidant protection. Bioelectromagnetics. 38:386–396, 2017.

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INTRODUCTION

For several decades, the biological effectiveness of extremely low frequency electromagnetic fields (ELF-EMF) has been investigated in in vivo and in vitro studies. It has been shown that many cellular systems, components, and processes can be affected by ELF-EMF exposure, such as genotoxicity, cell proliferation, apoptosis, cell cycle regulation and differentiation, metabolism, and various other physiological characteristics [Lee et al., 2015]. The latest study suggests that applied ELF-EMF reduces oxidative stress induced by global cerebral ischaemia and decreases possible negative effects that free radical species could have in the brain [Rauš Balind et al., 2014]. Currently, thrombolysis is the only approved effective treatment in the acute phase of stroke. Therefore, there is a need to develop novel therapies for ischaemic stroke patients, especially in early stages of the disease. Recent evidence suggests that ELF-EMF could be an additional therapy in early post-stroke rehabilitation due to its effects on key

processes involved in brain ischaemic damage and regeneration [Cheng et al., 2015]. Damage induced by strokes cannot be undone. However, immediate and long-term rehabilitation is an important component of treatment for ischaemic stroke patients due to the brain's own plasticity [Pruszyńska et al., 2015; Shahbazi-Gahrouei et al., 2016]. The electromagnetic

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field affects the organism at each level: molecular, subcellular, and cellular. The therapeutic effect on the brain is mainly associated with improvements in neurotransmission—the field increases cerebral blood flow, which contributes to increased metabolism of nervous tissue and has the beneficial effect of regeneration [Basset, 1999; Sieroń et al., 1999; Sieroń et al., 2002; Woldańska-Okońska and Czernicki, 2007]. Oxidative stress is one of the main processes responsible for post-stroke neuronal damage [Abd-Elsameea et al., 2014; Chen et al., 2014; Lee and Won, 2014]. Moreover, the brain is characterized by a relatively low level of antioxidants and high lipid content, and consumes large amounts of oxygen.

ELF-EMF is widely used as an additional therapy in neurorehabilitation, but there is not much data about its effect on oxidative stress and functional status. Reactive oxygen species (ROS) can be generated from many sources, such as oxidation of catecholamines or haemoglobin, when a massive release of iron ions occurs. In addition, ROS are produced as a result of changes in mitochondrial electron transport and activation of xanthine oxidoreductase and NAD(P)H oxidase, as well as during the metabolism of arachidonic acid [Domański et al., 2006]. With ischaemia, there is an increase in enzymatic production of ROS, especially superoxide anion and hydrogen peroxide, in a reaction catalyzed by xanthine oxidase. Then, as a result of reperfusion, molecular oxygen is supplied, and hypoxanthine is converted into xanthine by xanthine oxidase. O_2^- is released in large quantities, which leads to generation of H_2O_2 . Decreased catalase activity then reduces the effectiveness of inactivation of H_2O_2 which is converted into highly toxic hydroxyl radicals [Sims and Anderson, 2002; Broughton et al., 2009].

The human antioxidant defense system is comprised of enzymatic and non-enzymatic components. Low molecular weight antioxidants and antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) protect the organism against both the production and spread of free radicals. Different endogenous antioxidants, such as glutathione, uric acid, and bilirubin, and exogenous antioxidants, such as vitamins C, D, and melatonin, are responsible for total antioxidative status (TAS) [Miller et al., 2010].

Clinical effects in post-stroke rehabilitation of patients using electromagnetic fields are well documented in numerous studies [Jurowska et al., 1997; Kochetkov et al., 2000; Sieroń et al., 2002; Sieroń and Cieślak, 2003; Woldańska-Okońska and Czernicki, 2007]. These include the reduction of previously increased muscle tone, decreased muscle weakness,

improvements in the range of passive and active movements of limb paresis, and reduced aphasia and pain. Also, development of efficiency in walking has been observed in patients after ELF-EMF therapy. Bryl et al. [2004] studied the effect of ELF-EMF on the reduction of somatic symptoms and cognitive improvement in patients with depression. The authors noticed an improvement of mood, increased mobility, a reduction in the escalation of somatic symptoms, and better quality of sleep [Bryl et al., 2004].

A brain stroke results in a high level of oxidative damage, which leads to a decrease in functional status as well as depressive and cognitive impairment [Cichon et al., 2015].

We suspect that the mechanism of action of the electromagnetic field, which improves psycho-motor functions of stroke patients, might be associated with a reduction in oxidative stress. This paper covers the determination of SOD activity, which catalyses the dismutation of O_2^- , and CAT activity participating in the reaction of decomposition of H_2O_2 to water and oxygen. Furthermore, we also examined the effect of ELF-EMF on the total activity of low molecular weight plasma antioxidants.

Our study was designed to investigate the effect of ELF-EMF not only on antioxidant status, but also on cognitive and functional status, as well as depressive syndrome in post-stroke patients.

MATERIALS AND METHODS

Study Subjects

For the study, 57 post-ischaemic stroke patients were enrolled. Patients were randomly divided into two groups: ELF-EMF ($n = 23$) and non-ELF-EMF ($n = 34$). For safety reasons, participants with electronic (pacemakers, etc.) or metal implants were excluded from the ELF-EMF group. In the non-ELF-EMF group, only two patients had pacemakers. The ELF-EMF group had already undergone ELF-EMF therapy. Both the ELF-EMF and non-ELF-EMF groups were seated for the same amount of time—15 min. However, the ELF-EMF patients were exposed to electromagnetic fields with specific parameters (magnetic induction of 7 mT, 40 Hz frequency, rectangular and bipolar pulse shapes), whereas the non-ELF-EMF subjects were only given blind sham exposure. The electromagnetic field was focused on the pelvic girdle.

Both subject groups were on the same therapeutic program consisting of 30 min of aerobic exercise, 60 min of neurophysiological routines, and 15 min of psychological therapy. Ischaemic stroke patients

(study group aged 68.0 ± 15.8 ; control group aged 70.9 ± 15.3) with moderate stroke severity according to National Institutes of Health Stroke Scale (NIHSS) scores of 5.8 ± 3.8 in the study group and 6.2 ± 2.7 in the control group, who consented to participation, were included in the study. Clinical demographic characteristics are shown in Table 1. Patients with a medical history of pre-stroke dementia, haemorrhagic stroke, decreased consciousness, significant acute or chronic inflammatory factors, or neurological illness other than stroke were excluded. The patients followed up at the Neurorehabilitation Ward III of the General Hospital in Lodz, Poland, undergoing neurorehabilitation for 4 weeks, in which time they received no immunomodulators, immunostimulators, hormones, vitamins, minerals, or any other substitutions with antioxidative properties. Prior to the study, all of the subjects had undergone medical checkups, including neurological and internal examinations. Blood samples were collected twice, at an interval of 10 days before and after a standard 10 sessions of ELF-EMF therapy. For additional analysis of TAS levels, blood samples were collected three times, at an interval of 10 days before, after 10 treatments, and

after 20 treatments (Fig. 1). All blood samples were taken in the morning (between 7 am and 9 am) under conditions of patient fasting, and stored using the same protocol. The Bioethics Committee of the Faculty of Biology and Environmental Protection of The University of Lodz, Poland approved the protocol with Decision No. 28/KBBN-UŁ/I/2015. All participants provided written informed consent prior to participation. Depression was screened in both groups using the Geriatric Depression Scale (GDS), a 30-item self-report assessment designed to identify depression in elderly people, which is considered a reliable and sensitive indicator of post-stroke depression [Sun and Murphy, 2010]. A trained psychologist researcher administered the GDS scale, the outcomes of which are described as normal status (0–9), mild depression (10–19), and severe depression (20–30). Cognitive status was estimated in a Mini-Mental State Examination (MMSE), which allows for thorough and systematic assessment of many areas of cognitive function: registration, recall, language, orientation, attention, and calculation. The maximum score is 30 points. Scores below 24 points can indicate mild (19–23), moderate (10–18), or severe cognitive impairment [Folstein et al., 1975]. We also examined post-stroke patients for functional status using the Barthel Index of Activities of Daily Living (ADL), which is a scale used to indicate the patient's independence during basic and complex activities of daily living, as well as their gait efficiency [Collin et al., 1988]. The GDS, ADL, and MMSE were administered either on the same day as the blood sampling, or on the afternoon before.

Magnetronic MF10 device. ELF-EMF was conducted according to accepted guidelines using a Magnetronic MF10 generator (EiE Elektronika i Elektromedycyna, Otwock, Poland). The MF10 can generate rectangular, triangular, and sinusoidal pulse shape. In our study, as in therapeutic use, a coil applicator was used with a diameter 550 mm and length 270 mm, with 5 layers of 187 turns of 1.45 mm twin-parallel wires. Intensity of the electromagnetic field inside the solenoid was not uniform. Distribution of the electromagnetic field was vertical, whereas the generator induction coils were mounted horizontally. The set value of magnetic induction (7 mT) was at the geometric center of the applicator. The change of intensity of the electromagnetic field increased with the proximity of the coil's surface, by about plus 1.4. Other effects of the EMF field that could be sensed by the subjects were eliminated. The electromagnetic field itself was controlled by a computer system.

TABLE 1. Demographic and Clinical Characteristics

	Non-magnetic field therapy (<i>n</i> = 34)	Magnetic field therapy (<i>n</i> = 23)	<i>P</i>
Demographics			
Age (mean $\pm$ SD)	70.9 $\pm$ 15.3	68.0 $\pm$ 15.6	0.05
Sex (%)	65.6	64.8	0.64
Living alone (%)	48.1	51.9	0.14
Vascular risk			
Hypertension (%)	95.7	94.7	0.08
Diabetes (%)	24.8	21.7	0.36
Dislipidemia (%)	74.9	67.9	0.14
BMI $\geq$ 30 (%)	32	22	0.82
Concomitant medications			
Antidepressants (%)	32	23	0.47
ASA (%)	72	67	0.39
NSAID (%)	21	24	0.72
Stroke characteristics			
Weeks since stroke (mean $\pm$ SD)	2.1 $\pm$ 3.7	1.8 $\pm$ 3.5	0.61
NIHSS scores mean $\pm$ SD	6.2 $\pm$ 2.7	5.8 $\pm$ 3.8	0.81
ADL mean $\pm$ SD	10 $\pm$ 2.9	8.8 $\pm$ 2.4	0.22
Lesion location			
Anterior (%)	13	14	
Posterior (%)	39	49	
Intermediate (%)	48	33	
Lesion side			
Left (%)	58	54	
Right (%)	42	46	

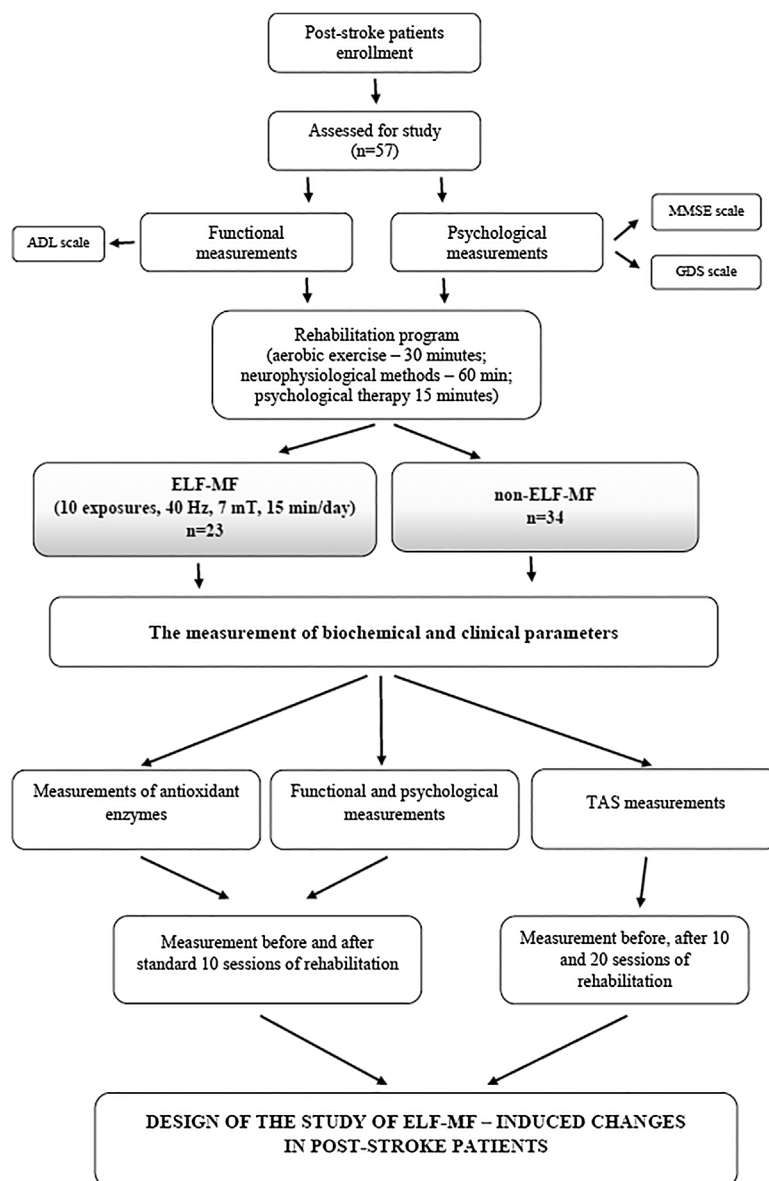


Fig. 1. Diagram of study.

Blood Sample Collection

Blood samples were collected into CPDA1 containing tubes (Sarstedt, Nümbrecht, Germany) and immediately centrifuged to isolate the plasma and erythrocytes. Erythrocytes were separated from the blood plasma by centrifugation (10 min, 710 g) at 4 °C and washed three times with 0.9% NaCl before examination.

Catalase Activity Estimation

Catalase activity in erythrocytes was estimated spectrophotometrically using the Beers and Sizer method [Beers and Sizer, 1952]. Absorbance measurement was performed with a 96-well microplate reader

(SPECTROstarNano, BMG Labtech, Ortenberg, Germany) at ambient temperature. Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of nM H₂O₂ consumed/min using a molar extinction coefficient of $\epsilon = 43600 \text{ M}^{-1} \text{ cm}^{-1}$ and shown as Bergmeyer units [Aebi, 1984], as well as a mean of the percentage of parameter changes for each study subject. (100% value was established for CAT activity level in each patient's blood before treatment; in each case the delta was calculated separately).

Superoxide Dismutase Estimation

SOD activity was determined using a spectrophotometric method [Fridovitch and McCord, 1969] with

a RANSOD kit (RANDOX Laboratories, Crumlin, UK). SOD activity was assayed based on the production of superoxide anions generated by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-9-5-phenyl-tertrazolium chloride (I.N.T.) to form a red formazan dye. The absorbance measurement was performed in a 96-well microplate reader (SPECTROstarNano, BMG Labtech, Ortenberg, Germany) at 37 °C. Changes in absorbance were recorded at 505 nm. The SOD activity was measured by the degree of inhibition of this reaction based on standard curve and showed as SOD units/ml of whole blood, as well as a mean of percent of parameter changes for each study subject. (100% value was established for SOD activity level in blood of each patient before treatment; in each case the delta was calculated separately).

Determination of Total Antioxidant Status (TAS) of Plasma

The TAS of plasma was measured spectrophotometrically with an “endpoint” protocol, at a wavelength of 734 nm for ABTS^{•+} assay [Re et al., 1999]. This method involves the reduction of the ABTS^{•+} (2,2'-azino-di-(3-ethylbenzthiazoline sulfate) (Sigma-Aldrich, St. Louis, MO), prepared with the reaction of ABTS with hydrogen peroxide (H₂O₂) in an acidic medium. Antioxidants contained in plasma have the ability to reduce ABTS^{•+}, causing discoloration of the solution in proportion to their content. The observed decrease of absorbance is a measure of the activity of antioxidants present in the sample [Erel, 2004]. The absorbance measurement was performed in a 96-well microplate reader (SPECTROstarNano, BMG Labtech). The TAS of blood plasma was calculated on the basis of standard curves, prepared with the use of Trolox (Sigma-Aldrich) as a reference antioxidant. Results are expressed in Trolox equivalents and a mean of the percentage of parameter changes for each study subject. (100% value was established for plasma TAS level in each patient before treatment; in each case the delta was calculated separately).

Data Analysis

All experiments were performed in duplicate and calculated as mean values. For all subjects, values of the parameters before the treatments were used as the output value (100%). Data from experiments performed on the same study subjects after appropriate treatments were expressed as a percentage of the output value. Values obtained in this way were expressed as mean ± SD. All the statistical analyses were performed using StatsDirect statistical software

v 2.7.2 (StatsDirect, Altrincham, UK). The results obtained were analyzed for normality with a Shapiro-Wilk test. The significance of differences between the values obtained for patients before and after treatments were analyzed using a test for normal distribution (paired *t*-Student), whereas the significance of differences between the ELF-EMF group and non-ELF-EMF group was determined using unpaired *t*-Student or U-Mann-Whitney tests.

For comparison of differences in clinical parameter changes between the ELF-EMF group and non-ELF-EMF group, we calculated changes in these parameters after appropriate treatments. Obtained values were also analyzed under the account of normality with Shapiro-Wilk test, and depending on results, an unpaired *t*-Student test or U-Mann-Whitney test were used.

Additionally, we performed correlation analysis between changes in both experimental and clinical parameters. For these analyses, Spearman's rank correlation and Spearman's rank correlation coefficient were used, and we also designated the probability of correlation [Hauke and Kossowski, 2011].

For all analyses, a level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Our comparative analysis shows that activities of CAT and SOD in hemolysates before rehabilitation have the same baseline level in both randomly selected ELF-EMF and non-ELF-EMF groups of patients, but after 10 treatments they were significantly higher in the ELF-EMF group (Table 2, Figs. 2 and 3). The increase of CAT activity (Δ CAT) after 10 sessions of ELF-EMF ($P < 0.05$) was about 20% higher in comparison to 10 sessions of non-ELF-EMF treatment (Fig. 2). SOD activity increased more than 40% in the ELF-EMF group ($P < 0.01$) and approximately 20% in the non-ELF-EMF group ($P > 0.05$) (Fig. 3).

The plasma determination of TAS shows that therapeutic programs have no impact on the antioxidant activity of low molecular weight antioxidants in plasma. In both groups, the change of TAS level after both 10 and 20 therapy sessions, with or without ELF-EMF, was low and not statistically significant (Table 2, Fig. 4).

In addition, we evaluated functional status, depression, and cognitive impairment using ADL, MMSE, and GDS in both groups of subjects. We observed a relationship between using ELF-EMF and improvements in clinical parameters. Functional status estimated in ADL (Δ ADL) was increased about

TABLE 2. Activity of Catalase and Superoxide Dismutase in Hemolysates and TAS of Plasma Obtained From ELF-EMF Group Versus Control Non-ELF-EMF Group

	Catalase			Superoxide dismutase			TAS	
	Non-ELF-EMF group	ELF-EMF group	<i>P</i>	Non-ELF-EMF group	ELF-EMF group	<i>P</i>	Non-ELF-EMF group	ELF-EMF group
	[Bermayer units] $\pm$ SD			[SOD units of whole blood] $\pm$ SD			[μ mol Trolox equivalents/l] $\pm$ SD	
Before treatments	5.06 $\pm$ 2.2	4.47 $\pm$ 1.5	0.108	187.9 $\pm$ 50.3	157.3 $\pm$ 56.8	0.279	3.5 $\pm$ 0.9	2.9 $\pm$ 1.2
Δ After 10 treatments [% of control]	1 $\pm$ 0.27	18 $\pm$ 0.20	0.023	17 $\pm$ 0.36	49 $\pm$ 0.33	0.041	6 $\pm$ 0.21	1 $\pm$ 0.25
Δ After 20 treatments [% of control]							3 $\pm$ 0.22	6 $\pm$ 0.10

20% in the ELF-EMF group compared with the non-ELF-EMF, wherein the baseline values of ADL before treatments in both groups were not statistically different (Table 3). Cognitive impairment measured in MMSE changed significantly, with Δ MMSE achieving about 40% better results in the ELF-EMF group than in non-ELF-EMF group of subjects. The baseline values of MMSE before treatments were also not statistically different in either group (Table 3). Moreover, the greatest improvement after ELF-EMF therapy was observed in depression syndrome estimation, assessed with the GDS, with a decrease of up to 60% (Table 3, Fig. 5).

Distribution of the results for ADL, MMSE, and GDS deviates from the norm in the study group, so the Spearman correlation method was suitable for use in these analyses. The obtained correlation parameters (Table 4) indicate a significant positive correlation between changes (Δ) of

CAT activity and Δ ADL. Moreover, correlation parameters indicate a significant positive correlation between Δ SOD activity and Δ MMSE, and a negative correlation between Δ SOD activity and Δ GDS. We have shown the detailed course of these dependencies, which confirms the correlation and numeric data presented in Table 4.

DISCUSSION

This study was designed to investigate the impact of ELF-EMF on both clinical parameters and antioxidative status in the blood of early-stage, ischaemic post-stroke patients.

Numerous methods have been adopted to reduce ROS formation and block ROS-mediated neuronal injury. However, the most serious problem of stroke still remains oxidative stress [Murakami et al., 1998; Suzuki, 2009]. For this reason, it is particularly

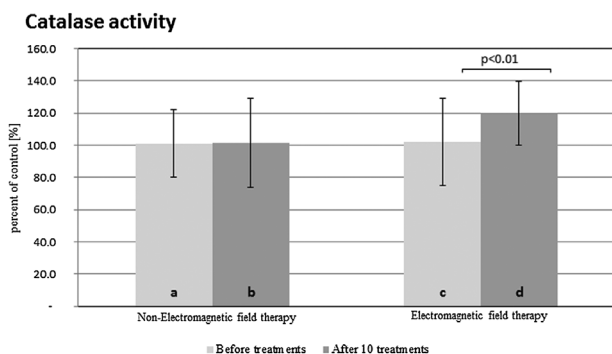


Fig. 2. Comparison of catalase activity in red blood cell hemolysates obtained from ELF-EMF group versus control non-ELF-EMF group. Statistical significance between both groups: b versus d— $P < 0.05$. Results were expressed as mean of parameter changes before and after treatment (100% was established for level of CAT activity in sample of each patient before treatments).

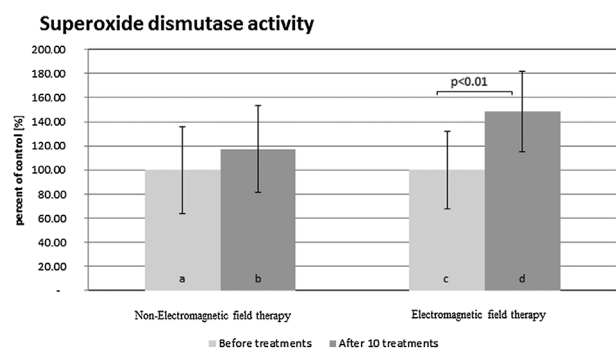


Fig. 3. Comparison of superoxide dismutase activity in red blood cell hemolysates obtained from ELF-EMF group versus control non-ELF-EMF group. Statistical significance between both groups: b versus d— $P < 0.05$. Results were expressed as mean of parameter changes before and after treatment (100% was established for level of SOD activity in sample of each patient before treatments).

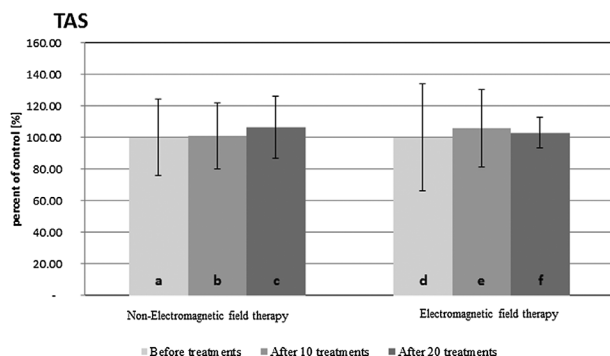


Fig. 4. Comparison of TAS of plasma obtained from ELF-EMF group versus control non-ELF-EMF group. Results were expressed as mean of parameter changes before and after treatment (100% was established for level of plasma TAS in sample of each patient before treatments).

important to find novel therapies that may reduce oxidative stress in stroke patients.

The consequences of stroke in terms of neuromuscular dysfunction include physical disabilities, such as functional impairment and cognition-related disabilities, and memory loss, speech impairment, poor problem-solving skills, and disorganized thoughts. Psychiatric diseases, including anxiety, depression, emotional instability, and fatigue, also occur [Annoni et al., 2006; Young and Tolentino, 2009]. Early admission to inpatient physical therapy is critical in reducing post-stroke disability.

ELF-EMF biological effects have not been elucidated. It is suggested that ELF-EMF causes changes in ion balance, which affects uncompensated magnetic spins of paramagnetic elements, free radicals, diamagnetic molecules, and the structure of tissues having piezoelectric and magnetostrictive properties [Brisdelli et al., 2014]. The electromagnetic field acts on the liquid crystal structure formed by cholesterol, its derivatives, and physicochemical properties of water, which is a basic environment—the principal filler of cells and tissue [Sulpizio et al., 2011; Yi et al., 2014]. This leads to spontaneous

depolarization of the cells, mainly through the influence of calcium channels [Morgado-Valle et al., 1998; Zmyslon, 2006]. There are limited data supporting the antioxidant effect of ELF-EMF, in particular its positive impact on improving the functioning of connective tissue's cells [Sosnowski et al., 1999; Kurzeja et al., 2013]. We have observed an increase of SOD activity in the hemolysate, with results that are consistent with data presented by Rauš Balind et al. [2014]. Those authors demonstrated that ELF-EMF decreased the level of oxidative stress in gerbil brains with induced global cerebral ischaemia, as demonstrated by amplified SOD activity [Rauš Balind et al., 2014]. Studies on mouse fibroblasts conducted by Kurzeja et al. [2013] showed a reduced level of oxidative stress markers, and normalization of the activity of SOD, GPx, and CAT enzymes, as well as a change of energy state in fibroblasts. In contrast, Sosnowski et al. [1999] observed a decrease in the concentration of H_2O_2 , without any irregularities in the activity of antioxidant enzymes. This previous research established an enhanced number of oxidative stress biomarkers and a lowered level of total antioxidants [Liu et al., 2011]. However, the kinds of changes in the activities of antioxidant enzymes remain controversial [Gariballa et al., 2002]. Additionally, the results of earlier studies do not provide a clear answer about the impact of electromagnetic fields on the body's red-ox system. This lack of clarity is due to the use of electromagnetic fields of different intensities and the variety of research models employed. Results obtained on cell lines or from laboratory animals cannot be explicitly related to human therapeutic methods using electromagnetic fields.

It is also worth mentioning that previous reports repeatedly suggest a significant attenuation in TAS in experimental model of global cerebral ischaemia. Ischaemia/reperfusion produces a significant decrease in brain TAS and an increase in the level of total oxidant status. It suggests an increased utilization by ROS as a contributing factor in decreasing TAS in

TABLE 3. Clinical Parameters Obtained From ELF-EMF Group Versus Control Non-ELF-EMF Group

	ADL [mean $\pm$ SD]			MMSE [mean $\pm$ SD]			GDS [mean $\pm$ SD]		
	Non-magnetic field therapy	Magnetic field therapy	<i>P</i>	Non-magnetic field therapy	Magnetic field therapy	<i>P</i>	Non-magnetic field therapy	Magnetic field therapy	<i>P</i>
Before treatments	10 $\pm$ 2.9	8.8 $\pm$ 2.4	0.217	22.3 $\pm$ 3.3	20.9 $\pm$ 2	0.128	12.4 $\pm$ 4.3	17.4 $\pm$ 3.6	0.0006
Δ After 10 treatments	4.7 $\pm$ 1.7	6.6 $\pm$ 1.7	0.0048	3.1 $\pm$ 1.7	4.7 $\pm$ 1.3	0.011	3.1 $\pm$ 2	7.8 $\pm$ 2	0.0001

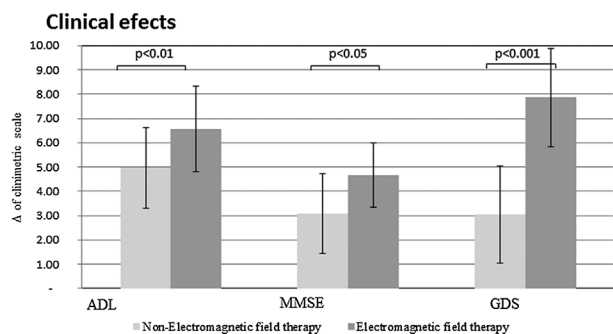


Fig. 5. Clinical parameters: ADL, MMSE, and GDS for ELF-EMF group versus control non-ELF-EMF group. Data were presented as delta of clinimetric scale before and after standard series of treatments (Δ ADL = increase of ADL; Δ MMSE = increase of MMSE; Δ GDS = decrease of GDS). Statistical analysis was performed using *t*-Student or U-Mann-Whitney test.

cerebral ischaemia [Jung et al., 2011; Simão et al., 2011; Uzar et al., 2012].

In addition to low molecular weight antioxidants, antioxidative enzymes protect the organism against overproduction of ROS. Our results indicate that ELF-EMF stimulates SOD activity and, to a lesser extent, CAT activity. The levels of both SOD and CAT activity after 10 exposures of ELF-EMF were approximately 20% higher (Table 2, Figs. 2 and 3). These results indicate that a low frequency electromagnetic field probably stimulates gene expression to antioxidative enzymes in hematopoietic cells in bone marrow. SOD is a metalloprotein that is found in almost all eukaryotic cells. The other potential mechanism of increased SOD activity in this group could be the interference of the electromagnetic field in electrostatic interactions of SOD molecules. Charged amino acid residues around the positively-charged SOD active site create an electric field gradient that provides the correct electrostatic driving force for O_2^- , directing this substrate into the site of

the active enzyme [Lévêque et al., 2001]. According to our predictions, ELF-EMF could affect this function's status, thereby changing SOD activity.

Catalase is not directly involved in the removal of ROS, but prevents Fenton's reaction, in which H_2O_2 is converted to highly toxic hydroxyl radicals. According to the literature, CAT activity decreases following a stroke, thereby reducing the effectiveness of the inactivation of H_2O_2 , which freely diffuses across the mitochondrial membrane and increases oxidative stress [Venarucci et al., 1999; Miller et al., 2011]. In the present study, we noticed that CAT activity in the erythrocytes of the ELF-EMF group increased by about 20% after 10 applications, while in the non-ELF-EMF group the CAT value remained unchanged (Fig. 2). The changes in the kinetic parameters of CAT and SOD confirm, for the first time, an interaction between ELF-EMF therapy and the human enzymological system. Significant changes in SOD/CAT activity determine the need for further studies, in order to establish events at the molecular level, so that possible effects of an ELF-EMF on human health can be better understood. Previous studies with rats exposed to ELF-EMF showed no significant changes in the activity of antioxidant enzymes (SOD and CAT). It should be noted that the irradiation of the animals resulted in their immobilization, which is in turn associated with stress [Martínez-Sámano et al., 2010; Glinka et al., 2013]. Stress activates a complex of physiological and psychological response mechanisms, leading to an imbalance of red-ox and overproduction of ROS [Passali et al., 2015]. Thus, it is not possible to directly compare our results with studies carried out using animals.

In our study, we also observed a dependence between ELF-EMF and improvement of clinical parameters of rehabilitated patients, as well as a relationship between oxidative stress factors and clinical parameters. Our findings show that the ADL value increased by about 20% more in ELF-EMF

TABLE 4. Correlation Coefficient Values Obtained for Change in Antioxidative Enzyme Activities (CAT and SOD) and Parameters of Clinimetric Scales (ADL, MMSE, GDS) After Electromagnetic Field Therapy

ADL	CAT activity		ADL	SOD activity	
	MMSE	GDS		MMSE	GDS
Rho = 0.5707 P = 0.0112 H1: positive correlation	Rho = 0.4184 P = 0.0684	Rho = -0.3732 P = 0.0707	Rho = -0.0072 P = 0.5044	Rho = 0.5006 P = 0.0347 H1: positive correlation	Rho = -0.5304 P = 0.0219 H1: negative correlation

Correlation was analyzed using Spearman's rank correlation method. Table consist Spearman's rank correlation coefficient (Rho), probability for correlation (p), and hypotheses verification (H1).

patients than in non-ELF-EMF patients (Table 3, Fig. 5), and there is a significant positive correlation between changes of CAT activity and Δ ADL (Table 4). The growth of parameters on the MMSE scale before and after ELF-EMF was about 25% (Fig. 5), and was significantly positively correlated with an increase in activity of SOD (Table 4). In contrast, we also proved a significant negative correlation between changes of GDS value and Δ SOD activity (Table 4).

Ciancarelli et al. [2013] investigated the influence of intensive neurorehabilitation on oxidative stress balance during recovery of neurological deficits in post-acute ischaemic stroke patients. They found that increased activity of Cu/ZnSOD correlated with improvement of the motor function (Katz Index) of post-stroke patients. The authors suggested that intensive neurotherapy might reduce oxidative stress and/or mobilize the endogenous antioxidant system, thereby decreasing neurological deficits [Ciancarelli et al., 2013]. Numerous studies have shown the relationship between the body's antioxidant status and level of dementia [Delibas et al., 2002; Torres et al., 2011; Arikanoglu et al., 2013; Picco et al., 2014; Baierle et al., 2015]. Post-stroke depression (PSD) is associated with increased disability and increased cognitive impairment, and leads to delay of the therapeutic process due to deterioration of patients' motivation and their low mood [Paolucci, 2008]. The molecular pathomechanism of PSD is unclear. In our previous studies, we showed a negative correlation between the activity of catalase and level of PSD, as well as correlations between level of oxidative damage of plasma proteins and the level of depression [Cichon et al., 2015]. Research conducted by Picco et al. [2014] reported that oxidative stress could play a role in impairing brain glucose utilization in elderly subjects with increasing severity of cognitive disturbance. Oxidative stress was determined by measuring the activity of CAT, SOD, and GPx activity. The authors demonstrated that reduction of antioxidant enzyme activity is associated with impairment of glucose utilization in the left temporal lobe structures, which leads to a deterioration of cognitive functions [Picco et al., 2014]. Therefore, in accordance with our assumptions, it is likely that improvements in cognitive function in post-stroke patients treated with electromagnetic fields may be due to increased activity of antioxidant enzymes.

In conclusion, ELF-EMF therapy is a non-invasive and safe treatment with the potential for promoting recovery in ischaemic stroke patients. The electromagnetic field increases the activity of antioxidant enzymes and improves the effectiveness

of post-stroke therapy. Increasing SOD and CAT activities after 10 treatments correlates with revised degrees of physical and mental disability determined on the basis of the three clinimetric scales used in this study. Therefore, these new insights might be important for in vivo modulation of ROS production in electromagnetic field treatment, which could open new avenues of biomedical research and therapeutic strategies for various neurological (and other) diseases. Ultimately, ELF-EMF seems to be a very useful additional tool in post-stroke therapeutic treatment.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in our studies using human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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