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The Effects of Electromagnetic Fields Generated from 1800 MHz Cell Phones on Erythrocyte Rheological Parameters and Zinc Level in Rats

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ABSTRACT

Objective: The aim of this study was to investigate the effects of the electromagnetic field generated from the 1800 MHz radiofrequency radiation (EF) on erythrocyte rheological parameters and erythrocyte zinc levels.

Material and Methods: Twenty-four male Wistar Albino rats were randomly grouped as follows: 1) two control groups and 2) study groups: i) Group A: EF exposed group (2.5 h/day for 30 days, the phone on stand-by), and ii) Group B: EF exposed group (2.5 min/day for 30 days, the phone ringing in silent mode). At the end of the experimental period erythrocyte rheological parameters such as erythrocyte deformability and aggregation were determined by an ectacytometer. Erythrocyte zinc level, which affects hemorheological parameters, was also measured by atomic absorption spectrophotometer.

Results: Erythrocyte deformability was decreased in both study groups but the decrease in group A was not statistically significant. Exposure to EF did not have any significant effect on erythrocyte aggregation. On the other hand, erythrocyte zinc level was significantly reduced in both study groups.

Conclusion: Exposure to EF may have decreased tissue oxygenation due to reduced erythrocyte deformability. Decrease in erythrocyte zinc level may have caused the impairment in erythrocyte deformability.

Key Words: Electromagnetic field, erythrocyte aggregation, erythrocyte deformability, mobile phone, trace element

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Introduction

Cell phones are not only communication devices, but also a source of electromagnetic fields induced by radiofrequency (RF) radiation. Therefore, the effects of mobile phones on human health is still controversial. Many researchers conducted studies on the effects of electromagnetic field induced by RF radiation with different Specific Absorption Ratios (SAR) on the nervous system, skeletal system, cardiovascular system and utero-placental functions (1-4).

Hemorheological parameters provide information on the flow properties of blood. Erythrocyte deformability, one of the basic rheological parameters of erythrocytes, is the ability of erythrocytes to alter their shapes in response to forces exerted upon them during blood flow (5). Another rheological parameter of the erythrocytes is their spontaneous tendency to aggregate at low shear velocities (5). Normally, blood flow causes dissolution of erythrocyte aggregates before they enter the capillaries, allowing adequate perfusion of the tissues (5, 6). Hemorheological parameters are related to homeostasis. Alterations in these parameters can be a result of as well as a contributor to the development of patho-physiological conditions (7).

Zinc plays structural and functional roles in biological membranes and its deficiency can cause copper deficiency, disruption in the functional use of iron, suppression of immune functions, decrease in high-density lipoprotein level, and disorders in DNA synthesis (8). Despite studies on the effects of electromagnetic fields on serum Zn level or metallothionein level that binds Zn in erythrocyte cytosol, there are no studies on its effects on erythrocyte Zn levels (9, 10). There are few studies investigating the relationship between Zn and hemorheological parameters. Khaled et al. showed that low serum Zn level was associated with high erythrocyte rigidity and blood viscosity in athletes (11). The same authors also reported that Zn replacement lowered the increase in blood viscosity induced by exercise and increased erythrocyte deformability (12).

The effects of cell phones on the cardiovascular system have been investigated by various researchers by examining parameters such as heart rate and blood pressure but the study results are controversial (13, 14). To the best of our knowledge, the effects of exposure to EF on hemorheological parameters have not been studied before. The aim of this study was to investigate the effects of EF on hemorheologi-

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cal parameters and erythrocyte Zn level which may alter the hemorheological parameters.

Material and Methods

Animals: The study was carried out on 24 adult, male Wistar Albino rats, weighing 250-300 g, obtained from the Experimental Research Unit of Pamukkale University. Approval was obtained by the Ethics Board for Animal Experiments of Pamukkale University. Animals were housed in plastic cages in a controlled environment with normal room temperature and 50% humidity, en-trained to a 12 h/12 h light/dark cycle and had ad libitum access to food and water.

Experimental protocols: Twenty four rats were divided into 4 groups with 6 rats in each group. The study was carried out during two types of exposure to cell phones (when the phone was switched on but not ringing i.e. on stand-by, and when the phone was switched on and ringing in silent mode). To imitate the duration of exposure in humans, time of exposure in Group A (Phone on stand-by) was kept longer than Group B (phone ringing). Rats in Group A were exposed to an electromagnetic field for a total of 2.5 hours for one month (30 minutes of exposure 5 times a day, with 5 minutes between each exposure, 7 days a week) while Group B rats were exposed to an electromagnetic field for a total of 2.5 minutes for one month (0.5 minutes of exposure 5 times a day, with 30 minutes between each exposure, 7 days a week). Two control groups, one for each study group, were also constructed, and comprised of animals placed in the same setting but without a cell phone present. In sum, 4 groups, namely A, B, CA, and CB were constructed.

RF Exposure system: PVC tubes with air vents were arranged in a radial manner, equidistant from the center, and 6 rats at a time were placed within the tubes (Figure 1). Before the experimental period, the animals were acclimated to the handling and restraint apparatus for a week. This exposure system was based on a previous report with some modifications (15). All the RF radiation exposure system was bordered with absorber material and based on a ground plane metal sheet. Moreover, all six objects were kept separated by shielded enclosures as seen in Figure 1. An experimental license is required to conduct animal exposure studies at these frequencies, in an unshielded environment, provided the experiment will not cause interference to any licensed wireless communications. Therefore, the experiments should be conducted in an RF-shielded room with an estimated attenuation of 100 dB or more to generally comply with existing RF emission limits for devices operating at these frequencies [FCC, 1993]. Therefore, we conducted our experiments in an RF-shielded room and measured shielding effectiveness was nearly 100 dB at 1800 MHz.

Background noise was under control during experiments and observed less than 0.01 mT electromagnetic field and 0.1 V/m, the value of total unwanted electric field intensity.

A digital Gauss/Tesla Meter (Unilab, Blackburn, England) was used for electromagnetic field noise. Uniformity and homogeneity of electric fields were tested by Holaday HI-3804 Electromagnetic Field Survey Meter-Industrial Compliance Meter and its probes (Maintan, England). The total vector sum of the electric field emitted from our experiment system was measured and confirmed by Portable RF Survey System (HOLADAY HI-4417, MN, USA) and Satellite level meter (PROMAX MC-877C, Barcelona, Spain). During the experiment, a spectrum analyzer/satellite reciever was used to investigate the reflections and background noises in this media. Also repetition time, frequency, and amplitude of spectrum of RF energy was investigated, observed, and verified by the instruments mentioned above.

In this study, the electromagnetic dosimetry solution refers to measured electric field density (V/m) and SAR. Our SAR calculation is based on the FDTD numerical code created in MATLAB software. The whole body SAR value obtained was 0.0083 W/kg for these physical and electrical properties (16-19).

Electrical properties, conductivity and relative dielectric permitivity constant were taken from the literature (20).

Hemorheological parameters

RBC deformability measurements: RBC deformability (i.e., the ability of the entire cell to adopt a new configuration when subjected to applied mechanical forces) was determined at shear stresses at 5.33 Pascal (Pa) by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics; Hoorn, The Netherlands). The system has been described elsewhere in detail (21). Briefly, a low hematocrit suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD, Sigma P 5288, ST. LOUIS, MI) was sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed cells was analyzed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated as: EI=(*L*-*W*)/(*L*+*W*), where *L* and *W* are the length and width of the diffraction pattern, respectively. An increased EI at a given shear stress indicates greater cell deformation

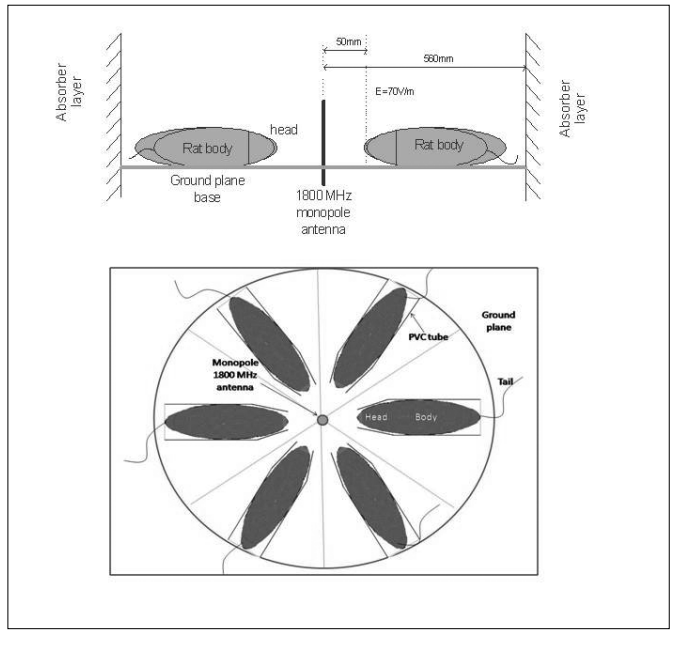

Figure 1. Exposure setup with physical dimensions

and hence greater RBC deformability. All measurements were carried out at 37°C.

RBC aggregation measurements: RBC aggregation was also determined by LORCA as described elsewhere (21). The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37°C. Back-scattering data are evaluated by the computer and the aggregation index (AI) is calculated on the basis that there is less light back-scattered from aggregating red cells. RBC aggregation measurements were evaluated at both native and standard (40%) hematocrit (Hct) and blood was fully oxygenated. The Hct of blood samples was adjusted to 0.4 L/L by adding or removing a calculated amount of autologous plasma obtained by centrifugation at 1,400 X g for 6 minute. An increased AI indicates greater RBC aggregation.

Erythrocyte Zn level measurement: Standard solutions of 0.5 µg/ml were prepared from 1000±0.002 mg/L stock solution (Titrisol, Merck) for Zn measurements. Measurements were performed with atomic absorption spectrophotometer (Perkin Elmer AAS 700, Ueberlingen, Germany), using Hollow Cathod Lamp (HCL) emitting light at a wavelength specific to each element with the following settings: 213.9 nm wavelength, 0.7 nm slit width and 30 mA current, air/acetylene flame, HCL and BGC (Back Ground Correction) modes on. The spectrophotometer was calibrated with blank and standard solutions (22). Whole blood samples were centrifuged, plasma and erythrocytes were separated and erythrocytes were frozen until the time of zinc measurement. Erythrocytes were thawed just before the measurements, diluted 1:20 in distilled water, which causes hemolysis and the Zn level in erythrocytes was measured (23).

Statistical analysis

Results were expressed as mean±standard deviation (SD). Statistical comparisons between groups were done by one-way ANOVA test, with p values <0.05 accepted as statistically significant. All analyses were carried out with the SPSS 10.0 statistical software (Statistical Package for Social Sciences, SPSS Inc).

Results

After a one month long exposure to electromagnetic field, erythrocyte deformability at 5.33 Pa shear stress was significantly decreased in Group B (0.48±0.00) compared to its control group CB (0.49±0.01) (Figure 2). On the other hand, erythrocyte deformability under the same shear force in Group A (0.47±0.01) was decreased in comparison to Group CA (0.48±0.02), but this decrease was not statistically significant (Figure 2).

Compared to the control groups, we found no statistically significant changes in aggregation parameters (AI and t 1/2) with exposure to electromagnetic field in either study group (Table 1).

When Groups A and B were compared in terms of aggregation and deformability parameters, we did not find statistically significant differences.

Erythrocyte Zn levels was significantly decreased in Group A (4.63±0.10) and Group B (4.89±0.77) compared to their control groups (6.61±1.32 and 9.57±1.42, respectively) (Figure 3). On the other hand, erythrocyte Zn levels compared to each other, group A and group B, was not statistically significant different.

Discussion

Erythrocyte deformability facilitates oxygen supply to the tissues by decreasing the viscosity of the blood and permitting erythrocytes to pass to the capillaries while blood flowed through large vessels (5). In the present study, we found that erythrocyte deformability under 5.33 Pa shear stress in Group B was significantly lower than the control. Erythrocyte deformability depends primarily on erythrocyte cytoskeleton, cytoplasmic viscosity and biconcave discoid shape. On the other hand, hemoglobin concentration predominantly determines the internal viscosity of erythrocytes (5). A study by Salem et al. showed that a static electromagnetic field increased hemoglobin concentration (10). It can be speculated that de-

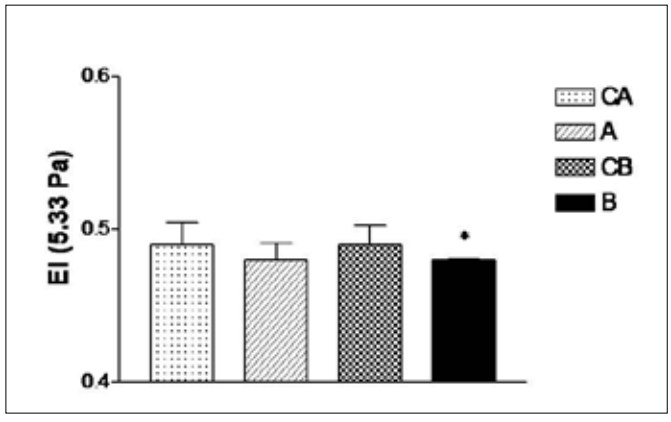

Figure 2. The effect of magnetic field for 2.5 hours (Group A) and 2.5 minutes (Group B) for one month on erythrocyte Elongation Index (EI) at 5.33 Pa shear stress of experimental groups, Mean±SD.; * :Difference from CB, p<0.05

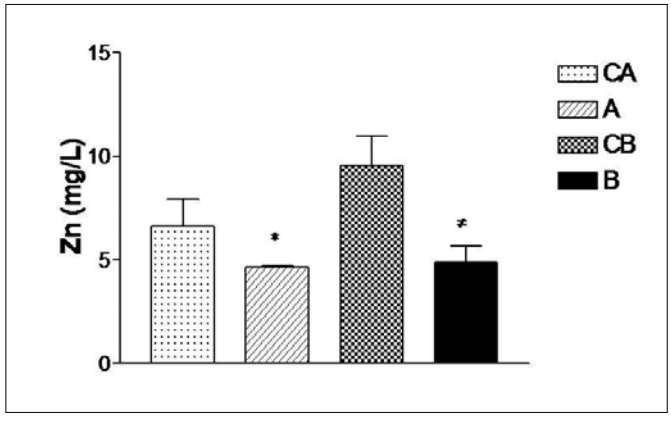

Figure 3. The effect of magnetic field for 2.5 hours (Group A) and 2.5 minutes (Group B) for one month on erythrocyte zinc (Zn) level, Mean±SD. *:Difference from CA, p<0.05; #:Difference from CB, p<0.05

Table 1. Erythrocyte aggregation parameters of experimental groups

	CA	A	CB	B
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
AI (%)	58.18±9.03	54.71±3.24	59.30±5.32	56.34±8.57
t ½ (s)	2.8±1.58	2.11±0.50	2.47±0.53	2.92±1.94

AI: aggregation index, t ½: aggregation half time

crease in deformability can be due to a possible increase in erythrocyte hemoglobin level in response to the electromagnetic field. Free radicals affect hemodynamics at various levels by altering the cellular properties of the erythrocytes or mechanical properties of the membrane (24, 25). Moustafa et al. (26) demonstrated that acute exposure to the electromagnetic field of cell phone origin increased lipid peroxidation, and decreased the Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSHPx) activities by causing oxidative stress. Even though oxidative parameters were not measured in the present study, a decrease in erythrocyte deformability that occurs in the presence of an electromagnetic field may also be related to increased free oxygen radicals.

We found in the present study that the Zn level in the erythrocytes of rats exposed to an electromagnetic field was lower than the controls. On the other hand, Akdag and colleagues' study found no significant effect of very low-frequency electromagnetic fields on serum Zn levels (9). The discrepancy in the results may be explained by the fact that the frequency of the electromagnetic waves used in the present study was much higher. Visco-elastic properties of the membrane are amongst the basic determinants of erythrocyte deformability (5). Zinc contributes to the visco-elastic properties of plasma membrane in mammals (27). It has been established that severe zinc deficiency increases the fluidity of the lipid layer of the membrane (28). It has also been argued that zinc deficiency can cause certain alterations in the structure and function of spectrin (29). A normal membrane skeleton is a sine qua non for erythrocyte deformability and in the presence of abnormal skeletal proteins, deformability is disrupted (5). Hence, disruptions in the functions of erythrocyte membrane skeleton or changes in membrane fluidity in zinc deficiency can impair deformability by altering the visco-elastic state of the membrane. Increased susceptibility of erythrocytes to hypotonic hemolysis has been reported in zinc deficiency (30). In a study by Kraus et al. (31), the authors noted that zinc deficiency decreased erythrocyte superoxide dismutase level but increased plasma Thiobarbituric Acid Reactive Substances (TBARS) and alanine levels, markers of oxidative stress, and that this increase was rectified by dietary antioxidants. Therefore, decrease in the erythrocyte deformability as a result of exposure to an electromagnetic field may also be related to a possible increase in oxidative stress as well as to zinc deficiency.

Erythrocyte aggregation depends on a balance between 3 different forces. These are the repelling force between negatively-charged erythrocytes, adhesion force between erythrocytes in the presence of plasma proteins and disaggregating shear forces induced by blood flow (5). Another hypothesis proposed for erythrocyte aggregation is the bridging hypothesis. According to this hypothesis, macromolecules in plasma are adsorbed onto the surface of the erythrocytes and decrease the disaggregating forces between them, holding the aggregates together (32). There are very few studies in the literature that addressed the relationship between an electromagnetic field and erythrocyte aggregation. Iino found that erythrocyte aggregation and, therefore, erythrocyte sedimentation rate increased when erythrocytes were exposed to a homogenous static field in their own plasma (33). The author attributed this result to alteration in cell orientation in response to electromagnetic field, stating that erythrocytes aggregate by forming bridges with plasma proteins, a process in which cell orientation plays a key role. In the present study, we did not find a significant effect of electromagnetic field on erythrocyte aggregation. This difference can be attributed to the fact that the whole body was exposed to electromagnetic field and to the duration of exposure.

Conclusion

This study showed that EF decreased erythrocyte deformability as well as Zn levels in rats, a trace element that affects the hemorheological parameters. It is possible that the EF exerts these effects by decreasing the Zn level in erythrocytes directly or by increasing oxidative stress by disrupting Zn absorption or metabolism. Another plausible mechanism is that the electromagnetic field decreases erythrocyte deformability by increasing erythrocyte hemoglobin levels. These possibilities need further studies for elucidation.

Conflict of Interest

No conflict of interest was declared by the authors.

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