

1 THE EFFECTS OF ELECTROMAGNETIC FIELD GENERATED FROM 1800 MHz
2 CELL PHONES ON ERYTHROCYTE RHEOLOGICAL PARAMETERS AND ZINC
3 LEVEL IN RATS

4 **ABSTRACT**

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

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

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

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

23 **Short title:** The effects of cell-phone on hemorheology

24 **Key words:** electromagnetic field; erythrocyte aggregation; erythrocyte deformability;
25 mobile phone; trace element

26 **1800 MHZ FREKANSLI CEP TELEFONUNDAN KAYNAKLANAN**
27 **ELEKTROMANYETİK ALANIN SIÇANLARDA ERİTROSİT REOLOJİK**
28 **PARAMETRELERİ VE ÇİNKO SEVİYESİ ÜZERİNDEKİ ETKİSİ**

29 **ÖZET**

30 **Amaç:** Bu çalışmanın amacı 1800 MHz radyofrekans radyasyonun oluşturduğu
31 elektromanyetik alanın (EF) eritrosit reolojik parametreleri ve eritrosit çinko (Zn)
32 düzeyi üzerindeki etkilerini araştırmaktır.

33 **Gereç ve Yöntemler:** Yirmidört adet, erkek Wistar Albino sıçan, iki kontrol ve iki
34 çalışma grubuna (A grubu: 30 gün boyunca, 2.5 saat/gün, telefon stand-by
35 pozisyonundayken EF' ye maruz kalan grup; B grubu: 30 gün boyunca, 2.5
36 dakika/gün, sessiz modda telefon çalarken EF' ye maruz kalan grup) ayrıldı.
37 Deneysel periyodun sonunda bir ektasitometre kullanılarak eritrosit reolojik
38 parametrelerinden eritrosit deformabilitesi ve agregasyonu, atomik absorpsiyon
39 spektrofotometresiyle ise eritrosit Zn düzeyi ölçüldü.

40 **Bulgular:** EF maruziyetinin eritrosit deformabilitesi üzerinde, kendi kontrol gruplarına
41 göre, B grubunda istatistiksel olarak anlamlı, A grubunda anlamlı olmayan azalmaya
42 yol açtığı bulundu. Eritrosit agregasyonunda ise gruplar arasında istatistiksel olarak
43 anlamlı bir değişiklik bulunmadı. Diğer taraftan eritrosit Zn seviyesi iki çalışma
44 grubunda da kontrollerine göre anlamlı biçimde azaldı.

45 **Sonuç:** EF' ye maruziyet doku oksijenasyonunu eritrosit deformabilitesini azaltmak
46 yoluyla bozabilir. Eritrosit Zn seviyesindeki düşüş eritrosit deformabilitesinin
47 bozulmasının bir nedeni olabilir.

48 **Kısa başlık:** Cep telefonunun hemoreolojiye etkisi

49 **Anahtar Kelimeler:** elektromanyetik alan; eritrosit agregasyonu; eritrosit
50 deformabilitesi; cep telefonu; eser element

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INTRODUCTION

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54 Cell phones is not only a communication devices, but also a source of
55 electromagnetic field induced by radiofrequency (RF) radiation. Therefore, the
56 effects mobile phones on human health is still controversial. Many researchers
57 conducted studies on the effects of electromagnetic field induced by RF radiation with
58 different Specific Absorption Ratio (SAR) on the nervous system^[1], skeletal system^[2],
59 cardiovascular system^[3], utero-placental functions.^[4]

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

70 Zn plays structural and functional roles in biological membranes and its
71 deficiency can cause copper deficiency, disruption in the functional use of iron,
72 suppression of immune functions, decrease in High-density lipoprotein level, and
73 disorders in DNA synthesis.^[8] Despite studies on the effects of electromagnetic field
74 on serum Zn level^[9] or metallothionein level that binds Zn in erythrocyte cytosol^[10],
75 there are no studies on its effects on erythrocyte Zn level. There are few studies

76 investigating the relationship between Zn and hemorheological parameters. Khaled
77 et al. showed that low serum Zn level was associated with high erythrocyte rigidity
78 and blood viscosity on athletes.^[11] Same authors also reported that Zn replacement
79 lowered the increase in blood viscosity induced by exercise and increased
80 erythrocyte deformability.^[12]

81 The effects of cell phones on the cardiovascular system have been investigated
82 by various researchers by examining parameters such as heart rate and blood
83 pressure but this study results are controversial.^[13,14] To the best of our knowledge,
84 exposure to EF on hemorheological parameters have not been studied before. The
85 aim of this study was to investigate the effects of EF on hemorheological parameters
86 and erythrocyte Zn level which may alter the hemorheological parameters.

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MATERIALS AND METHODS

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90 **Animals:** The study was carried out on 24 adult, male Wistar Albino rats, weighing
91 250-300 g, obtained from Experimental Research Unit of Pamukkale University.
92 Approval was obtained by the Ethics Board for Animal Experiments of Pamukkale
93 University. Animals were housed in plastic cages in a controlled environment with
94 normal room temperature and 50% humidity, en-trained to a 12 h/12 h light/dark
95 cycle and had ad libitum access to food and water.

96 **Experimental protocols:** Twenty four rats were divided into 4 groups with 6 rats in
97 each group. The study was carried out during two types of exposure to cell phones
98 (when the phone was switched on but not ringing i.e. on stand-by, and when the
99 phone was switched on and ringing in silent mode). To imitate the duration of
100 exposure in humans, time of exposure in Group A (Phone on stand-by) was kept

101 longer than Group B (phone ringing). Rats in Group A was exposed to
102 electromagnetic field for a total of 2.5 hours for one month (30 minutes of exposure 5
103 times a day, with 5 minutes between each exposure, 7 days a week) while Group B
104 was exposed to electromagnetic field for a total of 2.5 minutes for one month (0.5
105 minutes of exposure 5 times a day, with 30 minutes between each exposure, 7 days
106 a week). Two control groups, one for each study group, were also constructed,
107 comprised of animals placed in the same setting but without a cell phone present. In
108 sum, 4 groups, namely A, B, CA, and CB were constructed.

109 **RF Exposure system:** PVC tubes with air vents were arranged in a radial manner,
110 equidistant from the center, and 6 rats at a time were placed within the tubes (Figure
111 1). Before the experimental period the animals were acclimated to the handling and
112 restraint apparatus for a week. This exposure system was based on a previous report
113 ^[15] with some modifications. All RF radiation exposure system was bordered with
114 absorber material and based on a ground plane metal sheet. Moreover, all six objects
115 were kept separated by shielded enclosures as seen in Figure 1. An experimental
116 license is required to conduct animal exposure studies at these frequencies, in an
117 unshielded environment, provided the experiment will not cause interference to any
118 licensed wireless communications. Therefore, the experiments should be conducted
119 in an RF-shielded room with an estimated attenuation of 100 dB or more to generally
120 comply with existing RF emission limits for devices operating at these frequencies
121 [FCC, 1993]. Therefore, we conducted our experiments in an RF-shielded room and
122 measured shielding effectiveness was nearly 100 dB at 1800 MHz.

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

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

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

140 Electrical properties, conductivity and relative dielectric permittivity constant
141 were taken from the literature. ^[20]

142 **Hemorheological parameters:**

143 *RBC deformability measurements:* RBC deformability (i.e., the ability of the entire cell
144 to adopt a new configuration when subjected to applied mechanical forces) was
145 determined at shear stresses at 5.33 Pascal (Pa) by laser diffraction analysis using
146 an ektacytometer (LORCA, RR Mechatronics; Hoorn, The Netherlands). The system
147 has been described elsewhere in detail. ^[21] Briefly, a low hematocrit suspension of
148 RBC in an isotonic viscous medium (4 % polyvinylpyrrolidone 360 solution; MW 360
149 kD, Sigma P 5288, ST. LOUIS, MI) was sheared in a Couette system composed of a
150 glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A

151 laser beam was directed through the sheared sample, and the diffraction pattern
152 produced by the deformed cells was analyzed by a microcomputer. On the basis of
153 the geometry of the elliptical diffraction pattern, an elongation index (EI) was
154 calculated as: $EI = (L - W)/(L + W)$, where L and W are the length and width of the
155 diffraction pattern, respectively. An increased EI at a given shear stress indicates
156 greater cell deformation and hence greater RBC deformability. All measurements
157 were carried out at 37°C.

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

169 **Erythrocyte Zn level measurement:** Standard solutions of 0.5 µg/ml were prepared
170 from 1000±0.002 mg/L stock solution (Titrisol, Merck) for Zn measurements.
171 Measurements were performed with atomic absorption spectrophotometer (Perkin
172 Elmer AAS 700, Ueberlingen, Germany), using Hollow Cathod Lamp (HCL) emitting
173 light at a wavelength specific to each element with the following settings: 213.9 nm
174 wavelength, 0.7 nm slit width and 30 mA current, air/acetylene flame, HCL and BGC
175 (Back Ground Correction) modes on. The spectrophotometer was calibrated with

176 blank and standard solutions.^[22] Whole blood samples were centrifuged, plasma and
177 erythrocytes were separated and erythrocytes were frozen until the time of zinc
178 measurement. Erythrocytes were thawed just before the measurements, diluted 1:20
179 in distilled water, which cause hemolysis and Zn level in erythrocytes was
180 measured.^[23]

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

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RESULTS

188 After one month long exposure to electromagnetic field, erythrocyte
189 deformability at 5.33 Pa shear stress was significantly decreased in Group B
190 (0.48 ± 0.00) compared to its control group CB (0.49 ± 0.01) (Figure 2). On the other
191 hand, erythrocyte deformability under the same shear force in Group A (0.47 ± 0.01)
192 was decreased in comparison to Group CA (0.48 ± 0.02) but this decrease was not
193 statistically significant (Figure 2).

194 Compared to the control groups, we found no statistically significant changes in
195 aggregation parameters (AI and $t_{1/2}$) with exposure to electromagnetic field in either
196 study group (Table 1).

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

199 Erythrocyte Zn levels was significantly decreased in Group A (4.63 ± 0.10) and
200 Group B (4.89 ± 0.77) compared to their control groups (6.61 ± 1.32 and 9.57 ± 1.42 ,

201 respectively) (Figure 3). On the other hand, erythrocyte Zn levels compared to each
202 other group A and group B was not statistically significant differences.

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DISCUSSION

205 Erythrocyte deformability facilitates oxygen supply to the tissues by decreasing
206 the viscosity of the blood and permitting erythrocytes to pass to the capillaries while
207 blood flowed through big vessels.^[5] In the present study, we found that erythrocyte
208 deformability under 5.33 Pa shear stress in Group B was significantly decreased than
209 the control. Erythrocyte deformability depends primarily on erythrocyte cytoskeleton,
210 cytoplasmic viscosity and biconcave discoid shape. On the other hand, hemoglobin
211 concentration predominantly determines the internal viscosity of erythrocytes.^[5] A
212 study by Salem et al. showed that static electromagnetic field increased hemoglobin
213 concentration.^[10] It can be speculated that, decrease in deformability can be due to a
214 possible increase in erythrocyte hemoglobin level in response to electromagnetic
215 field. Free radicals affect hemodynamics at various levels by altering the cellular
216 properties of the erythrocytes or mechanical properties of the membrane.^[24,25]
217 Moustafa et al. demonstrated that acute exposure to electromagnetic field of cell
218 phone origin increased lipid peroxidation, and decreased the Superoxide Dismutase
219 (SOD) and Glutathione Peroxidase (GSHPx) activities by causing oxidative stress.^[26]
220 Even though oxidative parameters were not measured in the present study, decrease
221 in erythrocyte deformability that occurs in the presence of electromagnetic field may
222 also be related to increased free oxygen radicals.

223 We found in the present study that, Zn level in the erythrocytes of rats exposed
224 to electromagnetic field was lower than the controls. On the other hand, Akdag and

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

245 Erythrocyte aggregation depends on a balance between different 3 forces.
246 These are repelling force between negatively-charged erythrocytes, adhesion force
247 between erythrocytes in the presence of plasma proteins and disaggregating shear
248 forces induced by blood flow.^[5] Another hypothesis proposed for erythrocyte
249 aggregation is bridging hypothesis. According to this hypothesis, macromolecules in

250 plasma are adsorbed onto the surface of the erythrocytes and decrease the
251 disaggregating forces between them, hold the aggregates together.^[32] There are very
252 few studies in the literature that addressed the relationship between electromagnetic
253 field and erythrocyte aggregation. Iino found that erythrocyte aggregation and,
254 therefore, erythrocyte sedimentation rate increased when erythrocytes were exposed
255 to homogenous static field in their own plasma.^[33] The author, stating that
256 erythrocytes aggregate by forming bridges with plasma proteins, a process which cell
257 orientation plays a key role, attributed this result to alteration in cell orientation in
258 response to electromagnetic field. In the present study, we did not find a significant
259 effect of electromagnetic field on erythrocyte aggregation. This difference can be
260 attributed to the fact that the whole body was exposed to electromagnetic field and to
261 the duration of exposure.

262 In conclusion, this study showed that EF decreased erythrocyte deformability as
263 well as Zn levels in rats, a trace element that affects the hemorheological
264 parameters. It is possible that the EF exerts these effects by decreasing Zn level in
265 erythrocytes directly or by increasing oxidative stress by disrupting the Zn absorption
266 or metabolism. Another plausible mechanism is that the electromagnetic field
267 decreases erythrocyte deformability by increasing erythrocyte hemoglobin levels.
268 These possibilities need further studies to elucidate.

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271 **Conflict of Interest**

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273 No conflict of interest declared by the authors.

274

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426 **Table 1:** Erythrocyte aggregation parameters of experimental groups.

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	CA	A	CB	B
	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)
AI (%)	58.18 \pm 9.03	54.71 \pm 3.24	59.30 \pm 5.32	56.34 \pm 8.57
t $\frac{1}{2}$ (s)	2.8 \pm 1.58	2.11 \pm 0.50	2.47 \pm 0.53	2.92 \pm 1.94

436 AI, aggregation index; t $\frac{1}{2}$, aggregation half time

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FIGURE LEGENDS

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Figure 1. Exposure setup with physical dimensions.

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