

## Effects of Radiofrequency Radiation on Human Ferritin: An *In Vitro* Enzymun Assay

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### ABSTRACT

Ferritin is a macromolecule and is responsible for the long term iron storage function in human serum and plasma. Recent studies have highlighted the role of cell phone exposure on central nervous system, immune function and reproduction. The aim of this study was to investigate whether the human serum ferritin level could be interfered by the exposure to the 900 MHz GSM cell phones. Fifty human serum wells from 25 normal healthy donors were labeled with ruthenium to form a sandwich complex based on an immunoassay technique. All of them were placed into two batches, and the well heads in the first batch were exposed to 900 MHz exposure emitted from a speech mode cell phone (Nokia, Model 1202, India) for 30 min. Unexposed batch was served as the control sample under identical conditions and was compared with the exposed one in quantitative determination of ferritin using the Wilcoxon test with criterion level of  $P=0.050$ . Human serum wells in the exposed batch showed a significant decrease in serum ferritin relative to the control batch ( $P=0.029$ ). The average  $\pm$  SD ferritin level in the exposed batch was  $84.94 \pm 1.04 \mu\text{g/L}$  while it was  $87.25 \pm 0.83 \mu\text{g/L}$  for the unexposed batch. Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress and rapid diffusion of the human ferritin level in an *in vitro* enzymun assay. Also, the enzyme activity can be affected. Effects of exposure from mobile phones must be considered further.

**Key words:** Human ferritin, immunoassay test, radiofrequency radiation, ruthenium complex

### INTRODUCTION

Iron (Fe) is an essential micronutrient in biological systems and plays an important role in several cellular processes, such as adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and neurotransmitter synthesis.<sup>[1,2]</sup> Tissue iron is stored inside a porous protein capsule called ferritin.<sup>[3]</sup> Ferritin is a macromolecule and is responsible for the long term iron storage function mainly in the liver, spleen, and bone marrow. Many diseases are associated with iron overload or iron deficiency. Determination of ferritin is a suitable method for ascertaining the iron metabolism situation which provides a representative measure of body's iron reserves.<sup>[4]</sup> For determination of ferritin, human serum is labeled with ruthenium to form a sandwich complex based on an immunoassay technique.<sup>[4]</sup>

In recent years, tremendous development and use of mobile phone telecommunication has drastically increased the amount of human exposition from the microwaves (MWs)

radiation in everyday life. In this regard, a growing concern about the possible health hazards have increased considerably, since mobile phones MW may have health effects on almost everyone in the world, even those who do not have such phone.<sup>[5]</sup>

The World Health Organization (WHO) established the International Electro Magnetic Field (EMF) Project in 1996 to assess health and environmental effects of exposure to EMF in the frequency range from 0 to 300 Giga Hertz (GHz).<sup>[6,7]</sup> The mobile phone technology uses 880 and 1800 MHz.<sup>[8]</sup>

Many studies on different levels of organization (human populations, animal and plant, organs and sub-cellular) have been carried out or are in progress about the various effects of radiation emissions regarding the behavior, cancer, central nervous system, sleep, children, cardiovascular system, immune function, reproduction and development.<sup>[5,9-12]</sup>

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The emitted microwaves have been shown to have many effects upon the mammalian brain,<sup>[12]</sup> sperm motility and morphology,<sup>[9]</sup> neurological pathologies syndrome<sup>[13]</sup> and immune functions.<sup>[5]</sup> However, many potential harmful effects of cell phone radiation remain controversial. Nittby *et al.* have investigated that this energy affects the mammalian blood-brain barrier permeability 7 days after exposure to the radiation from a 900 MHz global systems for mobile (GSM) communications.<sup>[14]</sup> Hinrikus *et al.* have shown that, the human brain EEG beta rhythms energies were increased by exposure to 450 MHz MW.<sup>[15]</sup> Lai *et al.* investigated the effects of microwave exposure on cholinergic systems in rat brain showing that, it is possible to establish a dose-response relationship for each brain region when different microwave power densities are used.<sup>[16-18]</sup> Recent experimental studies in mice have shown that exposure to EMF led to significant testicular germ cell apoptosis and morphological changes.<sup>[19-21]</sup> Lu *et al.*, have found that household use of air conditioners, which generate significant amounts of EMF, has been associated with low semen quality.<sup>[22]</sup> Recently Shahbazi *et al.* have investigated that, mobile phones exposure results in reduction of chorionic gonadotropin level in human serum during the immunoenzymometric assay in laboratory.<sup>[23]</sup>

In addition, it is now generally accepted that weak EMF in the power frequency range can activate DNA to synthesize proteins.<sup>[24]</sup> Furthermore, recent studies have shown that long-term exposure decreases thyroid stimulating hormone (TSH) and tri-iodothyronine-thyroxine (T3-T4) levels.<sup>[25,26]</sup> Moreover, de Tommaso *et al.* found that, 900 MHz GSM exposure induces changes of contingent negative variation in healthy volunteers, especially reducing the arousal and expectation of warning stimulus.<sup>[12]</sup> Cespedes *et al.* found that, exposed proteins to the radio frequency (RF) magnetic fields of 1 MHz and 30 mT for several hours released more iron than control samples.<sup>[27]</sup> Using the same exposing protocol, in another research, Cespedes *et al.* showed that the rates of iron chelation with ferrozine, were reduced by up to a factor of 3 in the exposed proteins. They stated that, the observed effect was non-thermal and depended on the frequency-amplitude product of the magnetic field.<sup>[28]</sup> In addition, considering the natural superparamagnetic ferrihydrite nanoparticles of protein ferritin, Cespedes *et al.*, demonstrated that superparamagnetic nanoparticles increased their internal energy when exposed to radio frequency magnetic fields due to the lag between magnetization and applied field.<sup>[29]</sup>

According to the best of our knowledge, no published articles have presented a study on this issue with the methodology and analysis described here on the effects of cell phone radiation on the immunoassays for the *in vitro* quantitative determination of human serum ferritin. The aim of this study was to investigate whether the ferritin

levels in the complex could be interfered by the exposure to the 900 MHz GSM cell phones in the laboratory.

## MATERIALS AND METHODS

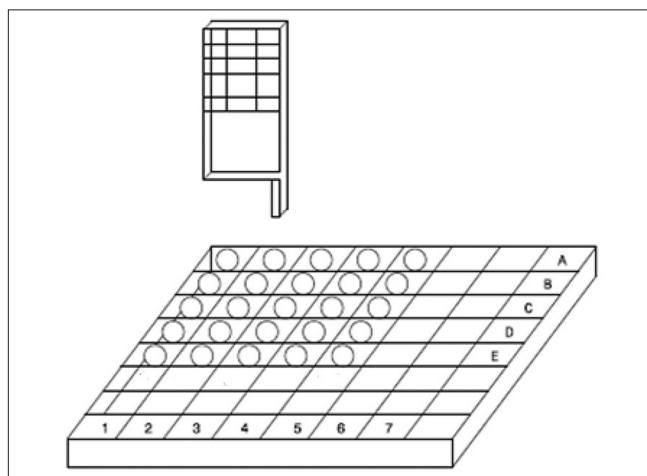
The study was approved by the Pathology Research Board at the Ahvaz Jundishapour University of Medical Sciences, Khozestan, Iran.

### Data Collection and Exposure

This study was performed based on an immunoassay technique for the *in vitro* quantitative determination of human serum ferritin in a ruthenium sandwich complex. Human serum samples were collected from 25 donors and were labeled with ruthenium. Each sample was divided to two aliquots and was placed into two batches: The first batch (exposed group) was exposed to a commercially available cell phone (Nokia, Model 1202, India) with 1.09 Watt per kilogram (W/kg) of the tissue locally in the head specific absorption rate,<sup>[30]</sup> which produces 900 MHz RF radiation, to represent the exposure of GSM. Phone was in the talk mode during expose. The phone had a hidden antenna placed on the top back of its handset. The distance between the phone antenna and each specimen was kept at 2.5 cm [Figure 1]. The duration of exposure was 30 min. For the second batch (control group), no radiation was applied to the samples and they completed the assay cycle under identical conditions during the study period.

### Immunoassay with Ruthenium Complex

For the assay two monoclonal antibodies M-4.184 and M-3.170 (Cobas, Roche Diagnostics Ltd, Mannheim, Germany) were used to form the sandwich complex. In the first incubation, 10  $\mu$ L of each sample, a biotinylated monoclonal ferritin-specific antibody and a monoclonal



**Figure 1:** Specimens were exposed to radiation emitted from the speech mode mobile phone at 2.5 cm distance

ferritin-specific antibody were labeled with ruthenium to form a sandwich complex. In the second incubation, after addition of streptavidin-coated microparticles, the complex was became bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Following application of a voltage to the electrode the induced chemiluminescent emission was measured by a photomultiplier (Hitachi High-Technologies Corporation, Tokyo, Japan). All specimens (aliquots) were kept at room temperature to avoid the effect of temperature on the assay. Finally, results were determined via a calibration curve which was generated instrument-specifically with a master curve via the reagent barcode.

All experiments were performed comparing 1.09 W/kg exposed batch with the unexposed control batch. To avoid the variability inherent to the assay used, all tests were performed for two independent experiments.

### Statistical Analysis

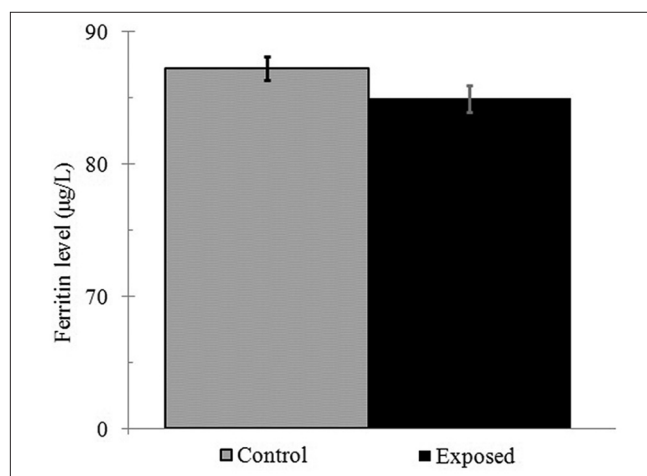
Mean values and standard deviations were calculated and statistical significance of the differences between exposed samples and controls was evaluated. A computer program (SPSS version 16.0, Chicago, IL, USA) was used for statistical analysis. Data were analyzed by Wilcoxon test (Nonparametric version of paired samples T-test). All hypotheses tested using a criterion level of  $P = 0.05$ .

### Power Density

According to the International Commission for Non-Ionizing Radiation Protection (ICNIRP)<sup>[31]</sup> and the Federal Communications Commission (FCC)<sup>[32]</sup> the reference level for exposure of RF-EMW is peak power density. It is a commonly used term for characterizing an RF electromagnetic field. There were some irradiation sources in the laboratory (i.e., the wireless networks in the laboratory). Since, the study was performed in a distinct place of the laboratory and background radiation for all the batches (exposed and control groups) were identical, power density of these sources was not monitored during the study.

## RESULTS

Radiation exposure from mobile phones altered the serum levels in the exposed wells. Using the Wilcoxon test, a significant decrease in serum ferritin in the exposed group compared to the control group was seen ( $P = 0.029$ ). The final average  $\pm$  SD of ferritin level in the exposed group was  $84.94 \pm 1.04$   $\mu\text{g/L}$  while it was  $87.25 \pm 0.83$   $\mu\text{g/L}$  for the control group [Figure 2]. In other words, the present study



**Figure 2:** A comparison of average  $\pm$ SD serum ferritin, among control and the exposed groups

showed that, the final ferritin measured level in the assay was affected by 30 min radiation exposure from 900 MHz mobile phone at 1.09 W/kg SAR.

## DISCUSSION

Results of this study showed that, human serum ferritin levels labeled with ruthenium were affected following mobile phones exposure. The reason could be due to oxidative stress and rapid diffusion at high electromagnetic irradiation and field caused by mobile phone. Moreover, the enzyme activity can be affected.

Serum ferritin is widely used in diagnosing and monitoring neurodegenerative disorders-Parkinson's and Alzheimer's disease, progressive supranuclear palsy and neuroferritinopathy.<sup>[33]</sup> As most iron in the human is present as ferritin-bound iron, it is obvious that any change in the structure or function of ferritin may be related to oxidative stress and damage. However, the mechanisms and regulation of ferritins iron release and reutilization have not yet been fully elucidated.<sup>[34]</sup> For determination of ferritin, immunoassay technique based on sandwich complex of human serum and ruthenium is used.

It has been shown that RF fields at 300 MHz to several GHz, at which significant local and non-uniform absorption occurs, induce torques on molecules that can result in displacement of ions from unperturbed positions, vibrations in bound charges (both electrons and ions), and rotation and reorientation of dipolar molecules such as water.<sup>[5]</sup>

The findings here were in good agreement with previously published literature<sup>[31-33]</sup> Zagorskaia and Rodina, found that lowered concentrations of thyroid hormones labeled with Iodine-125 during 2 months after a single exposure of rats to 20 mT extremely low frequency EMF<sup>[35]</sup> Diem

*et al.*, reported DNA single- and double-strand induced breaks due to 1800 MHz RF-EMF exposure at 1.2 W/kg SAR.<sup>[36]</sup> Ammari *et al.*, investigated the effects of a chronic GSM 900 MHz exposure on glia in the rat brain. They have concluded that chronic exposure to 900 MHz MW may induce persistent astroglia activation in the rat.<sup>[10]</sup> Barteri *et al.*, studied the *in vitro* interaction between RF radiation and proteins of different species. They have demonstrated that mobile phone exposure affects the structural and biochemical characteristics of an important CNS enzyme.<sup>[37]</sup> Nittby *et al.* have investigated that albumin extravasation enhanced in the rats which were exposed to mobile phones at 12 mW/kg SAR.<sup>[14]</sup> While, Lantow *et al.* demonstrated that RF-EMF exposure of human monocytes and lymphocytes did not have any activating capacity to induce hsp70 expression.<sup>[38]</sup> Gurisik *et al.* found no significant differences between sham-exposed and RF-exposed cells in any of the assays or conditions examined.<sup>[39]</sup> Lee *et al.* reported that 1763 MHz RF radiation alone did not elicit any stress response.<sup>[40]</sup> Yuasa *et al.*, investigated that the pulsed EMF field emitted by a mobile phone for 30 min has not short-term effects on the human somatosensory evoked potentials.<sup>[41]</sup> Possible reasons for these may be the lack of statistical power due to a small sample size, shorter exposure time and lower levels of SAR and serum concentrations. It has been shown that exposure at 1.09 W/kg SAR, especially in the higher levels of the human chorionic gonadotropin concentrations (>100 mIU/mL), caused a significant loss compared to 0.69 W/kg SAR exposure.<sup>[23]</sup> Moreover, considering the exposure time, an increased response in the alkaline comet assay in exposed human fibroblasts has been detected at 16 h compared to 4 h exposure time.<sup>[36]</sup> Therefore, perhaps, the trends seen for decrease in serum ferritin would have reached more statistical significance if exposure times had been longer.

Moreover, it should be noted that since each laboratory has its own conditions and instruments, results obtained in individual laboratories may differ from each other. Considering immunoenzymometric ferritin assay used in this study, it has been reported that repeatability and precision of the results by using different test methods and analyzers may varied from 1.9% and 2.7% to 3.0% and 4.4%, respectively.<sup>[39]</sup> At this work, serum ferritin level in the exposed group showed 2.7% decrease compared to the control group, which was within the used method's precision ( $P = 0.029$ ).

The results of this study were obtained by using a commercial 1.09 W/kg at 900 MHz mobile phone to reproduce the reality of the human exposition. Today, an average SAR of 2 W/kg is selected, because it is the safety limit for the mobile-phone microwave-radiation emission as defined by ICNIRP.<sup>[31,42]</sup> However, results of this study suggested that the allowed radiation emission levels for mobile phones might

not be sufficient to prevent biological effects. Of course, further investigations on SAR level dependence and dose dependence are required.

Mobile phones can affect the immunoassay a MW specific, non-thermal action, and a thermal molecular effect, or a combination of these mechanisms. Few studies that made the measurements before and after but not during exposure to EMF have ever demonstrated a significant effect of non-thermal levels of electromagnetic radiation on brain.<sup>[43]</sup> Panagopoulos *et al.*, studied the mechanism of EMF action on cells plasma membrane using a biophysical model. Their results showed that, an external EMF causes forced-vibration of all the free ions on the membrane surface.<sup>[44]</sup>

The present results are consistent with our previous study which found significant effects of the mobile phone use on the immunoenzymometric assays.<sup>[23]</sup> Even though we studied only one aspect of the human serum, using available *in vitro* methods, we conclude that short term effects can be detected on the human ferritin after 30 min mobile phone exposure.

Due to limitations and difficulties associated with *in vivo* studies, at this work an *in vitro* experiment was performed to investigate the effects of exposure to the cell phone on the ferritin level. Nevertheless, *in vivo* studies, whether on animals or human beings, may provide more convincing evidence of the EMF effects.<sup>[12]</sup>

More accurate follow-up studies are needed for the evaluation of the effects of the mobile phone use. The results here should be confirmed in larger series, employing repeated exposure-dose related effect design and providing a detailed assessment of EMF magnetic fields produced by the phones.

On the basis of the present results, we can believe that mobile phone exposure may affect human long term iron storage function by reducing the ferritin level in the human plasma and serum. Considering that this is the first study dealing with the possible effects of EMF on the *in vitro* immunoassays for the quantitative determination of ferritin, further *in vivo* studies on mammals are of utmost importance. However, the evaluation of the possible effects of cell phones radiation and the emitted MWs on the living organism is a complex process that needs the combined contributions of many scientific regulations.

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