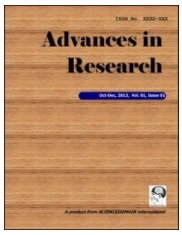


*Advances in Research*
*2(9): 478-493, 2014, Article no. AIR.2014.9.001*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# *In vitro* Effects of Low Intensity 1.8 Ghz Electromagnetic Radiation on Peripheral Blood Leukocytes from Healthy Donors: A Morphometric and Morphological Study

**E. Jirillo[1], S. Boffola[1], R. Stefanelli[1], T. Magrone[1], E. Vitale[1], M. T. Pappagallo[2], M. Lasalvia[3], G. Perna[3], V. Capozzi[3], A. Ermini[4], T. Ligonzo[5], L. Schiavulli[5] and P. F. Biagi[5*]**

*[1]Department of Basic Medical Sciences, Neuroscience and Sensory Organs, University of Bari, Bari, Italy.*
*[2]Blood Bank, Polyclinic Hospital of Bari, Bari, Italy.*
*[3]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy.*
*[4]Department of Industrial Engineering, University of Rome Tor Vergata, Rome, Italy.*
*[5]Department of Physics, University of Bari, Bari, Italy.*

***Authors' contributions***

*This a collaborative work conducted by all authors. Authors EJ, SB, RS, TM, EV and MTP attended to the acquisition of blood samples and performed all the morphometric and morphological analyses. The other authors set the electromagnetic radiation system and performed the experiments of blood exposure to EMR. All authors designed the study and participated either to the discussion of the results or the preparation of the manuscript draft. The Authors EJ, TM, VC and PFB arranged the final version. All authors read and approved the final manuscript.*

***Original Research Article***

***Received 17th March 2014***
***Accepted 12th May 2014***
***Published 23rd May 2014***

## ABSTRACT

**Aims:** Until now, studies related to the morphometric and morphological variations produced on peripheral blood leukocytes from healthy donors by exposure to 1.8 GHz electromagnetic radiation (EMR) yielded controversial results. The aim of this research work is to increase the statistics regarding the above mentioned variations.
**Methodology:** By using a reverberation chamber, which provides a controlled EMR intensity, 108 samples of human leukocytes from healthy donors were exposed to EMR of

**Corresponding author: E-mail: pierfrancesco.biagi@uniba.it;*

different intensities (12±4 V/m, 22±6 V/m, 42±9 V/m and 78±10 V/m) for times ranging from 5 min to 24 h. Sham exposed blood samples of the same donors were considered as controls. Using a computerized morphometric method, microscopic observations of the area size occupied by each cell were conducted; in each case the dimensional measurements were carried out on three different samples (from different donors). Besides, morphological observations were conducted staining smeared blood samples with May-Grünwald-Giemsa.

**Results:** Exposed and sham exposed leukocytes average size was compared using the Statistical GraphPad Prism 5.0 software. In 18% out of 108 cases examined, no effects dependent on EMR have been revealed. On the contrary, statistically significant variations in area of exposed leukocytes in comparison to non exposed cells were observed in 82% out of 108 cases examined. In 68% out of 108 cases an increase in leukocyte areas was demonstrated along with morphological variations of cells; in 14% out of 108 the cases, a decrease in leukocyte areas was observed.

**Conclusions:** Even though this study needs a functional evaluation of leukocytes exposed to EMR, our results suggest that 1.8 GHz EMR is able to produce increase in the leukocyte areas as well as morphological alterations.

*Keywords: Blood donors; electromagnetic radiation; leukocytes.*

## 1. INTRODUCTION

In the last years, several experiments have been carried out in order to investigate the effects of electromagnetic radiation (EMR) on biological systems. Results of these studies were sometimes discrepant and controversial and, therefore, this issue is still object of scientific debate.

In particular, an interesting item to point out concerns the investigation on the effects of electromagnetic radiation on peripheral blood cells, which circulate through the entire human body and, therefore, they are mainly involved into environmental exposure to EMR. Several works report on 1.8-2.4 GHz radiofrequency effects on peripheral blood cells after either *in vivo* or *in vitro* experiments. Most of these works discussed about radiofrequency field induced genotoxicity in peripheral blood leukocytes [1-13], by reporting conflicting results, whereas few of them deal with morphologic effect caused by radiofrequency field. In fact, morphology changes can be due to biochemical modifications occurring inside cells as a consequence of EMR action, which modifies the cell inner structure [14]. In particular, an early work of Cleary et al. reported no detectable effects of 2.45 GHz radiation at Specific Absorption Rate (SAR) values below 50 W/Kg on lymphocyte morphology and viability [15]. However, very recently Esmekaya et al. reported experiments about mutagenic and morphologic effects of 1.8 GHz modulated radiofrequency in human cultured peripheral blood lymphocytes at a SAR average of 0.21 W/kg. The main effects, such as destruction of organelle and nucleus structure cytoplasmatic, cytoplasmic lysis and destruction of membrane integrity resulted more pronounced with increasing exposure time up to 48 h, although chromatin change and the loss of mitochondrial crista were observed in cells exposed for 8 h and 24 h [16]. Detectable morphological effects after EMR radiation exposure at 1.8 GHz and SAR of 1.25 W/Kg were also reported by Aly et al. [17] indeed, they found changes in shape (cell shrinking, expanding and rolling) in human leukocytes following exposure.

Overall, the literature data about blood cells exposure to EMR have not definitely clarified the problem about modifications of cell morphology related to EMR action. Therefore, further experiments are necessary in order to increase the statistical data.

In this paper, we have exposed peripheral normal human leukocytes to EMR radiation at 1.8 GHz frequency commonly employed in the dual band mobile phones. Time of exposure ranged between 5 min and 24 h. These preliminary results show that a statistically significant variation of cell dimensions is detectable in exposed leukocytes regardless of intensities and time of exposure. Morphological observations also will also reveal alterations in cell shape.

## 2. MATERIALS AND METHODS

### 2.1 Radiation System at 1.8 GHz and Electric Field Measurement

In the framework of the low power microwave mobile phone communication researches, we have used an apparatus at 1.8 GHz EMR designed and implemented previously [18,19]. The apparatus uses a reverberation metallic chamber whose dimensions are larger with respect to the wavelength of the indoor EMR. The internal EMR is characterized by stochastic values, but it is averagely uniform and isotropic one [20]. In details, the apparatus consists of: a) a reverberation chamber (Fig. 1a) to be used as a radiated environment; b) a rack (Fig. 1b) with an appropriate instrumentation. A stick transmitting antenna 4.15 cm long (corresponding to $\lambda/4$ for 1.8 GHz) is fixed vertically on a side wall of the chamber. The rack contains an EMR generator (from 100 kHz to 2112 MHz) with an output power ranging from -140 $dB_m$ to +10 $dB_m$ and a first power amplifier (up to 5 W in the frequency range 1.5-2.0 GHz) connected to the transmitting antenna. Another power amplifier (up to 20 W for 1.8 GHz) is connected in cascade with the previous one. The generator and the power amplifiers are indicated as G, $P_1$ and $P_2$ in Fig. 1b. In addition one personal computer with a video-recording card for video data acquisition inside the chamber and one digital instrument to be connected to a humidity-temperature sensor placed in the chamber, are located on the rack. The apparatus was characterized at different input powers by measuring the electric field intensity (*E*) in different points by means of a Field Meter (PMM 8053B) connected with an optical fiber to an Electric Field Probe (PMM EP-600). The whole dimension of the probe is 5.3 cm and its weight is 90 g. The sensitivity and frequency ranges are 0.15-90 V/m and 100 kHz-9.25 GHz, respectively. The probe was calibrated in air at the PMM Calibration Laboratory.

### 2.2 Blood Collection

Peripheral blood was collected from healthy donors enrolled in the Blood Bank from Polyclinic Hospital (Bari, Italy). Healthy donors are usually screened either through a routine laboratory assessment or clinically and, thereafter, selected for blood donation. In such way factors as smoking, alcohol, acute and chronic diseases which may affect quantitatively and qualitatively the blood sample can be ruled out. Samples of citrated blood (1ml) were then dispensed into micro tubes for exposure to EMR.

### 2.3 Dosimetry and Cell Exposure Protocol

In a previous investigation [18] plexiglas boxes filled with 300 ml of physiological liquid were used as simple phantoms and *E* was measured inside the liquid by using the previous probe in a waterproof condition. Firstly, using the comparison between the values obtained by

numerical computation (performed with Microwave CST 5.0 software) and the experimental ones, a correction factor ε for the calibration of the probe inside the physiological liquid was estimated.

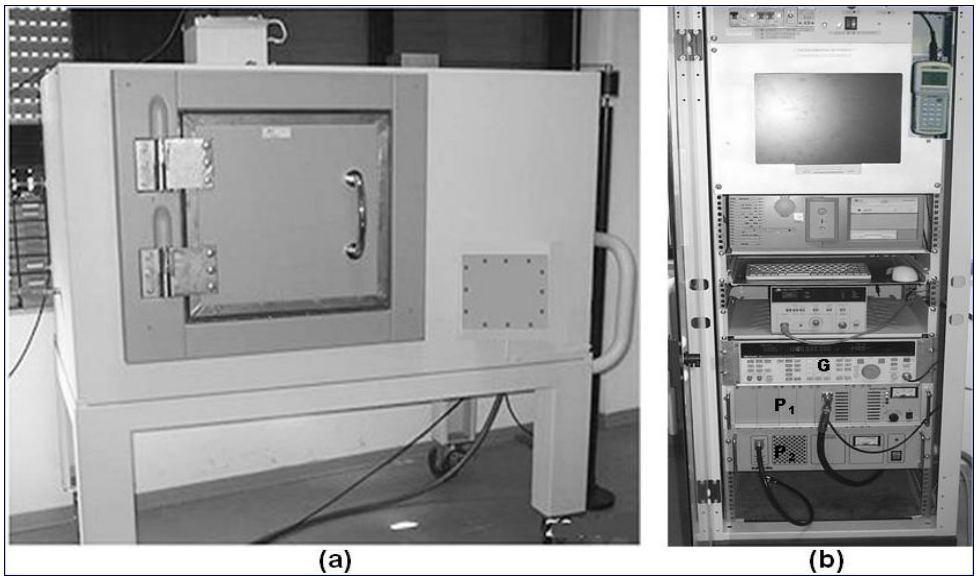


**Fig. 1. The EMR apparatus used in the experiments. (a) The reverberation chamber used as a radiated environment. (b) The rack with the appropriate instrumentation; the letters G, $P_1$ and $P_2$ indicate the generator and the two power amplifiers, respectively**

$\varepsilon = E_{me}/E_{in} = 1.3$ was obtained, where $E_{in}$ is the effective $E$ value obtained inside the liquid and $E_{me}$ is the measured value averaged over 6 min, indicated by the Field Meter. Then, measurements of $E$ were performed for different input powers; the correction factor was applied to the $E_{me}$ values of these measurements obtaining in such a way the effective $E_{in}$ values inside the physiological liquid. On the basis of these measurements, the SAR at the different powers was obtained by the relation:

$$SAR = \sigma E_{in}^{2}/\rho \quad (1)$$

where σ is the electrical conductivity and ρ the mass density of the physiological liquid. The values used were: σ= 1.585 S/m and ρ= 1006 kg/m$^3$. It was pointed out that the $E_{out}$ existing in the cage outside the phantom is about 12 times the corresponding $E_{in}$ inside the phantom. In a further investigation [19] proper oil-in-gelatine dispersion [21] mimicking human brain (gray matter) instead of the physiological liquid was used. By means of the previous procedure the SAR values at different powers were obtained using: σ= 1.870 S/m and ρ= 1009 kg/m$^3$. In this case it was found that $E_{out}$ was about 8 times the corresponding $E_{in}$ values into the phantom and a correction factor ε=1.5 was used.

For the blood samples four different radiation conditions of the empty environment were selected. Therefore, we adopted the procedure used for the physiological liquid and for each EMR condition several measurements of the $E$ were performed in different sites obtaining the following mean values: 12±4 V/m, 22±6 V/m, 42±9 V/m and 78±10 V/m, respectively. The previous two lower values of $E$ represent usually existing levels [8] near the antennas of

the cellular phones when working. The other values are about twice larger than the previous two and they can be detected near the antennas of cellular phones when they are trying to connect to their service provider.

**Table 1. Mean values of the electric field intensity $E_{in}$ inside 100 ml of a blood sample and of the $E_{out}$ defining the corresponding exposure conditions. In the right column the relative SAR values are reported**

| $E_{out}$ (V/m) | $E_{in}$ (V/m) | SAR (W/Kg) |
|---|---|---|
| 12±1 | 0.9±0.1 | $1.1\ 10^{-3}$ |
| 22±3 | 1.5±0.1 | $3.1\ 10^{-3}$ |
| 40±4 | 2.7±0.2 | $9.4\ 10^{-3}$ |
| 72±7 | 4.8±0.4 | $2.9\ 10^{-2}$ |

At these exposure conditions, by means of the procedure above mentioned, taking into account a correction factor ε=1.2 the SAR values for 100 ml of a given blood sample were obtained using: σ= 1.250 S/m and ρ= 1060 kg/m$^3$ [22]. At the same time, in order to evaluate the *E* to which the blood sample was effectively exposed, *i.e.* the $E_{out}$ values, measurements of the *E* were performed in different points inside the chamber. The results are shown in Table 1 where the error represents the semi dispersion of the values.

From Table 1, it appears that: 1) $E_{out}$ is about 15 times the corresponding $E_{in}$ values; 2) the perturbation produced by the blood sample with respect to the empty environment (the *E* values of the selected four different radiation conditions) remains into the error range; 3) Our SAR values are lower than those generally reported in these investigations, which are comprised in the range 0.4-10 W/kg.

In this regard, we point out that our SAR values should be in the previous range using for the calculation the $E_{out}$ values instead of the $E_{in}$ ones. However, in such way a systematic error may be introduced. Taking into account the statement 2) we have reported in the following of this paper the empty environment *E* values instead of the $E_{out}$ ones.

In our experiments, we have used continuous exposures of 5, 15, 30 min, 1, 2, 3, 6, 12 and 24 h, with a 1.8 GHz frequency, instead of intermittent exposure cycles as frequently used in recent studies [10,11,23].

Blood samples were kept at room temperature (RT) ranging from 17ºC to 21ºC during the different measurements. For exposure of 6 h or longer, variations of ±1ºC often occurred in the room. Temperature inside the chamber and the blood samples was simultaneously checked, mainly during the longer exposure times, and never significant differences of values were recorded. Sham exposed blood samples maintained at RT for the same periods of time were considered as controls. For each exposure condition, the dimensional measurements were carried out on samples from three different donors and the same sample was used as control and for exposure to EMR.

## 2.4 Analyses

The effects of different EMR for different exposure times in leukocytes have been evaluated by means of morphometric and morphological analyses.

#### 2.4.1 Morphometric analysis

- A constant number of fields (50 leukocytes *per* field) on the smear were acquired by means of an optical microscope (DMRB model, Leica; 100X immersion objective).
- The size of the area occupied by each cell was determined by mean of the software SPOT 5.1.

The comparison between exposed and sham exposed leukocyte average area was made by the paired two tailed *t* test of Statistical Graph Pad Prism 5.0 software. Values have been considered to be significant for $p< 0.0001$ and $p<0.05$, respectively.

#### 2.4.2 Morphological analysis

- Smeared blood samples were stained with May-Grünwald-Giemsa.

At least 50 leukocytes *per* field (at least 3 fields) were read by two scientists with expertise in haematology and observations were independently recorded in a double blind fashion.

## 3. RESULTS

### 3.1 Morphometric Analyses

The results are reported in the different panels of Figs 2-3, where the cases of statistically significant differences are marked by asterisks and the three different samples for each exposure condition are indicated by letters from A to I.

In Fig. 2 (left side), results obtained after exposure of leukocytes to EMR with $E = 78 \pm 10$ V/m for different times are indicated in panel A. A significant increase in leukocyte areas occurred for all three samples following 24 h exposure to EMR. Following 12 h exposure, a significant increase in leukocyte areas was observed in only one case. After 6 h exposure, in two cases, a significant increase in leukocyte areas occurred. However, in one control sample larger leukocyte areas than those measured in exposed samples were detected, thus resulting in a statistically significant decrease after exposure. In synthesis, among exposed cases to EMR the increased cellular areas were observed in 67% of samples, the decreased cellular areas were observed in 11% of samples and no effect was detected in 22% of samples.

In panel B (Fig. 2, left side), after 3 h exposure, in two cases leukocyte areas significantly increased, but in one case areas of sham exposed leukocytes were higher than those of exposed samples. With regard to 2 h exposure, in all cases, leukocyte areas were larger than controls. Following 1 h exposure, in one case areas of control leukocytes were larger than those of exposed samples. In two cases, exposed leukocytes displayed larger areas than those measured in the controls. In summary, an increased effect by EMR on leukocyte areas was observed in 67% of cases and a decreased effect was observed in 33% of cases.

In panel C (Fig. 2, left side), as for 30 min exposure, in one case exposed sample had larger areas than those of controls; in one case no significant difference was observed, while in the last case control leukocytes exhibited larger areas than those determined in exposed samples. With reference to 15 min exposure, in two cases no significant differences were observed, while in one case the exposure to EMR led to a more significant increase in

leukocyte areas in comparison to controls. It is worth mentioning that after 5 min exposure, all samples were characterized by larger areas than those detected in the controls.

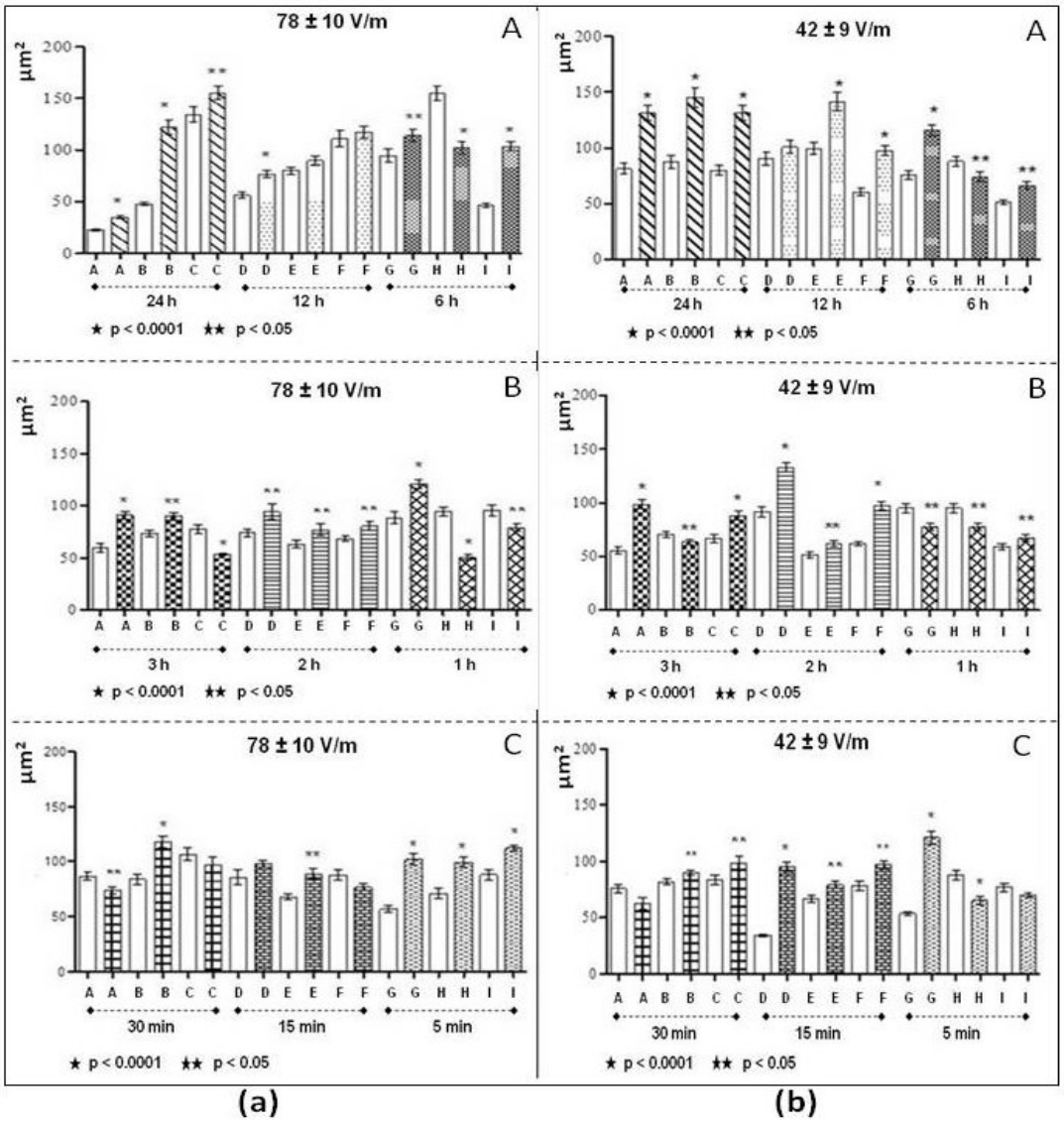


**Fig. 2. Effects on leukocyte areas in exposed and sham exposed samples with two different EMR intensities. (a) Left side: Average leukocyte area±SEM bars in exposed samples (weave columns) to EMR with *E* intensity of 78±10 V/m at different intervals of time and in sham ones (white columns), respectively. Panel A: intervals time from 24 h to 6 h; Panel B: intervals time from 3 h to 1 h; Panel C: intervals time from 30 min to 5 min. (b) Right side: The same of left side but related to EMR with *E* intensity of 42± 9 V/m**

In summary, the increased effect on leukocyte areas was observed in 56% of cases, the decreased effect was observed in 11% of cases and no effect was observed in 33% of cases.

Out of 27 cases, at E= 78±10 V/m exposure, leukocyte areas were statistically significant larger than those from controls in 64% of cases. The opposite behaviour occurred in 18% of cases and in 18% of cases, respectively, where no effect was revealed.

Fig. 2 right side shows the results obtained with exposure of *E*= 42±9 V/m at different times. In panel A, it is evident that a significant increase in leukocyte areas occurred for all three samples after 24 h exposure to EMR. Following 12 h exposure, a significant increase in leukocyte areas was observed in two cases, whereas in one case there was a negligible difference between exposed samples and controls. After 6 hours exposure, in two cases, a significant increase of cell areas was observed. However, in one case controls exhibited larger area than those detected in exposed samples. In summary, the increased effect on areas was observed in 78% of cases, the decreased effect as well as the absence of any effect was observed in 11% of cases.

In panel B, after 3 h exposure in two cases leukocyte areas significantly increased, while in one case there was a significant decrease. After 2 h exposure, in all cases, leukocyte areas were larger than those determined in controls. Following 1 h exposure, in one case areas of control cells were significantly larger than those of exposed samples and in the other two cases a significant decrease was detected. In summary, the increased effect on areas was observed in 67% of cases and the decreased effect was observed in 33% of cases.

In panel C, after 30 min exposure, in two cases exposed samples displayed larger areas than those from controls, whereas in one case controls exhibited larger areas than those of exposed samples. After 15 min exposure, in all three cases exposed leukocytes displayed larger areas than those detected in controls. Following 5 min exposure, in only one case exposed leukocytes showed larger areas than those measured in controls, in one case no significant difference between exposed and sham exposed cells were detected and in one case no effect was recorded. In summary, the increased effect on areas was observed in 67% of cases, the decreased effect was observed in 11% of cases and no effect was observed in 22% of cases.

Out of 27 cases, at *E*= 42±9 V/m exposure, leukocyte areas were statistically significant higher than those detected in controls in 71% of cases. The opposite situation occurred in 18% of cases, while no effects were observed in 11% of cases.

Fig. 3 (left side), showed results obtained after exposure to EMR with *E* = 22±6 V/m at different exposure times. In panel A, it was evident that a significant increased in leukocyte areas occurred for all three samples after both 24 h and 12 h exposure. Also After 6 h exposure, in two cases an evident increase in cell areas was observed, while no effect was evidenced in the other sample. In summary, increased effects on areas were observed in 89% of cases, while decreased effects were observed in 11% of cases.

In panel B (left side), after 3 h exposure, in two samples areas were significantly increased, while in one case there was no difference between exposed and not exposed cells. Following 2 h exposure, in all cases, leukocyte areas were statistical significantly higher than those detected in controls, whereas after 1 h exposure an increased effect was observed in two cases. Only in one case no effect was observed. In summary, increased effects on

cellular areas were observed in 78% of cases while in 22% of cases no effects were revealed.

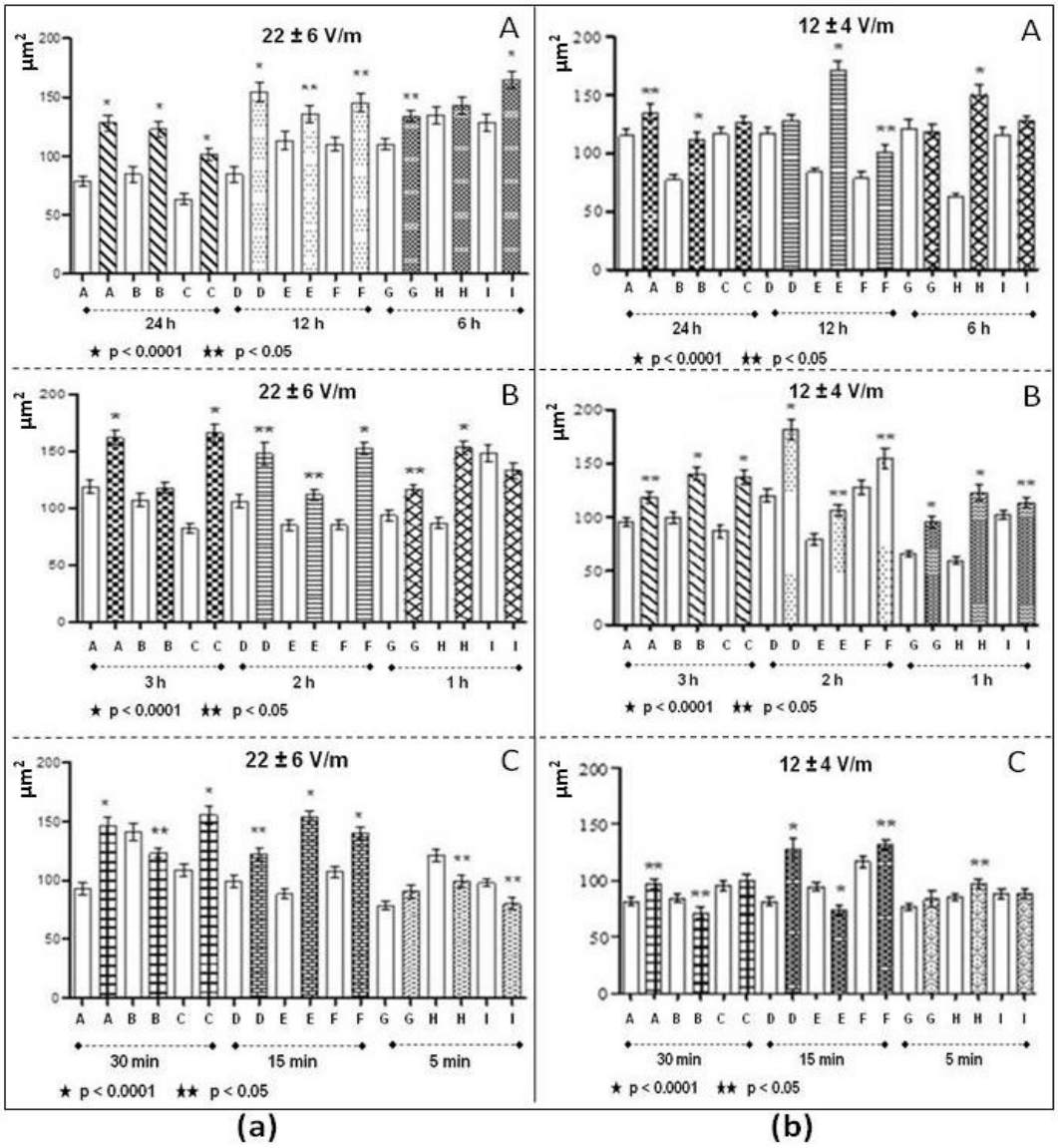


**Fig. 3. Effects on leukocyte areas in exposed and sham exposed samples with two different EMR intensities. (a) Left side: Average leukocyte area±SEM bars in exposed samples (weave columns) to EMR with *E* intensity of 22± 6 V/m at different intervals of time and in sham ones (white columns), respectively. Panel A: intervals time from 24 h to 6 h; Panels B: intervals time from 3 h to 1 h; Panel C: intervals time from 30 min to 5 min. (b) Right side: The same of left side but related to EMR with *E* intensity of 12± 4 V/m**

In panel C, at 30 min exposure, in one exposed sample there was a statistically significant decrease in areas in comparison to control areas, while in other two samples there was a significant increase in cellular areas. After 15 min exposure, all cases showed significant increase in cellular areas. Following 5 min exposure, in two cases a reduced area was evident and in the other one no difference in comparison to controls was observed. In summary, increased effect on areas was observed in 56% of cases, and the decreased effect was detected in 33% of cases. The lack of effects was observed in 11% of cases.

Out of 27 cases, with a 22±6 V/m exposure intensity, leukocyte areas were statistically significant higher in comparison to those from controls in 74% of cases. The opposite behaviour occurred in 11% of cases and it appeared only at minimum exposure time, while no effect in 15% of cases was revealed.

Fig. 3 (right side), showed results after *E*=12±4 V/m exposure at different exposure times. In panel A, it is evident that an increase in leukocyte areas occurred for all the three samples after 24 h, 12 h and 6 h exposure to EMR, respectively. Leukocyte areas were statistically significant higher than those from controls in 100% of cases.

In panel B, after 3 h exposure, in two cases leukocyte areas significantly increased, while in one case there was no difference between exposed and not exposed cells. The same situation occurred after 2 h exposure, whereas, after 1h exposure, an increase in leukocyte areas occurred in one case and in the other two cases no effect was observed. In summary, the increased effects on cellular areas were observed in 56% of cases while in 44% of cases no effect was revealed.

In panel C after 30 min exposure, in one case exposed samples exhibited larger areas in comparison to controls, in one case controls and exposed samples exhibited no significant difference and in one case there was a significant decrease. Following 15 min exposure, in two cases increased leukocyte areas in comparison to those from controls were observed, while in one case the opposite situation was observed. After 5 min exposure, one case showed a significant increase of leukocyte dimensions, while in the other two cases no effects were observed. In summary the increased effect was observed in 45% of cases, the decreased effect was detected in 22% of cases, while the lack of effects in 33% of cases.

Out of 27 cases with *E*= 12±4 V/m exposure, leukocyte areas were statistically significant higher than controls in 67% of cases. The opposite behaviour occurred in 7% of cases in correspondence of the lower exposure times. Finally, no effect was detected in 26% of cases.

## 3.2 Morphological Analyses

Samples which underwent morphometric analysis were also evaluated by microscopic observation (100X) after staining with May-Grünwald-Giemsa (Figs. 4-7).

In Fig. 4, samples with a shape that was statistically higher in comparison to controls are depicted. In panel a leukocytes of normal shape are shown. In particular, two neutrophils on the top side and one eosinophil on the bottom side are evident. Panel b shows a neutrophil with an ovoid larger shape than normal dimension, also exhibiting swollen nuclear lobes. Panel c shows irregular shaped leukocytes with dimension larger than the average in comparison to controls, nuclear material abnormally scattered in the cytoplasm and

presence of several vesicles. Panel d illustrates one leukocyte with irregular shape, thinned plasmatic membrane, and two dissociated nuclear segments. Finally, in panel e one leukocyte with abnormal shape, many vesicles scattered in the cytoplasm and irregularly shaped nucleus is shown.

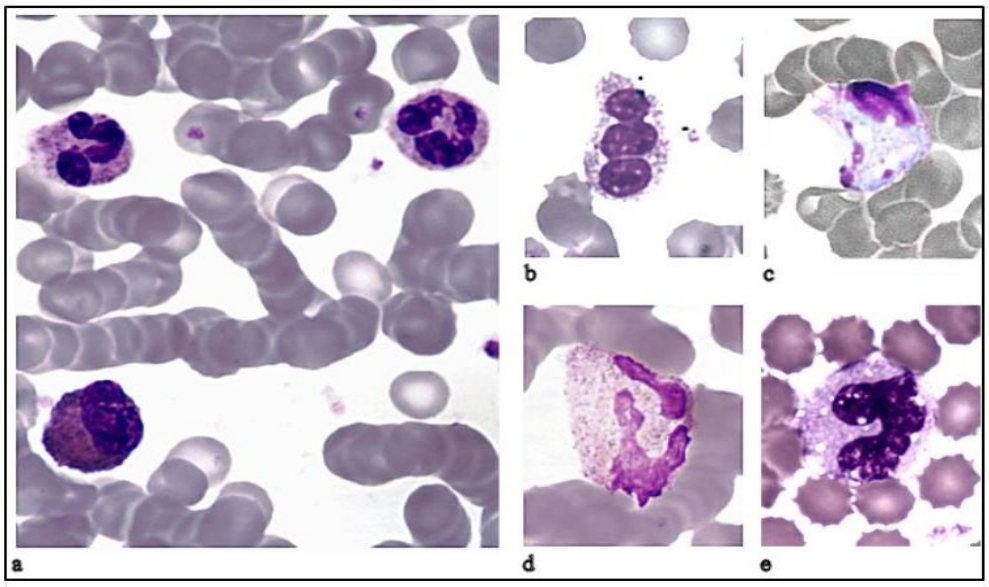


**Fig. 4. Morphological analysis of exposed and sham leukocytes to EMR. In panel a, sham leukocytes to EMR, are shown. In panels b-e, exposed to EMR are represented**

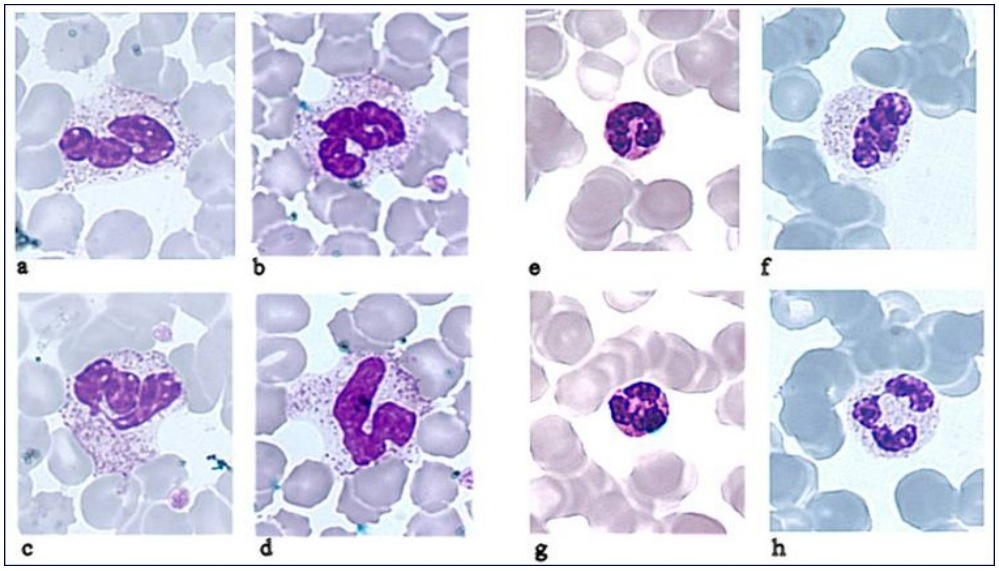


**Fig. 5. Morphological analysis of exposed and sham exposed leukocytes to EMR. In panels a-d, sham exposed leukocytes to EMR are shown. In panels e-h, exposed leukocytes to EMR are represented**

Fig. 5 shows leukocytes that according to morphometric analysis exhibited an increased average area before exposure, while same cells underwent a decrease in average area after exposure. In panels a, b and c neutrophils with shapes above the normal range with the presence of different vesicles in the cytoplasm are evident. In panel d one leukocyte with abnormal shape and irregular nucleus is shown. In panels e, g, and h neutrophils with reduced shape, but with normal nuclear and cytoplasmatic characteristics are shown. In panel f a normal neutrophil is shown.

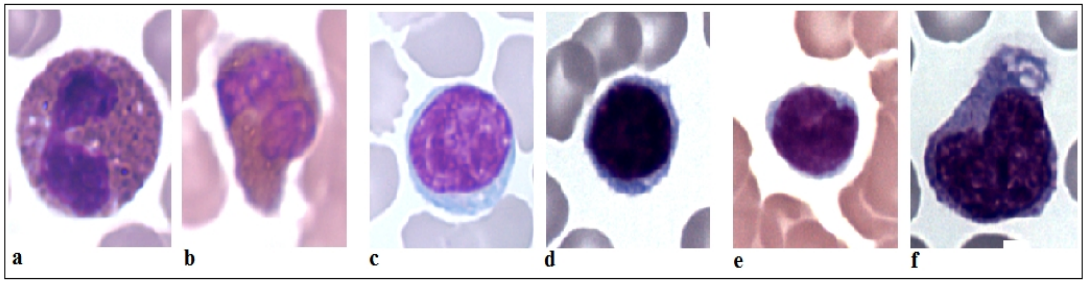


**Fig. 6. Morphological analysis of exposed and sham exposed leukocytes to EMR. In panel a sham exposed eosinophil is represented. In panel b an exposed eosinophil is depicted with irregular shape and presence of nuclei in the nucleus. In panel c a sham exposed lymphocyte is represented. In panel d an exposed lymphocyte is illustrated with more basophilic cytoplasm and larger nucleus. In panel e a sham exposed monocyte is represented. In panel f an exposed monocyte is depicted showing a larger size with abnormal shape and presence of nuclei in the nucleus**

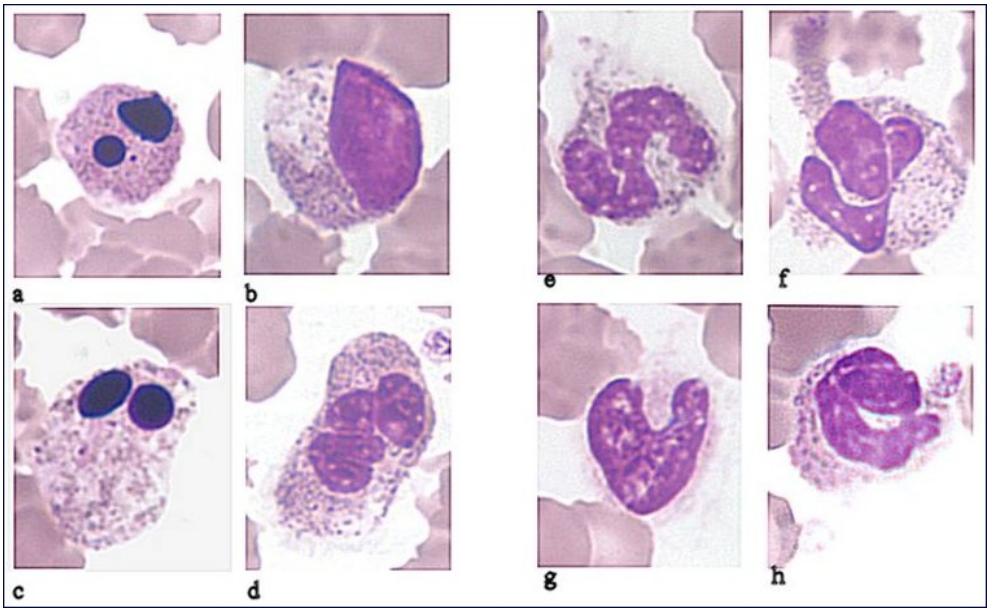


**Fig. 7. Morphological analysis of exposed and sham exposed leukocytes to EMR. In panels a-d, sham exposed leukocytes to EMR are showed. In panels e-h, exposed leukocytes to EMR are represented**

Fig. 6 represents other cell types which have changed shapes also showing irregular nucleus after EMR exposure: panels a and b: an eosinophil; panels c and d: a lymphocyte; panels e and f: a monocyte.

In Fig. 7, a selection of leukocytes which did not show any difference after exposure to EMR is reported. In fact, according to morphometric analysis, leukocytes before exposure exhibited larger area than normal average. Panels a and c show quite large cells with irregular edges, and with two distinct and separate nuclei. In panel b a blast cell is evident, while panel d shows a neutrophil with abnormal morphology and irregular nucleus. Panels e, f, g and h show leukocytes with a thinned plasmatic membrane and abnormal blast-cell like nuclei.

### 3.3 Temperature Influence

The previous results were obtained keeping blood samples at RT (17-21ºC), as mentioned in section 2.3. Some experiments were performed keeping blood samples at 37ºC. In order to obtain this effect, the inside of the reverberation chamber was ventilated with hot air. Then, we have exposed blood samples at our maximum $E_{out}$ (78±10 V/m) for 1 h and 2 h, respectively. The temperature in the chamber was continuously controlled and a value ranging ±1ºC around 37ºC was obtained. Under these experimental conditions, results were similar to those obtained at 17-21ºC. Under these experimental conditions, results (data not shown) were similar to those obtained at 17-21ºC.

## 4. DISCUSSION

In the present investigation, the availability of a calibrated reverberation chamber has allowed to carry out reproducible experiments on the exposure of human blood leukocytes to EMR.

Out of 108 cases examined with an exposure of *E* intensity ranging from [(78±10 V/m to 12±4)V/m)] and duration from 5 min to 24 h, no difference between exposed and not exposed cells has been recorded in 18% of the cases.

On the contrary, leukocyte areas have been statistically significant larger than those of controls in 68% of cases. The percentage does not seem to be affected by the exposure to *E* intensity, but it appears to be related to time exposure ranging from a mean value of 58% to a mean value of 83% by increasing the time from the minimum (30, 15, 5 min) to the maximum (24, 12, 6 h) range. In particular, as one can infer from Figs. 2 and 3, at 24 h exposure 100% increase in cellular areas has been attained, thus suggesting a dose effect mechanism.

The microscopic observations of stained blood films, clearly support the relationship between EMR exposure and modification in leukocyte shape. Mostly neutrophils seem to be affected by cytoplasmic and nuclear shape changes, even if evidences of morphological changes of eosinophils, lymphocytes and monocytes have been illustrated in section 3.2. In other cases, blast-like cells appear in the microscopic fields. However, their lineage of origin (myeloid, lymphoid or monocytic) is difficult to be identified under these experimental conditions.

At the present stage, EMR-mediated DNA modifications can be envisaged which may lead to the transformation of normal cells into abnormal cells, also undergoing putative apoptotic and/or autophagic processes for longer exposure times. In this respect, there are a few and conflicting data in literature. According to Xu et al. [11] various types of cell lines exposed to 1.8 GHz EMR undergo DNA damage. No cellular dysfunctions were observed in the experiments reported [17]. On the contrary, Szerencsi et al. [24] did not report DNA alterations of healthy human leukocytes exposed to magnetic resonance imaging. In the above mentioned paper, chromosome breakage and loss with formation of micronuclei were detected by comet assay. On the other hand, comet tail DNA% olive tail moment and micronucleus frequency ruled out DNA damage in human leukocytes exposed to EMR generated by 3T magnetic resonance. Observed discrepancies may depend on cell lines used and various procedures to irradiate cells.

In our study, morphometric and morphological evaluations do not allow at the moment to make any kind of speculation on putative EMR-mediated DNA alterations of leukocytes, as well their functional modifications, even including release of growth factors, cytokine and chemokines.

Another result of this study is the identification of those cases were following EMR exposure leukocyte area decreases with respect to that of not exposed cells. This event has occurred in 14% out of 108 cases we examined. Such percentage value seems to be mostly related to the exposure intensity than to the exposure time. In fact, the percentage ranges from 7% to 19% by increasing the *E* exposure intensity from the minimum (12±4 V/m) to the maximum (78±10 V/m) values. At present, we do not have any convincing explanation for this phenomenology. One can only speculate that we are dealing with previously stressed cells which, following *in vitro* EMR exposure, may undergo morphological and functional modifications. Even if blood donors are usually screened either through a routine laboratory assessment or clinically and, thereafter, selected for blood donation, a parallel investigation on putative occasional exposure to environmental EMR in the above cited subset of individuals is currently under way. In this respect, morphological study of leukocytes has revealed shape modifications of leukocytes from some blood donors before EMR exposure and resembling those of exposed cells.

## 5. CONCLUSION

The results we obtained indicate that exposure to 1.8 GHz EMR produces increase of the area of leukocytes and morphological alterations. The use of a reverberation chamber which allows a consistent generation of EMR seems to be a more innovative yet reproducible procedure. In addition in comparison to other reports where lymphocyte cultures were used [18], in our case all white blood cells have been screened after exposure to EMR.

Evaluation of DNA damage and functional tests on EMR-exposed leukocytes will be considered in future experiments.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi Prihoda TJ, Kuster N, Tillmann T, et al. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. International Journal of Radiation Biology. 2009;85(5):454-464.
2. Sommer AM, Bitz AK, Treckert J, Hansem VW, Lerchl A. Lymphoma development in mice chronically exposed to UMTS-modulated radiofrequency electromagnetic fields. Radiation Research. 2007;168(1):72-80.
3. Roberts NJ, Lu ST, Michaelson SM. Human leukocyte functions and the U.S. safety standard for exposure to radio-frequency radiation. Science. 1993;220(4594):318-320.
4. Roberts NJ, Michaelson SM, Lu ST. Mitogen responsiveness after exposure of influenza virus-infected human mononuclear leukocytes to continuous or pulse-modulated radiofrequency radiation. Radiation Research. 1987;110(3):353-361.
5. MaNamee JP, Bellier PV, Gajda GB, Levallèe BF, Lemay EP, Marro L, et al. DNA damage in human leukocytes after acute in vitro exposure to a 1.9 GHz pulse-modulated radiofrequency field. Radiation Research. 2002;158(4):534-537.
6. MaNamee JP, Bellier PV, Gajda GB, Levallèe BF, Lemay EP, Marro L, et al. No evidence for genotoxic effects from 24 h exposure of human leukocytes to 1.9 GHz radiofrequency fields. Radiation Research. 2003;159(5):693-697.
7. Capri M, Scarcella E, Bianchi E, Fumelli C, Mesirca P, Agostini C, et al. 1800 MHz radiofrequency (mobile phones, different Global System for Mobile communication modulations) does not affect apoptosis and heat shock protein 70 level in peripheral blood mononuclear cells from young and old donors. International Journal of Radiation Biology. 2004;80(6):389-397.
8. Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics. 2008;29(3):177-184.
9. Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, et al. Cytogenetic effects of exposure to 2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Research. 2009;29(11):4323-4330.
10. Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, et al. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicit. Radiation Research. 2013;179(2):243-253.
11. Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, et al. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. PLOS ONE. 2013;8(1):1-10.
12. Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicology and Environmental Safety. 2012;80(1):140-144.
13. Verschaeve L, Juutilainen J, Lagroye I, Miyakoshi J, Saunders R, de Seze R, et al. In vitro and in vivo genotoxicity of radiofrequency fields. Mutation Research. 2010;705(3):252-268.
14. Donnelan M, McKenzie DR, French PW. Effects of exposure to electromagnetic radiation at 835 MHz on growth, morphology and secretory characteristics of a mast cell analogue, RBL-2H3. Cell Biology International. 1997;21(7):427-439.
15. Cleary SF, Liu LM, Merchant RE. In vitro lymphocyte proliferation induced by radio-frequency electromagnetic radiation under isothermal conditions. Bioelectromagnetics. 1990;11(1):47-56.

16. Esmekaya MA, Aytekin E, Ozgur E, Guler G, Ergun MA, Omeroglu S, et al. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Science of Total Environment. 2011;410(41):59-64.
17. Ashraf A Aly, Safaai Bin Deris, Nazar Zaki. The effects on cell mobility due to exposure to EMF radiation. Advanced Computing: An International Journal. 2011;2(4):1-7.
18. Biagi PF, Castellana L, Maggipinto T, Maggipinto G, Ligonzo T, Schiavulli L, et al. A reverberation chamber to investigate the possible effects of *in vivo* exposure of rats to 1.8 GHz electromagnetic fields: a preliminary study. Progress in Electromagnetic Research. 2009;94:133-152.
19. Biagi PF, Righetti F, Maggipinto T, Maggipinto G, Ligonzo T, Schiavulli L, et al. Apparatus for *in vivo* exposure at 1.8 GHz microwaves. Journal of Instrumentation. 2011;6:1-11.
20. Corona P, Ferrara G, Migliaccio M. Reverberating Chambers as sources of stochastic electromagnetic fields. IEEE Transactions on Electromagnetic Compatibility. 1996;38(3):348-356.
21. Lazebnik M, Madsen EL, Frank GR, Hagness SC. Tissue-mimicking phantom materials for narrowband and ultrawideband microwave applications. Physics in Medicine and Biology. 2005;50(18):4245-4258.
22. Schwan HP. Electrical Properties of Blood and its Constituents: Alternating Current Spectroscopy, Blut. 1983;46:185-197.
23. Focke F, Schuermann D, Kuster N, Schar P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutation Research. 2010;683(1-2):74-83.
24. Szerencsi Á, Kubinyi G, Váliczkó É, Juhász P, Rudas G, Mester Á, et al. DNA integrity of human leukocytes after magnetic resonance imaging. International Journal of Radiatiation Biology. 2013;89(10):870-876.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=534&id=31&aid=4675*