JJBS

Volume 3, Number 1, January 2010
ISSN 1995-6673
Pages 21 - 28

Jordan Journal of Biological Sciences

No significant Cytogenetic Effects in Cultured Human Lymphocytes Exposed to Cell Phones Radiofrequencies (900MHz and 1800MHz)

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Abstract

The aim of this study isto examine whether radiofrequency (RF) used in cellular phone communications at a specific absorption rate (SAR) less than 1.2 w/kg could increase the spontaneous rate of sister chromatid exchanges (SCE) or elicit alterations both in the cell replication index (CRI) and mitotic index (MI) in human peripheral blood lymphocytes. Whole blood samples were obtained from twenty six healthy donors (male nonsmokers). Cultures were placed in sterile T-25 tissue flasks and sham-exposed or RF-exposed to 900 MHz or 1800 MHz radiation for 1 h, then incubated for 72 h at 37 °C. The mean SCE values of both RF exposure groups slightly increased, as compared with the sham-exposure group. However, there was no significant difference between the RF exposure groups and sham-RF exposure group. Compared with the negative control, both CRI and MI were non-significantly elevated after exposure to both frequencies, but to a lesser extent in case of 1800 MHz exposure. In view of the present guidelines of mutagenicity, which necessitates at least two-fold increase in SCE rate, RF is not considered as mutagenic.

الملخص

هدفت هذه الدراسة الى تقصي ما اذا كانت الموجات الراديوية المستخدمة في الهاتف النقال، عند معدل امتصاص محدد (اقل من 1,2 واط لكل كيلو غرام)، تزيد المعدل الطبيعي للتبادلات شق- الصبغية والى تغيير المستويات العادية لمعاملي تكاثر و انقسام الخلا يا اللمفية البشرية. و قد اخذت عينات دم من ستة وعشرين متطوعا من الرجال غير المدخنين. وتم تعريض مزارع الخلايا لمستويين من موجات الهاتف النقال(900 و 1800 ميغا هيرتز) لمدة ساعة واحدة. و بعد ذلك، اكملت مدة الزراعة ال اثنتين و سبعين ساعة على درجة حرارة 37 سيلسيس. و اظهرت نتائج البحث زيادة ضئيلة، ليست ذات دلالة احصائية، في معدلات التبادلات شق- الصبغية في الخلايا المتعرضة لأي من الترددين، مقارنة بالخلايا الضابطة, كما بينت الدراسة زيادة ملحوظة، لكنها ليست ذا معنى احصائي، في معاملي تكاثر و انقسام الخلايا، بالمقارنة مع المزارع الضابطة. وكانت هذه الزيادة اكثر و ضوحا عند مستوى التردد الا على . و في ضوء التعليمات العالمية الحالية المتعلقة بالسمية الوراثية، فانه يمكن الاستنتاج ان التعرض للمستويات المدروسة من اشعاعات الهاتف النقال لا تؤ ثر سلبا في المادة الوراثية.

Keywords: Human Lymphocytes; Sister-Chromatid Exchanges; Cell Replication Index; Mitotic Index; Cell Phone; Radio frequency Radiation..

1. Introduction

Cellular phones have been one of the fastest growing industries in modern history. The number of mobile phone users worldwide soared to over 3.3 billion by the end of 2007, a total penetration rate of 49 percent. Since their introduction in 1983, there have been remarkable changes in the cellular industry. The first generation mobile phone systems were analog and used 450 and 900 MHz portion of the RF spectrum. The second generation is digital and operates at frequencies 900 MHz and 1800 MHz. For example; the Global System for Mobile Communication (GSM) operates at 900MHz and 1800 MHz with digital modulation scheme and Time Division Multiple Access (TDMA). Recently, the third generation system such as the Universal Mobile Telecommunication System has been introduced and operates in the 1900-2200 MHZ range and uses digital modulation with Code Division Multiple Access (CDMA) (Moulder *et al*., 2005).

A great concern has been raised about the biological effects in persons using these devices. The relatively fixed position of the antenna to the head causes a repeated irradiation of a more or less fixed amount of electromagnetic (EM) energy (Radon *et al*., 2006). Results from present studies on use of mobile phones for 10 or more years give a consistent pattern of increased risk for

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acoustic neuroma and glioma. The risk is highest for ipsilateral exposure (Hardell *et al*., 2007). Furthermore, a statistically significant association between mobile usage and parotid gland (salivary gland) tumors has been reported (Sadetzki *et al*., 2008).

Children's brains absorb a greater proportion of the radiation emitted by a mobile phone (Christ and Kuster 2005; De Salles *et al*., 2006) and they are also likely to be at a greater risk due to their higher rate of cell division (than adults). Since normal cell growth is controlled by the genetic material (DNA), any change in DNA would be reflected on the general health of the living organism. In this regard, analysis on whole-genome cDNA arrays showed alterations in gene expression after various RF exposure conditions using different cell types, but no consistent RF- signature such as stress response could be identified (Remondini *et al*., 2006). In addition, modulation in gene regulation after RF fields exposure at SAR of 1.5 W/kg in p53-deficient embryonic stem cells has been described (Nikolova *et al*., 2005).

It has been reported that exposure of Molt-4 T-lymphoblastoid cells to the cell phone frequency of 836 MHz, consistently resulted in an observable variation of DNA damage (Phillips *et al*., 1998). Furthermore, DNA strand breaks were reported in human diploid fibroblasts and cultured rat granulosa cells (Diem *et al*., 2005) as well as in embryonic stem cell--derived neural progenitor cells (D'Ambrosio *et al*., 2002) after RF field exposure. In individuals, who used digital mobile phones for at least 2 years, with uplink frequencies at 935-960 MHz, there was a significant increase in SCE, but there was no change in cell cycle progression (Gadhia *et al*., 2003). Recently, a number of in vivo experiments have found mobile phone or simulated mobile phone radiation exposure can cause cell damage, reactive oxygen species (ROS) formation (which are the primary cause of DNA strand breaks), and cell death (Oktem *et al*., 2005; Ferreira *et al*., 2006; Oral *et al*., 2006; Panagopoulos *et al*., 2007). In vitro experiments have found an association between RF exposure and ROS production, and then subsequent DNA single and double strand breaks (SSB and DSB) (Nikolova *et al*., 2005; Friedman *et al*., 2007; Yao *et al*., 2008). Also, there have been a number of other cellular effects found, from cell mutations such micronuclei (MN) formation and cellular aneuploidy, following exposure to RF fields (Bisht *et al*., 2002; D'Ambrosio *et al*., 2002; Mazor *et al*., 2008; Schwarz *et al*., 2008) and even impaired cell repair or cell death (Joubert *et al*., 2008; Manti *et al*., 2008; Palumbo *et al*., 2008). Exposure to 900 MHz GSM radiation induced changes in gene expression, but not DNA DSB, in rat brain cells (Belyaev *et al*., 2006).

On the other hand, in vitro exposure of human blood leukocytes and lymphocytes to RF signals emitted by cellular telephones for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes (Tice *et al*., 2002). However, these researchers found that exposure of lymphocytes for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated cells. In cultured human blood cells, others (Zeni *et al*., 2005; Stronati *et al*., 2006) reported results that do not support evidence for any DNA damage [SCE, chromosome aberrations (CA), and MN] induced by extremely low frequency magnetic fields (ELFMF) exposure. Using the same cell system used by Diem *et al*. 2005), Speit *et al*. (2007) obtained clearly negative results. Their finding that RF radiations at cellular phone level do not induce DNA/ genetic damage has been recently confirmed (Tiwari *et al*., 2008). Furthermore, some papers could not find an association between RF exposure and ROS production as well as subsequent DNA SSB and DSB (Lantow *et al*., 2006; Valbonesi *et al*., 2008). No statistically significant difference in the level of DNA damage was observed between sham-treated Molt-4 cells and cells exposed to RF radiation for any frequency, modulation or exposure time (Hook *et al*., 2004). In mammalian cells, no direct cytogenetic effect of 835-MHz RF-EM field was found in the in vitro CA test (Kim *et al*., 2008).

Several studies investigated the influence of RF fields on cell cycle kinetics, but in the majority of the investigations no effects were detected (Capriet *et a*l., 2004; Zeni *et al* ., 2005; Lantow *et al*., 2006; Stronati *et al*., 2006; Chauhan *et al*., 2007; Huang *et al*., 2008). Similarly, no detectable changes in cell viability or incidence of apoptosis were observed in any of RF-field-exposed groups in a series of human-derived cell lines (Capri *et al*., 2004; Hook *et al*., 2004; Caraglia *et al*., 2005; Lantow *et al*., 2006; Chauhan *et al*., 2007; Huang *et al*., 2008), as well as in proliferating or differentiated murine neuroblastoma cells (Moquet *et al*., 2008). Alteration in cell proliferation was described only in a few reports (Pacini *et al*., 2002; Capri *et al*., 2004).

As stated by Özyalçin *et al.* (2002), one major reason for obtaining contradictory results is basically using ill-established experimental set up. Therefore, this study represents an attempt to further investigate the cytogenetic effects of RF field under specific experimental conditions which simulate the actual radiated power from GSM mobile phones; particularly applying the field at two frequencies, with fixed intensity, and vertical orientation of this field to cultured human lymphocytes.

2. Materials and Methods

Twenty six men (average age, 30 years) participated in this study. Each person completed a questionnaire to obtain relevant health history. All the subjects reported neither exposure to diagnostic x-ray nor treatment with drugs during the last three months before blood collection. Every man donated 1.0 ml of venous blood collected in heparinized tubes. Another 1.0 ml of blood was obtained from each of 6 randomly selected men of the study group to be used in control cultures.

A standard whole blood culture was prepared with slight modifications as reported previously (Khalil *et al*., 1993). Briefly, 0.5 ml of blood was added to 4.5 ml of complete RPMI 1640 culture medium (Sigma, USA) in a T25 polystyrene tissue culture flask. The medium was supplemented with 10 % new born calf serum (Eurolon, USA), 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml fungizone (Eurolon, USA). The culture medium was buffered with 15 mM HEPES at pH 7.2.

Two types of culture from each individual were made. The first was exposed for 1 h RF radiation at a frequency

of 900 MHz, while the second was exposed to radiation at a frequency of 1800 MHz. The exposure system consisted of GSM signal generator DLW-3000 xp which generates either 900 MHz or 1800 MHz. The signal generator was located at about 40 cm below the culture flasks. The Narda model 8616 and detachable probe (Model 8623D) were used extensively to measure the electric field and adjusted to be 24 V/m. This simulates the actual exposure levels from the available GSM mobile phones to the direction of the human tissues. Figure (1) shows the uniformity of the electric field distribution within the area where the cultures being located. This figure was obtained by simulating the exposure system using the HFSS software (HFSS, 2007). It is worth noting that the value of 24 V/m is equivalent to SAR value of 1.0 and 1.2 watts/kg for 900 MHz and 1800 MHz, respectively (Gandhi *et al*., 1996). In the two experimental protocols, the irradiation was performed while the flasks were kept in the incubator in a horizontal position at 37 °C in a humidified 5 ± 0.1 % CO2 atmosphere.

After the 1 h exposure to RF, the cultures were initiated by addition of 30 µg/ml bromodeoxyuridine (ACROS Organization, USA) and 5.0 µg/ml phytohemagglutinin-M (Sigma). Two additional sets of exactly matching cultures, except RF exposure, were included. The first consisted of six cultures with only culture medium added and served as negative control. The other, which was also of six cultures, constituted the positive control. To each of the positive cultures, 50 ng/ml of a chemical DNA damaging agent; mitomycin C (MMC, Janssen Chemical, Geel, Belgium) were administered. All cultures were incubated for a total of 72 h excluding the 1 h RF-exposure or sham -exposure period. During the last 3 h of the culture period, 10 µg/ml of colchicine (Park Scientific, Northampton, UK) were included.

Immediately following termination of cultures, cells were harvested and chromosome spreads were prepared, differentially stained and cytogenetically analyzed according to Khalil and Qassem (1991). The slides were coded and blindly scored for the three end points under investigation; sister chromatid exchanges (SCE), cell replication index (CRI) and mitotic index (MI). From each culture, the mean SCE frequency was determined in 25 to 30 well-differentiated second metaphases (each with at least 44 ± 2 chromosome complement). From the same preparations, 200 cells were classified as first (M1), second (M2), and third or further (M3) dividing cells. The CRI was calculated by multiplying the % of M1, M2 and M3 by 1, 2, or 3, respectively, then dividing the sum by 100. The proportion of mitotic nuclei was found in a total of 2000 nuclei per culture to figure out the MI.

Duplicates of each indicated experiment were performed.

3. Statistical analysis

ANOVA test was used to evaluate the collected data. Furthermore, a multiple comparison (LSD; Exact Fisher) test was performed to evaluate the differences between the 900 MHz or the 1800 MHz and the negative control values. To compare the effects of the 900 MHz and 1800 MHz treatments on the MI data, the independent *t*-test was employed.

4. Results

Descriptive statistics demonstrated no large variability within the data gathered from any group. Therefore, corresponding data for individuals of each group were pooled (Table 1, figures 2-4). When the cells were exposed either to 900 MHz or 1800 MHz, the individual SCE means were normally distributed as shown by the Kolmogorov-Smirnov (K-S) analysis. Thus, ANOVA test was used for the statistical analysis. The SCE means under exposure conditions were not significantly elevated in comparison to the negative control values ($p \leq 0.05$). A multiple comparison (LSD; Exact Fisher) test was performed to evaluate the differences between the 900 MHz or the 1800 MHz and the negative control values. Similarly, when the data obtained after using 900 MHz or 1800 MHz were analyzed, no statistical differences were observed under the two exposure conditions.

Also, ANOVA demonstrated clearly higher CRI values in both cases of exposure relative to the negative control. Further analysis did not show any significant difference between 900 MHz and 1800 MHz CRI data.

When ANOVA was applied to the MI data, obvious increases in MI values were detected under exposure conditions either to 1800 MHz or 900 MHz frequency over the negative control. To compare the effects of the 900 MHz and 1800 MHz treatments on the MI data, the independent *t*-test was employed. The mean MI values were not significantly different ($P \leq 0.5$) in cultures exposed to either frequency.

Finally, it should be reported that MMC (the positive control), at 50 ng/ml, resulted in significantly higher SCE levels, but significantly lower CRI and MI values than those of the experimental and the negative control ($P \geq 0.01$).

5. Discussion

Effects of RF fields on different biological systems have been investigated. Although the majority of studies have found no evidence of genotoxic effects, there are a few positive findings that should be followed up. Vijayalaxmi and Obe (2004) reviewed large number of investigations conducted during the years 1990–2003 using rodents, cultured rodent and human cells, and freshly collected human blood lymphocytes to determine the genotoxic potential of exposure to RF radiation. The results of most of these studies (58%) did not indicate increased damage to the genetic material (assessed from DNA strand breaks, incidence of CA, MN and SCE) in cells exposed to RF radiation compared to sham-exposed and/or unexposed cells. Some investigations (23%) reported an increase in such damage in cells exposed to RF radiation. Positive results means that epidemiological studies of people exposed to EM radiation are likely to show increased cancer, miscarriage and reproductive adverse effects. In fact many epidemiological studies have shown these effects (Szmigielski, 1996; Goldsmith, 1997; Sadetzki *et al*., 2008). The observations from other studies (19%) were inconclusive.

It is possible that certain cellular constituents altered by exposure to EMF, such as free radicals, indirectly affect DNA. Recently, Mcnamee and Bellier (Mcnamee and

Table 1: The effects of cellular phone radiofrequency fields; 900 MHz and 1800 MHZ on sister chromatid exchanges (SCE), cell replication index (CRI) and mitotic index (MI) in cultured human lymphocytes. Culture medium, negative control; mitomycin C (MMC), positive control (Mean ± standard error of the mean; SEM)

Treatment	SCE		CRI		MI	
	Mean	SEM	Mean	SEM	Mean	SEM
900 MHz	4.94	0.3	2.85	0.1	0.247	0.0
1800 MHz	5.35	0.2	2.56	0.3	0.235	0.0
Culture Medium	3.28	0.5	2.20	0.2	0.215	0.0
MMC	21.20	2.0	1.60	0.1	0.155	0.0

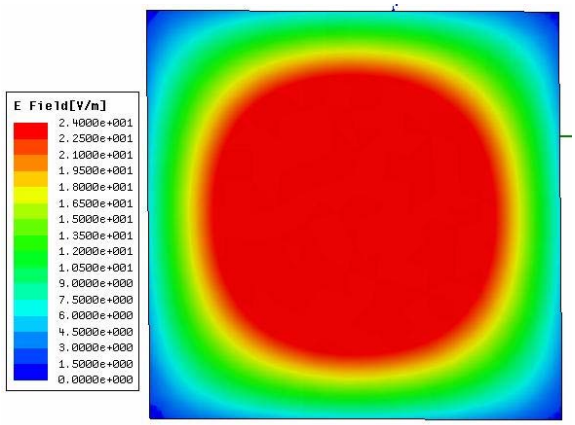

Fig.1.The electric field distribution within the culture area.

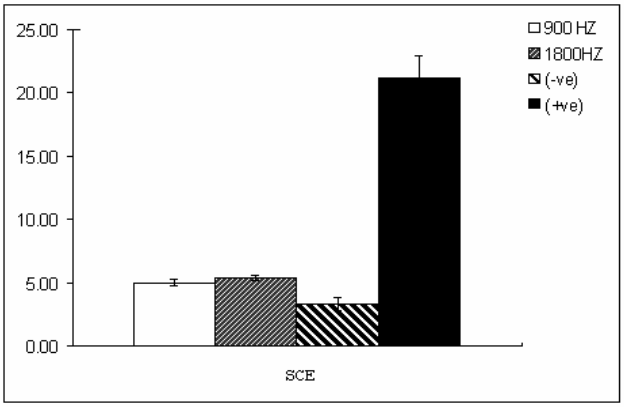

Fig.2 The effects of cellular phone radiofrequency fields at 900 MHz and 1800 MHz on sister chromatid exchange (SCE) in cultured human lymphocytes.

- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

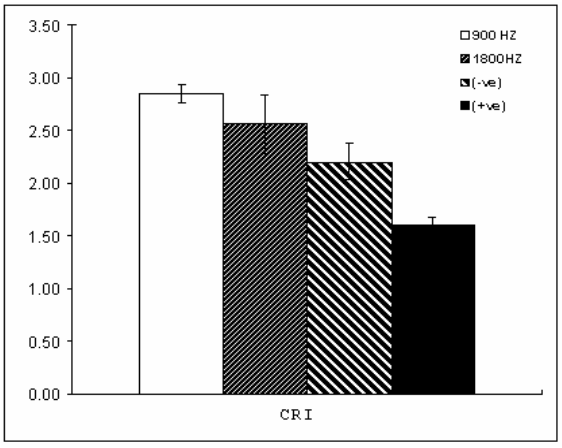

Fig.3 The effects of cellular phone radiofrequency fields at 900 MHz and 1800 MHz on cell replication index (CRI) in cultured human lymphocytes.- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

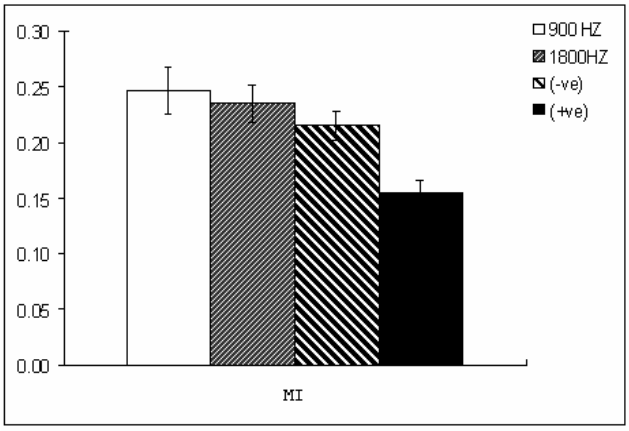

Fig.4 The effects of cellular phone radio frequency fields at 900 MHz and 1800 MHz on mitotic index (MI) in cultured human lymphocytes.- VE culture was treated with culture medium only. + VE culture was treated with 50 ng/ml mitomycin C (MMC).

Bellier, 1997) evaluated the biological and/or health effects of thermalizing and non-thermalizing RF radiation exposures and concluded that the scientific literature on this subject is full of conflicting results and the question of whether RF radiation exposure can contribute to cancer risk remains unresolved. Even with using the same experimental system, the genotoxic effects of exposure to RF-EM fields were not reproducible (Bisht *et al*., 2002).

Some in vitro studies including the present one provide evidence that gene expression is affected at RF exposure close to the guidelines. If these studies are confirmed they will be important for a mechanistic understanding of the interaction of RF fields with cellular tissue. While it seems appropriate to perform experimental studies using pure experimental RF fields, it may be needed to emulate the complex modulation patterns and intensity variations typical to real mobile phone use in future studies. This way data can be obtained which are better suited for comparison to epidemiologic studies.

Two plausible biological mechanisms involving free radicals are involved in this effect. The first involves increased free radical activity and genetic damage as a response to exposure. The second involves increased free radical activity and genetic damage because of an induced reduction of a free radical scavenger, e.g. reduced melatonin (Reiter, 1994). It is clear however, that both mechanisms have the same effect of damaging the DNA and chromosomes. Another established biological mechanism, EMR-induced alteration of cellular calcium ion homeostasis (Blackman, 1990) is also involved in cell regulation, cell survival and apoptosis, DNA synthesis and melatonin regulation.

The response is not likely to be linear with respect to the intensity of the radiation as it is seen from the present findings. Other parameters of RF radiation exposure, such as frequency, duration, waveform, and amplitude-modulation, etc, are also important determinants of biological responses and affect the shape of the dose (intensity)-response relationship. In this regard, the impact on calcium ions, which are important in maintaining normal health functions in brain tissues, was found in experiments. An exposure level of 30 mW / cm^2 is usually able to slightly raise the temperature over an hour. The Blackman's experiment was undertaken under isothermal conditions, with samples being kept within 0.4 ºC of 22 ºC.

The reductions in the MI reported in this study may be related to the impaired cell repair or cell death observed recently by other workers (Panagopoulos *et al*., 2007; Joubert *et al*., 2008; Palumbo *et al*., 2008). Furthermore, the present data that RF exposure results in depressions in the CRI is in agreement with some previous studies (Pacini et al.2002; Carpi *et al*., 2004), but not with others (Huang *et al*., 2008; Moquet *et al*., 2008).

According to the United Kingdom Environmental Mutagen Society (UKEMS) guidelines (UKEMS, 1990), at least a doubling in SCE frequency or a clear dose-response relationship should be accepted as a positive response. Thus, based on the data obtained from the present study and in reference to the UKEMES guidelines, it can be concluded that the 900 MHz and 1800 MHz exposure does not double the SCE frequency. However, the possibility that RF fields are weak mutagens can not be ruled out. Future studies in our laboratory are going to be carried out to examine the immunocytogenetic effect of RF-field exposure in both in vitro human and in vivo rodent model systems.

Acknowledgments

This work was supported by the Deanship of Research and Graduate Studies at Yarmouk University, Irbid, Jordan, grant number 28/2006.

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