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SELÇUK TIP DERGİSİ

The Effect of Mobile Phone on The Motility and DNA Integrity of Human Sperm

Cep Telefonun İnsan Sperm Motilitesine ve DNA Bütünlüğüne Etkisi

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Özet

Çalışmanın amacı farklı uzaklıklarda ve 1800 ile 900 MHz frekanslardaki cep telefonlarına maruz kalan insan sperm hücrelerinin motilite ve DNA bütünlüğünü değerlendirmektir. Hastalar 20'şer örnekten oluşan 2 ana gruba ayrılmışlardır ve 15 dk boyunca 900 ve 1800 MHz yayılımı olan aktif radyasyona maruz bırakılmışlardır. Her grup kendi içerisinde; Kontrol Grubu, 2,5 cm ve 10 cm olmak üzere 3 alt gruba ayrılmıştır. Ayrıca sperm motilitesi (WHO 2010 Kriteri) ve DNA bütünlüğü (Acridine Orange Boyama) bakımından değerlendirilmiştir. 2,5 ve 10 cm uzaklıktan radyasyona maruz kalan gruplarda motilite parametresi (Class A+B), hem 900 hem de 1800 MHz'lik frekanslarda istatistiksel olarak farklılık göstermemiştir ($p>0,05$). Fakat DNA bütünlüğü bakımından karşılaştırıldığında gruplararasında mesafe ile ters ilişkili olarak istatistiksel bakımdan anlamlı bir farklılık vardır ($p<0,05$). Çalışmamız radyasyon kaynağından uzaklık azaldıkça DNA bütünlüğündeki hasarın artacağını göstermektedir ve güvenlik için cep telefonlarının vücuttan uzak mesafede konumlandırılması önerilmektedir.

Anahtar kelimeler: Acridine Orange, Cep Telefonu, Spermatozoa, DNA Bütünlüğü, Radiofrekans Radyasyon

Abstract

To determine human sperm cell motility and DNA integrity exposed to mobile phones broadcasting at 900 MHz and 1800 MHz frequency bands according to different distances. Two main groups were created with each containing 20 samples. Samples were treated for 15 minutes with 900 MHz and 1800 MHz radiofrequency actively emitted radiation. Each group was divided into three subgroups as; control group, 2,5 cm and 10 cm distance exposed group. Each group was evaluated in terms of sperm motility (according to WHO 2010 criteria) and DNA integrity (Acridine Orange Staining). Statistically the motility parameter (Class A+B type) of 2,5 and 10 cm radiation exposed groups showed no any significant difference for both 900 MHz and 1800 MHz frequency bands ($p>0,05$). But for comparisons of DNA integrity there was a statistically significant difference for groups related inversely with the distance ($p<0,05$). Our data reveals that reduction of the distance from the source of radiation increases the rate of damage of DNA integrity. Therefore it can be suggested that it is as safer as one keeps mobile phone as long away from his/her body.

Key words: Acridine Orange, Mobile Phone, Spermatozoa, DNA Integrity, Radiofrequency Radiation.

INTRODUCTION

Currently, mobile phone usage is intense and world widely widespread. A common discussion is going on about the possible damage that the radiofrequency radiation (RF) emitted by mobile phone can exert on different organs and tissues. There are reports about the interaction of radiofrequency energy with biological tissues and the reports warning about the dangerous effects are increasing day by day. In the literature RF decreases sperm count and motility, and increases the oxidative stress (1-3). There are two kinds of waves in radiation band as ionizing and non-ionizing where mobile phone emissions are in non-ionizing. The emitted wavelength from mobile phones is at the low microwave spectrum so does not have enough energy to ionize atoms or molecules (3). Some authors claim that this is enough to consider mobile phone usage is not harmful for humans so is safety (4,5). Several experimental studies revealed the adverse effects of electromagnetic and static magnetic fields in many body systems including the reproductive system. One big concern is that they may disturb the testicular function. There are reports emphasizing a possible link between mobile phone usage with decreased semen quality (3-5).

Semen analysis remains the most important clinical laboratory test available for the evaluation of male infertility (6). As nearly 20% of infertility problems is due to male factors alone. 50% of infertility cases with male factor have low or even absence of spermatozoa concentration (7,8). In the present study, spermatozoa from normospermic samples were examined at light microscopic level after exposed to cell phone RF radiation with two distances. Two parameters were under our scope as sperm motility and DNA integrity (according to Acridine Orange Stain)

MATERIALS AND METHODS

Selection Criteria of Semen Samples

For this study, patients whom admitted to the unit of assisted reproductive technologies in Medical Faculty of Necmettin Erbakan University between February 2009-June 2009 was selected. The study was approved by the no: 2008/360 local ethical committee. 40

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normospermic patients were selected according to WHO 2010 criteria. After 3-5 day sexual abstinence samples were collected to sterile, polypropylene containers. After liquefaction macroscopic (color, volume and viscosity) and microscopic examinations were evaluated. Patients with normospermic values and those whom signed written consent entered the study.

Exposure to RF

Semen samples of patients were divided into three equal parts (1 ml of each) and were separated three different sterile containers. Each sample in the two containers expose to mobile phone RF radiation (at usage mode) from distance 2,5 and 10 cm during 15 min respectively. Last sample of the container was evaluated as control group (Group c=Gc). As the first 20 patients group of patients expose to 900 MHz frequency band GSM mobile phone which broadcasts (Group 1a,1b), the other group of 20 members expose to 1800 MHz frequency band GSM mobile phone which broadcasts (Group 2a,2b). Devices were mobile phones which can be found in the existing commercial market. 20 patients in each group were evaluated simultaneously and separately according to 2,5 (Group 1a,2a) and 10 cm parameters (Group 1b,2b). Created the following mechanism for these measurements (Figure 1). After exposed to RF radiation for 15 min, semen samples of distance 2,5 cm and 10 cm and semen parameters of control group were re-examined, then results were compared with the initial state.

Evaluation of Motility

Before and after RF radiation was performed motility was evaluated by Makler Counting Chamber (Sefi–Medical Instruments). 200 cells were counted for the assessment of motility. Showing progressive motion and at least advancing 3 square spermatozoa motility was evaluated as +4 (9,10).

The Evaluation of DNA Integrity with Acridine Orange Stain

Each sample of 3 groups was spread on a slide. After drying the smears, was fixed in 5 min +4 °C by Carnoy Solution which considered the best fixative to demonstrate DNA damage (11) then in the dark, 5 min were stained with 1% AO (10 ml 1% Acridine Orange Solution, 40 ml 0,1 M Citric Acid, 2,5 ml 0.2 M Na2HPO47H2O mixture, pH 2,5) and slides were rinsed with distilled water. Samples were closed without waiting for drying and immediately were examined in the dark room and dark area under Olympus BH-2 photo-attached microscope on filters with a wavelength of 515-530 nm. Acridine Orange is a metachromatic substance. In the cytochemistry staining, when AO interacted with normal spermatozoa with double-stranded DNA, emitted green fluorescence at 515-530 nm. denatured abnormal spermatozoa with one stranded DNA emitted yellow-orange-red fluorescence (10,12). Spermatozoa reflecting green fluorescence were counted under the fluorescence microscope, and then were determined as a percent.

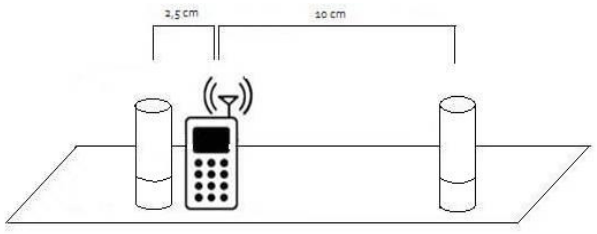
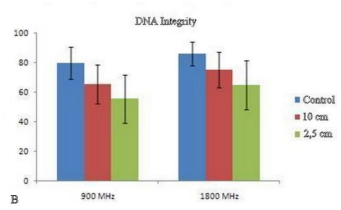


Figure 1.

Table 1. DNA structural integrity percent of sperm cells related to distance and radiofrequency properties with non-exposed control group. (A) Groups can be easily classified as, Group 1a being exposed to 900 MHz radiation at a 2.5 cm distance. (B) Bar representation of groups.

Group	2.5 cm (a)	10 cm (b)	Control (c)
900MHz (1)	55.5	65.4	79.7
1800MHz (2)	64.7	75.1	86.2

A



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Statistical Analysis

The results were evaluated in computer statistically by the "SPSS 13.0 for Windows" program. Data was summarized in the form of mean ± standard deviation, corporations between groups were done by using "independent samples T test" method. A meaningful difference was noted when the p value was smaller than 0.05.

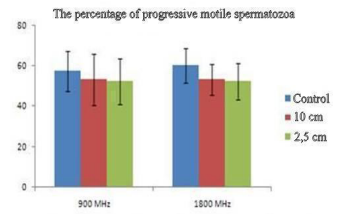
RESULTS

Our study revealed that sperm motility was unaffected from mobile phone radiofrequency while statistically important differences for a decrease in DNA integrity were observed according to the distance from mobile phone electromagnetic source. Sperm cells exposed to 900 MHz at a distance of 2.5 cm (G1a) and 10 cm (G1b) and control (G1c) respectively revealed 55,5 ± 16,4; 65,4 ± 13,3 and 79,7 ± 10,9 percent DNA integrity. When compared with control group (Gc), exposure groups

Table 2. The results of motility parameter (percents of A+B type motility). Our results reveal that there is no any significant difference between groups according to studied distances (Ga and Gb) from radiation source. B Bar representative of table 2A.

Group	2,5 cm (a)	10 cm (b)	Control (c)
900 MHz (1)	52,3	53,1	57,5
1800 MHz (2)	52,3	53,2	60,0

A



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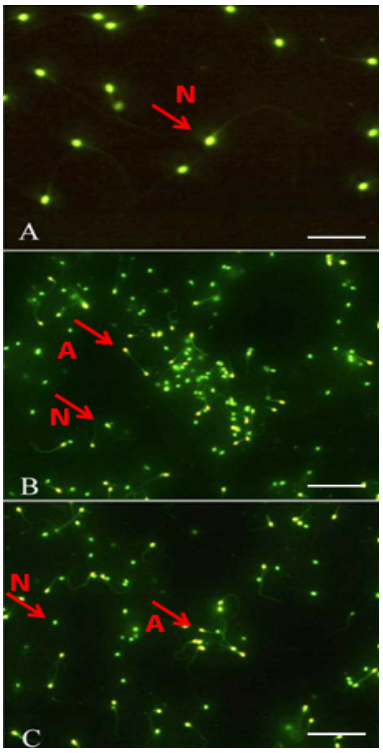

Figure 2. The DNA integrity of control group sperm cells, PMM; x400 (A), 10 cm distance exposure Groups, PMM x200 (B), 2,5 cm distance exposure Groups, PMM x200 (C), A: sperm with abnormal DNA structure; N: sperm with normal DNA structure. PMM: Photo Microscopic Magnification. Scale Bar: 17 µm

G1a and G1b is statistically different (p <0,00, p=0,01 respectively). Sperm cells exposed to 1800 MHz at a distance of 2.5 cm (G2a) and 10 cm (G2b) and control (Gc) respectively revealed 64,7 ± 16,7; 75,1 ± 12,3; 86,2 ± 8,1 percent DNA integrity. When compared with control group (Gc), exposure groups G2a and G2b is statistically different (p<0,00, p=0,02 respectively).

It can be concluded that at 900 MHz and 1800 MHz bands, as closer to the radiation source (mobile phone at speaking mode) the DNA integrity of the sperm cells deteriorate more significantly.

The second parameter of our study was to search the effect mobile phone radiation on sperm motility. The sum percent of A+B type motility was evaluated, the values are shown in Table 2. For group 1 (900 MHz exposure) according to statistical analysis there is no any difference between study and control groups for motility parameter even at different distances. The p values for the motility parameter (A+B percent) of control group and G1a and G1b were p: 0.74 and p: 0.1 respectively. Also the p value for radiation bearing groups was 0.7 which revealed no any significant difference.

For group 2 (1800 MHz exposure) according to statistical analysis there is no any difference between study and control groups for motility parameter even at different distances. The p values for the motility parameter (A+B percent) of control group and G2a and G2b were p: 0.28 and p: 0.69 respectively. Also the p value for radiation exposed groups was 0.81 which revealed no any significant difference.

DISCUSSION

As mobile phone usage wide spreads it as being a radio magnetic wave source gains more attention especially in health and medical related fields. The current published documents for interactions of mobile phone RF and biologic tissues are limited. As each biological tissue have unique properties to evaluate such mobile phone effect, especially for long time usage needs extensive research and data for a more precise conclusion. The potential of possible unexpected effects may or not have destructive effects on biological tissues even if the exposure parameters are under legal regulated values (13,14). One kind of effect of mobile phone radiation depends on its tissue heating but there are important differences between tissues for heat regulation such as eyes and testicular sites because of the low circulation properties (15). One of the first human studies of mobile phone RF interaction with sperms revealed a decrease in sperm number when mobile phone is carried at back pocket or waist when compared to any other body part carriage. This study was done on 52 males ages between 18-35 (16). In an 13 month term tracking study 221 males were selected (half number per group), divided to two groups according to one group as mobile phone usage (900-2000 MHz) and the other group was not using mobile phone during the time, when sperm numbers of the individuals were compared, the mobile phone using group had a 30% decrease in sperm number (17). In an other study rats were exposed to mobile phone RF in speaking mode and differences in brain, eye, ear, liver, lung, heart, kidney, intestines and skin tissue was revealed when compared to null control group but they especially report that the most effected tissue was testicular tissue with a low number of sperm count (18). There are other reports documenting sperm cell DNA fractures at single and double chains at exposures with the same RF (19-21).

Further, it is documented that males who speak with mobile phone more than several hours daily have lower sperm number, motility and viability; all parameters being proportional with usage time (22,23,24). There are authors strongly suggesting to use mobile phone as less as possible (25,26).There are also studies revealing controversial results as no any difference in sperm motility and morphology in rats exposed to mobile phone RF (23,27). In some studies, on rats exposed to mobile phone there were no any statistically significant difference compared with control group for sperm count and morphology parameters (28-30). In another study rats were exposed to mobile phone RF for 5 weeks (2 hours daily), they found an increase in apoptotic forms and a significant decrease in total number of sperms, in another study going 12 weeks (1 hour daily) 16 rats were studied in 13 of them hypospermatogenesis was found while 3 of them showed maturation arrest (31). Controversially another group exposed mobile phone RF 30 minutes daily and found no any anomaly in rats (32). Human spermatozoa with A+B> 50% were exposed to mobile phone radiation with SAR values 2.0 and 5.7; no any significant decrease was detected in motility by a research team (33) but when the same team further searched for fertilization capacity of sperms in 1 hour daily exposed males (without any problem with motility) the acrosome reaction and the head acrosome percent was significantly decreased (34).

It is supposed that mobile phone RF interacts with sperm cell membrane and generates oxidative stress which can cause DNA damages proportional to exposure scale. In our study we couldn't find

a statistically meaningful decrease in sperm motility according to two distances but we found DNA damage revealed by acridine orange staining inversely proportional to RF source under in vitro conditions (Figure 2). To use mobile phone as less as possible and keep it away from body seems to be important for long term personal health maintenance and when the concept is race fertility for long term public health. We suggest further studies are needed with wider subject numbers and updated RF parameters used in new generation mobile phones.

Competing interests

The authors declare that they have no competing interests.

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