

Possible impact(s) of cell phone electromagnetic radiation on human sperm parameters

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Background

Epidemiological in-vitro studies have highlighted the possibility that exposure to radiofrequency electromagnetic waves emitted from cell phones may negatively affect seminal parameters through altering the DNA integrity of sperm and releasing reactive oxygen species (ROS).

Purpose

To determine the hazardous effects of using a cell phone on semen quality in correlation with DNA damage and the release of ROS.

Patients and methods

The study was carried out at the Department of Dermatology and Andrology and Cleopatra-IVF center Ismailia, Egypt, on a total of one hundred with infertile men. They were divided into four groups according to their cell phone use: group A: no use ($n=15$); group B: less than 2 h/day ($n=30$); group C: 2–4 h/day ($n=25$); and group D: more than 4 h/day ($n=30$). Semen analyses were carried out using computer-aided sperm analysis both dynamically and morphologically according to WHO. DNA damage and ROS level were assessed using an alkaline comet assay and a thiobarbituric acid assay, respectively.

Results

The current study showed that the duration of cell phone usage correlated negatively with the proportion of grade A and grade B sperm motility, but correlated positively with the proportion of grade C and grade D motility. Group D showed an increased ROS level and altered DNA strands in the spermatozoa.

Conclusion

Prolonged use of cell phones could have hazardous effects on semen quality in men. Electromagnetic radiation can lead to breakage of DNA strands and elevation in the ROS level.

Keywords:

DNA damage, electromagnetic, mobile phone, sperm parameters

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Introduction

Recently, there has been controversy over the impact of radiofrequency electromagnetic waves (RF-EMW) emitted by cell phones on human health. The effects of these radiations on male reproduction are a key element of this debate as several studies have suggested a relationship between mobile phone usage and semen quality. The potential mechanisms involved have not been established; however, human spermatozoa are known to be particularly vulnerable to oxidative stress because of the abundant availability of substrates for free radical attack, for example high content of polyunsaturated fatty acids in their cell membrane and the lack of cytoplasmic space to accommodate antioxidant enzymes [1]. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes toward sperm DNA damage. The latter has, in turn, been linked to poor fertility and increased incidence of miscarriages [2].

Mobile phones communicate by transmitting radio waves through a network of fixed antennas. They are low-powered RF transmitters, operating at frequencies between 450 and 2700 MHz, with peak powers in the range of 0.1–2 W. The handset only transmits power when it is turned on. The power (and the RF exposure to a user) reduces rapidly with increasing distance from the handset [1]. The amount of RF energy absorbed from phones depends on the exposed tissues, called the specific absorption rate (SAR). The SAR of cell phones varies from 0.12 to 1.6 W/kg body weight depending on the model [3].

On the basis of these clinical data, attention has been focused recently on the environmental and genetic factors that might be involved in the etiology of DNA damage in the male germ line. These investigations have suggested that one of the environmental factors involved in the etiology of DNA damage in human spermatozoa is increased exposure to RF-electromagnetic radiation

(EMR) emitted from mobile phones. This association was suggested by an epidemiological study that found negative correlations between mobile phone usage and various attributes of semen quality, particularly motility [4]. This was followed by an experimental study involving exposure of male mice to RF-EMR, which showed a significant impact on the integrity of both the mitochondrial and nuclear genomes [5]. Many studies have also suggested that EMW emitted from cell phones could reduce the fertilizing potential of men [6–8].

Inhibition of sperm motility has been observed in humans and suggests that the oxidative stress induces damage to the motility apparatus. This reflects the *in-vivo* situation, where a strong inverse relationship has been observed between mitochondrial reactive oxygen species (ROS) generation and sperm motility despite the presence of glucose in the incubation medium to compensate for any defect in the ability of the mitochondria to generate ATP. These results are in agreement with previous studies that have shown an inverse relationship between the peroxidation status of human spermatozoa and their movement ability. However, mitochondrial activity is a potential source of the free radicals responsible for this damage and altered sperm motility [9]. Similarly, a study aimed at the determination of the effects of EMR from a cell phone on human sperm motility found a decrease in sperm motility in semen samples of 27 men exposed to a 900 MHz cell phone for 5 min [10]. ROS production in sperm suspensions, lipid peroxidation, and DNA oxidation are associated with poor sperm function, which causes infertility [11,12]. ROS can damage many biomolecules, including DNA, enzymes, and lipids. These findings also indicated that mobile phone radiations may lead to oxidative stress because of the overproduction of ROS in human semen [1]. In light of these data, there is an urgent need to determine whether exposure to RF-EMR emitted from mobile phones can affect human semen quality and cause DNA damage (*in vivo*).

Patients and methods

Patients

This a cross-sectional comparative study that was carried out during the period from January 2010 to December 2010 and included one hundred infertile men attending the Andrology Outpatient Clinic, Department of Dermatology and Andrology (FOM-SCU) and Cleopatra-IVF center, Ismailia, Egypt.

Inclusion criteria

The inclusion criteria were as follows: patients who had been using the mobile phone model GSM1800/1900 MHz for at least 1 year (except group A), nonsmokers, patients residing or working away from base station (EM tower), and those with no direct exposure to another source of EMW such as a computer or a microwave and with no time to watch television.

Exclusion criteria

The exclusion criteria were as follows: smokers, genitourinary infection, varicocele, and necrozoospermia.

Detailed history including questions on cell phone use habits, possession, daily stand-by position, and daily transmission times was determined carefully from all individuals enrolled in this study. They were subjected to a physical examination and divided into four groups according to their cell phone use per day: group A: no use ($n = 15$); group B: less than 2 h/day ($n = 30$); group C: 2–4 h/day ($n = 25$); and group D: more than 4 h/day ($n = 30$). The study was carried out in accordance with the guidelines of the Helsinki Declaration, and was performed after obtaining informed consent from all participants.

Calculation of the electromagnetic waves doses in the exposed population

Each patient agreed to visit the clinic every week for 1 month with his own mobile phone (without any limitations on its usual usage throughout the period of research) and the physician calculated the number of minutes of all phone calls and the duration of each call per week from the mobile records. Patients whose statements did not match with their mobile phone usage as shown in their mobile records were excluded from the study.

Semen analysis

Semen quality was assessed by the andrologist in terms of sperm concentration, motility, and morphology on the basis of the WHO criteria [13]. All the participants provided a semen sample by masturbation into a wide-mouthed plastic container in a room close to the semen laboratory. Sampling was carried out after 2–5 days of abstinence. The semen samples were marked with an anonymous serial number and were then placed immediately in an incubator at 37°C, where they remained during the entire analysis procedure. Analysis was carried out as soon as the ejaculates had liquefied. All ejaculates liquefied within 1 h. The volume was then aspirated into a 10 ml pipette, providing 0.1 ml accuracy. The pH was measured using a pH tape (pH 6.5–10.0) and recorded after 20 s. Sperm concentration and motility was determined using computer-aided sperm analysis (CASA, Hamilton Thorne, HTM-IVOS, version 12; Baumann Medical AG, Wetzikon, Switzerland). The setting for CASA was 30 frames, 50 Hz, and a minimal cell-size of three pixels at a minimum contrast of 80 (standardized unit by Hamilton). The microscope setting was an objective magnification of $\times 10$. A standard analysis chamber of 20 μm was filled and placed into the Hamilton (prewarmed at 37°C) [13]. Sperm motility was graded as follows: class a and b, fast and weak forward motility; class c, nonprogressive motility; and class d, immobile spermatozoa. Sperm viability was assessed by mixing one part of the processed semen sample with three parts of 1% aqueous eosin for 30 s on a precleaned, grease-free glass slide. Then, three parts of a 10% aqueous nigrosin solution were mixed with the eosin–semen mixture and

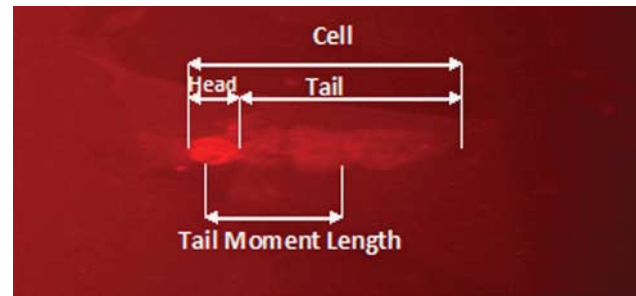
evenly spread onto fresh slides. A total of 200 sperm were counted at a magnification of $\times 400$ for live: dead ratios and morphology (head and tail abnormalities). The live sperm stained white and the dead sperm stained pink.

Alkaline comet assay (single-cell gel electrophoresis)

The comet assay was performed at the Genetic Unit and Oncology Diagnostic Unit (FOM-SCU). All chemicals and reagents used in this research were obtained from Sigma (Sigma Aldrich, Sigma Chemical Co., St. Louis, Missouri, USA) unless stated otherwise. Approximately 1×10^5 sperm in 10 μ l of PBS were mixed with 75 μ l of 0.5% low-melting-point (LMP) agarose at room temperature and then rapidly pipetted miniature agarose gels on microscope slides (which were precoated with 100 μ l of 1% normal melting point agarose in PBS and kept overnight to dry out or for at least 2 h before performing the assay) and covered with glass cover slips. Another layer of 0.5% LMP agarose was pipetted on top of the second solidified layer and allowed to solidify at room temperature [14,15]. Lysis of the cells *in situ* to remove DNA-associated proteins and allow the compacted DNA in the sperm to relax was carried out for 1 h at 4°C by removing the cover slip and immersing the slides in a Coplin jar containing a freshly prepared (within 2 weeks) cold lysis solution (2.5 mol/l NaCl), 100 mmol/l Na₂EDTA, 10 mmol/l Tris, pH 10, containing 1% Triton X-100, 40 mmol/l dithiothreitol (DTT), and proteinase K (Bioshop, Burlington, Canada) 100 μ g/ml added just before use. Finally, the slides were placed side by side in a freshly prepared electrophoresis solution (containing 300 mmol/l NaOH and 1 mmol/l EDTA, pH 13.0, at 12–15°C) and kept for 20 min to allow for unwinding of the DNA and the expression of alkali-labile damage. Electrophoresis was performed for 20 min at 25 V (0.714 V/cm) and 300 mA. Then, the slides were drained and flooded with three changes of neutralization buffer (0.4 mol/l Tris; pH 7.5), each for 5 min [16]. All steps of the comet assay preceding the electrophoresis were performed on ice to prevent DNA repair and the effects of metabolic processes. Furthermore, to avoid position effects during electrophoresis, two parallel replicate slides per sample were prepared, and each replicate was processed in a different electrophoretic run [17]. Ethidium bromide (20 μ g/ml) was used to stain the sperm DNA. Fifty cells from each slide were captured using a Leica DFC 425 camera (Heerbrugg, Switzerland), mounted on a Leica DM 1000 fluorescent epimicroscope (excitation filter of 515–560 nm from a 100 W mercury lamp and a barrier filter of 590 nm) using the Iplab spectrum software (Scanalytics Inc., Fairfax, Virginia, USA). The slides were marked vertically into five equal segments and 10 cells were randomly selected from each segment from top to bottom.

Analysis of comet parameters was performed using TriTek CometScore, version 1.5 (<http://www.autocomet.com>). It was performed blinded to sample group status. The DNA damage was quantified by measuring the displacement between the genetic material of the nucleus 'comet head' and the resulting 'tail'. Tail moment and tail DNA% are the two most commonly used parameters to analyze the

Figure 1



Tail DNA% = $100 \times \text{tail DNA intensity} / \text{cell DNA intensity}$. Tail moment can be measured using one of the following methods: (a) olive tail moment = tail DNA% $\times$ tail moment length, (b) extent tail moment = tail DNA% $\times$ length of tail. Tail moment length is measured from the center of the head to the center of the tail [19].

results of the comet assay [18]. The comet moment is the product of the fluorescence in the comet tail and the tail length measured from the edge of the comet head (Fig. 1). Duplicate slides were processed simultaneously from 20% of patients selected randomly. The coefficient of variation ranged from 0 to 3% for the tail parameters (comet length and moment) in all duplicate samples.

Thiobarbituric acid assay

Quantification of the lipid peroxide malondialdehyde (MDA) level in semen plasma was performed at the Forensic and Toxicology Department (FOM-SCU) using a commercially available spectrophotometric determination kit (www.bio-diagnostic.com). All the reagents and standard were supplied by the manufacturer. The reaction was started by the addition of 1 ml of the thiobarbituric acid (TBA) reagent (25 mmol/l) and concentrated acetic acid to 200 μ l of the samples, boiled in water for 30 min, and the TBA-reactive species were quantified by spectrophotometry (HALO DB-20 UV-VIS spectrophotometer; Dynamica GmbH, Australia) at 534 nm with 600 nm as the reference wavelength. The latter wavelength was used only for baseline correction. As samples contained a small constant background peak attributable to the fluorescence of TBA itself, the calculation of the MDA value for each sample was performed against its blank [MDA concentration (nmol/ml) = sampleAbs/standardAbs $\times 10$] [20].

Statistical analysis

Parametric and nonparametric tests were performed depending on the distribution of the data. All normally distributed data were subjected to a parametric analysis of variance. Continuous trait data are shown as mean $\pm$ SEM unless otherwise specified. Comparison of the frequencies between groups was carried out for statistical significance using the standard Pearson χ^2 -test. Multivariate stepwise regression analysis was used to establish the relationships between comet parameters and semen analysis. Data were analyzed using TexaSoftWINKS4.651 software (Texas, USA). Statistical significance was assumed for *P* values less than 0.05.

Table 1 Relation between the semen pattern of the men ($n = 100$) and the duration of cell phone usage (h/day) in the studied groups

Semen parameters	Mean $\pm$ SEM			
	Group A ($n = 15$)	Group B ($n = 30$)	Group C ($n = 25$)	Group D ($n = 30$)
Volume (ml)	2.8 $\pm$ 0.2	3.6 $\pm$ 0.3	3.6 $\pm$ 0.2	3.2 $\pm$ 0.2
Concentration (million/ml)	35.5 $\pm$ 8.7	21.5 $\pm$ 3.5	27.5 $\pm$ 4.1	21.6 $\pm$ 3.9
Count/ejaculate (million)	90.3 $\pm$ 19.5	76.2 $\pm$ 14.0	90.8 $\pm$ 10.9	73.1 $\pm$ 12.6
Motility ratio*	45.2 $\pm$ 5.6	47.7 $\pm$ 1.9	42.6 $\pm$ 2.0	31.7 $\pm$ 3.8
Progressive motility (%)*	35.7 $\pm$ 5.5	32.8 $\pm$ 1.7	26.9 $\pm$ 1.9	25.4 $\pm$ 3.7
Normal sperm (%)	50.3 $\pm$ 4.5	44.2 $\pm$ 2.9	42.6 $\pm$ 3.8	42.7 $\pm$ 3.3

* $P < 0.05$.**Table 2 Relation between the period usage of cell phones and the grades of sperm motility in men of the studied groups**

Cell phone use (years)	Grades of sperm motility				Total
	Grade A	Grade B	Grade C	Grade D	
1–2					
Count	19	8	0	0	27
Total (%)	22.4	9.4	0.0	0.0	31.8
2–5					
Count	5	7	9	9	30
Total (%)	5.9	8.2	10.6	10.6	35.3
> 5					
Count	2	5	11	10	28
Total (%)	2.4	5.9	12.9	11.8	32.9
Total					
Count	26	20	20	19	85
Total (%)	30.6	23.5	23.5	22.4	100.0
Statistics		$\chi^2 = 40.6$		$P = 0.000$	

Grade A, rapid progressive motility; grade B, slow progressive motility; grade C, sluggish motility; grade D, immotile sperm.

Results

The median age of the men recruited was 36 years (range 25–60). Comparison of the various semen parameters of the groups studied indicated statistically significant differences in the motility ratio and the percentage of progressive motility, which were significantly lower in group D ($P < 0.05$, Table 1). This group showed, in addition, a lower mean count/ejaculate (million) and an increase in dead sperm and atypical forms when compared with the other groups, but these did not reach statistical significance ($P > 0.05$, Table 1). The relation between the period of cell phone usage and grades of sperm motility in the studied groups is presented in (Table 2). Interestingly, 28/85 participants (32.9%) who had a cell phone for more than 5 years, 11 (12.9%) were in grade C and 10 (11.8 %) were in grade D.

In terms of the relation between cell phone usage and grades of sperm motility in the men of the groups studied, it was found that 15/37 (17.6%) of men who used to place the cell phone close to their waists were in grade D, whereas only 1/26 (1.2%) and 3/22 (3.5%) men who were in this grade used to hold the cell phone in their hand and used a hands-free cell phone, respectively ($P < 0.001$, Table 3).

Relation of radiofrequency electromagnetic radiation and DNA damage

Many researchers have assessed tail length, percentage DNA, and moment; however, the results from the

Table 3 Relation between the usage of cell phones and grades of sperm motility in men of the studied groups

Usage of cell phone	Grades of sperm motility				Total
	Grade A	Grade B	Grade C	Grade D	
Held in the hand					
Count	14	7	4	1	26
Total (%)	16.5	8.2	4.7	1.2	30.6
Hands-free cell phone					
Count	10	6	3	3	22
Total (%)	11.8	7.1	3.5	3.5	25.9
Placed close to the waist					
Count	2	7	13	15	37
Total (%)	2.4	8.2	15.3	17.6	43.5
Total					
Count	26	20	20	19	85
Total (%)	30.6	23.5	23.5	22.4	100.0
Statistics		$\chi^2 = 28.4$		$P = 0.000$	

Grade A, rapid progressive motility; grade B, slow progressive motility; grade C, sluggish motility; grade D, immotile sperm.

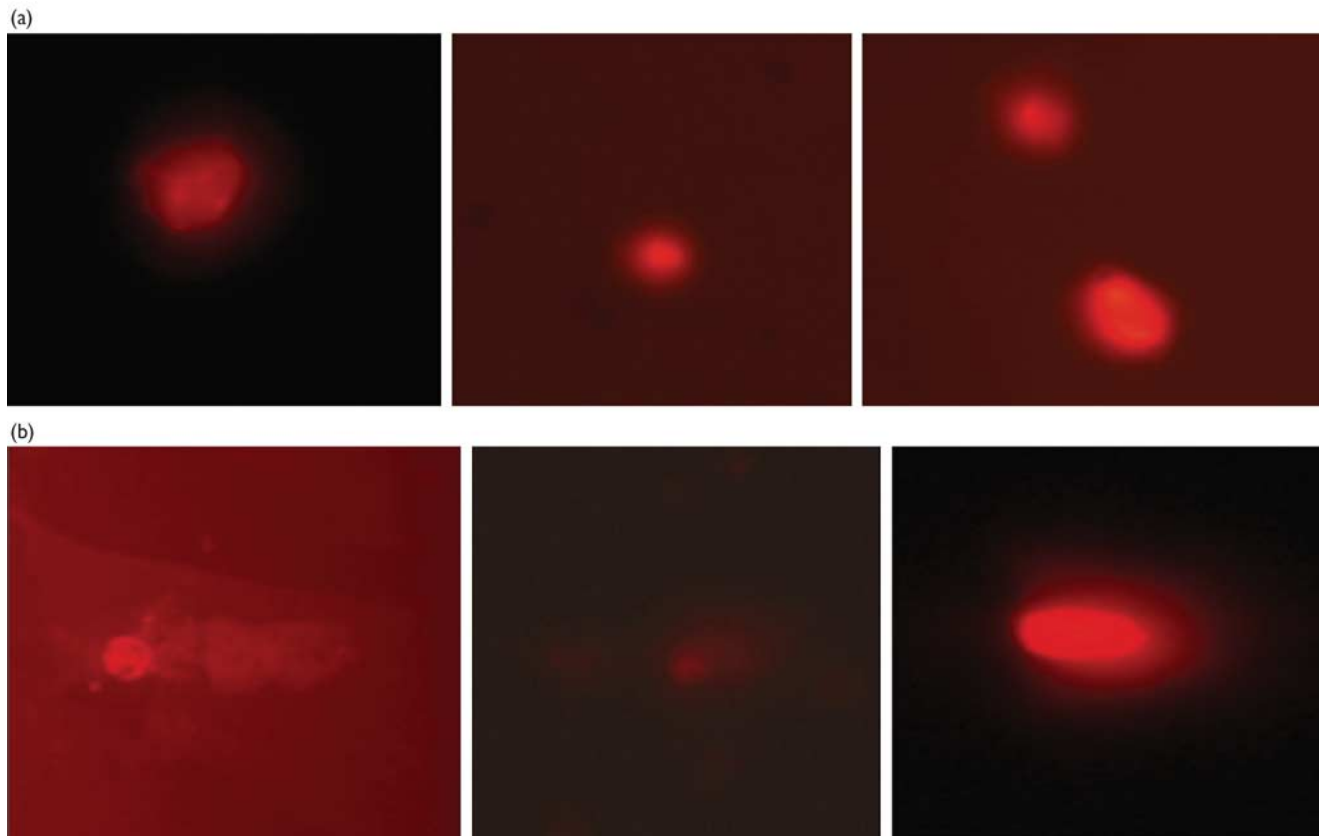
percentage tail DNA measurements have been documented, presumably because this measure yielded better discrimination in their analysis [21]. Although all three measurements are inter-related, we chose to correlate our data with the tail moment, because in the current study, this measure exaggerated the differences between patients. The differences in DNA damage between ejaculates were highly significant using the comet tail moment. This may perhaps be expected as both the length of migration of the DNA and the amounts released from the head are independently increased by DNA damage [19]. Upon inspection of the DNA damage moments and tail lengths in the sperm populations, there was a wide range of damage distribution profiles (Fig. 2). All sperm produced measurable comet parameters and its relation with the duration of cell phone usage (h/day) in the studied groups is presented in Table 4.

On using multivariate stepwise regression analysis to establish the relationships between comet parameters and semen analysis, no statistically significant correlation was found (data not shown).

Relation of radiofrequency electromagnetic radiation and reactive oxygen species production

The mean MDA level (nmol/ml) in group D was 25.8 $\pm$ 12.7 (mean $\pm$ SD); these patients showed significantly ($P < 0.001$) higher mean values compared with the patients in groups A, B, and C (14.1 $\pm$ 3.6, 15.9 $\pm$ 4.8, and 16.9 $\pm$ 5.1, respectively) (Fig. 3).

Figure 2



(b) Examples of damaged DNA (containing cleavage and strand breaks) that have migrated (alkaline electrophoresis conditions, 25 V/300 mA for 20 min) further than intact DNA (a) and produce a 'comet tail' shape.

Table 4 Relation between comet measurements for the cohort of men ($n=100$) investigated and the duration of cell phone usage (h/day) in the studied groups

	Mean $\pm$ SD				P value
	Group A ($n=15$)	Group B ($n=30$)	Group C ($n=25$)	Group D ($n=30$)	
Tail moment	18.1 ± 8.1	20.3 ± 9.6	19.7 ± 7.5	24.9 ± 8.9	0.04
Tail length (μm)	72.5 ± 18.7	75.8 ± 20.1	74.9 ± 18.6	85.6 ± 20.8	0.09
Tail DNA%	40.5 ± 15.3	43.6 ± 10.6	41.3 ± 13.6	49.6 ± 10.5	0.03

Discussion

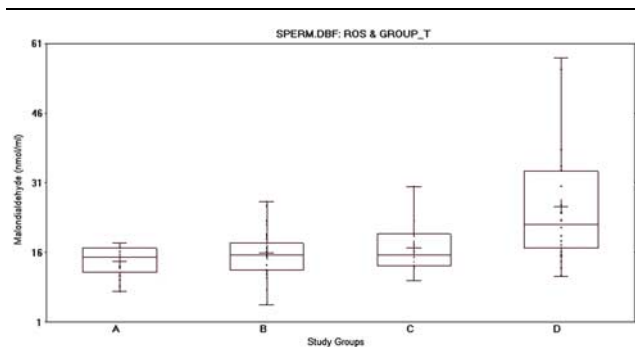
The increase in the popularity of cell phones has been accompanied by a growing concern regarding the harmful effects of cell phone radiation. The effects of cell phone exposure on male fertility have been studied extensively in the last few years [22,23]. Recent studies also have highlighted the role of cell phone exposure on sperm motility, morphology, and viability, thus proposing a reduction in male fertility [24]. Other reports suggest that RF-EMW may lead to DNA damage and chromosomal instability [2]. The effects on sperm parameters have been evaluated in many animal and human studies both *in vitro* and *in vivo*, but the results are still inconclusive.

In the present study, our results showed that increased duration of cell phone usage (h/day) was negatively correlated with the proportion of grade A and grade B sperm motility, but positively correlated with the

proportion of grade C and grade D motility. These results are in agreement with a study of the influence of electromagnetic waves on sperm motility, which reported a reduction in the proportion of rapid progressive sperm motility (grade A) from 32.3 to 26.1% after 1 month of cell phone usage for 6 h daily in their study population [6]. Similar results have been reported, wherein 65.7% of men not using cell phones had greater than 50% (grades A + B) sperm motility, whereas only 35.4% of men who frequently used cell phones had greater than 50% (grades A + B) sperm motility [25].

In the current study, it was found that there was no statistically significant decrease in the sperm concentration in the three groups (B, C, and D) that used a cell phone. This result was in agreement with a study carried out to determine the effects of cell phone emissions on sperm motility in rats; it was reported that exposure of

Figure 3



Box and whiskers plot of the reactive oxygen species level among the studied groups. The median and interquartile range (25th–75th percentile) are shown as solid horizontal lines and the mean of all groups is shown as (+). Participants exposed more to radiofrequency waves (group D: >4 h/day) had a significantly higher mean malondialdehyde level (nmol/ml) compared with the other groups ($P < 0.001$).

rats to a 1.9 Hz cell phone from a distance of 1 cm for 6 h/day for 18 weeks did not lead to a significant decrease in the sperm concentration [24]. However, in another study, a significant decrease in the sperm count was found in men who used cell phones for more than 4 h/day ($n = 114$, count $50.3 \pm 41.9 \times 10^6/\text{ml}$) as compared with those who did not use cell phones at all ($n = 40$, count $85.8 \pm 35.5 \times 10^6/\text{ml}$) ($P < 0.0001$) [1].

EMR exerts both thermal and nonthermal effects on living cells. There is no consensus among authors as to which effect predominates [26,27]. An increase in testicular or body temperature on exposure to EMW may cause a reversible disruption of spermatogenesis, which leads to a decrease in the sperm count and concentration [28]. However, recent studies have reported that RF-EMW emitted from commercially available cell phones exert no thermal effects at a SAR of 1.6 W/kg [24].

In terms of the relationship between the duration of cell phone usage and semen quality in the current study, our results are in agreement with the results of a small prospective study of 13 men with normal semen analysis; it was found that the use of GSM phones for 6 h a day for 5 days decreased the rapid progressive motility of spermatozoa [6]. In addition, another study on 371 men undergoing infertility evaluations found that the duration of usage and the daily transmission time of cell phones correlated negatively with the proportion of rapidly progressive motile spermatozoa (grade A) [2]. In terms of the relation between the use of cell phones and grades of sperm motility, our result was in agreement with a study that represented the image content influences on men's semen quality and reported that the placement of cell phones close to the waist has been found to decrease sperm motility and concentration as compared with men not using cell phones at all or keeping them elsewhere [7]. In addition, it is important to note that many men carry their cell phones in the trouser pocket (or clipped to their belts on the waist) while using a hands-free device such as Bluetooth. This technology exposes the testes to more

high power density radiation than a cell phone in the 'Stand-by mode' in a trouser pocket [28].

Significant changes in sperm morphology have not been reported in animal studies carried out to determine the effect of whole-body exposure of rats to microwaves emitted from a cell phone [29,30]. Similarly, no significant alterations in morphology were reported by another group that performed animal experiments [24]. However, the same group reported that 80% of the slides in the exposed group showed large clumps of sperm cells that were only able to turn about in their position and could not break free. Also, there were no effects of RF exposure on DNA strand breakage (assessed by a comet assay), unstable chromosomal alterations (assessed by metaphase analysis), or alterations in the speed of in-vitro cell cycling (assessed by the nuclear division index) in lymphocytes in their experiment involving exposure of human blood samples to RF (24 h, 935 MHz) [31].

However, to our knowledge, this is the first in-vivo study to show that the prolonged use of cell phone more than 4 h/day results in an increased percentage of abnormal sperm heads (although this did not reach statistical significance) together with altered DNA strands ($P < 0.05$) in the spermatozoa in Group D. The correlation between cell phone use and the changes in the motility parameters suggests that these effects accumulate. Also, the present study showed a statistically significant increase in the ROS levels in group D.

A study examining the origins and consequences of DNA damage in male germ cells reported that the common causative mechanism for the disruption of sperm-fertilizing potential and the concomitant presence of high levels of DNA damage in the sperm nucleus is oxidative stress [32]. It has been known to limit the fertilizing potential of human spermatozoa by causing peroxidative damage to the sperm plasma membrane [33]. A study showed that mobile phone radiation induces the production of ROS and DNA damage in human spermatozoa, and reported that RF-EMR in both the power density and the frequency range of mobile phones enhances the generation of mitochondrial ROS by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and ultimately DNA fragmentation [2]. These could explain our findings of a significantly increased MDA concentration and DNA damage as shown by comet measurements in group D of the current study. This could be because of either direct EMW-mediated effects or a defect in DNA repair mechanisms. Also, it has clear implications for the safety of extensive mobile phone use by men of reproductive age, potentially affecting both their fertility and the health and well-being of their offspring. A similar study has also reported that RF-EMR emitted from mobile phones enhances the generation of mitochondrial ROS by human spermatozoa, decreasing the motility and vitality of these cells and inducing DNA fragmentation [2].

A study of the effects of low-frequency electromagnetic fields on DNA has formulated a hypothesis that aids the

interpretation of our results, reporting that the EMR of cell phones may cause DNA breakage in cells in the male genital tract, which can occur in a low-frequency electromagnetic field. In-vitro studies are warranted to examine the increased numbers of chromosome aberrations of the micronuclei in human leukocytes and DNA breakage [34]. Also, a moderate correlation has been found between sperm motility and the sperm chromatin structure, which are probably because of distorted epididymal protamination [35].

Excess exposure to RF-EMR from sources such as mobile phones can be considered to be one of the key environmental factors involved in the stimulation of sperm mitochondria in the production of high levels of ROS. The outcome of oxidative damage induced by EMF will therefore depend on various factors including the oxidative status of the cell, the ability of endogenous antioxidation enzymes and processes to counteract free radical buildup, the availability of exogenous antioxidants, the parameters of exposure (e.g. generated power, duration of exposure, and wave shape), and whether the oxidative damage is cumulative [3].

Conclusion and recommendations

This study concludes that, with the use of mobile phone model GSM1800/1900 MHz, RF-EMF induces a decrease in the sperm count and motility, and abnormal forms that may lead to infertility. These findings indicated that the changes observed could be because of the overproduction of ROS induced by EMW, which can trigger DNA fragmentation. Men of reproductive age who have a high level of mobile phone usage should limit their exposure to RF by limiting the length of the calls, by using 'hands-free' devices to keep mobile phones away from the body, and should not keep their phones in the receiving mode below the waist level. Usage of the phone in areas of good reception also decreases exposure as it allows the phone to transmit at reduced power and permits use of mobile phone accessories.

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Conflicts of interest

There are no conflicts of interest.

References

- Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, *et al.* Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertil Steril* 2009; 92:1318–1325.
- De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. *PLoS ONE* 2009; 4:e6446.
- Kesari KK, Kumar S, Behari J. Mobile phone usage and male infertility in Wistar rats. *Indian J Exp Biol* 2010; 48:987–992.
- Fejes I, Závaczki Z, Szölösi J, Koloszar S, Daru J, Kovács L, *et al.* Is there a relationship between cell phone use and semen quality? *Arch Androl* 2005; 51:385–393.
- Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. *Int J Androl* 2005; 28:171–179.
- Davoudi M, Brössner C, Kuber W. The influence of electromagnetic waves on sperm motility. *J Urol Urogynakol* 2002; 9:18–22.
- Kilgallon SJ, Simmons LW. Image content influences men's semen quality. *Biol Lett* 2005; 1:253–255.
- Lewis SEM, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res* 2005; 322:33–41.
- Koppers AJ, De Iuliis GN, Finnie JM, McLaughlin EA, Aitken RJ. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. *J Clin Endocrinol Metab* 2008; 93:3199–3207.
- Erogul O, Oztas E, Yildirim I, Kir T, Aydur E, Komesli G, *et al.* Effects of electromagnetic radiation from a cellular phone on human sperm motility: an in vitro study. *Arch Med Res* 2006; 37:840–843.
- Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ Jr., Agarwal A. Relationship between oxidative stress, semen characteristics and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril* 2000; 73:459–464.
- Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003; 79:829–843.
- World Health Organization (WHO). *WHO laboratory manual for the examination and processing of human semen*. 5th ed. WHO, Geneva: World Health Organization (WHO); 2010.
- Haines G. DNA damage in human and mouse spermatozoa after in vitro-irradiation assessed by the comet assay. *Adv Exp Med Biol* 1998; 444:79–91.
- Donnelly ET, O'Connell M, McClure N, Lewis SEM. The effect of smoking and eating habits on DNA damage in Indian population as measured in the comet assay. *Mutat Res* 2001; 474:121–128.
- Dhawan A, Mathur N, Seth P. The effect of smoking and eating habits on DNA damage in Indian population as measured in the comet assay. *Mutat Res* 2001; 474:121–128.
- Kašuba V, Rozgaj R, Gamulin M, Trošić I. Assessment of cytogenotoxicity of irinotecan in V79 cells using the comet, micronucleus and chromosome aberration assay. *Arh Hig Rada Toksikol* 2010; 61:1–9.
- De Boeck M, Touil N, De Visscher G, Vande PA, Kirsch Volders M. Validation and implementation of an internal standard in comet assay analysis. *Mutat Res* 2000; 469:181–197.
- Olive PL, Banath JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the 'comet' assay. *Radiat Res* 1990; 122:86–94.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95:351–358.
- Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ. DNA integrity in human spermatozoa: relationships with semen quality. *J Androl* 2000; 21:33–44.
- Deepinder F, Makker K, Agarwal A. Cell phones and male infertility: dissecting the relationship. *Reprod Biomed Online* 2007; 15:266–270.
- Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. *Fertil Steril* 2008; 89:124–128.
- Yan JG, Agresti M, Bruce T, Yan YH, Granlund A, Matloub HS. Effects of cellular phone emissions on sperm motility in rats. *Fertil Steril* 2007; 88:957–964.
- Wdowiak A, Wdowiak L, Wiktor H. Evaluation of the effect of using mobile phones on male fertility. *Ann Agric Environ Med* 2007; 14:169–172.
- Cook A, Woodward A, Pearce N, Marshall C. Cellular telephone use and time trends for brain, head and neck tumours. *NZ Med J* 2003; 116:1175.
- Weisbrot D, Lin H, Ye L, Blank M, Goodman R. Effects of mobile phone radiation on reproduction and development in *Drosophila melanogaster*. *J Cell Biochem* 2003; 89:48–55.
- Desai NR, Kesari KK, Agarwal A. Pathophysiology of cell phone radiation: oxidative stress and carcinogenesis with focus on male reproductive system. *Reprod Biol Endocrinol* 2009; 7:114.
- Dasdag S, Akdag MZ, Aksent F, Yilmaz F, Bashan M, Dasdag MM, *et al.* Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. *Bioelectromagnetics* 2003; 24:182–188.
- Dasdag S, Ketani MA, Akdag Z, Ersay AR, Sari I, Demirtas ÖC, *et al.* Whole-body microwave exposure emitted by cellular phones and testicular function of rats. *Urol Res* 1999; 27:219–223.
- Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, *et al.* 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. *Int J Radiat Biol* 2006; 82:339–346.
- Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. *Reprod Biomed Online* 2007; 14:727–733.
- Shen HM, Ong CN. Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility. *Free Radic Biol Med* 2000; 28:529–536.
- Ivancsits S, Diem E, Jahn O, Rüdiger HW. Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way. *Int Arch Occup Environ Health* 2003; 76:431–436.
- Giwerzman A, Richthoff J, Hjøllund H, Bonde JP, Jepson K, Frohm B, *et al.* Correlation between sperm motility and sperm chromatin structure assay parameters. *Fertil Steril* 2003; 80:1404–1412.