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ORIGINAL ARTICLE

The detrimental effect of cell phone radiation on sperm biological characteristics in normozoospermic

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Abstract

Radiofrequency electromagnetic radiation emitted from cell phone has harmful effects on some organs of the body, such as the brain, heart, and testes. This study aimed to assess the effects of cell phones on sperm parameters, DNA fragmentation, and apoptosis in normozoospermic. Normal sperm samples were divided into two groups of control and case. The samples from the case were placed for 60 min at a distance of approximately 2.5 cm from the cell phone set in the active antenna position. Control samples were exposed to cell phones without active antennas. All specimens were analysed by World Health Organization criteria. Sperm viability, sperm with chromatin abnormality and maturity, DNA fragmentation, and apoptosis were examined. Viability and motility in the case were significantly lower than the control ($p < .001$, $p = .004$ respectively). The percentage of apoptotic sperms and DNA fragmentation were significantly higher in the case when compared with the control ($p = .031$, $p < .001$ respectively). The other parameters studied such as morphology, chromatin abnormality, and maturity showed no significant difference between the case and control groups. Cell phone waves had a detrimental effect on human sperm's biological features. Therefore, it is recommended to keep the cell phone away from the pelvis as much as possible.

KEYWORDS
cell phone, electromagnetic radiation, sperm, apoptosis

1 | INTRODUCTION

Today, we are facing an increase in infertility in human societies and this increase is related to various factors, such as lifestyle, diet, and electronic devices, and radiation. The use of innovative technologies, such as the Internet, email, social networks, and smartphones, has increased dramatically in modern societies (Habeeb et al., 2020). One of the bad habits of people is frequent and prolonged contact with devices that emit radiofrequency electromagnetic radiation (RF-EMR) such as cell phones (Hammoud et al., 2006). Putting a mobile phone in the pocket for a long time causes the electromagnetic waves emitted by these devices to contact the reproductive system (Gautam et al., 2020). Mobile devices have units for sending and receiving radio signals by electromagnetic waves (EMW). These waves are usually limited to 900–1800 GHz (Mahaldashtian et al., 2021) and have less energy than atoms and ionizing molecules (Habeeb et al., 2020). Following the increase in the use of mobile phones in the world, there is a possibility of the destructive effect

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of electromagnetic waves on reproduction (FALAHATI et al., 2011; Kesari et al., 2018; Merhi, 2012).

Radiation can be classified as ionized or non-ionized radiation. Non-ionizing radiation consists of electromagnetic waves that are not sufficiently active and cannot separate electrons from atoms or molecules. These waves include radio waves and microwaves. Mobile waves are a type of radio wave with a maximum wavelength of 300 GHz (Alkayed et al., 2020; Kesari et al., 2018). The frequencies of these radio waves are low and classified in the range of low-frequency waves. Therefore, this radiation is non-ionized and the emitted energy cannot break the chemical bonds in the biological system. In contrast, the energy in high-frequency electromagnetic waves (1,000,000 MHz), known as an ionizing wave, such as X-rays can break chemical bonds and molecules, causing serious damage to cell structures (Bhat, 2013). Harmful effects of electromagnetic radiation have been proven in previous studies. For example, prolonged exposure to electromagnetic waves reduced sperm production, impairs motility and morphology, and increases oxidative stress in the testes (Kesari et al., 2018). Excessive production of reactive oxygen species (ROS) causes hormonal imbalance, gonadal dysfunction, tissue and cell damage (Pandey et al., 2017). The results of different studies showed that electromagnetic waves have various effects on different parts of the body. While some researchers have reported that EMR reduces fertility, others reported that these waves can reduce both the quantity and quality of spermatozoa (Gautam et al., 2020; Kesari et al., 2018). These waves also cause hormonal changes in the testes and interrupt the spermatogenesis process, in addition to causing foetal death and growth disorders in the embryonic period (Odacı & Özyılmaz, 2015). However, there are controversial results in studies on the electromagnetic waves of mobile phones on male fertility parameters (Adams et al., 2014; Feijo et al., 2011; Liu et al., 2014; Radwan et al., 2016; Rago et al., 2013). Moreover, most previous studies were done on the effect of mobile waves on animal models. To the best of our knowledge, studies on the effects of RF-EMR emitted from cell phones on human spermatozoa are mainly limited to sperm parameters as well as chromatin quality and DNA fragmentation. Therefore, this study aimed to assess the direct effect of RF-EMR emitted from the cell phone on the biological characteristics of human spermatozoa using various cytological assays in normozoospermic cases.

2 | MATERIALS AND METHODS

2.1 | Design of study

This study was approved by the ethics committee of Birjand University of Medical Sciences (IR.BUMS.REC.1395.12). In this study, 60 normal semen samples (normozoospermic) were randomly selected. The status such as orchitis, varicocele, hydrocele, diabetes mellitus, hypertension, and cardiac, neural, and nephritic diseases was excluded. Also, an informed consent form including the method of investigation and confidential information was obtained from each participant. The semen samples were obtained by masturbation following 3 days of abstinence. After collecting the specimens, they were kept in the incubator (37°C) to liquefy for 30 min. After sperm evaluation by an automatic sperm analyzer (CASA, Germany), each sample was divided into two equal parts as the case and control groups. Then, the samples from both groups were put in an Incubator for 1 hr. During this period, a cell phone (Sony Ericsson W300i, Japan) was placed in active antenna mode at 2.5 cm away from the case samples. Control samples were exposed to cell phones without active antennas (Baste et al., 2008; Oktem et al., 2005).

2.2 | Sperm evaluation

WHO criteria were used for semen analysis (Organization, 2010). The rates of sperm morphology and motility were recorded in each group. Sperm viability was tested using eosin–nigrosin staining. Also, the percentage of apoptotic sperms and DNA fragmentation were tested by TUNEL and SCD method respectively. Aniline blue (AB) and toluidine blue (TB) staining was used for determining sperm cells with additional histones and stable chromatin respectively.

2.3 | Sperm morphology staining

The Diff–Quick staining was used to evaluate sperm morphology according to the WHO protocol (Organization, 2010). The dried coloured slides were scanned under a light microscope at 100× magnification to look for morphological anomalies. A total of 200 spermatozoa per sample were classified according to their morphology (i.e. normal and abnormal heads, middle pieces, and tails). The sum of the abnormal spermatozoa was expressed as a percentage (Doostabadi et al., 2021).

2.4 | Sperm motility

Motility was expressed as the percentage of progressive and non-progressive spermatozoa (Organization, 2010). The progressive motility (PR) indicated spermatozoa moving actively, either linearly or in a large circle, regardless of speed; the non-progressive motility (NP) pointed out all other patterns of motility with an absence of progression (Doostabadi et al., 2021).

2.5 | Sperm viability

Sperm vitality was estimated by assessing the membrane integrity of the cells. The eosin–nigrosin staining was used to evaluate sperm viability according to the WHO protocol (Organization, 2010). Spermatozoa with red (D1) or dark pink (D2) heads were considered dead (damaged membrane), whereas spermatozoa with whiteheads or light pink heads (L) were considered alive (intact membrane) (Doostabadi et al., 2021).

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2.6 | Aniline blue staining for chromatin maturity

Aniline blue (AB) staining is a cytochemical test for the detection of residual histones and therefore indirectly evidences the presence of lower amounts of protamine in the sperm nucleus (Nasr-Esfahani & Tavalaee, 2021). For each stained smear, 200 spermatozoa were evaluated with a light microscope (100x objective). Spermatozoa with unstained nuclei were considered normal (mature chromatin), while those blue-stained were considered abnormal (immature chromatin).

2.7 | Toluidine blue test for chromatin abnormality

Toluidine blue (TB) stain is a basic nuclear dye used for metachromatic and orthochromatic staining of chromatin (Nasr-Esfahani & Tavalaee, 2021). This stain is a sensitive structural probe for both sperm DNA structure and sperm chromatin packaging because the test measures the accessibility of the sperm chromatin DNA phosphate residues for dye molecules, which is dependent on both the protein condition and DNA integrity. The quality of the chromatin of the spermatozoa was determined by light microscopy at 100× magnification as a function of the metachromatic coloration of the sperm heads according to the following scores: score = 0 light blue (good chromatin); score 1 = dark blue (light abnormal chromatin); and score 2 = purple and violet (severe chromatin anomaly). The total sperms with scores 1 and 2 were considered TB+or sperm with abnormal chromatin, while sperms scoring 0 as TB− or sperm with normal chromatin.

2.8 | TUNEL assay for sperm apoptosis

Terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling assay (TUNEL) was first explained by Gorczyca and colleagues (1993) and used for direct detection of DNA fragmentation in mammalian spermatozoa. The principle of the TUNEL test is labelling the 3'ends of fragmented DNA with biotinylated dUTP using recombinant terminal deoxynucleotidyl transferase (TdT) enzyme in a template-independent manner. These incorporated labelled nucleotides can be distinguished in spermatozoa using flow cytometry, fluorescence microscopy, and also light microscopy (Avendaño et al., 2009). The nuclei of spermatozoa with fragmented DNA (TUNEL+) showed a bright green color, the nuclei of normal cells (TUNEL–) were place green.

2.9 | Sperm Chromatin Dispersion test for DNA fragmentation

Sperm chromatin dispersion (SCD) test is an easy, fast, and reliable test for detecting sperm DNA fragmentation. During the test, controlled acid denaturation of the DNA and removal of nuclear proteins of the semen sample takes place. This assay is according to the fact that sperm cell with fragmented DNA fails to create the feature 'halo' of dispersed DNA loops that are seen in non-fragmented sperm DNA after acid denaturation and removal of sperm nucleoproteins (Jose Luis Fernández et al., 2003; Talebi, 2011). As a result, a large halo can be seen in sperm with intact DNA, while spermatozoon with dispersed chromatin materials or fragmented DNA will not produce a halo. SDF cut-off value was taken as ≥30% to differentiate between infertile and fertile men using a standard protocol (José Luis Fernández et al., 2005). The SCD test gives a similar predictive value for sperm DNA fragmentation as the TUNEL assay. So, this technique is considered a relatively simple alternate of 'sperm chromatin structural assay' for the assessment of sperm DNA. The major advantage of the SCD test is that it does not dependent on the colour or fluorescence intensity of chromatin. In this test, the percentages of spermatozoa with no dispersed nuclei (with very small halos or none at all) and dispersed nuclei (with medium or large sized halos) are determined and can be easily and reliably evaluated. The result of a recent study showed a significant correlation between sperm DNA fragmentation rate detected by SCD test and fertilization rate and also embryo quality.

2.10 | Sample size

The sample size was estimated using the formula of $N = \frac{Z2Pq}{0/d2}$, in which N= number of cases, Z = value of confidence level = 95%, P = estimated average prevalence of infertility, $q = 1-P$, and d = accepted error precision around the mean of the population. Therefore, the sample size was estimated as $N = (0.95)^2(0.15)(0.85)/0.002 = 57$. To ensure the collection of the estimated sample size, three more cases were added to increase the power of analysis.

2.11 | Data analyses

The homogeneity of data was evaluated by the Shapiro–Wilk test. Accordingly, the independent T test and Mann–Whitney test were used to compare the variables between the groups. SPSS software (version 22) was used for the statistical analysis, and the differences were considered significant if $p < .05$.

3 | RESULTS

The rate of spermatozoa with abnormal morphology in the case group decreased when compared with controls ($p = .64$, Table 1). Comparison between sperm motility in the control and case groups showed that this parameter was significantly reduced in the latter (68.55 ± 13.64 versus. 61.40 ± 13.98; $p = .004$). Also, the rate of

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Parameters	Groups		
	Case	Control	*p* value
Abnormal morphology (Diff–Quick)	25.95 ± 9.88	26.55 ± 9.91	$p = .64$
Sperm motility	61.40 ± 13.98	68.55 ± 13.64	$p = .004$*
Sperm viability (Eosin–nigrosin)	68.26 ± 5.47	75.01 ± 5.21	$p < .001$*
chromatin maturity (AB)	43.28 ± 14.69	44.15 ± 11.84	$p = .38$
chromatin abnormality (TB)	38.41 ± 10.83	38.56 ± 10.45	$p = .96$
Apoptotic sperm (TUNEL)	30.68 ± 9.81	26.90 ± 9.17	$p = .031$*
DNA fragmentation (SCD)	27.93 ± 6.62	24.46 ± 6.02	$p < .0001$****

TABLE 1 The mean ± *SD* of sperm quality parameters in the studied groups

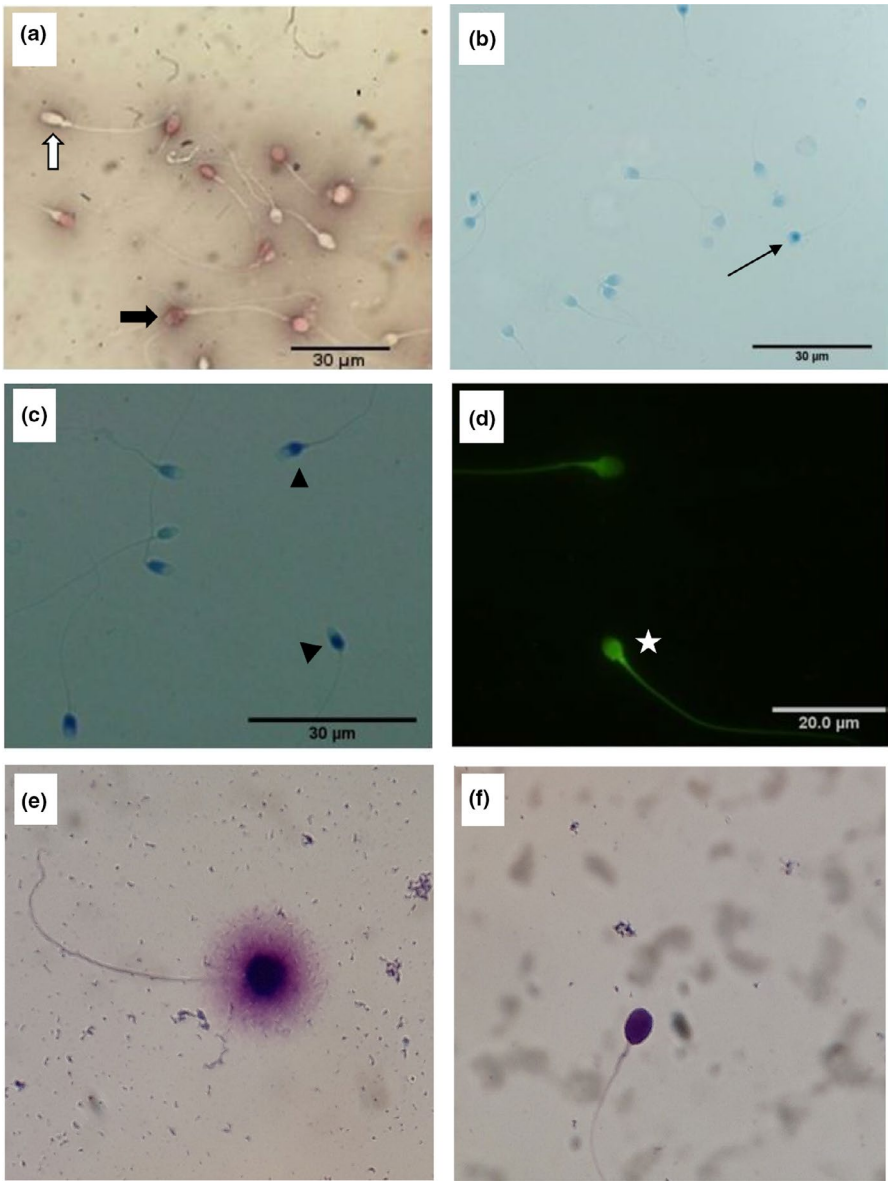


FIGURE 1 Photomicrographs of sperms stained with eosin–nigrosin (a), aniline blue (b), toluidine blue (c), TUNEL (d), and SCD (e, f). The white thick arrow represents live sperm, while black thick arrow shows stained dead spermatozoa. The thin arrow shows sperm with immature chromatin, arrowheads show spermatozoa with abnormal chromatin, *Shows apoptotic spermatozoa, Image e, halo sperm with no fragmentation in DNA, while image f, sperm without halo shows intense DNA fragmentation

live sperms in the case was significantly lower than in the control group (68.26 ± 5.47 versus. 75.01 ± 5.21; $p < .001$, Table 1; Figure 1a). Differently, the AB and TB staining methods were used to evaluate sperm chromatin maturity and sperm chromatin abnormality, indicating that there were no significant differences between the two groups ($p > .05$; Table 1 and Figure 1b,c). Furthermore, the TUNEL assay showed that the percentage of apoptotic sperms in the case group was significantly higher than in the control group (30.68 ± 9.81 versus. 26.90 ± 9.17, respectively; $p = .031$; Table 1; Figure 1d). Finally, the SCD test showed

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that the rate of DNA fragmentation in the case group was significantly higher than the controls (27.93 ± 6.62 versus. 24.46 ± 6.02; $p < .0001$) (Figure 1e,f).

4 | DISCUSSION

Many researchers believe that the harmful effects of the cell phone can be caused by the generated heat, also EMWs emitted from the devices (Black & Heynick, 2003). In the present study, EMWs emitted from cell phones are not able to affect sperm morphology. However, in studies conducted by Gorpinchenko et al. (2014), Yildirim et al. (2015), and Al-Bayyari (2017) the cell phone usage (short and long times) did not affect sperm morphology. In contrast, others proved that men who use mobile phones for a long time had an abnormal rate of sperm morphology (Kesari & Behari, 2012).

In addition, both sperm motility and viability were significantly decreased in our case group. Our results were consistent with the above-mentioned studies in which they compared the use of daily cell phones in two different groups based on the longer and shorter duration of use. They found that long-duration use of mobile phones significantly decreased sperm viability as well as motility (Al-Bayyari, 2017; Gorpinchenko et al., 2014; Yildirim et al., 2015). Moreover, others showed that sperm viability in the case group exposed to radio electromagnetic waves at frequencies of 1.8 GHz for 16 hr was significantly lower than the controls (De Iuliis et al., 2009). On the other hand, the percentage of sperm motility in the case group exposed to mobile waves for 1 hr was insignificantly lower than that of the controls (Zalata et al., 2014). Results from several articles show that keeping phones in pants or belt pockets shows sperm progression (Al-Bayyari, 2017; Kilgallon & Simmons, 2005). Several groups prove that men who use cell phones for a long time have decreased sperm concentration, motility, normal morphology, and viability (Ashok Agarwal et al., 2011; Erogul et al., 2006; Kesari & Behari, 2012; La Vignera et al., 2012; Yan et al., 2007).

In the present investigation, the result of TUNNEL and SCD tests showed that the mean percentage of apoptotic sperms and DNA fragmentation in the case group increased significantly compared to the controls. Similarly, a significant increase in sperm DNA damage was noted in men who were exposed to microwave at 2.45 GHz for 2 hr a day for 45 days by measuring the tail length of the DNA and the movement of the tail using the comet method (Meena et al., 2014). Prolonged exposure to RF-EMF waves breaks DNA in the testicles. Also, any change in the DNA of sperm can cause mutagenic or tumorigenic effects. The results of studies showed that the use of electromagnetic waves with a frequency of 900 MHz and 1.7 GHz caused the breakage of sperm DNA and embryonic stem cells in mice (Baverstock, 2000; Nikolova et al., 2005) In contrast to our findings, Agarwal et al. (2009) showed that comparing the amount of sperm with damaged DNA and cell death between groups that were exposed to mobile waves for 1 hr and the control showed no significant differences (Agarwal et al., 2009). In male germ cells, the amount of damage caused by EMF is very high due to the high compaction of the DNA. However, short-term exposure to RF cannot alter the level of the genome, and if does, long-term exposure may cause DNA damage. It is also suggested that the main cause of DNA fragmentation in sperm may be the presence of high levels of oxidative stress. The reason for differences in the results can be using electromagnetic waves with different wavelengths or duration of exposure.

Our results also showed that spermatozoa with chromatin maturity and chromatin abnormality decreased in the case group with respect to controls. There have been no studies to evaluate the effects of EMWs on sperm chromatin and histone changes in the literature. Concerning the possible mechanisms of EMWs on sperm cells, it is clear that EMWs cause oxidative stress, which may lead to increased cellular damage and death (Moustafa et al., 2001). During the interaction of ionizing radiation with biological systems, free radicals are generated which react with the water molecule and produce a wide range of ROS (Free et al., 1976). ROS has a high affinity for reaction with all cell components so that the main goal for radicals OH is DNA, lipid, and protein. As a result, the interaction of the radical OH with the cellular genome causes a variety of cellular abnormalities that ultimately lead to cell death (Free et al., 1976). The ionizing radiation, due to free radical productions, can break a chemical bond and triggering changes in biological events, including mutations and damage to cell membranes (Benkhaled et al., 2006). EMWs impose their destructive effects in different ways, such as changes in the material released from the cell membrane, the destruction of protein binding bands, disturbance in the synthesis of macromolecules, and the alteration of nucleotides in DNA (Barnes, 1992; Levin, 2003).

5 | CONCLUSION

The cell phone waves can reduce the sperm's biological characteristics, such as morphology, motility, viability, DNA integrity, and an increase in apoptosis in normozoospermic men. Therefore, it is recommended for men to keep the cell phone away from their pelvic.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

AUTHOR'S CONTRIBUTION

MR Dostabadi and MM Hassanzadeh-Taheri assisted in the design of the project and wrote the first draft. A Hosseini Nejad assessed the data. Hosseini M and Zardastam supervised the statistical analysis. Khalili MA and Dostabadi MR designed the study and interpretation of the data and presented a critical revision of the draft.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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