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Impact of Non-Ionizing Electromagnetic Emission from Cell Phones on Semen Parameters and DNA Integrity in Men

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ABSTRACT

Background: Various lifestyle and environmental variables are associated with male infertility, and one of those variables is radiation exposure. The most common source of non-ionizing radiation affecting male fertility is cell phones, which are certainly a necessary component of everyday life.

Methods: Ninety- seven semen samples were classified into normozoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia groups. Standard seminal analysis was conducted to measure semen parameters and DNA fragmentation rate according to WHO 2021 guidelines. Participants received antioxidant treatment including Vitamin E and Coenzyme Q10 (CoQ10) for two months, and semen parameters and DNA fragmentation index were evaluated before and after treatment.

Results: The samples affected by electromagnetic waves emitted from cell phones exhibited a substantial reduction in semen parameters and DNA integrity. The normozoospermic group showed a significantly higher sperm agglutination ($p = 0.048$) and DNA fragmentation index ($p < 0.001$) in patients exposed to > 4 hours of non- ionizing electromagnetic waves. Sperm concentration was significantly lower in the oligozoospermia group with more exposure time ($p = 0.031$); on the contrary, the DNA fragmentation index was significantly higher in these patients ($p = 0.050$). In the asthenozoospermia and teratozoospermia groups, the only significant finding was higher DFI with higher exposure time ($p = 0.043$, $p = 0.048$).

Conclusion: The quality of semen and DNA integrity are both negatively correlated with electromagnetic waves released by cell phones. It is concluded that men of reproductive age should refrain from carrying their phones in their front trouser pocket or hip pocket, since this might harm spermatozoa parameters and reduce the reproductive capability of males.

INTRODUCTION

Reproductive diseases and infertility are significant worldwide issues, and the male factor is becoming an important factor in designing assisted reproductive technologies and diagnosis methods [1]. Recent increases in infertility must be attributed to a variety of environmental and behavioral factors. Excessive exposure to heat and radiation is likely to have an impact on male infertility [2]. Mobile phones, microwave ovens, and laptops are the most widespread causes of nonionizing radiation, which may play a role in an increase in male infertility [3]. Mobile phones are one of the main sources of non- ionizing radiation exposure daily. Because of improved services like the internet and smartphone devices, a mobile phone now essentially controls our everyday life [4]. However, the adverse effects on health associated with their use are routinely disregarded [5]. Recent studies have shown that mobile phone use has a major impact on sperm as a whole, seminal tubules, and testicular stromal cells [6].

Non- ionizing electromagnetic waves (EMW) can impact several factors, including oxidative stress (OS), sperm count, sperm motility, semen concentration, and sperm morphology, causing a negative impact on reproductive health. OS is the main source of DNA damage and sperm dysfunction [5]. Infertile males are more probably to have sperm DNA fragmentation, which can impact pregnancy success [7]. Although the seminal fluid analysis results were normal, a number of infertile men were found to have an increase in DNA fragmentation [8]. Consequently, the contemporary study focuses on the effects of cell phone- emitted electromagnetic wave exposure on semen parameters in samples of Iraqi men. The aim of the study is to investigate the influence of long-term exposure to non-ionizing EMWs emitted by mobile phones on human male fertility and quality of sperm through DNA fragmentation index and reproductive parameters.

METHODS

Subjects and Study Design

Ninety-seven fertile and infertile males (All participants were informed about the objective of the questionnaire) attended the consultation clinic of the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University in Baghdad, from November 2022 to May 2023.

Questionnaire

The self- designed questionnaire consisted of questions related to personal information and daily habits, including name, age, occupation, weight, height, date of marriage, type of infertility (primary or secondary), duration of infertility, any previous investigations & treatments of infertility, medical history, previous surgical, and exposure history to chemicals or radiation. Information related to cell phone utilization was also present, such as location, duration of use, and where the mobile phone was carried.

Collection and analysis of samples

The semen sample was taken from each subject in a sterile wide-mouth container in a private room near the laboratory. Before sample collection, the participants were orally informed that they should abstain from sexual activity for 2 to 7 days. After that, the specimen was put in an incubator at 37°C and analyzed after its liquefaction. Appearance and liquefaction of the semen samples were visually assessed. A graduated container was used to find out the volume of seminal fluid. Ejaculate viscosity was measured by a wide-bore plastic pipette, and the semen's pH was measured by using a pH strip. Semen concentration, progressive and non-progressive sperm motility, immotile sperm, agglutination, and round cells were measured according to the manual of WHO 2021 [9,10]. Sperm morphology was evaluated by using a Testisimplets slide [11]. 10 µL of semen was put into the slide, covered with the typical cover slip, and left for 5 minutes at room temperature. The detailed assessment was done under light microscope, and at least 200 spermatozoa were examined to estimate the morphology of semen sample; the normal sperm morphology score was 30% by staining (WHO 1999) or above. The sperm DNA fragmentation index (DFI) was detected through the acridine orange stain according to WHO 2021 and Al-Dujaily (2015) [9,12]. The DFI was divided into classification of 15% or less excellent sperm integrity DNA fragmentation, 15-30 % moderate/ elevated, and more than 30% high/ severely elevated DFI [13].

Acridine orange protocol

Acridine orange protocol was implemented according to Tejada (1984) [14]. An amount of 10 µL semen sample was created on the slide, followed by 20 minutes of air drying. To fix the slide, use Carnoy's solution for 2-24 hours. Wait for it to completely dry before staining. The pH of the stain was adjusted to 2.5, and all of the preparations were carried out at room temperature and in dark. The stain of 2–3 mL was used for 5 minutes on a slide, which was allowed to dry after being cleaned carefully with distilled water. After staining, the slide was examined using a fluorescent microscope (BEL Company, Italy) with a 40× objective lens. 200 spermatozoa were examined for DNA integrity. Each sample's spermatozoa's DNA was tested and given a score based on whether it fluoresced yellow, green, or red. Spermatozoa with green fluorescence were considered normal, but sperm heads with yellow or red fluorescence were considered abnormal. DNA fragmentation index (DFI) can be calculated from the given equation [9,12-14]:

DFI = red fluorescence / (red fluorescence + green fluorescence)

Statistical analysis

Data was organized using Microsoft Office 2010 and analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. The data were described by measuring range, standard error, mean, and frequency. Depending on the nature of the data, the groups were compared by applying chi-square, ANOVA, independent sample t-test, and paired t-test. At a p-value equal to or less than 0.05, the results were considered significant.

RESULTS

Classification of the studied groups

Ninety-seven males were enrolled in this study. These patients were classified into four groups according to the seminal fluid analysis. Group 1 included 40 males with normozoospermia; the second group involved 25 with asthenozoospermia; Group 3 included 16 patients with oligozoospermia, and the fourth group included 16 males with teratozoospermia. The patients' results are expressed in mean ± SEM.

Comparison of demographic features among the studied groups

According to the results of the demographic feature comparison among 4 study groups (Table 1), there were no noteworthy alterations in mean patient age (p = 0.863), mean body mass indices (p = 0.530), duration (p = 0.871), and types of infertility (p = 1.00).

Seminal fluid analysis parameters and DFI% comparison according to contact time of non-ionizing radiation in the normozoospermia group

There was significantly higher sperm agglutination (12.11 ± 3.27 vs. 5.29 ± 1.13; p = 0.048) and DNA fragmentation index (17.47 ± 1.78 vs. 8.71 ± 0.88; p < 0.001) in individuals exposed to non-ionizing radiation for more than four hours.

There were no significant differences between the two exposure periods in the concentration of sperm (p = 0.857), sperm motility (p > 0.05), morphologically normal sperm (p = 0.317), and round cells (p = 0.485), as illustrated in Table 2.

Seminal fluid analysis parameters and DFI% comparison according to exposure time of non-ionizing EMW in the asthenozoospermia group

In the asthenozoospermia group, the only significant finding was higher DFI% with higher exposure time (19.45 ± 2.86 vs.13.20 ± 1.93; p = 0.043); however, seminal fluid parameters showed no significant differences according to exposure time of EMW (Table 3).

Comparison of seminal fluid analysis parameters and DFI% according to exposure time of non-ionizing EMW in the oligozoospermia group

Sperm concentration was significantly lower in patients with more exposure time (6.42 ± 0.63 vs. 9.67 ± 0.33; p = 0.031), on the contrary DFI was significantly increased in seminal fluids of these patients (17.23 ± 2.94 vs. 5.00 ± 1.00; p = 0.050), although sperm motility, morphologically normal sperm, round cells and sperm agglutination were statistically insignificant between two groups of patients in the oligozoospermia group as presented in Table 4.

Comparison of seminal fluid analysis parameters and DFI% according to exposure time of non-ionizing EMW in teratozoospermia group

The single significant finding was higher DFI% in patients with higher exposure time (17.83 ± 2.91 vs. 9.25 ± 2.75; p = 0.048); meanwhile, all seminal analysis parameters showed no significant difference between the two patient groups with teratozoospermia as demonstrated in Table 5.

Comparison of seminal fluid analysis parameters and DFI% before and after treatment in the normozoospermia group

In the normozoospermia group there was significant improvement in morphologically normal sperm and DNA fragmentation index after treatment (35.00 ± 1.97 vs. 39.00 ± 1.80; p = 0.003) and (23.20 ± 2.02 vs. 11.40 ± 2.26; p < 0.001) respectively; however, no significant differences were noted in sperm concentration, sperm motility, round cells and sperm agglutination after treatment.

Comparison of seminal fluid analysis parameters and DFI% before and after treatment in the asthenozoospermia group

The results in asthenozoospermia showed improvement of sperm motility after treatment, in which there was an increased percentage of progressively motile sperm (11.67 ± 2.04 vs. 31.33 ± 5.37; p = 0.006) and decreased percentage of immotile sperm (53.33 ± 5.20 vs. 39.22 ± 4.48; p = 0.017). There was also improvement in DNA fragmentation index (29.11 ± 3.71 vs. 15.44 ± 2.10; p = 0.001).

Comparison of seminal fluid analysis parameters and DFI% before and after treatment in the oligozoospermia group

There was significant improvement after the treatment in both sperm concentration (7.60 ± 0.60 vs.17.40 ± 2.14; p = 0.011) and DFI% (28.40 ± 3.64 vs. 19.20 ± 3.34; p = 0.001); however, there were no significant differences in sperm motility before and after treatment, morphologically normal sperm (p=0.070), round cells (p=1.00), and sperm agglutination (p=1.00).

Comparison of seminal fluid analysis parameters and DFI% before and after treatment in teratozoospermia group

There was only a significant improvement in DNA fragmentation index (22.67 ± 6.12 vs. 18.33 ± 6.57; p = 0.004) after therapy in teratozoospermia group. On the contrary, there were no significant changes in all seminal fluid analysis parameters before and after the treatment.

Tables

Parameter	Normozoospermia group	Asthenozoospermia group	Oligozoospermia group	Teratozoospermia group	p value
Age (years)	33.80 ± 1.28	35.08 ± 1.61	32.93 ± 2.46	33.44 ± 2.16	0.863∀ NS
BMI (kg m^{-2})	27.37 ± 0.67	27.46 ± 0.55	28.54 ± 1.11	29.01 ± 1.55	0.530∀ NS
Duration of infertility (years)	4.30 ± 0.53	3.84 ± 0.52	4.50 ± 0.72	4.56 ± 0.84	0.871∀ NS
Types of infertility N. (%)	Primary 27 (67.5%)	Primary 17 (68.0 %)	Primary 11 (68.8 %)	Primary 11 (68.0%)	1.00 € NS
	Secondary 13 (32.5%)	Secondary 8 (32.0 %)	Secondary 5 (31.3 %)	Secondary 5 (31.3 %)	

Table 1: Comparison of demographic features among the studied groups. Values are expressed as Mean ± SEM, NS: Not significant (p > 0.05), ∀: ANOVA test, and €: Chi square

Semen parameters		Exposure time of non-ionizing EMW		*p* value
		< 4 hours/day	⩾ 4 hours/day	
Sperm concentration ($\times 10^6$ mL^{-1})		44.05 ± 3.89	43.05 ± 3.83	0.857 Ŧ NS
Sperm motility %	Progressive motile %	47.48 ± 4.12	43.90 ± 4.24	0.549 Ŧ NS
	Non progressively motile %	22.71 ± 2.59	27.68 ± 3.20	0.231 Ŧ NS
	Immotile sperm %	27.81 ± 2.73	28.42 ± 1.74	0.854 Ŧ NS
Morphologically normal sperm %		39.43 ± 2.14	36.74 ± 1.49	0.317 Ŧ NS
Round cells ($\times 10^6$ mL^{-1})		3.67 ± 0.30	4.00 ± 0.37	0.485 Ŧ NS
Agglutination %		5.29 ± 1.13	12.11 ± 3.27	0.048 Ŧ S
DNA fragmentation index %		8.71 ± 0.88	17.47 ± 1.78	< 0.001 Ŧ S

Table 2: Comparison of seminal fluid analysis parameters & DFI% according to exposure time in the normozoospermia group. NS: Not significant (p> 0.05); S: Significant (p ≤ 0.05); Ŧ: Independent sample t-test

Semen parameters		Exposure time of non-ionizing EMW		*p* value
		< 4 hours/day	⩾ 4 hours/day	
Sperm concentration ($\times 10^6$ mL^{-1})		35.00 ± 3.86	35.90 ± 3.65	0.907 Ŧ NS
Sperm motility %	Progressive motile %	13.00 ± 3.00	16.50 ± 1.59	0.329 Ŧ NS
	Non progressively motile %	37.00 ± 7.00	35.00 ± 3.26	0.789 Ŧ NS
	Immotile sperm %	47.60 ± 8.79	48.25 ± 4.04	0.944 Ŧ NS
Morphologically normal sperm %		35.80 ± 7.39	42.50 ± 3.65	0.422 Ŧ NS
Round cells ($\times 10^6$ mL^{-1})		3.20 ± 0.73	3.00 ± 0.37	0.811 Ŧ NS
Agglutination %		3.00 ± 2.00	2.00 ± 0.92	0.636 Ŧ NS
DNA fragmentation index %		13.20 ± 1.93	19.45 ± 2.86	0.043 Ŧ S

Table 3: Comparison of seminal fluid analysis parameters & DFI% according to exposure time in asthenozoospermia group. NS: Not significant (p> 0.05); S: Significant (p ≤ 0.05); Ŧ: Independent sample t-test

Semen parameters		Exposure time of non-ionizing EMW		*p* value
		< 4 hours/day	⩾ 4 hours/day	
Sperm concentration ($\times 10^6$ mL^{-1})		9.67 ± 0.33	6.42 ± 0.63	0.031 Ŧ S
Sperm motility %	Progressive motile %	30.00 ± 5.00	26.92 ± 8.68	0.872 Ŧ NS
	Non progressively motile %	21.67 ± 11.67	27.15 ± 5.88	0.691 Ŧ NS
	Immotile sperm %	46.67 ± 14.53	45.92 ± 8.14	0.968 Ŧ NS
Morphologically normal sperm %		20.00 ± 2.89	24.62 ± 3.50	0.552 Ŧ NS
Round cells ($\times 10^6$ mL^{-1})		2.66 ± 0.67	2.85 ± 0.39	0.841 Ŧ NS
Agglutination %		3.33 ± 2.33	1.15 ± 0.83	0.351 Ŧ NS
DNA fragmentation index %		5.00 ± 1.00	17.23 ± 2.94	0.050 Ŧ S

Table 4: Comparison of seminal fluid analysis parameters & DFI% according to exposure time in oligozoospermia group. NS: Not significant (p> 0.05); S: Significant (p ≤ 0.05); Ŧ: Independent sample t-test

Semen parameters		Exposure time of non-ionizing EMW		*p* value
		< 4 hours/day	⩾ 4 hours/day	
Sperm concentration ($\times 10^6$ mL^{-1})		25.00 ± 7.77	22.34 ± 3.55	0.730 Ŧ NS
Sperm motility %	Progressive motile %	28.25 ± 2.78	27.75 ± 5.70	0.962 Ŧ NS
	Non progressively motile %	26.00 ± 6.49	21.66 ± 3.53	0.555 Ŧ NS
	Immotile sperm %	45.25 ± 8.56	50.58 ± 7.46	0.709 Ŧ NS
Morphologically normal sperm %		19.00 ± 3.11	14.92 ± 2.89	0.460 Ŧ NS
Round cells ($\times 10^6$ mL^{-1})		3.75 ± 0.95	4.00 ± 0.33	0.749 Ŧ NS
Agglutination %		5.00 ± 2.89	1.67 ± 1.12	0.207 Ŧ NS
DNA fragmentation index %		9.25 ± 2.75	17.83 ± 2.91	0.048 Ŧ S

Table 5: Comparison of seminal fluid analysis parameters & DFI% according to exposure time in teratozoospermia group. NS: Not significant (p> 0.05); S: Significant (p ≤ 0.05); Ŧ: Independent sample t-test

DISCUSSION

The demographic features of the studied group

There was no significant difference in demographic features, namely, age, weight, type of infertility, and the period of infertility between the four studied groups (normozoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia). This result was in agreement with a study published by Carkci *et al.* (2017) [15]. The current study recorded no significant

association between weight gain and semen quality among infertile men in the studied groups. By this finding, the BMI factor was discarded from being effective on the results of the four groups (27.3-29.1). A similar finding was reported by Rufus (2018) and MacDonald (2010) [16-17].

Noticeably, there was no significant difference in the duration of infertility between patients in these four groups. Nevertheless, it has been noticed that the duration of infertility (DI) had a negative effect on sperm parameters in primary infertile men. Sperm concentration was negatively related to DI, and patients with prolonged DI reported higher rates of azoospermia. Furthermore, DI was also significantly related to a higher risk of oligozoospermia [18]. These findings indicated that the men in the four groups had comparable demographic features, which were important to eliminate any variable affecting the results.

Influence of non-ionizing EMW from cell phone exposure on semen parameters

The data of the present study revealed, there was a comparison of semen parameters according to non-ionizing EMW exposure time (> 4 hours and < 4 hours) in the normozoospermia group. Although there was no significant difference between the sperm concentration (progressive, non- progressive), motile sperm, immotile sperm, normal morphology, and round cells when using the mobile for less than 4 hours and more than 4 hours, there was a significant difference in the sperm agglutination and DFI rate. It has been reported that anti- sperm antibody (ASA) induced by any defect in the blood near the testes may cause a rise in the proportion of sperm agglutination [19], and the present study found such negative effects by exposure to a cell phone for more than 4 hours/ day. At the same time, it has been noticed that DNA damage increases with cell phone exposure [20], which is comparable to the current work result. In the asthenozoospermia group, there was no significant difference in semen parameters in both groups of mobile phone usage duration (except for a significant increase in DNA fragmentation rate). This agrees with the study by Kim (2021) [8], who demonstrated that the decrease in sperm quality parameters after exposure to the RF-EMW was not significant, even with the rise in cell phone use. However, it has been postulated that exposure to cell phone radiation decreases sperm motility and viability [21].

It is also shown in the current study that a decrease in sperm concentration occurs in the oligozoospermia group that uses mobile phones for more than 4 hours than those that use mobile phones for less than 4 hours. These results agree with research done by Agarwal (2008), who observed that mobile phone usage reduces the quality of semen parameters in men by decreasing motility, sperm count, and damaging morphology [22]. Additionally, only the rate of DNA fragmentation was significantly increased in the oligozoospermic group.

Noticeably, there was no substantial variance in semen parameters of the teratozoospermia group in both durations of mobile phone usage. This finding is consistent with another study by Hassanzadeh (2021), who demonstrated that cell phone usage (short or long term) has no effect on the morphology of sperm [23]. In contrast, different researchers pointed towards abnormal sperm morphology rates in people who use cell phones for a longer duration of time [24]. However, there was a significant elevation in DNA fragmentation rate when using the mobile for more than 4 hours.

The effect of non-ionizing EMW from cell phone exposure on DNA integrity

In the current study, a significant difference in DNA damage was observed in all four seminal fluid groups (normozoospermia, asthenozoospermia, oligozoospermia, and teratozoospermia) between the subcategories of less and more than 4 hours of mobile phone use. Through the results, it was clear that DNA damage can be caused by excessive exposure to mobile phone radiation. This was in agreement with the study by Rago (2013), who suggested that excessive exposure to RF-EMF emitted from cell phones for more than four hours daily caused sperm DNA damage [25]. Agarwal (2009) concluded that DNA damage due to exposure to the RF-EMW is significant, but this damage may be the result of collective recurrent exposure and is not detected in cases of short-term exposures [26].

Comparison of semen parameters and DNA fragmentation before and after treatment

The results obtained after undergoing treatment indicate a substantial enhancement in the semen parameters and DNA integrity among people who used their mobile phones daily after undergoing treatment for a period of 1-2 months [27]. The evidence proves that EMWs, particularly from cell phones, cause DNA damage through DNA adduct formation and increased mitochondrial ROS production [26]. The treatment included two parts, the first was based on giving the appropriate treatment by the attending physician, and the second was instructions to the patient to keep the mobile phone away from the pants/ hip pocket or places close to the genital tract and reduce its daily use. Numerous other studies show the useful impacts of antioxidants on Sperm DNA Fragmentation (SDF) in infertile men [28].

In the present study, there was a significant difference in DNA fragmentation between before and after treatment in normozoospermia, asthenozoospermia, oligozoospermia, and teratozoospermia groups. There was a significant difference in normal morphology in the normozoospermia group between before 35.000 ± 6.236 and after 39.000 ± 5.6765 treatment, and there was a significant improvement in progressive sperm motility between before 11.67 ± 8.0467 and after 31.333 ± 16.1090 treatment. The treatment of the current study depends mainly on taking antioxidants for about 2 months, containing mainly L- carnitine, L- arginine, Lycopene, selenium, N- acetyl L- cysteine, Coenzyme Q10, vitamin E, and C. This finding was inconsistent with Agarwal (2020) [27]. Interestingly, the antioxidant treatment of the oligozoospermia group resulted in a significant improvement in sperm concentration [29].

L-carnitine and Coenzyme Q10 were essential and important antioxidants that were found in all of the body's tissues and abundant in sperm mitochondria, where they play an important role in generating energy. Their importance in cell respiration and energy generation supports usage as an antioxidant and pro-motility substance. The levels of CoQ10 in the seminal plasma have been demonstrated to have a linear relationship with the count and motility of the sperm [30-31].

It is recommended to use the DFI test with seminal fluid analysis when diagnosing fertility in men. Men who are planning for fatherhood, particularly when recorded fertility problems occur, it would be best to refrain from holding a cell phone in places close to their genitals. Doing so will surely decrease the negative impact of cell phone radiation on fertility potential.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest concerning the publication of this manuscript.

AUTHOR CONTRIBUTIONS

Supervised, checked, and proofread by Professor Dr. Saad Salih Al- Dujaily. Dr. Hayder M. Al-Dabaaj followed up the patients participating in this study before and after treatment. Enaam. J. Husain prepared the first draft, conducted data analysis, wrote, and revised the manuscript.

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