IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Enaam et al., Vol. 13, Issue 1, Pp. 73-85 (2023) Original Research
DOI: http://doi.org/10.28969/IJEIR.v13.i1.r6.23 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Effect of non-ionizing radiation from mobile phones in different time periods exposure on DNA integrity of infertile men.

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ABSTRACT

Exposure to radiation of various forms is one of the many environmental, health, and lifestyle variables that have been linked in recent years to an increase in the rate of male infertility. According to data from several recently published studies, mobile phone use may negatively affect sperm parameters, which may lower male fertility .Ninety-seven samples of semen from fertile and infertile individuals were sent to the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. Al-Nahrain University in Baghdad, between November 2022 and May 2023. The samples were split into four groups: normozoospermia (n = 40), asthenozoospermia (n = 25), oligozoospermia (n = 16), and teratozoospermia (n = 16). Each group was further divided into two groups based on the amount of time spent using a mobile phone each day: less than 4 hours per day and equal to or more than 4 hours per day. A routine seminal fluid analysis was carried out to measure the sperm parameters and test the deoxyribonucleic acid fragmentation rate using an acridine orange stain .Samples exposed to electromagnetic waves of cell phones showed a significant decrease in DNA normality. deoxyribonucleic acid fragmentation index ($p < 0.001$) in normozoospermia patients exposed to more than 4 hours/day of non-ionizing electromagnetic waves. The results of deoxyribonucleic acid fragmentation index after treatment were shown a significant improvement compared to before the treatment in all fertile and infertile groups. Electromagnetic waves emitted from cell phones are associated with a decrease of deoxyribonucleic acid normality when exposure for <4hours/day. The study recommend that men of reproductive age avoid keeping their cell phones in the hip pocket and front pants pocket which may negatively affect spermatozoa deoxyribonucleic acid integrity and impair male fertility.

Received: 07-Apr- 2023
Accepted: 30-Apr-2023
Published: 08-May-2023

How to cite:

Enaam J. Husain, Hayder M. Al- Dabbach , Saad S. AL-Dujaily ; Effect of non-ionizing radiation from mobile phones in different time periods exposure on DNA integrity of infertile men; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2023); 13 (1): 73-85.
Doi: http://doi.org/10.28969/IJEIR.v13.i1.r6.23

KEYWORD

Electromagnetic Waves, Male Infertility, DNA Fragmentation Index

1. Introduction

An increase in the rate of infertility during recent years has become the main focus of many studies, which may be attributed to many factors, including heat, a renewed lifestyle, and radiation emitted from devices that have become an integral part of the daily routine, especially the mobile phone (Kesari KK et al., [1]). People of all ages often use mobile phones, and they use them for a variety of reasons for extended periods. Phones generate radiofrequency-electromagnetic waves (RF-EMWs), low-level RF between 800 and 2200 MHz that may be absorbed by a person's body and may negatively impact their brain, heart, endocrine system, and reproductive system (Al-Bayyari N [2]).

Several studies have demonstrated that exposure to RF-EMWs radiated by cell phones drastically reduces sperm count, vitality, and motility (Agarwal A et al., [3]). The RF-EMWs increase the creation of reactive oxygen species in human spermatozoa, which reduces motility and vitality. They also stimulate the synthesis of DNA base adducts, which causes DNA fragmentation (Okechukwu CE [4]). DNA fragmentation may have negative consequences, including fetal problems, a rise in miscarriage rates, and a decline in fertility (Kim S et al., [5]). New research has revealed that cell phone use affects sperm, seminal tubules, and testicular stromal cells (Zhang S et al., [6]). The negative health effects linked to their usage, however, are commonly ignored (Okechukwu CE [4]).

Men who used their phones for more than four hours each day while carrying them in their pants pockets saw severe sperm DNA damage (Rago R et al., [7]). Males who have sperm DNA damage are more likely to have trouble getting pregnant.

Despite the normal sperm parameters results (Kim S et al., [5]). Therefore, the goal of the current study was to determine how exposure to electromagnetic waves from mobile phones affected sperm DNA normality in samples of Iraqi men.

2. Patients and Methods

1- Patients and Study Design:

In this study includes ninety-seven fertile and infertile males (All participants were understanding of the questionnaire's objective) attending the consultancy of the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies. Al-Nahrain University in Baghdad, during the period from 2022 to 2023. Inclusion criteria include fertile normozoospermic men, infertile idiopathic men, and unexplained infertile men. Exclusion criteria include azoospermic men, alcoholics, heavy smokers, semen infection, diabetes mellitus, Hyperprolactinemia, genetic anomalies, and men with varicocele.

2- Questionnaire:

A self-designed questionnaire was used in this study. The questionnaire consisted of many 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 history and exposure history to chemicals or radiation. Information related to mobile phone usage, such as the daily duration use of mobile phone and the location, and where the mobile phone was carried .

3-Semen collection and analyses: Semen was collected in a sterile wide-mouth container by masturbating; the specimen was put in an incubator at 37 C° and mixed gently, and analyzed after it was liquefied, the liquefaction and appearance of the semen were mostly assessed visually. Semen volume was measured by measure pipette, ejaculate viscosity was

measured by a wide-bore plastic pipette, and the semen's pH was measured by using a pH strip (WHO, 2021 [8]). Semen concentration, sperm motility (progressive, non-progressive, immotile), sperm agglutination, and round cells was performed according to the manual of WHO (WHO, 2021 [8]), and morphologically normal sperm according to the manual of (WHO, 1999 [9]). Sperm morphology was evaluated by using a test implant slide (WALDECK Company, Germany). According to SA, the patients were divided into four groups. Group one included 40 males with normozoospermia; the second group involved 25 with asthenozoospermia, 16 patients with oligozoospermia (Group 3), and another 16 males with teratozoospermia (G 4). Each group was divided according to the period of mobile exposure per day into either < 4 hours/day or > 4 hours/day. Acridine orange stain was used to detect the sperm DNA fragmentation index (DFI) According to (WHO, 2021 [8]) & (Al-Dujaily SS et al. [10]). After a two-month course of antioxidant (CoQ10 400 IU & vitamin E 400 IU) treatment period, the semen analysis and the DNA fragmentation test were performed once more.

4- Acridine orange protocol:

Acridine orange protocol was done according to (Tejada RI et al. [11]). On the slide, a smear of (10µl) semen sample was created, and it was left to air-dry for around 20 minutes. Then, the slide was fixed with Carnoy's solution for 2-24 hours. After that, it was left for a short while to dry completely before staining. For 5 minutes, 2–3 ml of the acridine orange stain was applied to the slide. After being carefully cleaned with distilled water, the slide was allowed period to dry and then was examined using a fluorescent microscope (BEL Company, Italy) using a (40X) objective lens. Two hundred spermatozoa were examined for DNA normality. Each

sample's spermatozoa's DNA was tested and given a score based on whether it fluoresced green, red, or yellow. The sperm head with green fluorescence was noted as being normal, but the sperm head with yellow-red fluorescence was reported abnormal. DNA fragmentation index (DFI) was calculated from the equation DFI= red fluorescence / (red fluorescence + green fluorescence) (WHO, 2021 [8]) & (Varghese AC et al., [12]).

5- Statistical analysis:

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010. The descriptive statistics, including frequency, range, mean, and standard error, were measured to describe the data. Depending on the nature of the data, the groups were compared by applying analysis of variance (ANOVA), independent sample t-test, paired t-test, and Chi-square. The results were considered significant when p-value was equal to or less than 0.05

3. Results

1- Classification of the studied groups

Ninety-seven infertile males out of 100 semen samples were enrolled in the present study; the patients were divided into four groups (Figure 1). The patient's result is expressed in mean ± SEM.

2- Comparison of demographic features between the studied groups

The comparison of demographic features between the four studied groups was demonstrated in (Table 1). According to the results, there were no significant differences in the mean patient's mean age (p=0.863), mean body mass indices (p=0.530), duration (p=0.871), and types of infertility (p=1.00) between the studied groups.

3- Comparison of DFI% according to exposure time of non-ionizing EMW in the four groups

There was a significantly higher Sperms DNA fragmentation index in patients exposed to more than 4 hours/day compared to those exposed to less than 4 hours/day (17.47 ± 1.78 vs. 8.71 ± 0.88; p < 0.001)of non-ionizing in the normozoospermic group. In the asthenozoospermia group, the only significant finding was higher DFI% with higher exposure time compared to less exposure one (19.45 ± 2.86 vs.13.20 ± 1.93; p=0.043), DNA fragmentation index was significantly higher in seminal fluids of oligozoospermia patients (17.23 ± 2.94 vs. 5.00 ± 1.00; p=0.050). Finally, 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) in teratozoospermia as shown in (Table 2).

4- Comparison of DFI % before and after treatment in the studied groups

In the normozoospermia group, there was a significant improvement in DNA fragmentation index after treatment compared to before treatment (11.40± 2.26 vs. 23.20 ± 2.02; p < 0.001). There was also an improvement in the DNA fragmentation index between before and after treatment (29.11 ± 3.71 vs. 15.44 ± 2.10; p=0.001) in the asthenozoospermia group. The comparison of DFI % before and after treatment in the oligozoospermia group according to the results was significant improvement after the treatment in DFI % (28.40 ± 3.64 vs. 19.20 ± 3.34; p=0.001), and there was a significant improvement in DNA fragmentation index (22.67 ± 6.12 vs. 18.33 ± 6.57; p=0.004) after therapy in teratozoospermia group.

Table *(1):* Comparison of demographic features between the studied groups

Demographic Parameters	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/m²)	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 %)	

Vales are express as Mean ± SEM ; NS: Not significant ($p > 0.05$); ∀: ANOVA test; €: Chi square

Table (2): Comparison of DFI% according to the exposure time of non-ionizing EMW in studied groups

DNA fragmentation index	Exposure time of non-ionizing		*p*-value
	< 4 hours/day	≥ 4 hours/day	
Normozoospermia group	8.71 ± 0.88	17.47 ± 1.78	< 0.001 ₸ S
Asthenozoospermia group	13.20 ± 1.93	19.45 ± 2.86	0.043 ₸ S
Oligozoospermia group	5.00 ± 1.00	17.23 ± 2.94	0.050 ₸ S
Teratozoospermia group	9.25 ± 2.75	17.83 ± 2.91	0.048 ₸ S

S: Significant ($p \leq 0.05$); ₸: Independent sample- t-test

***(Table 3)*:** Comparison of DFI% before and after treatment in studied groups

DNA fragmentation index	Before treatment	After treatment	*p*-value
Normozoospermia group	23.20 ± 2.02	11.40 ± 2.26	< 0.001 ¥ S
Asthenozoospermia group	29.11 ± 3.71	15.44 ± 2.10	0.001 ¥ S
Oligozoospermia group	28.40 ± 3.64	19.20 ± 3.34	0.001 ¥ S
Teratozoospermia group	22.67 ± 6.12	18.33 ± 6.57	0.004 ¥ S

S: Significant ($p \leq 0.05$); ¥: Paired sample- t test

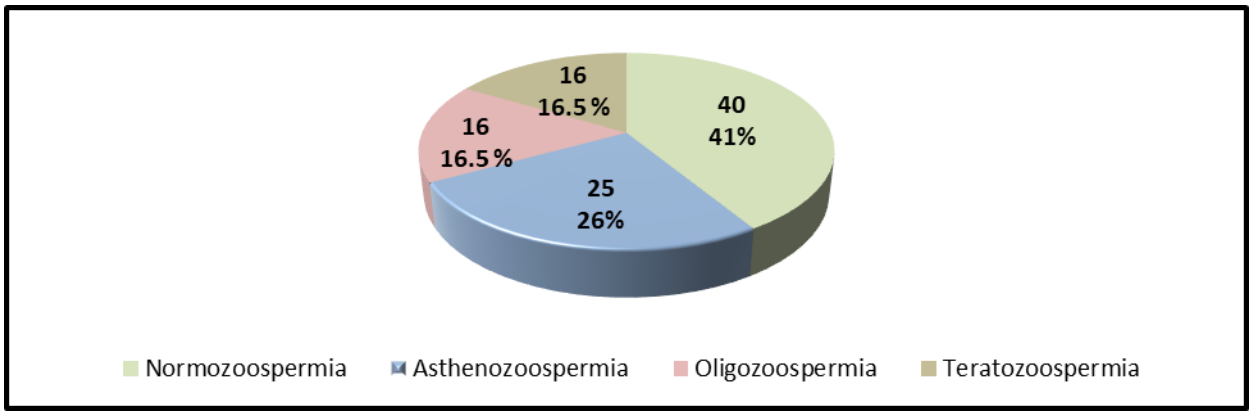

Figure (1): Classification of the studied groups

4. Discussion

1- The demographic features of the studied group

The current study revealed no significant difference in demographic features, namely, age, weight, type of infertility, and the duration of infertility between the four studied groups (normozoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia) as illustrated in (Table 1). There was no significant

difference in the age factor between the four studied groups (the mean from 32-35 years) as the present study determined the range of men's ages enrolled in the research. This result was in agreement with a study published by (Carkci et al., 2017 [13]). It has been mentioned that although there may be a gradual decline in male reproductive potential with age, the average paternal age has been increasing in recent years for various socioeconomic reasons. Furthermore, many males ask to father children at older ages, and a detailed understanding of changes in the male reproductive system associated with aging is of critical importance (Avellino G et al., [14] & Sharma R et al., [15]). The current study found 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 O et al., 2018 [16] & MacDonald A et al., 2010 [17]). Even though the overweight seemed documented in all four groups, there was no impact of the weight on semen parameters, which found no association between the BMI and semen parameters. In contrast, weight was demonstrated to have a deleterious impact on reproductive function, particularly in obese males, involving problems with subclinical prostatitis, poor quality of sperm, and erectile function. Importantly, obesity has a deleterious impact on sperm characteristics such as concentration, motility, morphology, and viability. Furthermore, obesity impacts chromatin condensation as well as the rising fragmentation of DNA, increasing apoptosis, and epigenetic alterations that can be passed down to children (Leisegang K et al., [18]).

Noticeably, there was no significant difference in the duration of infertility between patients in these four groups. Nevertheless, it has been noticed that

duration of infertility (DI) had a negative effect on sperm parameters in primary infertile men. Sperm concentration was negatively related with DI, and individuals with prolonged DI reported higher rates of azoospermia. Furthermore, DI was linked to an increased chance of oligozoospermia (Boeri L et al., [19]). These findings indicated that the men in the four groups had comparable demographic features, which were important to eliminate any variable that may affect the results of the study (Table 1) .

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

In the current study, the results were shown a significant difference in DNA damage between less and more than 4hrs/day usage of mobile phones in all four seminal fluids groups (normozoospermia, asthenozoospermia, oligozoospermia, and teratozoospermia) as shown in the (Table 2). Through the results, it was clear that excessive exposure to radiation from mobile phones can cause DNA damage. This was in agreement with the study by (Rago R et al., 2013[7]), who was suggested that excessive exposure to RF-EMF emitted from cell phones for more than four hours daily caused sperm DNA damage. (Agarwal A et al., 2009 [3]) concluded that DNA damage due to exposure to the RF-EMW is significant, but this damage may be the result of cumulative repeated exposure and not detected after short-term exposures.

3- Comparison of semen parameters and DNA fragmentation before and after treatment

The results obtained after undergoing treatment indicate a significant improvement in DNA integrity in the people who used their mobile phones daily after undergoing treatment for a period of 2 months (Agarwal A. et al., [21]). The evidence proves that electromagnetic waves,

particularly from cell phones, increase mitochondrial ROS production and DNA adduct formation, causing DNA damage (Agarwal A et al., 2009 [3]).

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 clinical research has shown that antioxidants have a favorable effect on sperm DNA fragmentation in infertile males (Micic S et al., [21]). In the present study, there was a significant difference in DNA fragmentation between before and after treatment in normozoospermia, asthenozoospermia, oligozoospermia, and teratozoospermia groups, as shown in (Table 3). This observation is in agreement with (Kim GY, 2018 [22]) report when he pointed out that if a male has a high level of DNA damage, he should aim to reduce it through lifestyle change and antioxidant treatment (Micic S et al., [21]).

6- Conclusion

It has been determined that using a cell phone for longer than four hours per day caused several abnormalities in male fertility, particularly in the normalcy of sperm DNA. Therefore, when diagnosing male fertility who use a mobile device, the DFI test should be combined with seminal fluid analysis. Increase the social culture media's attention to the detrimental effects of cell phone radiation on the ability to conceive.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Enaam J. Husain performed the study, and Hayder M. Al-Dabbach Saad S. AL-Dujaily supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure .

Ethical Clearance

The study was approved by the Ethical Approval Committee

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