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Original Article

Association between daily exposure to electromagnetic radiation from 4G smartphone and 2.45-GHz wi-fi and oxidative damage to semen of males attending a genetics clinic: a primary study

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Abstract: We evaluated the semen quality and oxidative stress attack on spermatozoa nuclear DNA after men were exposed to a combination of electromagnetic radiation from an 1800-MHz 4G smartphone network and 2.45-GHz wi-fi. According to the daily network exposure time, the subjects involved were divided into three groups, namely group one: less than 30 min; group two: 31-120 min; and group three: more than 121 min. Routine semen tests, reactive oxygen species levels, total antioxidant capacity, glutathione peroxidase (GSH-Px) and superoxide dismutase, 8-hydroxy-2'-deoxyguanosine expression, and urine and comet assays were conducted for all participants and the results for the three groups were compared. With extended exposure time, reactive oxygen increased, while total antioxidant capacity, glutathione and superoxide dismutase, and superoxide dismutase decreased. Increased expression of 8-OHdG and sperm DNA fragments (head DNA% decreased while tail DNA% and Olive tail moment increased) was observed. Sperm count, vitality, and motility decreased significantly. Electromagnetic radiation may induce oxidative stress, damage sperm nuclear DNA, and eventually reduce sperm quality; these factors negatively affect male fertility.

Keywords: Oxidative stress attack, combinative electromagnetic radiation, low quality of semen, sperm nuclear DNA

Introduction

Approximately 14% of married couples suffer from difficulties in conception in developed countries [1]. In the past 50 years, studies have shown that human semen quality has decreased worldwide [2]. Because male factors affect half of the infertility cases [3], many studies have focused on the causes of decreased semen quality. Along with chemical exposure [4], irradiation in the environment has recently caused concerns.

An increasing number of studies has shown that electromagnetic radiation plays a deleterious role in semen quality. In human sperm, the electromagnetic radiations emitted from cell phones have adverse effects on semen quality, including sperm count [5], motility [6], and morphology [7]; wireless fidelity exposure [8] and low-frequent electromagnetic field [9] also have negative effects. In experimental animals, the harmful impacts of electromagnetic radiation on the male reproductive system include histological changes in the seminiferous tubule in rats [10], damage to the mitochondrial and nuclear genomes of epididymal spermatozoa in mice [11], interference with spermatogenesis in rats [12], poor sperm motility in rats [13], and irregular sperm morphology [14]. Ultimately, decreased semen quality leads to male infertility [15].

Studies on the non-thermal biological effects of electromagnetic radiation have clearly revealed that oxidative stress undermines semen quality [16]. The production of reactive oxygen species (ROS) and antioxidants caused by all types of

Table 1. Characteristics of Exposure Index factors of subjects involved

Exposure Index factors	Characteristics
Geographic area	Shaanxi province
Population categories	Adults (Han people)
User load profiles	Game, website surfing, chatting
The environment of usage	Indoor (office and home)
Radio access technologies	4G-LTE [1]plus 2.45 GHz Wi-Fi;
Posture of usage	Sitting, standing
Devices of usage	Mobile, PC, Laptop
Data transmission styles	Voice call and data transmission
Possible using scenarios	Access points in office and home
Mean power density in city areas (Equitment: TS/001/UB Taoma base unit[2])	2400 uW/m^2

1. Long term evolution; 2. Product Co: Tecnoservizi, Rome, Italy.

electromagnetic radiation exposure has been observed in animal-based male production studies [17]. An imbalance between oxidation and antioxidant capacity after electromagnetic radiation exposure induces oxidative stress, triggering the disruption of intercellular structures and molecules [18]. Targets of oxidative attack include lipids [19], proteins [20], and nucleic acids [21, 22]. These factors affecting sperm genotoxicity involve the oxidative DNA damage marker 8-hydroxy-2'-deoxyguannosine (8-OHdG) [23] and DNA fragmentation [24]. Thus, we can use various parameters to evaluate the effects of electromagnetic radiation on human sperm quality.

In the present study, we evaluated the effects of a combination of electromagnetic radiation from a 1800-MHz smart phone network service and 2.45-GHz wireless fidelity (wi-fi) rather than signal exposure from cell phone conversations or other types of electromagnetic exposure alone. In addition to clinical data, we assessed ROS levels, total antioxidant capacity, glutathione, and superoxide dismutase to determine the extent of oxidative stress in semen. 8-OHdG and fragments of sperm DNA produced following oxidative injury indicate electromagnetic radiation damage to semen. We evaluated the causes of decreased semen quality as well as oxidative stress in semen following exposure of men to an electromagnetic field from smart phones and wi-fi.

Materials and methods

Individual exposure assessment

EI (exposure index) was evaluated in this study. Factors associated with EI were considered, such as time period per day, geographic sites, population categories, user load profiles, environment of usage, radio access technologies, posture of usage, devices used, data transmission styles, and usage scenarios. Cumulative doses of radiofrequency electromagnetic exposure include downlink exposure from a base station and access points, while uplink exposure results from all individual wireless communication devices and excludes irradiation from frequency modulation radio and digital terrestrial television transmitters. The habits of the subjects are shown in **Table 1**. Subjects in the three groups showed no differences, except for time period per day, which was used as a criterion for grouping. After comparing the conditions of the districts in different cities reported previously [25, 26] and the actual exposure situation in our region, the approximate EI to the whole body was estimated as $3.19\pm0.07 \times 10^{-7}$ W/kg (mean EI = 3.19 W/kg).

Subjects

The study strictly adhered to the rules and regulations of the Ethics Administration of Xi'an Jiaotong University, and all subjects consented to participation in the study. The project was approved by the Chinese Ethics Committee of Registering Clinical Trials (ChiCTR-OCH-14004802). Males (270) attending the genetics clinic at the First Affiliated Hospital and Shaanxi Maternal and Child Care Service Center from June 2014 to October 2014 were enrolled in this study. Subjects were of an active reproductive age. Subjects that were affirmed to have chronic disease such as diabetes mellitus, hypertension, cardiac neural and nephritic disease; genetic disease; reproductive endocrinological disease; urologic infection history; urologic tumor and operation history; orchitis; varicocele; consumption of tobacco and alcohol; medicine-taking; or occupational exposure to poison and irradiation were excluded from the study. Self-reporting questionnaires addressing several aspects such as age, body mass index, abstinence time, income, and education were administered to all subjects

and collected 30 min after two trained colleagues explained the details of the study. The average daily exposure time of an individual was summed using software (Wifi History View, NirSoft) and records from mobile network companies. Subjects were divided into three groups according to their daily network exposure time: group one, less than 30 min per day (n = 89); group two, 31-120 min per day (n = 104); group three, more than 121 min per day (n = 77).

Routine semen test

Semen samples from subjects in each group were collected by masturbation in a sterile container after an abstinence duration of 3-7 days, and seven sperm parameters, including volume, pH, sperm count, liquefaction time, viability, progressive motile (PR) sperm, and immotile (IM) sperm were analyzed using a QingHuaTongFang computer-assisted sperm analysis system (CASAS-QH-III GK-9900) following WHO 5th Edition guidelines. Trained technicians in the laboratory were blinded to subjects' information.

Semen ROS, TAC, GSH and SOD measurements

First, 200 μL of each unprocessed liquated semen sample was added to 5 μL of 5 Mm luminol (5-amino-2, 3 dihydro-1, 4 phthalazinedione, dissolved in dimethyl sulfoxide, both from Sigma, St. Louis, MO, USA), and the ROS level in each sample was measured with an FLx800™ analyzer (BioTek, Winooski, VT, USA) within 15 min. The results were expressed as RLU/s/× 10^6. After centrifugation (300 × g, 5 min), the seminal plasma was collected and frozen at -80°C. Under low ambient light conditions, three wells for each sample were prepared: (1) Three total activity wells contained 240 μL StabilZyme Stabilizer (Neobioscience, Shenzhen, China), 30 μL Signal Reagent (0.1 M Tris-HCL, 12 M fresh hydrogen peroxide, 41.8 mM 4-iodophenol, and 282.2 mM luminol, Neobioscience), and 30 μL horseradish peroxidase (HRP) (Bioss Antibodies, Woburn, MA, USA). (2) Three nonspecific background wells contained 220 μL StabilZyme Stabilizer, 30 μL Signal Reagent, and 50 μL sample. (3) Three Trolox wells contained 190 μL StabilZyme Stabilizer, 30 μL Signal Reagent, 30 μL HRP, and 50 μL of each concentration of Trolox standard (12.5-100 μM). (4) Three sample wells contained 190 μL StabilZyme Stabilizer, 30 μL Signal Reagent, 30 μL HRP, and 50 μL sample. The wells were analyzed with an FLx800™ analyzer. A standard curve for Trolox was generated from the output and antilog of the sample results to determine Trolox equivalent values (in μM). GSH-Px and SOD activities in each sample were measured with a GSH-Px kit and SOD kit (Jiancheng, Nanjing, China).

Immunofluorescence staining of 8-OHdG in spermatozoa

For each sample in each group, 2 μL liquefied semen was placed on three silicified glass slides slightly followed by fixation with 200 μL 4% (w/v) paraformaldehyde (Guoan Biotech, China) for 30 min after air-drying the samples. Slides were washed with 0.01 M PBS three times, blocked with goat serum (Bioss) containing 0.005% Triton X-100 (Guoan Biotech) for 60 min, and incubated with 1:200 rabbit-anti-human multiclonal antibody against 8-OHdG (Proteintech, Rosemont, IL, USA) at 4°C overnight. PBS was used for control samples in place of antibodies. Cy5-labeled goat anti-rabbit IgG (Bioss) at a 1:500 dilution was incubated with the slides for 40 min at 37°C overnight. The slides were visualized under a fluorescence microscope (BX41, Olympus, Tokyo, Japan) to detect green fluorescence (515-560 excitation filters). Fluorescence images were transferred into grey images to determine fluorescence intensities.

Sperm DNA fragments by comet assay and CASP analysis

Frosted glass slides were pre-coated with 200 μL 0.5% (w/v) normal-melting-point agarose (Biowest, Nuaillé, France) in PBS and covered with coverslips for 15 min to allow for solidification. The concentrations of all semen samples were adjusted to 6 × 10^6/mL with PBS, and the coverslips were removed. Next, 10-μL adjusted samples were placed into microcentrifuge tubes containing 75 μL 0.5% (w/v) low-melting-point agarose (Biowest) and then pipetted into a normal-melting-point agarose gel. The coverslips were replaced and removed 15 min later, and then the slides were immersed in fresh 4°C lysis solution containing 2.5 M sodium chloride, 100 mM sodium EDTA, 10 mM Tris-HCL, and 1% Triton X-100 (pH 10) for 60 min at 4°C. The slides were removed and incubated in a solution containing 1.25 mL DTT for 30 min at 4°C. The slides were removed and drained, followed

Table 2. The one way ANOVA analyses of general background data and semen routine parameters among three groups

Groups involved	Group One	Group Two	Group Three
Age (years)	28±5	27±6	28±5
BMI (kg/m^2)	22.85±2.68	22.51±2.59	22.81±2.22
Abstinence time (days)	5±1	5±1	5±1
Educational level (years)	17±5	16±4	18±5
Income (yuan/mon)	3530±380	3375±444	3634±411
Semen volume (ml)	3.3±1.3	3.3±1.1	3.5±1.6
Semen pH	7.46±0.12	7.46±0.13	7.49±0.12
Liquefaction time (min)	22.79±10.00	23.77±7.78	24.53±6.81
Sperm count (/10^6)	74.20±3.70*	56.90±7.77**	24.10±1.47***
Sperm viability (%)	65.37±14.20*	55.49±13.39**	39.10±17.78***
Progressive sperm (%)	63.12±14.62*	56.87±40.63**	37.25±17.47***
Immobility sperm (%)	34.31±14.20*	44.37±13.34**	60.97±17.88***

One way ANOVA *represents $p < 0.05$ between group one and two, **represents $p < 0.05$ between group two and three and ***represents $p < 0.05$ between group one and three.

by electrophoresis in a horizontal electrophoresis tank with fresh alkaline electrophoresis solution consisting of 0.3 M sodium hydroxide and 1 mM EDTA at a current of 300 mA, after which the tank was flooded with neutralization buffer (0.4 M Tris) for 5 min. The drained slides were stained with 50 μL of 200 μg/mL EtBr solution for 2 h and covered with coverslips. Images on the slides were captured using a 40 × Olympus fluorescence microscope equipped with a 515-560 excitation filter and 50 comets per slide were analyzed with CASP software.

Statistical analyses

After checking the normality of each variable by the Kolmogrov-Smirnov test, normally distributed continuous variables (age, body mass index (BMI), abstinent time, income, educational years, volume of semen, semen pH, and liquefaction time) were compared by one-way analysis of variance, whereas other variables were analyzed by the Kruskal-Wallis test. Moreover, through the log-normal transformed method to improve the normality of the data involved, multiple linear regression models were built to estimate the association between electromagnetic irradiation exposure time and semen parameters showing significant differences between each group, as well as oxidative conditions and oxidative damage to sperm DNA. Confounding factors entered in the regression model included age (years), body mass index, abstinent time (days), income (yuan/month), and education (years). The equation for linear regression before adjusting for confounding factors was as follows: dependent variable = crude β × exposure time level + constant. After adjusting for confounding factors, the equation was as follows: dependent variable = adjusted β × exposure time level + a × age + b × BMI + c × educational level + d × income + e × abstinence time + constant. A p value < 0.05 was considered to indicate the significant influence of the independent variable (exposure time level).

All statistical analyses were conducted using SPSS statistical program (version 22.0 software, SPSS, Inc. Chicago, IL, USA) and p values less than 0.05 were regarded as statistically significant.

Results

Decreased semen quality with same demographic background because of extended exposure time

The demographic data, abstinence time, and results of the routine semen assay in three groups are presented in **Table 2**. No significant differences were observed in ages, body mass index, education level, income situation, and abstinence time in each group. With increasing exposure time in the three groups, sperm count, sperm vitality, and percentage of progressive sperm deceased dramatically, while the percentage of immobile sperm gradually increased. We investigated the changes in the routine semen test results in the three groups. There were no significant changes in semen volume, semen pH, and semen liquefaction time between each group; however, the Kruskal-Wallis test revealed significant differences in sperm count, sperm, percentage of progressive motile sperm, and percentage of immobile sperm in each group (**Table 2**, *represents $p < 0.05$ between groups one and two, **represents $p < 0.05$ between groups two and three, and ***represents $p < 0.05$ between groups one and three).

Table 3. Oxidative stress condition among three groups along with extended exposure time

Groups involved	Group One	Group Two	Group Three
ROS (RLU/10^6/ml)	5.55±2.06*	7.31±1.80**	9.17±1.98***
TAC (μMtrolox equivalent)	72.55±11.15*	56.14±13.78**	23.61±10.78***
GSH-Px (IU/ml)	50.80±8.41*	39.93±10.49**	23.96±6.30***
SOD (IU/ml)	13.20±3.52*	9.88±2.78**	5.76±1.23***

One way ANOVA *, ** and ***represent as same as **Table 2**.

Longer electromagnetic exposure induced increased oxidative stress in semen

The oxidative stress situation in semen is shown in **Table 3**. Along with exposure to electromagnetic radiation in the three groups, the production of reactive oxygen species increased gradually. However, the seminal plasma total antioxidant capacity declined apparently; so did the amounts of enzymatic antioxidants such as GSH-Px and SOD. Using the Kruskal-Wallis test analysis, significant differences were determined in each of the three groups (**Table 3**: *, ** and ***represent $p < 0.05$).

Higher expression of 8-OHdG with longer electromagnetic exposure time

Expression of the oxidative damage biomarker 8-OHdG is shown in **Figure 1**, and the levels of 8-OHdG in the three groups were identical. In spermatozoa, DNA is distributed in the head, neck, and small parts of the tail, and 8-OHdG is produced following DNA damage. The antibody for 8-OHdG, which produced a green fluorescent signal, identified the location of DNA or 8-OHdG. By measuring fluorescence signals, we evaluated the extent of sperm DNA damage by evaluating 8-OHdG expression.

The green signals in three groups and negative control samples were subjected to relative average gray intensity analysis to determine the level of DNA damage. 8-OHdG expression, corresponding to the three images (**Figure 1A**, **1C** and **1E** and under light field), is shown in **Figure 1B**, **1D** and **1F**. The relative average gray intensity increased modestly with a longer exposure time to electromagnetic radiation in the three groups. **Figure 1G** and **1H** show control images under light field and green fluorescence, respectively. **Figure 1I** shows all data from the relative average gray intensity as box images to illustrate the differences between each group. Importantly, significant differences in the average gray intensity in each group were revealed by the Kruskal-Wallis test (**Figure 1**, *, ** and ***represent $p < 0.05$, as in **Table 2**).

Less head DNA, more tail DNA, and Olive tail moment resulted from longer electromagnetic exposure time

The results of the comet assay to detect nuclear DNA damage in the sperm head (**Figure 2A-C**) were quantified using CASP software with three indices: head DNA, tail DNA, and Olive tail moment. The percentage of sperm head DNA clearly decreased, while the percentage of sperm tail DNA and sperm Olive tail moment increased following increased exposure to electromagnetic radiation. Additionally, the differences in the head DNA, tail DNA, and Olive tail moment in each of the three groups were significant according to the Kruskal-Wallis test (**Figure 2D-F**; **Figure 2**, *, ** and ***represent $p < 0.05$, as in **Table 2**).

Negative association between electromagnetic irradiation exposure and semen quality in the three groups

As shown in **Table 4**, semen parameters following electromagnetic exposure, such as low sperm count, decreased sperm viability, and motile sperm percentage, and immobile sperm percentage, were negatively correlated with the exposure time. After adjusting for confounding factors by multiple linear regression, the adjusted β coefficients of the exposure time level to sperm count (β = -0.499), sperm viability (β = -0.218), motile sperm proportion (β = -0.226), and immobile sperm proportion (β = 0.284), were significant ($p < 0.001$).

Positive association between electromagnetic irradiation exposure and oxidative stress in semen of the three groups

The balance between oxidation and antioxidants in semen was disrupted by electromagnetic exposure. ROS (β = 0.247, $p < 0.001$) levels were positively associated with exposure time in the multiple linear regression model, while TAC (β = -0.442), the amount of GSH-Px (β

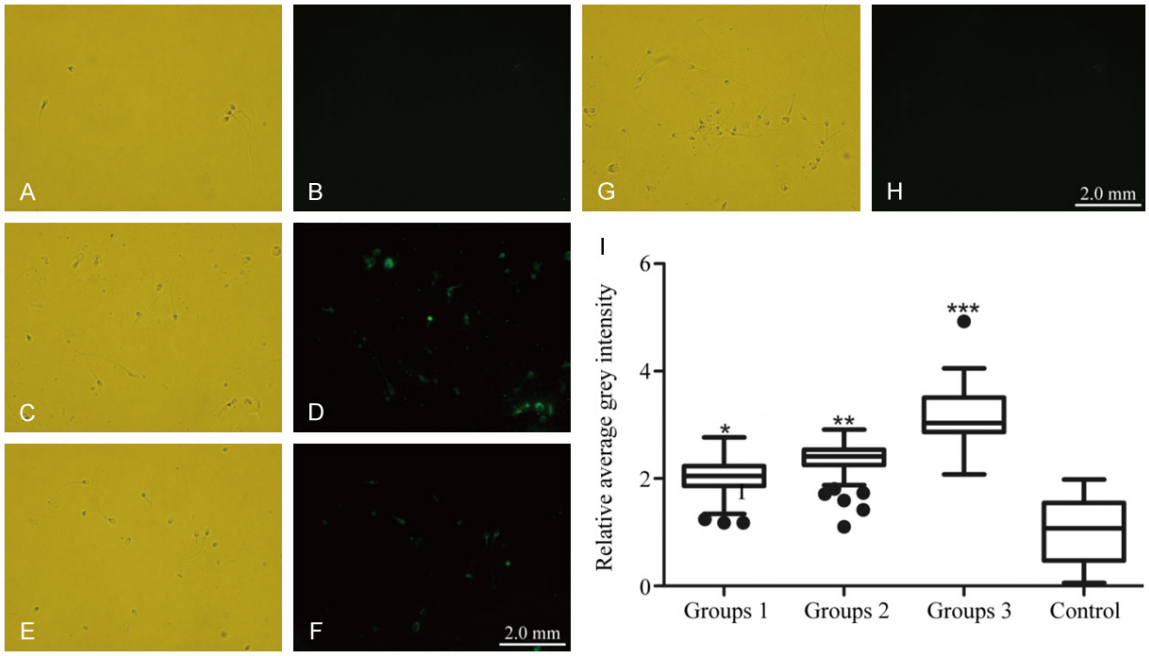

Figure 1. Expression of 8-OHdG in three groups. (A) Sperm smear from Group One under light field (× 400). (B) 8-OHdG staining of (A) identical smear under green color fluorescence (515-560 excitation filters) (× 400). (C) and (D) Are from Group Two. (E) and (F) Are from Group Three. (G) and (H) Are from Control. (I) Relative average fluorescence-transferred grey intensities in three groups and Group Control. *it represents significant differences between Group One and Group Two using Kruskal-Wallis test. **it represents significant differences between Group Two and Group Three using Kruskal-Wallis test. ***it represents significant differences between Group One and Group Three using Kruskal-Wallis test.

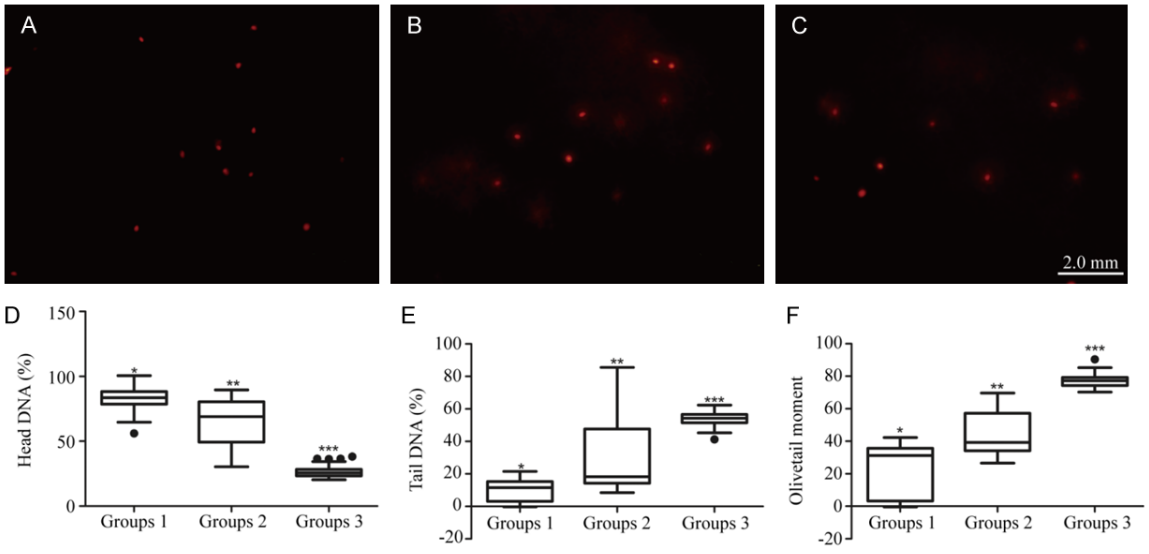

Figure 2. Comet assay Images and CASP analysis of DNA fragments. A. Sperm DNA from Group One under green color fluorescence (515-560 excitation filters) (× 200). B. Group Two. C. Group Three. D. CASP results of sperm head DNAs in three groups. E. CASP results of sperm Tail DNAs in three groups. F. CASP results of sperm Oliver tail moments in three groups. The symbols*, ** and ***are as same as what are showed in **Figure 1**.

= -0.281) and the amount of SOD (β = -0.323) exhibited the opposite relationships in the same situation in **Table 5**. All adjusted β coefficients were significant.

Table 4. Negative association of electromagnetic irradiation exposure timelevel with semen routine assay in three groups (Kruskal-Wailis analyses of variances were compared the median between groups $P < 0.05$) before and after adjusting for confounding factors

Exposure time level	Sperm count (/10^6)	Sperm viability (%)	Progressive sperm (%)	Immobile sperm (%)
Crude β coefficients	-0.503	-0.224	-0.230	0.285
95% CI	(-0.574, -0.432)	(-0.266, -0181)	(-0.277, -0.183)	(0.231, 0.340)
P-value	0.001	0.001	0.001	0.001
Adjusted β coefficients	-0.499	-0.218	-0.226	0.284
95% CI	(-0.570, -0.427)	(-0.260, -0.176)	(-0.272, -0.179)	(0.229, 0.339)
P-value	0.001	0.001	0.001	0.001

Regression coefficients were adjusted for age, body mass index, education level, income situation and abstinence duration. Before adjusting for confounding factors, Dependent Variable (sperm count, sperm viability, progressive sperm and immobile sperm) = Crude β × Exposure time level + Constant. After adjusting for confounding factors, the equation was changed into Dependent variable(sperm count, sperm viability, progressive sperm and immobile sperm = Adjusted β × Exposure time level + a × Age + b × BMI + c × Educational level + d × Income + e × Abstinence time + Constant.

Table 5. Positive association of electromagnetic irradiation exposure time level with oxidative condition in three groups before and after adjusting for confounding factors

Exposure time level	Reactive oxygen species (/10^6/ml)	Total antioxidant capacity	GSH-Px (IU/ml)	SOD(IU/ml)
Crude β coefficients	0.249	-0.444	-0.284	-0.323
95% CI	(0.215, 0.283)	(-0.483, -0.405)	(-0.315, -0.253)	(-0.355, -0.292)
P-value	0.001	0.001	0.001	0.001
Adjusted β coefficients	0.247	-0.442	-0.281	-0.323
95% CI	(0.213, 0.281)	(-0.481, -0.404)	(-0.311, -0.250)	(-0.355, -0.291)
P-value	0.001	0.001	0.001	0.001

Regression coefficients were adjusted for age, body mass index, education level, income situation and abstinence duration. Before adjusting for confounding factors, Dependent Variable (ROS, TAC, GSH and SOD) = Crude β × Exposure time level + Constant. After adjusting for confounding factors, the equation was changed into Dependent variable (sperm count, sperm viability, progressive sperm and immobile sperm = Adjusted β × Exposure time level + a × Age + b × BMI + c × Educational level + d × Income + e × Abstinence time + Constant.

Table 6. Positive association of electromagnetic irradiation exposure time level with oxidative damage to sperm DNA in three groups before and after adjusting for confounding factors

Exposure time level	8-OHdG (gray intensity)	Head DNA (%)	Tail DNA (%)	Olive tail moment
Crude β coefficients	0.154	-0.340	0.779	0.794
95% CI	(0.137, 0.170)	(-0.373, -0.307)	(0.729, 0.829)	(0.738, 0.851)
P-value	0.001	0.001	0.001	0.001
Adjusted β coefficients	0.152	-0.336	0.780	0.797
95% CI	(0.136, 0.169)	(-0.369, -0.304)	(0.730, 0.829)	(0.741, 0.854)
P-value	0.001	0.001	0.001	0.001

Regression coefficients were adjusted for age, body mass index, education level, income situation and abstinence duration. Before adjusting for confounding factors, Dependent Variable (8-OHdG, head DNA, tail DNA and olive tail moment) = Crude β × Exposure time level + Constant. After adjusting for confounding factors, the equation was changed into Dependent variable (sperm count, sperm viability, progressive sperm and immobile sperm = Adjusted β × Exposure time level + a × Age + b × BMI + c × Educational level + d × Income + e × Abstinence time + Constant.

Positive association between electromagnetic irradiation exposure and damaged sperm DNAs of three groups

The damage to sperm nuclear DNAs due to electromagnetic exposure involved increased expression of 8-OHdG, higher tail DNA percentage and Olive tail moment, and lower head DNA percentage in the comet assay. A positive association between exposure time and 8-OHdG (β = 0.152, $p < 0.001$), accompanied by a negative relationship with head-DNA (%) (β = -0.336,

$p < 0.001$), showed significantly deleterious influences following exposure of the sperm DNA. Extended exposure time was positively related to tail-DNA (%) ($\beta = 0.780$, $p < 0.001$) and Olive tail moment ($\beta = 0.797$, $p < 0.001$), which play an aggressive role in damaging sperm DNA (**Table 6**).

Discussion

We found that with increased exposure time in the three groups, the level of ROS in the semen increased significantly, whereas the level of antioxidants in the semen decreased, including that of enzymes such as GSH-Px and SOD and non-enzymes such as TAC. Because of the increased oxidative stress, the antioxidants cannot meet the demand for consumption of ROS. Additionally, oxidative damage markers of sperm DNA were observed as 8-OHdG and DNA fragments. With longer exposure time, 8-OHdG expression increased in the three groups, with sperm DNA fragments detected in the comet assay. In our study, the sperm count, vitality, and motile sperm decreased with increasing exposure dose in the three groups. The results of multiple linear regression supported the oxidant indices and further damage to sperm DNA, which decreased bad sperm quality and were strongly related to electromagnetic irradiation exposure. These results indicate that electromagnetic radiation from 1800-MHz smart phone network service and 2.45-GHz wi-fi signaling has harmful effects on semen quality such as sperm DNA damage, caused by an imbalance between ROS generation and antioxidants in the semen. The electromagnetic field may disable spermatozoa in the pool of over-oxidative substances. The response to stress induced by the 1800-MHz smart phone network service and 2.45-GHz wi-fi signaling disturbs oxidative metabolism in the semen and alters the spermatozoa.

A study by De Iuliis et al. [27] showed that ROS levels increase following short-term exposure to electromagnetic radiation because of enhanced NADH activity. Increased ROS production due to electromagnetic radiation decreases human semen quality through NADH [28]. Several *in vivo* animal studies [29] showed that cellphone radiation causes oxidative stress by increasing the production of ROS or by decreasing antioxidant activity (catalase, GSH-Px and SOD). A recent study [30] focusing on damage caused to DNA by electromagnetic radiation reported single-strand DNA breaks in developing brain cells in rats exposed to 2.45-GHz and 16.5-GHz fields [31] and double-strand DNA breaks in mouse embryonic stem cells under 1.7-GHz fields [32]. Furthermore, Lai and Singh demonstrated that free radical scavengers block electromagnetic radiation from damaging DNA [33].

Some previous studies have shown conflicting results regarding whether electromagnetic radiation impairs semen parameters. With respect to sperm count, Dasdag et al. [10] and Wdowiak et al. [7] found that exposure to electromagnetic radiation from cell phones did not lead to decreased sperm concentrations. Yan et al. [34] found no significant difference between the electromagnetic radiation exposure group and control group mice.

There were a few limitations to our study. First, the information for exposure time was not validated and was self-reported by the subjects. Moreover, the standby possession of mobile phones producing electromagnetic waves was not considered. Finally, we may have ignored other elements with beneficial or negative effects on sperm quality.

Following electromagnetic radiation exposure, oxidative stress in the semen increased and semen quality decreased in an exposure time-dependent manner. In future studies, we will examine animal models to determine the time sequence of effects from electromagnetic radiation from an 1800-MHz mobile cyber network and 2.45-GHz wi-fi on semen quality.

Disclosure of conflict of interest

None.

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