MEDICINE AND HEALTHCARE REPORTS

Effects of Wi-Fi (2.45 GH z) Exposure on Sperm Parameters in NMRI Mice

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Article Information

Article Type: Review Article
Journal Type: Open Access
Volume: 2 Issue: 3
Manuscript ID: MHR-2-116
Publisher: Science World Publishing

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Citation:

Rahimi M (2021). Effects of Wi-Fi (2.45 GH z) Exposure on Sperm Parameters in NMRI Mice. Med Healthcare Rep. 2(3); 1-5

Recieved Date: 27 February 2021
Accepted Date: 12 March 2021
Published Date: 18 March 2021

ABSTRACT

In today's universe, one of the waves that humans possess to deal with today, 2.45-GHz radio-frequency radiation of wireless devices connected to the Internet. This survey proposed to investigate the Sperm motility and sex hormones adult male NMRI Mice during long-term exposure of Wi-Fi radiation. This is an animal experimental work, which was conducted in Malayer University, Malayer, IRAN, from March to May 2019. Two-month-old male NMRI Strain mice (30±2 gr) (n=27) were exposed to the 2.45 GHz radiation in a bedroom with two Wi-Fi antennas on walls. Animals were divided into the three following groups: I. Control group (n=9) including healthy animals without any exposure to the antenna, II. 4-hour group (n=9) exposed to the 2.45 GHz Wi-Fi radiation for 4 hour per day during two months and III.8-hour group (n=9) exposed to the 2.45 GHz Wi-Fi radiation for 8 hours per day during 3 months. Sperm motility and concentration of sex hormones with Eliza were evaluated in the exposed and control animals. Sperm motility and average hormone concentration Testosterone levels and LH decreased significantly in 8-hour groups in comparison to the mastery group. But this parameter was not significantly decreased in group (4 hour/day/2 months) compared to control group.

KEYWORDS: Electromagnetic Radiation; Testis; Spermatogenesis; Testosterone

INTRODUCTION

Due to the introduction of electronic devices into the daily lives of humans and the wide range of applications in telecommunications, data transmission, industry and wireless systems, being safe from their side effects inevitable in today's world. Wireless Fidelity (Wi-Fi) has come out as the favorite route of internet Wireless communication and connectivity [1]. Wi-Fi signal operates in an unlicensed spectrum range of 2.45 to 5GHz [2] which minimizes its operation cost. For this reason, Wi-Fi becomes a daily necessity and is widely used in various devices. It has been proposed that male infertility during the past several decades is related to the direct or indirect exposure to certain environmental elements such as Radio-Frequency Electromagnetic Waves (RF-EMW) [3]. Kumar et al. Showed pulsed electromagnetic field (100 Hz) exposure causes significant increases in caspase and creatine kinase and significant decreases in testosterone

and melatonin in male Wistar rats (70 days old) [4]. In vitro work on human spermatozoa by Aventine et al showed that Wi-Fi radiation decreased motility and induced DNA fragmentation of human sperm by a non-thermal effect [5]. In-vitro pilot study of Oni et al demonstrated a negative outcome of Radio Frequency Radiation (RFR) from 2.4 GHz laptop antenna on human semen [6]. In experiment Gannes F was not observed for 1h per day's exposure to the 2450 MHz Wi-Fi signal, deleterious effects on rat male and female reproductive organs and fertility [7]. Hi et al observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group, indicating DNA damage due to exposure also decreased levels of catalase and glutathione peroxidase activity which may have been due to radiofrequency effects on enzyme activity [8]. Also Shokri et al showed that both 1-hour and 7-hour exposed to the 2.45 GHz Wi-Fi radiation during 2 months caused a decrease in sperm parameters in a time dependent pattern [9]. Okay et al reported that wireless (2.45 GHz) EMR caused oxidative damage in testis of rat by increasing the levels of lipid peroxidation and decreasing in vitamin A and E levels [10]. Kim et al showed Long-Term Exposure of Rats (4 weeks of age) to 2.45 GHz caused the negative effects on history pathological changes and apoptosis status of rat testis [5]. Wireless devices have been widespread used in our living and working environments for longer exposure times than wireless phones which may have an untoward influence on health [11]. According to the Bioinitiative report (http://www.bioinitiative.org/), current safety guidelines for Electromagnetic Field (EMF) exposure are not sufficient and should be revised based on data from various toxicological tests [12]. Due to whole body exposure to the RF, we tried to analyze potential effects of 2.45 GHz Wi-Fi radiation from a wireless antenna on the reproductive system of freely moving NMRI male mice's for 4-hour exposed to the 2.45 GHz Wi-Fi radiation for 4 hour per day during two months and 8-hour exposed to the 2.45 GHz Wi-Fi radiation for 8 hours per day during 3 months.

MATERIALS AND METHODS

ANIMALS

This is an animal experimental study, which was conducted in the Department of biological Sciences, Faculty of Science, Malayer University, Malayer, Iran, from March to May 2019. Animals, 3-month old mice (n=27), were maintained as national guidelines and protocols approved by the Institutional Animal Ethics Committee. All experimental protocols were approved by the Ethics Committee of Malayer University of Sciences, Malayer, Iran. Healthy adult male mice weighing (31±2 gr), were randomly selected and housed under environmentally controlled conditions. The mice were fed with a standard laboratory diet and clean drinking water.

EXPOSURE SYSTEM

The exposure system was a chamber (190cm×80 cm ×80 cm), designed for whole-body exposure of free-moving rats to a Wi-Fi signal. Two Wi-Fi antennas (--) were placed at the center of two sides of the chamber. Animals were divided into three following groups (n=9 per each group): I. control group including healthy animals without any exposure to the antenna, II. 4-hour group including animals exposed to the 2.45 GHz Wi-Fi radiation one hour per day during two months (4 hour/day/2 months) and III. 8-hour group including animals exposed to the 2.45 GHz Wi-Fi radiation eight hours per day during three months (8 hours/day/3 months). All exposure conditions were coded and analyzed in a blind manner.

ANALYSIS HORMONES

Mice were extracted from their hearts using an insulin syringe. Blood samples were collected by centrifugation and centrifuged at 3000 rpm for 20 minutes. Serum samples were kept in the freezer for further examination. Subsequently, the levels of LH, testosterone hormones in the Malayer medical laboratory were determined by ELISA (Iranian Preventive Medicine Kit LOT 89001 and LOT 88005).

SPERM CHARACTERISTICS

Animals were anesthetized intra peritoneally with a mixture of ketamine (Sigma- Aldrich, Germany) and xylazine (Sigma Aldrich, Germany). The weight gain of animal in each group was defined as the differences between initial and final body weights. Testis, were accurately weighed. Caudal part of epididymis was dissected out and chopped in the 5 ml of Ham's F10 medium solution (GIBCO, USA). Epididymal sperm were collected following 5 minutes incubation at 37˚C to allow sperm to swim out of the epididymal tubules. One drop of sperm sus-pension was placed on a microscope slide and cover slipped. At least 10 microscopic fields were observed at ×40 magnification by a phase contrast microscope (Olympus BX51, Tokyo, Japan). The sperm motility parameters were recorded according to the World Health Organization (WHO) recommendations. The percentages of progressive, motile, and immotile sperm were expressed as the ratio to the total counted sperm. The sperm count parameters were also obtained by the method described in the WHO recommendations [13]. Briefly, 5 µl aliquot of epididymal sperm was diluted with 95 µl of diluents (0.35% formalin containing 5% NaHCO3 and 0.25% trypan blue, Merck, Germany), and approximately 10 µl of this diluted specimen was transferred to the counting chambers of the haemocytometer. The cells were counted with a light microscope at ×40 magnification.

ANALYSIS DATA

The data were expressed as mean ± Standard Errors of the Mean

(SEM). The variables were analyzed by one-way ANOVA. When a significance found, Tukey post hoc tests were performed. All analyses were performed using the SPSS (SPSS Inc., Chicago, IL, USA) version 16. The statistical significance level was set at P≤0.05.

RESULTS

ANALYSIS HORMONES OF LH AND TESTOSTERONE

LH and testosterone levels were not significantly decreased in group II (4 hour/day/2 months) compared to control group, while in group III (8 hours/day/3 months) LH and testosterone level significantly decreased compared to control group (Figure 1).

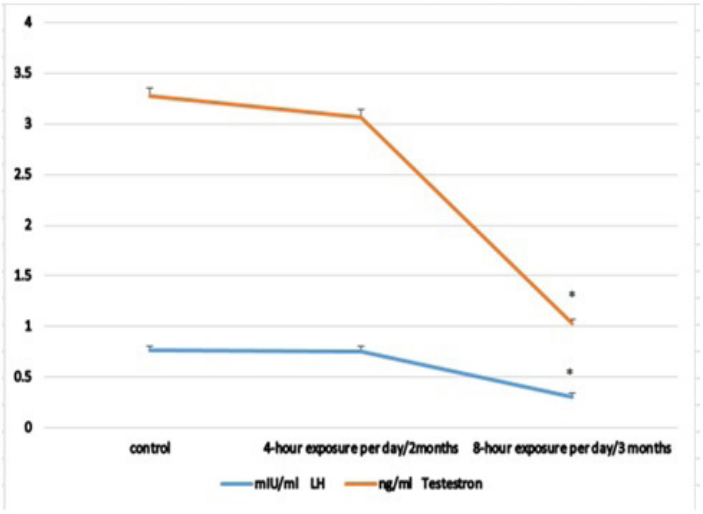

Figure 1: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on LH and testosterone levels. Values are expressed as mean ± standard errors of the mean (SEM). *; P value≤0.001.

SPERM CHARACTERISTICS

WEIGHT OF TESTICLES

Despite 4hour exposure to the 2.45 GHz Wi-Fi radiation caused no significant changes in the relative weight of testicles. The relative weight of both right and left testicles reduced significantly (P≤0.001) following three months exposure of animals to the 2.45 GHz Wi-Fi radiation for 8 hours per day (Table 1).

Table 1: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on the weights of testis in male mics. Values are expressed as mean ± standard errors of the mean (SEM).

Groups	Relative right testis weight(%)	Relative left testis weight(%)
Control	0.52±0.01	0.50±0.01
4-hour group	0.50±0.01	0.49±0.01
8-hour group	0.39±0.01	0.38±0.01

SPERM MOTILITY

We examined the sperm motility in group II and group III compared to control group. 8 hours/day/3 months exposure to the 2.45 GHz Wi-Fi radiation caused significant changes on the sperm motility parameters (Figure 2). Although changes on the sperm motility in group II showed no significant differences in the experimental groups, compared to control group. Therefore, our findings showed a significant reduction in the percentage of motile sperm in 8 hours/day/3 months groups as compared to control group.

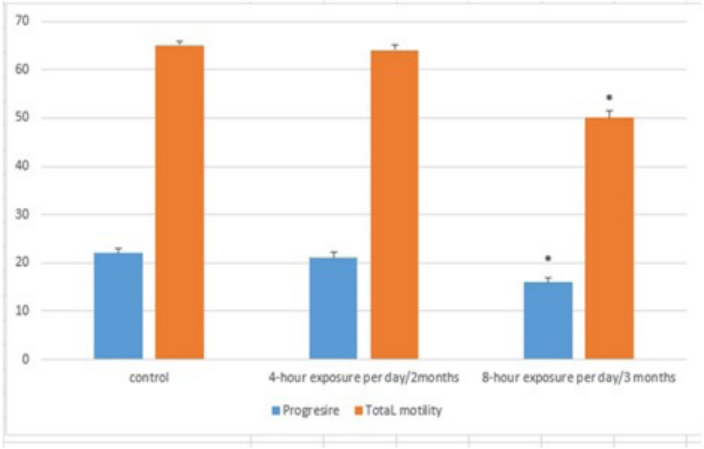

Figure 2: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on total percentage of progressively motile sperm. Values are expressed as mean ± standard errors of the mean (SEM). *; P value≤0.001.

SPERM COUNT

Table 2 shows that exposure to the 2.45 GHz Wi-Fi radiations showed a clear negative impact on the concentration parameters. Sperm samples from 8-hour groups (P≤0.001) exhibited a significant lower concentration as compared to the control group. In parallel with the sperm count reduction, the proportion of normal to abnormal sperm showed a similar reduction in 8-hour groups.

Table 2: The effect of exposure to the 2.45 GHz Wi-Fi radiations on the concentration parameters. Values are expressed as mean ± standard errors of the mean (SEM). The 4-hour and 8-hour groups were compared to the control ones. P≤0.001.

	Control	4-hour group	8-hour group
Sperm count(x10^6/ml)	4.48±1.60	4.08±2.01	2.88±1.6
Normal Sperm(%)	90.24±1.07	89.98±1.08	80.68±0.80

DISCUSSION

The past several decades, Failure in male fertility is considered as a major concern [14]. Various studies have been conducted to evaluate the effect of Wi-Fi transmitter on male reproduction health. However, there are conflicting findings between studies [15]. It

has been suggested that direct or indirect exposure to Wi-fi as the main environmental factor plays a dominant role on sperm motility in addition to Long-time wi-fi exposure Caused behavioral or structural changes of the basal cell [16, 17]. Zentai et al in 2015 showed that 60 min Wi-Fi exposure does not alter human oscillatory brain function or objective measures of sustained attention [18]. Also we showed that of 2.4 GHz Wi-Fi exposure on the body weight and reproductive organ weights in the 4 hour/day/2 months group no deleterious effects while decreased significantly in 8 hour/day/3 months group in comparison to the control group. This present result is in line with previous reported animal experiment that demonstrated no adverse effects of 2.45 GHz radio-frequency exposure on the body weight [7]. But khaki et al. showed that 50 Hz non-ionizing radiation during two months caused pathological changes that lead to sub fertility and infertility [19]. In 2010 researcher showed that Long-term RF-EMF exposure leads to reduction in serum testosterone levels in experimental animals [20]. Testosterone is a primary male gender hormone and any change in the normal levels may be result in pathological changes that lead to sub fertility and infertility also alter epithelial proliferation in the seminal vesicles [21]. Kumar et al. showed that long-term exposure of 2.45 GHz radiation can reduce the level of serum testosterone in rats [4]. Yuksel et al in 2016 showed that Long-term exposure to Wi-Fi devices decreases plasma prolactin, progesterone, and estrogen levels in rats [22]. Also we showed that effects of wi-fi on LH and testosterone levels were dependent on the longevity of exposure per day. In vitro studies of Oni et al in 2011 showed exposure of the 2.45 GHz RFR on sperm human caused changes in the motility and DNA fragmentation [6]. Our findings showed effects of Wi-Fi exposer on percentage of motile sperm were dependent on the longevity of exposure per day. Also Sperm motility decreased significantly in8 hour/day/3 month's group in comparison to the control group. But this parameter were not significantly decreased in group (4 hour/day/2 months) compared to control group. Zha et al in 2019 showed that Low-intensity short-term or intermittent exposure to EMR has little adverse effect on reproductive organs and sperm That many antioxidant and anti-free radical agents, can protect some special populations from EMR [23]. According to the results of Kamali et al in 2018, oxidative defense system in rats exposed to Wi-Fi signal was significantly affected compared to the control group [24]. Also Ceyhan et al in 2012 demonstrated the role of oxidative mechanisms in EMR-induced skin tissue damages that β-glucan could ameliorate oxidative skin injury via its antioxidant properties [25]. Also Yüksel et al in 2016 showed that Long-term exposure to Wi-Fi devices increases uterine oxidative stress in pregnant rats and their offspring [22]. we suggest the effects of long term exposure of wi-fi were mainly due to the elevation of testicular temperature and oxidative stress activity.

CONCLUSION

In conclusion, exposure long term towards 2.45 GHz RF-EMR emitted by Wi-Fi transmitter is hazardous on the male reproductive system and Wi-Fi users to avoid long-term exposure of emissions from Wi-Fi equipment. Further studies are needed to better understand the possible biological mechanisms of Wi-Fi device and relevant outcomes.

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