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ORIGINAL ARTICLE



What is adverse effect of wireless local area network, using 2.45 GHz, on the reproductive system?

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ABSTRACT

Aim: To investigate the inflammatory effect and testicular damage on rats exposed to low level of electromagnetic fields (EMF) at 2.45 GHz microwave radiation.

Methods: Twenty two Wistar rats were divided into two groups. Group 1 was the control group and not exposed to EMF. Group 2 was exposed to low level EMF (average E-field 3.68 ± 0.36 V/m, whole body average SAR, 0.0233 W/kg, in 10 g tissue) at 2.45 GHz for 1 hour/day for 30 consecutive days. At the end of the study, interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-32 (IL-32), C-reactive protein (CRP) were measured in rat serum and IL-6, IL-10, IL-32 were measured in rat testis tissue. Furthermore, testicular tissues were evaluated histopathologically in terms of spermatogenesis and coagulation necrosis.

Results: Serum IL-6 and CRP levels were found to be significantly different in the study group compared to the control group ($p < .05$), but no significant difference was found in serum IL-10, IL-32 levels and testis tissue IL-6, IL-10, IL-32 levels compared to the control group ($p > .05$). On the other hand, histopathological evaluation of testicular tissue revealed a significant difference in necrosis and spermatogenesis when compared with the control group ($p < .05$).

Conclusions: It may be concluded that low level EMF at 2.45 GHz increases inflammation and testicular damage and negative impact on male reproductive system function.

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Introduction

Microwave radiations are a part of high frequency region in electromagnetic spectrum of non-ionizing electromagnetic radiations present in the environment such as mobile phones and wireless fidelity (Wi-Fi) devices and this poses a health risk (Kesari and Behari 2010). In recent years, the use of portable computers, connected to local area networks, known as Wi-Fi has increased dramatically. The use of these devices facilitates daily life, but also brings with several adverse effects associated with electromagnetic fields (EMF). For the most part, laptop computers and wireless local area networks (WLAN) operate at 2.45 GHz frequency. These effects are usually a result of long-term absorption of electromagnetic radiation field (EMF) at low levels (Desai et al. 2009). Microwave radiation which may result in exposure in a low level EMF may affect organ systems in the body because of near or far field and chronic exposure. On the other hand, portable computers incorporated with Wi-Fi are commonly used on the lap therefore exposing the genital area to EMF as well as high temperatures (Sheynkin et al. 2005; Paulius et al. 2008; World Health Organization 2011).

The EMF effects on biological system depend on some physical parameters as electric field, specific absorbed rate

(SAR) dose, dielectric properties of biological tissue, for example tissue conductivity, of biological system. The SAR dose has been used for standard assessment of EMF effects on biological system. The SAR limit value has been recommended to 4 W/kg by some commissions as American National Standards Institute (ANSI), National Council on Radiation Protection and Measurements (NCRP), The International Commission on Non-Ionizing Radiation Protection (ICNIRP). As reported by many reviews and reports, these parameters may cause thermal effect depending on thermal stress at very high peak SARs (tens of W/kg) and non-thermal that electric field is below from 10 V/m and/or SAR is below from 0.1 W/kg (ICNIRP 1998, 2009; Yakymenko et al. 2011, 2016; Manna and Ghosh 2016). Therefore the exposure studies and the reports of high frequency Electromagnetic Radiation on biological system at low level electric field, low level SAR, in other words non-thermal effects are going on and are get becoming very significant.

Spermatogenesis is highly dependent on optimal conditions, and is essential for reproduction. This process is highly organized and can be affected at all levels, such as proliferation, differentiation of male germ cells, intratesticular and extratesticular spermatogenesis regulatory mechanisms

(Sharlip et al. 2002). This adverse effects on spermatogenesis can be occur result of environmental such as diet, toxic elements, infection and inflammation (Azenabor et al. 2015), EMF (Deepinder et al. 2007) and leading to infertility in men.

Systemic inflammatory response is a situation caused by a variety of conditions, considered the evidence of common inflammatory pathways resulting from the production and interaction of various humoral and cellular mediators including cytokines. Cytokines play a major role inducing inflammatory responses. A major regulator of cytokine induction is the nuclear factor kappa B (NFkB). NFkB binds to the nuclear DNA and activate the cytokines or stress genes synthesis. NFkB activated by agents such as cytokines, mitogens and free radicals. Indeed, it has been reported that some of the helpful effects of different antioxidants are related with their ability for inhibit NFkB activation. The role of free radicals as stimulator of cytokine synthesis has been reported in different studies (Costa-Pereira et al. 2011). Oxidative stress may increase cytokine production by several different mechanisms. Reactive oxygen species (ROS), produced by oxidative stress act as a second messenger that activates transcription factors such as NFkB and protein-1 (AP-1), leading to transcription of genes encoding cytokines, growth factors and extracellular matrix (ECM) proteins (Closa and Folch-Puy 2004; Elmarakby and Sullivan 2012; Azenabor et al. 2015). Further, these oxidative adverse effects suggested that chronic exposure to high frequency EMF may lead to infertility via free radical species and inflammation by adverse effect on sperm production.

In male gonads, cytokines are produced physiologically and have been reported to be interested in the normal function of the gonads. The main source of cytokines in the male gonad is testicular macrophages, although the production of certain cytokines by Leydig and Sertoli cells have been reported by some authors (Hales et al. 1999; Soder et al. 2000; Maegawa et al. 2002; Diemer et al. 2003). We aimed to show the possible inflammatory effects that independent from thermal effect of EMF by investigating IL-6, IL-10, IL-32, CRP levels in the serum, IL-6, IL-10, IL-32 levels in testis tissue and by histopathological evaluating on the testis tissue of rats, exposed to low level EMF at 2.45 GHz at average E-field 3.68 ± 0.36 V/m, whole body average SAR, 0.0233 W/kg, in 10 g tissue, used in WLAN systems in this study.

Material and methods

Animals

A total of 22 male Wistar rats aged between five and six months and weighting 250–300 grams were obtained from Surgical Research Laboratory, Ondokuz Mayıs University. The rats were kept in standard cages and they fed with standard food and water. They were put in cylinder restrainers for 1 hour/day for a week before the study to eliminate the stress factors on the rats. Following the adaptation period, they were divided into two groups, randomly (Group 1, 2). Group 1 was the control group, not exposed to low level EMF at 2.45 GHz. Group 2 exposed to low level EMF at 2.45 GHz (at for 1 hour/day for 30 days. All rats of Group 2

were kept in plexiglass cages during low level EMF exposure on the other hand rats of Group 1 were kept in plexiglass cage in the other room without EMF. Illumination system was created to provide 12 hours of day and 12 hours of night conditions. Temperature was fixed at 22 ± 1 °C.

The study was approved by Ondokuz Mayıs University Ethical Committee (HADYEK/81, 23.09.2009).

Application of the electromagnetic field

In this study, Microwave system with monopole antenna (2004X-RF, Everest Co., Adapazari, Turkey) was used to produce the low level EMF at 2.45 GHz. Microwave radiation generator can radiate at 2.45 GHz frequency, with a range of 0–1 Watt output power, and produce continuous or pulsed (modulated at 217 Hz) radiation. To document any reflections and background noise, the electric field measurements of medium before exposure of rats at 2.45 GHz was measured by the Department of Electrical and Electronic Engineering at the Ondokuz Mayıs University using Portable Field Meter and EP-330 electric field probe (PMM 8053 Portable Field Meter and EP-330 electric field probe Costruzioni Elettroniche Centro Misura Radioelettriche S.r.l., Italy). The electric field measurement device can measure instant measurements at X,Y,Z cartesian coordinates and time axis and the EP-330 electric field probe (level range: 0.3–300 V/m, size: 317 mm length, 58 mm diameter) was able to perform measurements at 100 kHz–3 GHz frequency range. Because of the probe's level range background electric fields lower than 0.3 V/m were not detected in the experimental setting. An exposure design of rats, low level EMF at 2.45 GHz applications and the electric field measurements during exposure applications were made by the Department of Biophysics at the Ondokuz Mayıs University. The exposure design of rats is given as Figure 1. Here, a galvanized plate with 1 mm thick was placed for grounding of the static field at the bottom of a pie cage plexiglas restrainer that was specially designed. E-field measurements were conducted at the top surface of plexiglas restrainer using Portable Field Meter and EP-330 electric field probe (PMM 8053 Portable Field Meter and EP-330 electric field probe Costruzioni Elettroniche Centro Misura Radioelettriche S.r.l., Italy). The height of the antenna from the ground (bottom of galvanized plate) was about 15 cm. To supply an equal distribution of the electric field to rats, the antenna was located in the middle of the pie cage restrainer in vertical position and the coaxial cable-rat distance in the horizontal position was 6 cm (Figure 1). Before this study for a week to eliminate the stress factors on the rats, they were put in restrainers for 1 hour/day.

The average E-field and the SAR measurement

In this study, the 2004X- RF microwave emitter was limited at 0.2 W output power to obtain about 3 V/m average E-field and to exposure low level EMF at 2.45 GHz. Before the 2004X- RF microwave emitter was set a continuous wave and the rats placed to restrainer. During the low level EMF exposure at 2.45 GHz, the electrical field measurements were



Figure 1. Electromagnetic field exposure system.

taken from the head, dorsal and tail regions of 11 rats for 1 minute. These obtained data (33 average E-field data) were recorded from the PMM 8053 Portable Field Meter memory to a computer for every day. A whole body average E-field value was calculated from means of these 33 electric fields values and at the end of 30 days, the average of all the 30 total whole body electric field values (average E-field) was calculated (SPSS for Windows, version 15.0, SPSS Inc., Chicago, IL, USA) and were expressed as means $\pm$ standard deviations (SD). Average E-field minimum and maximum values and mean $\pm$ SD for 30 days was 3.17–4.88 V/m and 3.68 ± 0.36 V/m, respectively.

In order to simulate experimental setup Computer Simulation Technology (CST) Studio Suite (2018) (www.cst.com) was used. CST Studio Suite is an electromagnetic field solver based on finite integration technique (FIT) for numerical simulations which is very similar to finite-difference time domain (FDTD) technique. It performs discretization on non-orthogonal grids using integral form of Maxwell's equations. Simulated E-field values were compared to those of measured E-field values which were obtained with the E-field probe, and close corroboration was achieved. The distributions of E-field on rat were simulated using CST Studio Suite and E-field values were given in Figure 2.

CST Studio Suite was used to calculate whole body average SAR value on the rat. Using voxel rat model in CST Studio Suite and Wi-Fi antenna which was modeled with equipment's transmit power, SAR calculations have been performed and the results were given in Figure 3. 10 g average SAR level of whole body was found as 0.0233 W/kg.

Biochemical examination

At the end of 30 days, the rats were anesthetized by intraperitoneal ketamine and 4–5 cc intracardiac blood was collected by thoracotomy in normal biochemistry tubes. Thereafter, the blood was centrifuged at 3000xg (Shimadzu UV160A, SNo: 28006648, JAPAN) for 10 minutes for obtaining serum. One of the testis tissue samples were collected after reperfusion with normal saline (0.9% NaCl). Subsequently, tissue samples were manually homogenized by liquid nitrogen and keep up in eppendorf tubes containing 1 ml phosphate

buffered saline (PBS) (pH 7.4, 10 mM). Finally, homogenized samples were sonicated (220 V, METU electromechanical, SNo: 30607, GERMANY) for 1 minute. All samples were stored at -40°C until they were studies. Also the other testis was stored in formaline for pathological examination.

IL-6, IL-10, IL-32 levels were measured both in the serum samples and testis tissue samples but CRP levels were measured only in the serum samples.

IL-6, IL-10, IL-32 Measurement: IL-6, IL-10, IL-32 levels were measured by using commercial measurement ELISA kit (Cat.No: FCK-E30646, CK-E30651, CK-E30040, HANGZHOU, EASTBIOPHARM CO.LTD) and was measured by using ELISA reader (BIOTEK SYNERGYTM 4, Serial No:233513,USA) and, the results were expressed as pg/mg protein for testis tissue IL-6, IL-10, IL-32 levels and pg/mL for serum IL-6, IL-10, IL-32 levels.

CRP Measurement: CRP levels were measured in serum by using commercial measurement ELISA kit (Cat.No: CK-E30459, HANGZHOU, EASTBIOPHARM CO.LTD) and was measured by using ELISA reader (BIOTEK SYNERGYTM 4, Serial No: 233513, USA). The results were expressed as ng/mL.

We were chose IL-6 as a proinflammatory indicator, IL-10 as an anti-inflammatory indicator, IL-32 associated with cell differentiation, apoptosis, and CRP associated with acute inflammatory response.

Histopatological examination

Testicular tissues were fixed in the 10% neutral formaline. After tissue tracing, tissues were embedded in the paraffine and 5 micron thick sections were taken. Sections stained with hematoxylin eosin were examined with an Olympus BX51 optic microscope (Olympus Optical Co., Japan) by an experienced pathologist who did not know the experimental design.

Coagulation necrosis (CN) and spermatogenesis of the testicular tissue were evaluated. The grade of coagulation necrosis was scored as 0–3. If there is no necrosis in the seminiferous tubules, it was evaluated as Grade 0 whereas if the seminiferous tubulus necrosis is less than 25%, between 25%–75% and more than 75%, were evaluated as Grade1, Grade2 and Grade3 respectively. Twenty seminiferous tubules

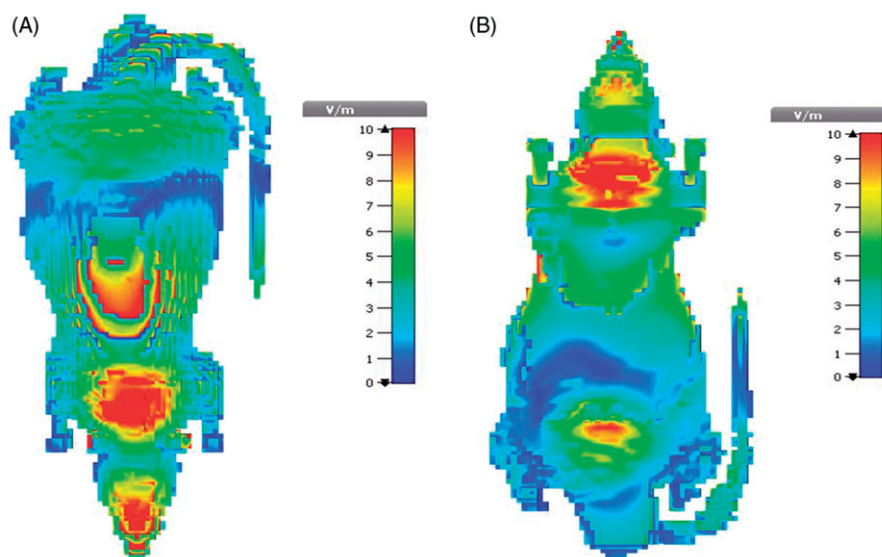


Figure 2. E-field values A: Top view, B: Bottom view.

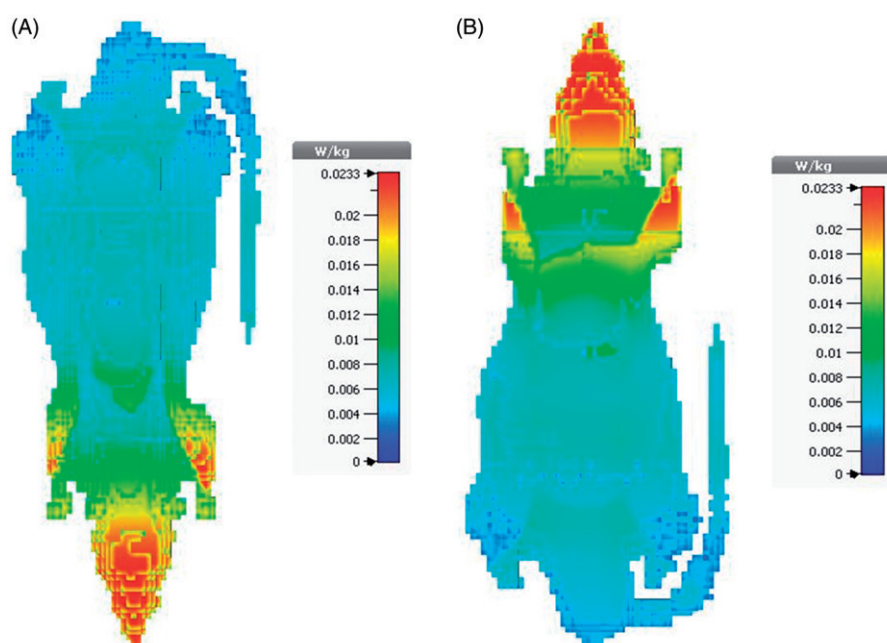


Figure 3. SAR values (10 g. avg) A: Top view, B: Bottom view.

were evaluated for spermatogenesis for each testis tissue and Modified Johnsen Scoring System (JS) was used. For each seminiferous tubule observed in the JS, a score is given and their sum is divided by the number of seminiferous tubules observed in the preparation. A score of 1–10 was given to each tubule according to the maturity of the germ cells. The histological findings of the seminiferous tubules and their corresponding scores are as follows: 10: Germinal epithelium is very ordered, there are many spermatozoa; 9: Germinal epithelium dislocates and lumen accumulates correctly, there is spermatozoa; 8: Germinal epithelium is very ordered but there are less than 10 spermatozoa in the lumen; 7: No spermatozoa, there are a lot of spermatids; 6: No spermatozoa, less than 10 spermatids; 5: No spermatozoa or spermatids, many spermatocytes; 4: No spermatozoa, no spermatids, less than 5 spermatocytes; 3: Germ cell has only

spermatogonia; 2: No germ cells, only Sertoli cells; 1: No cells in the seminiferous tubulus. For normal testes, the Johnsen score average is 9.39 and at least 60% of the tubules should have a score of 10 (Parlaktas et al. 2014; Dieckmann et al. 2007; Asghari et al. 2016).

Protein Assay: The protein assay was applied according to method described by Lowry et al. (Lowry et al. 1951).

Statistical analysis

Statistical analysis was performed by using SPSS 15.0 software. Shapiro–Wilk test was used for analysis of distribution. Student *t* tests were used for analysis of data with normal distribution (serum and tissue IL-6, IL-10, IL-32 and serum CRP) whereas Mann–Whitney *U* test was performed for

analysis of data of histopathological scoring. Results are presented as median and minimum value and maximum value for nonnormal distribution whereas mean $\pm$ SD and minimum value and maximum value for normal distribution. p value less than .05 was considered as significant in all tests.

Results

It was found that low level EMF at 2.45 GHz significantly increase IL-6 and CRP levels in serum ($p < .05$) but not found significant difference in levels of serum IL-10, IL-32 (Table 1) and tissue IL-6, IL-10, IL-32 ($p > .05$) (Table 2). On the other hand, histopathologically examination of testicular tissues in terms of necrosis and spermatogenesis were significantly different in study group compared with the control group ($p < .05$) (Table 3, Figure 4).

According to the coagulation necrosis scoring between grades 0–3; in the control group seven samples were evaluated as 0 and four samples as 1; in the study group, five samples were evaluated as 2 and five samples evaluated as 1 and only one sample was evaluated as 3. When spermatogenesis were assessed according to JS score, in the control group nine samples showed complete spermatogenesis and two samples showed slightly damaged spermatogenesis. On the otherhand, in the study group, only three samples had full spermatogenesis while four samples had slightly damaged spermatogenesis and three samples had less than five spermatozoa in each tubule.

Discussion

In this study, low level EMF (at 2.45 GHz, whole body average SAR value in 10g tissue, 0.0233 W/kg, 1 h/day for 30 consecutive days) nonthermal effects on the testicular inflammatory damage were investigated by measured some cytokines (IL-6, IL-10, IL-32) and CRP in serum and testicular tissue (IL-6, IL-10, IL-32) and by evaluated histopathologically of testicular tissue. It was found that low level EMF significantly increased IL-6 and CRP levels in serum. On the other hand, histopathological examination of testicular tissues in terms of necrosis and spermatogenesis showed that significantly increased coagulation necrosis and decreased spermatogenesis.

The reproductive organs, especially testes, are sensitive to EMF exposure. Thus, this study is focused on the effects of electromagnetic radiation, emitted from a wireless network, on testicular tissue inflammation and spermatogenesis.

Current studies focus on adverse effects of 2.45 GHz EMF on the reproductive systems. Some of these studies have used high SAR values (more than 10 W/kg electric field and/or 0.1 W/kg SAR), which may also cause thermal effects (Saygin et al. 2016, 2011). Saygin et al. (2016), investigated the effect of 2.45 GHz EMF (3 h/day for 30 days, at 3.21 W/kg SAR) on oxidative damage and inflammation according to malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), vascular endothelial growth factor (VEGF) levels in serum and histopathological examination and prostaglandin E2 (PGE2), calcitonin gene-related peptide (CGRP) levels in

Table 1. Comparison of parameters in serum.

	Groups	n	Mean $\pm$ SD	Min/Max	p
IL-6 (pg/mL)	1	11	167.75 $\pm$ 31	111–205	<.05
	2	11	243.90 $\pm$ 100	96–362	
IL-10 (pg/mL)	1	11	24.60 $\pm$ 9.69	15.39–40.59	>.05
	2	11	26.11 $\pm$ 5.81	19.19–39.64	
IL-32 (pg/mL)	1	11	29.48 $\pm$ 4.36	21.23–33.84	>.05
	2	11	30.00 $\pm$ 7.19	21.42–41	
CRP (ng/mL)	1	11	72.43 $\pm$ 24.23	34.66–107.77	<.05
	2	11	104.68 $\pm$ 21.50	56.19–135.25	

Group 1: Control group; Group 2: Low level EMF at 2.45 GHz exposed group; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-32: Interleukin-32; CRP: C-Reactive Protein; $p < .05$ compared with Group 1 (Control).

Table 2. Comparison of parameters in testis tissue.

	Groups	N	Mean $\pm$ SD	Min/Max	p
IL-6 (pg/mg prot)	1	11	56 $\pm$ 28	11–90	>.05
	2	11	76 $\pm$ 11	61–90	
IL-10 (pg/mg prot)	1	11	15 $\pm$ 6.6	4–25	>.05
	2	11	14.9 $\pm$ 2.6	11–19	
IL-32 (pg/mg prot)	1	11	10 $\pm$ 5	5–19	>.05
	2	11	12 $\pm$ 3	8–19	

Group 1: Control group; Group 2: Low level EMF at 2.45 GHz exposed group; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-32: Interleukin-32; CRP: C-Reactive Protein; $p > .05$ compared with Group 1 (Control).

Table 3. Comparison of histopathological parameters of testis tissues corresponding JS and coagulation necrosis (CN) in testis tissue samples.

	Groups	n	Median	Min/Max	Q1	Q3	IQR	p
JS	Group 1	11	10	9–10	9.5	10	0.5	<.05
	Group 2	11	9	5–10	8	10	2	
CN	Group 1	11	0.00	0–1	0	1	1	<.05
	Group 2	11	1.5	1–3	1	2	1	

Group 1: Control group; Group 2: Low level EMF at 2.45 GHz exposed group; Q1: Median of the first half, Q3: Median of the second half, IQR: Interquartile range; $p > .05$ compared with Group 1 (Control).

rat testis tissue. They found that increased MDA and TOS, decreased TAS and VEGF in serum and vascular congestion, interstitial edema, tubular degeneration, reduction in spermatogenesis in the seminiferous tubules. Saygin et al. (2011), investigated 2.45 GHz microwave effects (1 h/day for 28 days, at 3.21 W/kg SAR) on the apoptosis via evaluating TNF α , caspase 3, caspase 8 and testicular leydig cell count and spermatogenesis corresponding Jhonsen score in the other work. They found that reduced leydig cell and spermatogenesis increased caspase 3, caspase 8 but not TNF α and they suggested that increased extrinsic apoptosis in the rat testis tissue.

Studies have also been reported in the literature investigating possible non thermal effects on the reproductive system (Oksay et al. 2014; Shahin et al. 2014; Kesari and Behari 2010; Ozorak et al. 2013). Oksay et al. 2014, showed that increased lipid peroxidation, reduced GSH (glutathione) and GSH-Px (glutathione peroxidase) in the rat testis tissue exposed 2.45 GHz EMF at 0.1 W/kg SAR value during 60 min/day for 30 days. Shahin et al. 2014, reported that 2.45 GHz microwave radiation at 0.018 W/kg SAR during 2 h/day for 30 days decrease sperm count and decrease seminiferous tubule diameter and degeneration of seminiferous tubules in mice. Also they noted reduction in testicular 3 β -HSD activity and plasma testosterone levels. Kesari and Behari 2010,

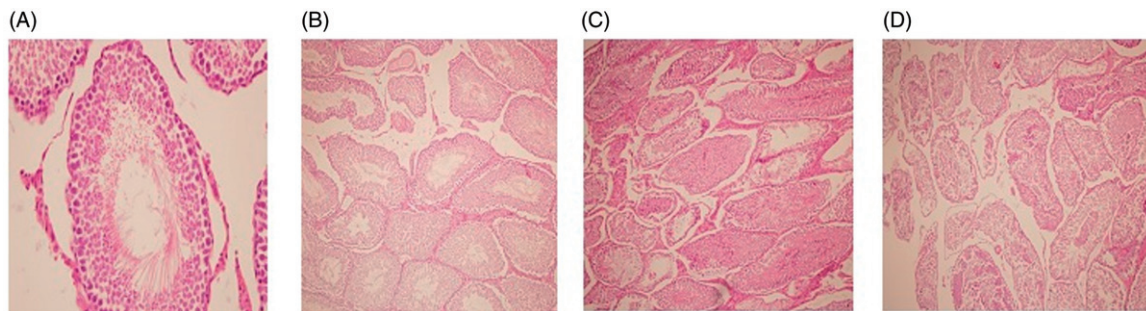


Figure 4. Coagulation necrosis of testis tissue (HEX100). A: Full spermatogenesis in control group, B: Grade 1, C: Grade 2, D: Grade 3.

investigated the oxidative and apoptotic effects of microwave radiation on the rat reproductive system according to superoxide dismutase (SOD), glutathion peroxidase (GPx), catalase (CAT) activities and flow cytometric apoptotic and viable cell counts. Rats were exposed to 2.45 GHz, at 0.11 W/kg SAR value during 2h/day for 35 day. They found that decreased GPx, SOD activities and increased CAT activity whereas increased apoptotic cell and decreased sperm count in collected sperm samples.

Avendano et al. 2012, evaluated the effects of laptop computers connected to local area networks (2.45 GHz) on human spermatozoa with ex vivo study. Sperm samples were incubated and exposed to an active laptop computer during 4h, under controlled temperature conditions. They found that decreased sperm motility and increased sperm DNA fragmentation. On the other hand Ozorak et al. 2013 did not showed significantly different on sperm viability with in vivo rat study. But they, found that increased lipid peroxidation and decreased GSH-Px activity in the testis tissue of adult rats exposed to 2.45 GHz at 0.18 ± 0.07 W/kg SAR for 60 min/day during gestation up to six weeks of age (five days per week). In this study we found a significant reduction in spermatogenesis in the study group compared to control group, consistent with the above studies. Also coagulation necrosis was determined in rat testis tissues exposed to low level EMF at 2.45 GHz (Table 3, Figure 4). In addition cell culture studies about of 2.45 GHz microwave were also reported in literature.

Yutong et al. (2010) used N9 microglial cells that exposed to EMF at 2.45 GHz. Their results showed that activated signal transducer and activator of transcription 3 (STAT3), STAT3 DNA-binding ability, increased transcription levels of the inflammation-associated genes, iNOS and TNF- α and they suggested that EMF exposure at 2.45 GHz can initiate the activation of microglia cells and STAT3 pathway related inflammation and malignancy.

Findings of all these mentioned above studies about of non thermal effects of EMF at 2.45 GHz on the reproductive system, suggested that EMF increases oxidative stress and decreased antioxidant capacity. Further reported studies show that increased apoptosis and DNA fragmentation, decreased spermatogenesis, sperm motility, sperm count. Also cell culture studies reported that EMF induces STAT3 pathway associated with inflammatory response and malignancy.

Inflammation is known to be important in the pathogenesis of many species of malignancies. STAT consist of a family of seven transcription factors that mediate signal

transduction from the extracellular environment to the nucleus. STAT3 is activated by a number of cytokines involved in injury responses, including IL-6 and many growth factors. The mechanisms of STAT3 activation include both autocrine and paracrine production of IL-6 leading to STAT3 phosphorylation. The studies proposed that STAT3-dependent tumorigenesis is mediated by IL-6 signals from the tumor microenvironment (Brumberg and Wang 2009; Yutong et al. 2010). Especially dysregulated IL-6 production has been reported in association with different diseases and cancers (Klein et al. 2016; Costa-Pereira et al. 2011; Guo et al. 2012; Tanaka and Kishimoto 2014). Klein B. et al. (2016) found that significantly increased proinflammatory cytokines such as IL-6 and TNF- α , anti-inflammatory cytokines such as TGF- β and chemokines in testicular neoplasia. One of the most important outcomes of these studies is the crucial role of IL-6 in testicular cancer that introduces potential novel diagnostic and/or therapeutic aspect for testis cancer.

IL-6 is produced by many different cells: T cells, B cells, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glia cells and keratinocytes. IL-6 is responsible for the initiation of the acute-phase response in human hepatocytes via increasing protein synthesis such as fibrinogen, α 1-antichymotrypsin, ceruloplasmin, haptoglobin, α 1-acid glycoprotein, α 1-antitrypsin, C-reactive protein and complement factor B (Akdis et al. 2011). In our study we found that significantly increased IL-6 levels in serum but not testis tissues in study group. There is no study in literature involved with effects of 2.45 GHz EMF on the cytokine levels. This increased IL-6 levels may be related with increased free radicals and their inflammatory stimulator effects (Tables 1, and 2).

CRP is pentraxin family protein and is produced by hepatocytes as part of the non-specific acute phase response, although other sites of local CRP synthesis and secretion have been suggested. CRP production is stimulated by proinflammatory cytokines, especially IL-6. CRP is widely used as marker for inflammation, infection and tissue damage in daily rutin. However, recent evidences indicate that CRP also plays a role in oncology. CRP binds with highest affinity to phosphocholine residues, but it also binds to a variety of other ligands, such as modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles, and apoptotic cells (Pepys and Hirschfield 2003; Janik et al. 2017). Effects of 2.45 EMF on the CRP levels have not

been reported in literatur. In our study we were found significantly increased CRP serum levels but not testis tissues in study group (Tables 1, and 2).

IL-32 plays role in control of cell differentiation, stimulation of pro- or anti-inflammatory cytokines and apoptosis, and it has pleiotropic effect in the pathogenesis of various disorders, such as infectious, cancerous and inflammatory diseases (Khawar et al. 2016). IL-10 is antiinflammatory cytokine and mainly produced by monocytes, T cells, B cells, macrophages and dendritic cells. Also it was suggested that mast cells produce IL-10 and limit the rate of leukocyte infiltration, inflammation and skin pathology in the situation of contact dermatitis or chronic UVB irradiation. It has been defined that immunosuppressive effects of IL-10 exerts through interference with the NF- κ B activation pathway through inhibition of NF κ B DNA binding activity. It appears that the primary mechanism of IL-10-induced anti-inflammatory effects is mediated by the activation of STAT3 (Akdis et al. 2011). We did not found significantly different between study and control group corresponding to IL-10 and IL-32 levels in serum and testis tissues (Tables 1, and 2). There is no study investigating the association between 2.45 GHz EMR and IL-10 and IL-32 in literature. Only one investigation about of cell phone suggested that EMR decreases IL-10 levels in saliva (Siqueira et al. 2016).

In conclusion, current experimental findings indicate that low level EMR impairs male reproductive system via inducing oxidative stress and suggested that EMF has been toxic effect on reproductive system especially in testes. It is obvious that EMF may also impose health risk. In accordance with these studies we found necrosis in rat testis tissue and decreased spermatogenesis. Also we have found that exposure to a 2.45 GHz low level-EMF at 2.45 GHz increased CRP and IL-6 levels in rat serum. We suggest that EMR generated to inflammatory effect via increased oxidative radical, may be related tissue damage. Therefore, even if it makes our life easier, long-term continuous exposure to low levels non-ionizing EMF should be minimized. However many studies have been done, this current study was done with rats and additional human studies are needed. This investigation was limited by the small number of inflammatory/anti-inflammatory markers; therefore, studies with more inflammatory/anti-inflammatory markers may provide more information. Further studies are required.

Disclosure statement

No potential conflict of interest was reported by the authors.

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