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Effects of Low Magnetic Irradiation on Morphology and Ultrastructure of Parotid Glands in Rats and Amelioration by Vitamin E

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Abstract

Background: Human is daily exposed to electromagnetic field from different source in modern societies with associated health impacts. This experiment aims to study the histological structure of parotid gland after exposure to extremely low frequency magnetic field ELF-MF associated with utility grid 50/60 Hertz (Hz) and to detect the possible role of Vitamin E supplementation.

Materials and methods: Thirty adult male albino rats were classified into three groups: Group (I) was control animals, group (II) and (III) were exposed to ELF-MF (50 Hz and 100 – 300 mili Tesla (mT)). Animals of group III were supplemented with daily doses of vitamin E directly before exposure to ELF-MF. Light and electron microscope examination of parotid glands were done.

Results: Degeneration of parotid gland acini and ducts together with cellular infiltrations, fibrosis and mucous transformation of the acinar cells were detected after exposure to ELF-MF. These changes were limited with vitamin E supplementation in group III. So, exposure to ELF-MF leads to parotid gland affection which could be tolerated with vitamin E supplementation.

Keywords: Low frequency magnetic field; Rat parotid gland; Histology ultrastructure; Morphometry

Introduction

Electromagnetic fields (EMFs) are a class of non ionizing radiation that is invisible line of force present everywhere in our environment and produced by electrically charged objects [1]. A magnetic field results from the flow of current through wires or electrical devices. Human is daily exposed to EMFs from natural sources [2]. Increase in the level of environmental exposure to EMFs was reported during the twentieth century with the spread of the utilization of electricity in almost every aspect of civil and domestic life [3]. In modern society, it is impossible to avoid exposure to EMFs produced by power lines and many kinds of electrical appliance and underground cables [4].

Most of the research on the biological effects of non-ionizing radiation is done at one of two frequency ranges; extremely low frequency (ELF) associated with utility grid 50/60 Hertz (Hz) and radio frequency (RF) associated with wireless systems (800 MegaHertz (MHz) to 2.5 GigaHertz (GHz) range [5]. People who lived or worked near transformers, electrical closets, circuit boxes telephone line workers television repairers and other high current electrical equipment had higher average EMFs exposure [6]. Examples of devices that emit ELF-MF include power lines and electrical appliances, such as electric shavers, hair dryers, computers, televisions, electric blankets, and heated waterbeds. Most electrical appliances have to be turned on to produce a magnetic field. The strength of a magnetic field decreases rapidly with increased distance from the source [1].

Potential health effects from exposure to electric fields from power lines is typically not of concern since electric fields are effectively shielded by materials such as trees, walls, etc., therefore, the majority of the information related to EMFs focuses primarily on exposure to magnetic fields (MFs) from power lines. EMFs were associated with an increase in childhood leukemia, adult brain cancer, miscarriage and fertility in both male and female [1,7]. Radiation effects on salivary glands are of particular interest, where the altered composition of saliva results in distress, often irreversible complications such as oral dryness, nocturnal oral discomfort, and susceptibility to oral infections and dental caries [8]. However, few studies reported that MF associated with wireless system (RF-MF) caused disturbed parotid physiology including salivary rate and protein content [9] and even parotid tumor were reported [10]. No previous experimental studies on the effect of ELF-MF on parotid gland were found.

Based on higher radiosensitivity of the parotid gland over the submandibular when exposed to radiation [11] and the association of oxidative stress with the mechanism of EMFs induced damage [12,13] and radioprotective effect of vitamin E [8] together with its role as antioxidant against EMFs [14], this study aimed to detect the histological changes of parotid gland after exposure to ELF-MF and the possible role of using antioxidant vitamin E in prevention of hazards of exposure.

Materials and Methods

Thirty adult healthy male albino rats (6-8months) weighing 200-250 gm were supplied by the laboratory animal unit, Faculty of Medicine, Zagazig University, Egypt. The animals were fed standardized hard rat chew pellets and tap water ad libitum. Before starting the experiment, all animals were allowed to acclimate to their housing environment and to exclude diseases for a period of 2 weeks. Animals of all groups were kept under the same environmental conditions of temperature, illumination, acoustic noise and ventilation and received the same diet

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during the course of the experiment. This study was done according to the Faculty ethical committee of care and use of laboratory animals.

Expose facility (magnetic field generator system)

Magnetic field (MF) was produced by homogenous magnetic field generator, which designed in Physics Department, Faculty of Science, Cairo University, Egypt. The system formed of a solenoid consisting of 320 turns from electrically insulated 2 mm cupper wire thickness wound in a homogenous way around a cupper cylinder 1.5 mm thick, 40 cm diameter and 25 cm length. The cylinder wall earthed to eliminate electric field components effects. The magnetic field generator was temperature controlled during the exposure period by using a water pump. The cage of the rats was put in the middle of the coil by using supports inside it to get a homogenous and higher magnetic fields strength. The ends of coil are connected to variac fed from the mains (220v, 50Hz). The field strength was 20 G (gauss) and adjusted by changing the voltage through the coil. [15]

Experimental protocol

The thirty animals were equally classified into three groups: Group I (control group): was kept under the same conditions in plastic cage without exposure to ELF-MF. They were subdivided into two equal subgroups: *Subgroup Ia:* received no treatment and *subgroup Ib:* injected with vitamin E (tocopherol acetate, pharco Egypt) daily at doses of 50mg/kg body weight (BW) intramuscular (IM) [16]. Animals of group II were put in the generator system and exposed to 50 Hz and 100 – 300 miliTesla (mT) ELF-MF 4 hours/day at the same time (from 8am-12pm) for successive 30 days. This is the least frequency for MF exposure used by Rajkovica et al. [17]. Animals of group III were injected with daily doses of vitamin E in a dose of 50mg/kg BW IM directly before putting in the system and exposure to ELF-MF.

At the time of sacrifice, the animals had to refrain from eating and drinking water for a minimum of 90 minutes before sample taking [8] then animals were anaesthetized by ether inhalation and perfused through the heart with a mixture of 1% paraformaldehyde and 1.5% glutaralheyde buffered with 0.1 M Sodium cacodylate to pH 7.4. With the head extended, parotid glands were found in the front of the ear. Right and left parotids were dissected out from each animal carefully and one member of each pair was sectioned longitudinally into two halves for light microscope examination. Other member of each gland pair was cut into 1 mm slices and processed for electron microscope examination. For light microscope examination, specimens were processed to prepare 5 µm thick paraffin serial sections at 400 µm interval and stained with Haematoxylin and Eosin (H & E) to verify histological details, Mallory's trichrome (MT) for detection of collagen fibers and Periodic acid Schiff's (PAS) reaction for histochemical detection of glycoprotein according to Bancroft and Gamble [18] and for transmission electron microscope examination according to Glauert and Lewis [19].

Quantitative morphometric measurements

Serial sections stained with H&E, PAS and Mallory's trichrome sections were morphometrically analyzed for detection of diameter of the parotid ducts; area % of blue stained collagen fibers in the septa, around duct and blood vessels and optical density of PAS stained sections using Leica Qwin 500 image analyzer computer system (Cambridge, England). The measuring frame of a standard area is equal to 7286, 78µm^2. For each parameter ten different non overlapping fields from ten different specimens were examined in each group.

The obtained data from morphometrical analysis were subjected to one-way analysis of variance (ANOVA) and post hoc test using Statistical Package for the Social Sciences (SPSS) version 11.

Results and Discussion

In spite of the presence of studies on the effect of RF-MF on parotid gland associated with higher frequencies [9,10], studies on the effect of ELF-MF on structure and function of parotid gland are limited. In the present study, the effect of ELF-MF on parotid gland morphology and ultrastructure and protective role of vitamin E is investigated.

Examination of H&E stained sections (Figure 1) showed that control subgroups (Ia and Ib) had the same structures. Figures of subgroup Ia are used for comparison with other groups. The parenchyma of parotid gland was formed of serous acini and ducts with normal structure. The serous acini consist of wedge-shaped secretory cells with basal nuclei surrounding a lumen. Striated ducts are prominent in the section (Figure 1A). The results of the present work provide evidence that ELF-MF induces different forms of degenerative changes in parotid gland of group II. Most of the serous acini had irregular outlines and were widely separated. Some acinar cells contained darkly stained nuclei and cytoplasmic vacuoles (Figure 1B). Degenerated structures were replaced with homogenous acidophilic areas (Figure 1C). There were cellular infiltrations inside connective tissue septa (Figures 1D). Parotid gland of vit E supplemented group showed that most of acini and ducts were apparently normal histological features (Figure 1E).

Significant dilatation of the parotid ducts was evident by morphmetrical analysis of diameters of ELF-MF exposed group (Table 1). Moreover, the amorphous acidophilic areas which detected in the gland after ELF-MF exposure were described in other study as hyaline degeneration of duct cells [20]. Affection of the parotid ducts are related to accumulation of ROS in their lining cells [21]. Structural and functional affection of the parotid gland were reported with different forms of irradiation including ionizing radiation [22,23].

Mallory's trichrome stained sections (Figure 2) clarified the presence of extensive blue colored collagen fibers in parotid gland

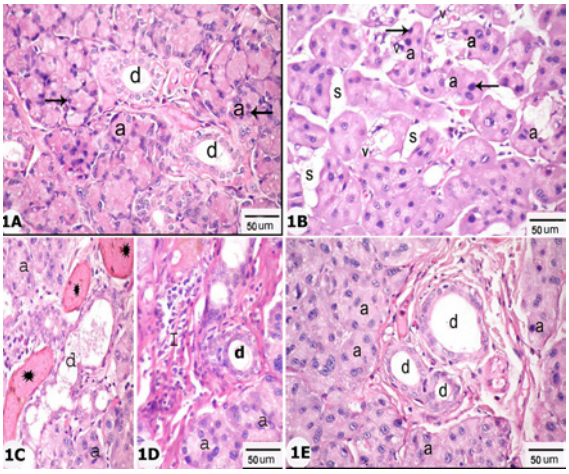

Figure 1: H&E stained sections of control subgroup Ia (Figure 1A); group II (Figures. 1B,C,D) and group III (Figure 1E): showing the normal serous acini (a) and striated ducts (d) of parotid gland of control group in 1A. Most of the acini of group II have irregular outlines, widely separated (s) and contain darkly stained nuclei (arrows) and vacuoles in Figure (1B). Homogenous acidophilic areas (*) and dilated interlobular ducts (d) are seen in (Figure 1C). Cellular infiltration (I) inside connective tissue septa is prominent in Figure 1D. Figure (1E): showing apparent normal acini and ducts of group III X 400.

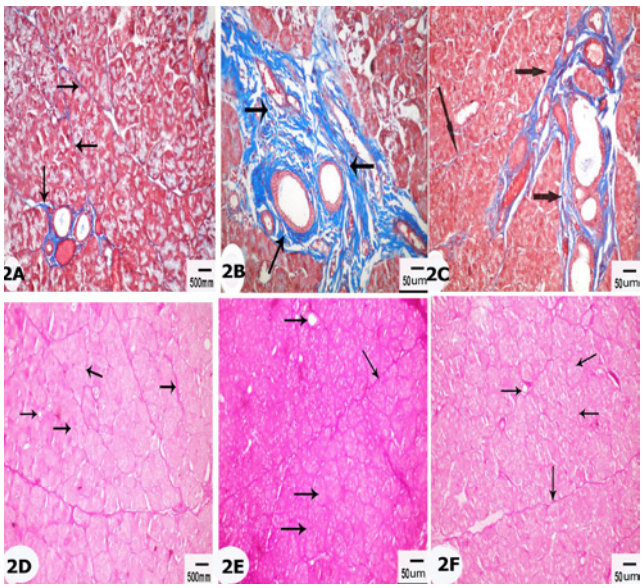

Figure 2: MT stained sections showing blue stained collagen fibers (arrows) in between the lobules, around interlobular ducts and blood vessels in control subgroup Ia (Figure2A), group II (Figure 2B) and group III (Figure 2C) X200. PAS stained sections showing positive magenta red PAS reaction in the cytoplasm and basement membrane of acinar and ductal cells (arrows) in control group (Figure 2D), strong reaction in group II (Figure 2E) and decreased reaction intensity in group III (Figure 2F) X200.

of group II (Figure 2B). Collagen fibers were apparently decreased in group III in comparison to group II (Figure 2C). Fibrosis of the gland was confirmed by morphometrical results (Table 1). Signs of inflammation as cellular infiltrations and fibrosis which detected after ELF-MF exposure is explained by increasing evidence for the immunologic role and oxidative stress in EMFs effect [24]. There is possibility that EMFs activated macrophages to become high secretory cells and release several factors, such as: interleukin-1, tumor necrosis factor, prostaglandins, ROS, lipid peroxides. These metabolites were injurious to other cells and their release was chemotactic for other cells as neutrophils or to lymphocytes. In addition, oxidative stress stimulates the expression of genes involved in collagen biosynthesis [25].

PAS stained sections (Figure 2) revealed strong positive reaction in acinar and ductal cells cytoplasm and basement membrane of group II (Figure 2E) in contrast to mild positive reaction that detected in group III (Figure 2F). These observations were confirmed with statistical analysis of optical density of PAS reaction (Table 1). Similar increase in PAS reaction was observed after EMFs exposure in different tissues [23-26] and explained by actual mucous transformation indicative of adaptive change taken by serous acini in response to the stressful effect induced by the EMFs with increased secretion of cortisol and accumulation of glycogen in the cells. This finding was confirmed by electron microscope finding of multiple electron lucent secretory granules in ELF-MF exposed group (Figure 3 B). Santoro et al. [27] found that EMFs interfere with protein phosphorylation and modify the plasma membrane structure and interfere with the initiation of the signal cascade pathways for protein synthesis. They also observed that decreased protein content may be due to ruptured cellular organoids. Affection of parotid gland secretion and decreased protein content was found in parotid gland adjacent to handheld mobile with RF-MF of higher frequencies [9].

Examination of the ultrathin sections (Figure 3) showed that serous acinar cells of the control parotid gland contain densely packed endoplasmic reticulum in addition to the electron dense secretory granules and other cytoplasmic organelles (Figure 3A). The striated and secretory ducts consist mainly of columnar cells with deep basolateral invaginations and intercellular interdigitations of the plasmalemma accompanied by numerous large, elongated mitochondria (Figure 3D). Exposure to ELF-MF induced degenerative change in acinar and duct cells. Many acinar and duct cells appeared apoptotic with irregular heterochromatic nuclei, dilated rough endoplasmic reticulum (RER) and electron lucent secretory granules (Figure 3B,3E). While parotid gland of vitamin E supplemented group showed that some acinar and duct cells were apparently normal. Other acinar cells were still affected (Figure 3C,3F).

The previous degenerative changes of parotid gland in light and

Groups / Parameters	group (I) X±SD Median	group (II) X±SD median	group (III) X±SD median	P value
Diagonal diameters of duct	20.19 ± 6.05 20.43	33.01± 6.02* 31.86	21.36 ± 2.6≠ 21.91	<0.001
Area % of collagen	7.08 ± 3.05 6.14	22.78 ± 5.72* 22.46	8.92 ±4.11≠ 9.06	<0.001
Optical density of PAS	0.52 ± 0.047 0.54	0.76 ± 0.025* 0.76	0.56±0.049≠ 0.56	<0.001

*significant with control
≠significant with group II

Table 1: Showing the changes in diagonal diameters of parotid duct, area % of collagen fibers and optical density of PAS reaction in all studied groups.

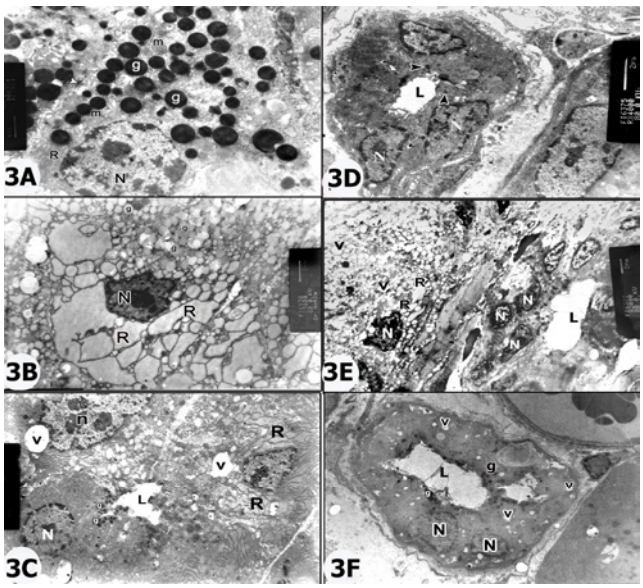

Figure 3: Ultrastructure photomicrographs showing control acinar cell with apical slectron dense secretory granules (Figure 3A X6000). Acinar cell of group II appears with irregular heterochromatic nucleus, dilated rough endoplasmic reticulum and electron lucent secretory granules (Figure 3B X 3000). Some acinar cells of group III contain irregular nuclei (n), dilated rough endoplasmic reticulum (R) and multiple vacuoles (v) (Figure 3C X 4000). Interlobular duct cells appears normal in control group (Figure 3D X4000) and group III (Figure 3F X5000) while epithelial cells lining interlobular ducts and acini of group II are seen with heterochromatic nuclei, electron dense granules and vacuoles (Figure 3E X2000) *(m) mitochondria, (g) secretory granules, (R) rough endoplasmic reticulum, (arrow heads) desmosomes, (v) vacuoles, (N) nucleus, (L) lumen.*

electron microscope examination could explained by selective damage of EMF to the plasma membrane of the secretory cells of the salivary glands and increase of Na+ influx into the cell followed by water which in turn causes distention of RER and appearance of cytoplasmic vacuoles with leakage of granules and subsequent lysis of acinar cells [21]. Also, free oxygen radicals that produced after exposure to MFs acting as oxidizing agents and able to increase cellular damage [28] and cause decomposition of membranes with leakage of damaging lysosomal hydrolytic enzymes [29]. Similar apoptotic changes were detected in liver cells after exposure to EMFs and explained by decreased tissue concentration of glutathione enzyme [30].

Similar protective role of vitamin E against EMFs induced oxidative stress was previously reported in different tissues [14,31]. The protection by vitamin E may also due to the reduction of lipid peroxidation. Thus, vitamins E inhibited the EMFs-induced tissue damage and supported the hypothesis that superoxide radicals are involved in its pathogenesis. Also, vitamin E caused a significant increase of antioxidant enzymes which decreased in EMFs exposed animals [32].

The previous results concluded that exposure to MF even at the lower frequency (ELF-MF) lead to variable degenerative changes in parotid salivary glands of adult male albino rats with relative limitation of these changes in vitamin E supplemented group. This may constitute an argument for further more complex studies on biological and health consequences of exposure to ELF-MF, to attest the necessity to change the actual safety international standards with special recommendation to study using vitamin E as a protective measure.

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