

Mobile Phone Electromagnetic Fields Affecting the Hepatocytes in the White Leghorn Chicken Embryo: An Ultrastructural Study

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This study was designed to measure the adverse the effects of electromagnetic waves on the hepatocytes in vivo. For this purpose, a developing chick embryo model was selected. 40 fertilized chick eggs were used. An egg incubator was used for egg development. In the exposed group, 20 fertilized eggs were exposed to mobile phone electromagnetic waves by placing a mobile phone inside the incubator in silent mode, while in the control, the mobile phone was removed. Mobile phone received calls from outside for 50 minutes in 24 hours. Liver was removed for electron microscopy. Control group revealed normal hepatocytes with big central nucleus, well-developed cristae in normal looking mitochondria, rounded nucleus, scattered ribosomes, few glycogen vacuoles and rough endoplasmic reticulum. Normal looking sinusoids lined by simple squamous epithelium and few Kupffer cells. Sinusoids were containing many nucleated RBC. On tenth day of development, exposed group revealed marked proliferation of mitochondria. At day 15, electron-dense mitochondria which were either swollen or dumbbell shaped and degenerated invisible cristae were apparent. Marked infiltration of fatty vacuoles and myelin-like figures in the cytoplasm, and derangement of classic hepatic lobule and sinusoids was observed. We conclude that electromagnetic waves affected the proliferation of hepatocytes in the chick embryo.

Keywords: Electromagnetic waves, hepatocyte, fatty change, mitochondria, lipid droplets, chick embryo

Smart phones have added a new dimension into our lives. We are more preoccupied with our mobiles than talking to persons seated next to us. Parents give mobiles to their two-year olds to play with instead of toys. Before traveling we make sure that the mobile charger is not left behind. Kids and teenagers spend more time playing with mobiles than playing outside. Do we know that we are risking our health if spending too much time with smart phones? Smart phones use

electromagnetic waves which are now believed to be harmful to living cells. WiFi is connected through electromagnetic waves when in use during calling and downloading data from the internet^{1,2}. Newly born are affected more due to the presence of larger number of embryonic stem cells^{3, 4,5,6,7}. Now a days a newly born is exposed to these waves soon after birth and will continue until he dies. Prenatal and postnatal cell phone exposure leads to behavioral problems in children^{8,9}.

Hypothesis of this research conducted at National University of Science and Technology, College of Medicine, is that the smart phone electromagnetic waves affect normal structure and function of liver cells in chick embryo model.

Objectives

To study the ultra-structure of the liver of the developing chick embryo in normal developing chick and compare it to liver exposed to mobile phone electromagnetic waves.

MATERIAL AND METHODS

Animal Experiment

Sohar poultry provided zero-day fertilized chicken eggs of breed 'Cobb' (*Gallus gallus domesticus*) for this experimental study. A strict pre-fixed inclusion and exclusion criteria was applied to these fertilized eggs. The chick embryo model was previously extensively used as an animal model to access the effects of electromagnetic waves¹⁰⁻¹⁹. Egg incubator Model EH-35, Sino-PFE Company, China, was made available for this experiment (Fig. 1a). All the eggs were placed in such a way that the farthest egg was within a radius of 16cm from the mobile phone placed inside the incubator. The temperature was set at 37 degrees, humidity at 50-60% and automatic egg rotation at ten rotations per day.²⁰ eggs were placed at one time in the egg holders. The experiment was performed twice and the specimens were sent for histological and electron microscopy preparations. It was partially blinded. The two groups will be randomly allotted a number of 20 eggs; control group and the experimental group. One incubator was used carrying 20 eggs at a time; one wave

consisted of an exposed group experiment and one wave of control group. The eggs were exposed electromagnetic waves of the mobile phone during egg development while eggs were not exposed to waves in the control group. The mobile company and mobile phone set used for this study was using 1800 MHz frequency, power of 0.47 W/kg body and SAR 1.10 w/KG (head). Electromagnetic exposure and strength was confirmed by using a TriField Meter, model 100XE (Fig. 1b).

Experimental Group

20 fertilized eggs were exposed to electromagnetic field inside the incubator when the mobile was rung from outside. To prevent noise interference, the mobile was kept in silent mode and vibration disabled. All the eggs were within one wavelength (approximately 16.5 cm) of the mobile phone electromagnetic waves 14. A schedule was made with calling times and duration. 10 calls were made each 24 hours; one-call duration was 5 minutes. Nights were calling free. 50 minutes of exposure were given s in 24 hours starting from day 1; total exposure time was 500 minutes at day 10 and 750 minutes at day 15. 10 eggs each were scarified at day 10 and 15. After making a small hole in the shell, it was carefully cut by scissors and the embryos were carefully dissected from the membranes after cutting the umbilical cord. Survivability was noticed by observing the beating heart and movements of limbs (Fig. 1c). After exposure the abdominal cavity the liver was removed and fixed in 10% glutaraldehyde solution for EM preparation.

Control Group

Same experiment was repeated with 20 eggs but the mobile phone was not placed inside the

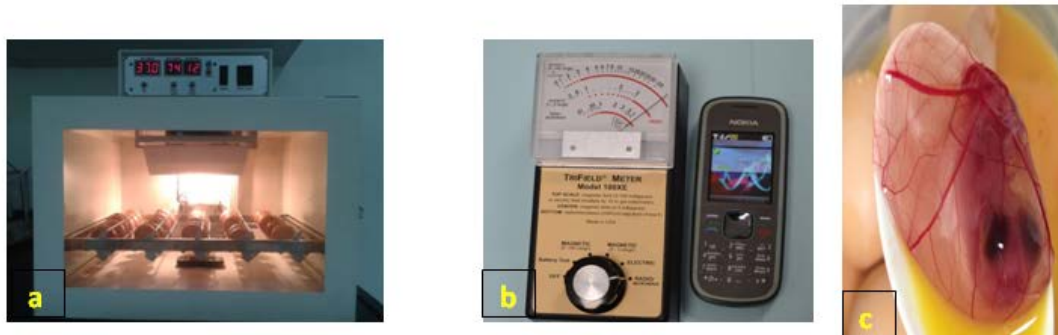


Fig. 1. a) Egg incubator b) Mobile phone in calling mode and the Trifield meter c) Developing chick embryo showing blood vessels and the membranes

incubator. The eggs were sacrificed at days 10 and 15, liver specimens were removed, placed in 10% glutaraldehyde solution for electron microscopic preparation.

RESULTS

There was no mortality observed in all the groups at day 10 and 15.

Control Group

Electron microscopic findings both at day 10 and 15, revealed rows of hepatocytes forming

hepatic lobules with lying in between the rows lined by simple squamous epithelium. (Fig.2). Hepatocytes well formed with a rounded nucleus in the center showing well developed chromatin. Nuclear double layered membrane with pores was clearly seen. Mitochondria with well-arranged cristae were also observed, surrounded by rough endoplasmic reticulum and free ribosomes. There were few glycogen vacuoles can be seen in the cytoplasm. Sinusoids can be seen in between the hepatocytes lined by simple squamous epithelium and Kupffer cells. Sinusoids were filled with RBCs

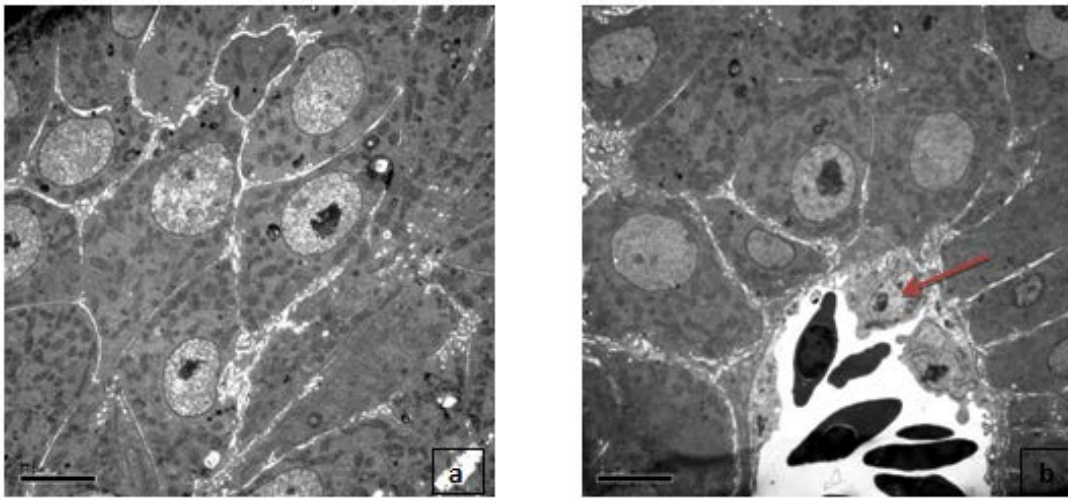


Fig. 2. Control group at day 10: a) well-formed hepatocytes with rounded nucleus, well-formed canaliculi in between the hepatocytes, b) Sinusoids lined by epithelial cells and kupffer cells (arrow) and showing well-formed RBCs in its lumen

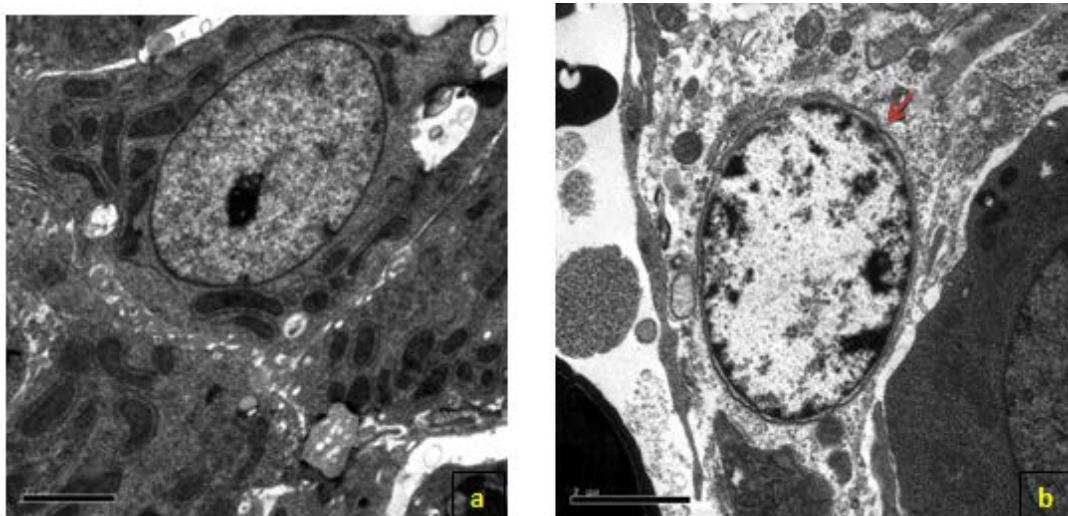


Fig. 3. Control Day 15 a) the hepatocytes showing oval mitochondria well-formed rounded nucleus, b) double layered nuclear membrane with nuclear pores (arrow)

having an oval nucleus. Canaliculi can also be seen in between the hepatocytes (Fig.3)

Exposed Group

Hepatocytes revealed marked increased in number of mitochondria at day 10, some of them were swollen and surrounded by rough endoplasmic reticulum (Fig.4a). At day 15, mitochondria became electron-dense, some were rounded while

others were elongated and dumbbell shaped. Few mitochondria and cristae showed degeneration. A prominent layer of rough endoplasmic layer around the mitochondria could also be seen. A rounded central nucleus could be seen clearly. The double layered nuclear membrane was blurred and pores were not clearly seen (Fig.5b). Few myelin-like figures were observed in the cytoplasm at day 15

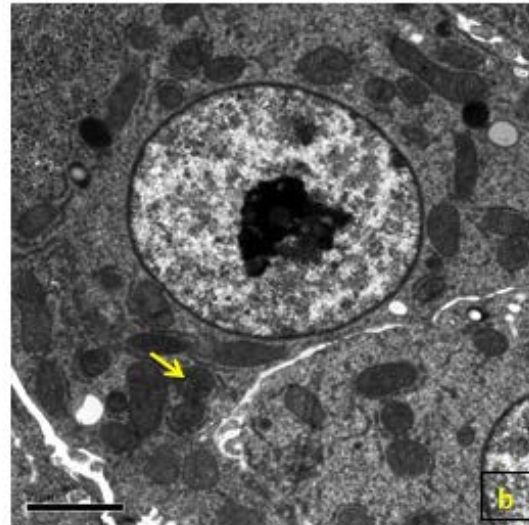
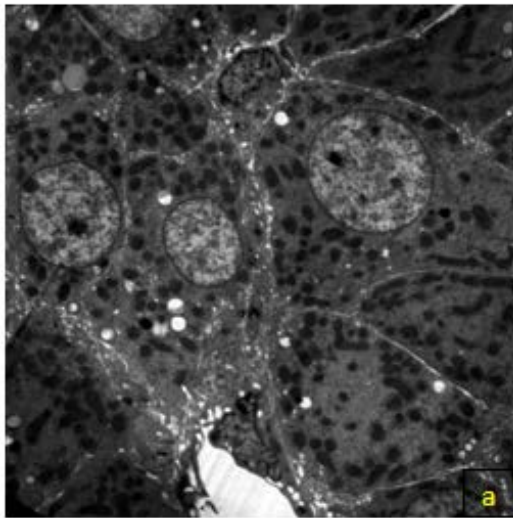


Fig. 4. a) Exposed group Day 10: Marked proliferation of mitochondria and infiltration of fat vacuoles in the cytoplasm of the hepatocytes. b) Mitochondria changed shape and become rounded, no change seen in the nuclei which remained rounded with intact bilaminar membrane

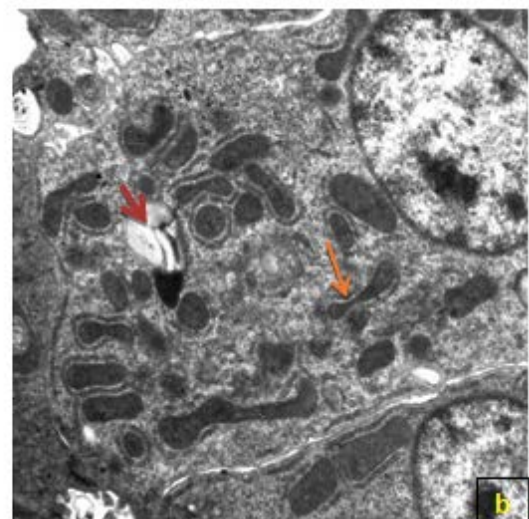
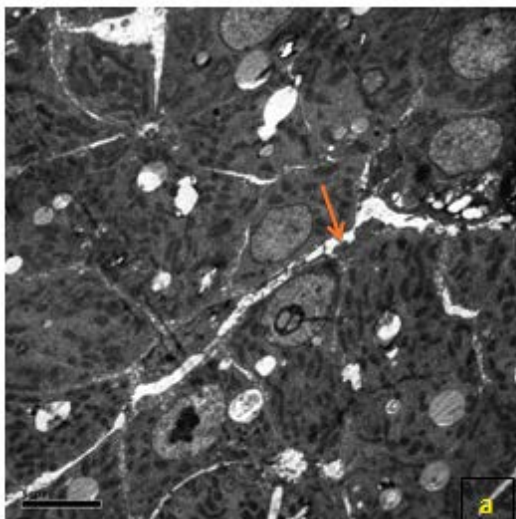


Fig. 5. Exposed group Day 15: a) marked infiltration of fat vacuoles was apparent, shape of the nucleus becomes slightly irregular, the double layer nuclear membrane cannot be seen clearly and pores were not seen. The canaliculi becomes widened (arrow) b) the mitochondria becomes dumbbell shaped (arrow) and myelin figures (short arrow) were seen

(Fig.5b). Lipid filled vacuoles increased in number from day 10 to day 15 and sinuses were dilated (Fig.5a).

DISCUSSIONS

Many studies used developing chick embryo to observe the effects of exposure of electromagnetic waves. Most of the studies studied gross morphological changes or histological effects on different tissues, however there are few ultrastructural studies. This is another study looking at the changes occurring inside the hepatocytes when exposed to radio waves. Liver is one of the organs which are affected very early in response to any external or internal stress. The radio waves are a source of external stress to the living cells. We have chosen day 10 and 15 day old chick embryo because most of the scientist has chosen these two days so it will be easy to compare with our study.

First observation was an increase number of mitochondria in the exposed group at both, day 10 and day 15. Oxidative stress induced by H₂O₂ in human lung is known to increase number of mitochondria and mtDNA¹⁵. Electromagnetic wave exposure after 10 days affected the mitochondria which became swollen. Mitochondrial membrane permeability is dependent on interaction between Ca²⁺ and ROS system, and an increased sympathetic activity is considered a primary cause of electromagnetic wave-induced calcium influx into the mitochondria¹⁶. Mitochondria swelling is caused by free O² produced by ROS inside the mitochondria to invade the thiol protein which produces transition pores in the mitochondrial membrane to open and cause increase permeability¹⁶. Attia *et al.* also reported mitochondrial in the study done on rat hepatocytes after electromagnetic exposure¹⁷. Voyedovodskata reported that paramagnetic particles are naturally located in the mitochondria of liver cells which make the hepatocytes sensitive to magnetic field exposure and leading to modification of haemostasis by altering mitochondrial respiration in the hepatocytes¹⁸. Moreover, Gorczynska and Wegrzynowicz reported that mitochondria are the most sensitive organole to stress-generating factors and observed mitochondrial swelling in such conditions¹⁹.

Most of the mitochondria became elongated; dumbbell shaped, and showed the process of degeneration at day 15 in the exposed group. Rough endoplasmic reticulum and free ribosomes were seen surrounding the mitochondria. Mitochondria are known for free radicals production in human sperm after electromagnetic wave exposure²⁰. Oxidative DNA damage may be due to electron leakage from the mitochondrial electron transport chain.

Lipid Droplets

Second Significant Observation was increase number of lipid filled vacuoles in the cytoplasm of the hepatocytes in the exposed groups. Disturbances in lipid inclusions and fat metabolisms were reported to increase the cytoplasmic vacuolation. In any pathological conditions, blockage of gluconeogenesis due to free radicals disturbs lipids and protein metabolisms and cause cell damage¹⁶. Rough endoplasmic reticulum surrounding the mitochondria may be damaged and fragmented and contributing in fat droplets accumulation.

Hepatocytes of the developing chick embryos revealed fatty changes after electromagnetic waves exposure which is due to oxidative stress when exposed to electromagnetic waves^{21, 22, 23}. The damage is dose dependent¹⁴. Similar results were reported by Lahijani *et al.*¹⁶. Similar results were also reported by other authors in rats and rabbits^{17, 24}. Radiation exposure may affect the breakdown of fat in the liver, similar to pregnancy, alcoholism, and malnutrition and poisoning. Fatty change is the initiation of damage to the hepatocytes, which is shown by an increase in the number of vacuoles filled with triglyceride fat. This is a sign of abnormal metabolism which may be due to an increase in Oxygen radicles species (ORS) production by the hepatocytes^{21, 22, 23}. Increase proliferation of mitochondria in hepatocytes and marked derangement of its internal structure in cardiac muscles is a sign of oxidative stress which was observed by other authors^{15, 20, 25}. Many authors have reported different effects of electromagnetic waves on the chick embryo which increases mortality of the developing chick embryo and resulted in malformations^{10-14, 26-30}.

Nuclei

Control group revealed rounded nuclei at both day 10 and 15. On the other hand, in the

exposed group, it was circular at day 10, but at day 15 the shaped became oval or irregular but no change was observed in chromatin. Double layered nuclear membrane and pores was clearing seen control groups, however, it has become blurred and the pores were not clearly seen in the exposed groups. Electromagnetic exposure leads to a damage of pores in the nuclear membrane, or it becomes irregular which may result in release of nuclear material into the cytosol¹⁶.

Different theories believe that mobile phone radiation produces reactive oxygen species (ROS) and DNA damage which was observed in human sperm. It also affects genes, cell membrane function and signal transduction^{31, 32, 33}. Rao *et al* recently provided new evidence that radio waves affect the plasma membrane³⁴. Radio waves also induce oxidative stress through NADH oxidase enzyme stimulation, which may be a major cause of various cellular adverse effects, observed in *in vitro* studies³⁵⁻⁴². As a consequence of increased levels of free radicals, various cellular and physiological processes can be damaged including gene expression, release of calcium from intracellular storage sites, cell growth, and apoptosis. Many scientist reported radio wave effects on genes resulting in signal transduction which resulted in alterations in membrane structure and function, and changes in metabolism associated with free-radical production^{40-41, 42}.

CONCLUSION

Electromagnetic wave exposure has induced many changes to the hepatocytes of chick embryo, especially mitochondria and nucleus, and increased fat deposition in the cytosol suggesting fatty change in the liver. Hence, it is scientifically documented that these electromagnetic waves are causing damage to living cells. Further studies should be carried out to fully understand the mechanism of this fatty change in hepatocytes mitochondrial damage when exposed to RFW.

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