Effect of Electromagnetic Mobile Radiation on Chick Embryo Development

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Abstract: The widespread use of mobile phones in the last decade has increased the concern about its potential effects on human body. This research aims to study the effect of electromagnetic waves emitted from mobile phones on chick embryos development. Fertile hen eggs were divided into two groups control and treated group. Both groups were incubated at 37.5 ◦C. The treated group had an active mobile device (900MHz- 1800MHz) during incubation. The mobile was rang 4 times daily for 15 minutes each time. Embryos were extracted on days 7, 10, and 14 of incubation. Congenital malformations were seen in treated embryos (bigger embryos, subcutaneous bleeding, and brain malformation) compared to the controls. Also increased eye growth in 7 and 10 days, significant increase in neural retina thickness in all studied ages, significant increase in retina lipid peroxidase and significant decrease in glutathione level in 10 and 14 days treated chick embryo retina compared to the controls. It was concluded that mobile phone electromagnetic waves (900MHz- 1800MHz) might induce embryonic eye growth till 10 days of incubation, and then it might cause brain malformation with reduced body and eye growth in chick embryo.
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Key words: mobile phones, chick embryo, eye development, retina thickness

1. Introduction

The number of mobile phone users worldwide is increasing tremendously. This led to increase the worries about possible health risks, related with mobile phone use and position near the body. Inhabitants living near mobile phone base stations. Suffered from; headache (23.5%), memory changes (28.2%), dizziness (18.8%), tremors (9.4%), depressive symptoms (21.7%), and sleep disturbance (23.5%) (Abdel-Rassoul *et al.,* 2007). Studies showed reproductive side effects caused by the use of mobile phones. Extremely low frequency electromagnetic field was able to reduce the fertilization rate in swine animal model, and negatively affect early embryo development. **(Bernabo *et al.*, 2010).** Some effects showed negative alterations of the nervous tissue in the brain and some sensory organs. Rapid cellular molecular alterations was seen in the rat brain after exposure to 900-MHz pulsed microwaves and power of 6 W/kg for 15-min. **(Bonnefont *et al.*, 2004).** Also electromagnetic waves emitted from mobile phones (900 MHz) reduced glutathione level in brain tissue and blood of exposed guinea pigs **(Meral *et al.*, 2007).** Electromagnetic field (EMF) emitted by a mobile phone with frequency 900 MHz caused derangement of chick embryo retinal differentiation **(Zareen, *et al.*,2009).** Low frequency electromagnetic fields caused chick embryos to have abnormal brain ventricles, spina bifida, eye malformation, and growth retardation **(Lahijani *et al.*, 2007).** The thermal effects of mobile phones on the eye induced cataracts, corneal edema, endothelial cells loss and retinal degeneration **(Vignal *et al.*, 2008).**

On the other hand electromagnetic waves were found to stimulate proliferation and differentiation of embryonic cells **(Parivar *et al.*, (2006).**

When using mobile phones the person is exposed to electromagnetic fields from the mobile phone and the work station. These electromagnetic fields might alter the cell structure beginning with the plasma membrane and its receptors to the different biomolecules present within the cell which might cause genotoxicity. This alteration might have its effect on cell proliferation in terms of increasing or reducing proliferation rate thus playing an important role during early embryonic development **(Panagopoulos, *et al.*, 2004, Zareen, *et al.*, 2009).**

Regarding the widespread of mobile phone use during pregnancy, it was very important to conduct series of studies to detect, analyze and understand the potential teratogenic effects of the use of mobile phones before and during pregnancy. Chick embryos were used as a model in this study for the ease of exposing them to a stable dose of EMF.

2. Material and Methods

This study was given approval for the methodology and other ethical issues concerning the work by Biology Department, – Science Faculty - King abdulaziz University.

Fertilized chicken eggs were obtained from a private home farm chicken raised by the researcher.

Average weight of eggs was (39.5) gm. The mobile phone used in the present study was on Extended Global System for Mobile Communication (EGSM) 900 MHz and 1800 MHz networks and Specific energy Absorption Rate (SAR) 2.0 watts/kilogram which is the limit stated by International Commission on Non-Ionizing Radiation (ICIRP) guidelines. Fertile hen eggs were incubated in an electrical incubator from Al-sloli foundation Kingdom of Saudi Arabia model number ME4A / ME4M in two batches. One for control group and the other for treated group. Each batch contained 30 eggs. Both were incubated under identical standard conditions temperature 37.5^{o}C, and suitable ventilation and humidity. For the treated group a mobile phone on silent was placed at the upper center of the eggs which were organized as a square circumference having the mobile at the center of the square. The distance between the mobile and eggs was ranging from 10.5 to 19 cm (.See Figure 1). The mobile phone was rang for 15 minutes every 6 hours daily from any other mobile phone, which is 60 minutes per-day. Embryos were collected from all groups at the following incubation days 7, 10 and 14. The embryos were extracted and washed in warm saline solution then they were dried and weighed, and photographed.

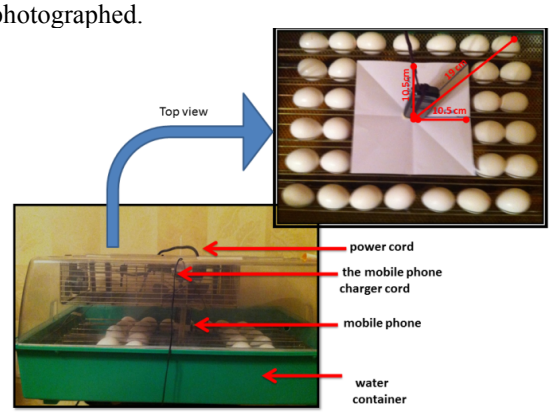

Figure 1: Showing the experimental set-up

At least 10 embryos from each group of each age were fixed in 10% formalin their eyes were removed for histological studies. The eyes were then dehydrated, cleared in xylene, embedded in soft paraffin wax and cut at 5µm thickness then stained by Haematoxylin and eosin (to examine the general structure of the retina), also 5 embryos of age 10 and 14 days of each group were frozen for Glutathione (GSH) and Lipid peroxide (LPO) assays.

Photographing:

Each embryo was photographed using a Lumix Panasonic camera as shown in Figure (2). a ruler was put near the embryo to be used as a scale when performing morphometrics using the photos. The camera zooming and distance between the camera and specimen was the same for all whole body photos. The head and left and right eye of each embryo were photographed using an Olympus SZx10 stereo microscope with DPZ-BSW camera.

Slides were photographed using a compound Olympus Bx51 microscope connected to an Olympus DP72 camera and a compound Nikon DS-Fil microscope connected to a Nikon ECLIPSC 80i camera.

Figure 2 : Showing the photographing methodology
Morphometric studies

Measurements of all alive control and treated specimens were taken. Full embryonic length measurements and head length were taken from the photos taken by the Lumix Panasonic camera using a computer program "Image tool" (http://ddsdx.uthscsa.edu/dig/itdesc). Length of the beak, and eye measurements, were taken by DPZ-BSW software. The measurements of the eye retina were taken from the histological slide photographs using Image tool program for measurements. See figure 3 for measurement method. At least 18 slides were done from 20 eyes of controls and each treatment. Each slide contained 3-4 sections. From each section 3 measurements were taken from 3 different areas. All readings were saved in Excel 2003.

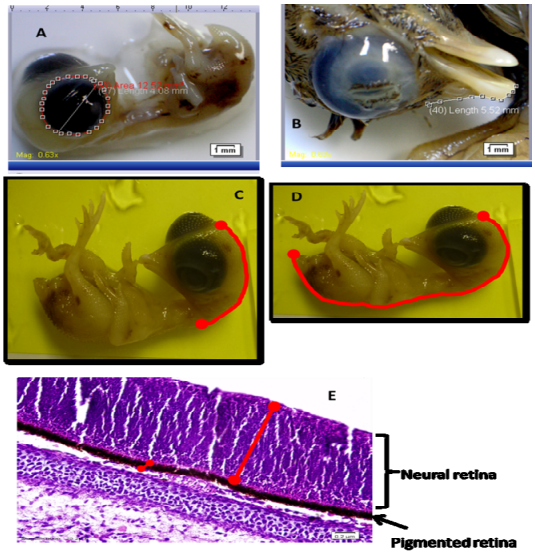

Figure 3: Showing morphometric measurements methodology. (A) eye measurements, (B) beak length, (C) head length, (D) whole body length, (E) retina thickness.

Glutathione (GSH) and Lipid peroxides (LPO) Assays:

Embryos of age 10 and 14 days were frozen immediately after extraction Eye retina of the frozen embryos was removed by making a slit at the back of each eye extracting the vitreous body and lens. The retina was then detached from the sclera. Retinas of the same age treatment were gathered till reaching the weight of 0.1 mg for each batch.

Reduced glutathione assay

Reduced GSH estimation was performed by the method of (Beutler *et al*., 1963). Retinas were homogenized in 1 ml of 1.1% KCl cooled, then homogenate (100 ll) was mixed with 750 ll of precipitate solution (1.67 g of glacial meta-phosphoric acid, 0.2 g of EDTA and 30 g of NaCl in 100 ml of D.W) and 900 ml of D.W. Homogenated tissue were centrifuged at 2000g for 15 min to precipitate proteins. Protein-free supernatant (250 ml) was added to 1 ml of Na^2 HPO4 (0.3 M) solution and the reaction was initiated by adding 125 ml of DTNB (6 mM) and the absorbance of 5-thio-2-nitrobenzoic acid (TNB) formed was measured at 412 nm. The level of GSH was obtained by standard curve and expressed as mmole per g eye tissue.

Lipid peroxidation assay:

The extent of LPO was estimated as the concentration of thiobarbituric acid (TBA) reactive product malondialdehyde (MDA) by using the method of Ohkawa *et al.* (1979). Two hundred fifty microliters of eye tissue homogenate were added to 1.5 ml of 1% phosphoric acid (pH 2.0) and 1 ml of 0.6% of TBA in air–light tubes and were placed in a boiling water bath for 25 min. After incubation, the sample was cooled to room temperature and MDA-TBA was extracted with 2.5 ml of butanol. Organic phase was separated by centrifugation for 5 min at 2000g and measured at 532 nm. MDA concentrations were determined using 1,1,3,3-tetraethoxypropane as standard and expressed as l mol/g eye tissue.

Statistical analysis:

Data was analyzed using SPSS 13. The test used with normal distribution was Anova, Student-Neuman Keul test. In case of abnormal distribution Man-Whiteney U test was used from the non parametric test. Significance was at $p<0.05$.

3. Results

Subcutaneous bleeding was seen in several treated embryos of age 7 and 10 days (Figure 4). The 14 days embryos were covered with feathers so it was not possible to examine them for subcutaneous bleeding. However some congenital malformations were seen in 14 days embryos these were; development of one eye or no eye development (anophthalmia),brain malformation, abdominal hernia and beak malformation(. See Figure 5).

An increase in whole body weight and whole body length in 7 and 10 days treated embryos was seen compared to the controls, however the increase was only significant in 10 days treated embryos (P=0.012) for both weight and length. On the other hand 14 day treated embryos showed a non significant decrease in whole body weight and whole body length compared to the controls (See Figure 6).

Treated embryonic beak length showed an increase in 7 day embryos compared to the controls, however the increase was not significant. On the other hand 10 and 14 days treated embryos showed a significant increase in embryo beak length P=0.049 for both compared to the controls (See Figure 6)

Treated embryo right and left eye growth parameters (eye weight, eye diameter, eye area, eye perimeter) showed an increase in 7 and 10 day embryos compared to the controls, however the increase was only significant in 10 days treated embryos (p= 0.000 for right and left eye weight, p= 0.041. p= 0.049 for right and left eye diameter respectively, p= 0.001. p = 0.000 for right and left eye area respectively, p=0.002, p= 0.007 for right and left eye perimeter respectively. On the other hand left and right eye growth parameters of 14 day treated embryos decreased non-significantly (See Figure 7).

Significant increase in neural retina thickness was seen in all studied ages 7,10, and 14 day of

treated chick embryo compared to the controls *P*=0.000. (See Figure 8)

No difference in pigmented retina thickness was seen in 7 and 10 day treated embryos. On the other hand 14 day treated embryos showed a significant increase in pigmented retina thickness compared to the controls *P*=0.020 (See Figure 8).

A significant decrease in retina glutathione was seen in treated embryos in days 10 and 14 of incubation compared to the controls *P*=0.000. See (Figure 9).

A significant increase in retina lipid peroxidation was seen in treated chick embryos in days 10 and 14 of incubation compared to the controls *P*=0.000. See (Figure 9).

On statistically comparing neural retina thickness of treated 7 day chick embryo and control 10 day chick embryo no significant difference was seen between neural retina thickness of treated 7 day chick embryo and control 10 day chick embryo *P*=0.63, on the other hand when statistically comparing between neural retina thicknesses of controls 7 day chick embryo and control 10 day chick embryo a high significant different was seen between neural retina thickness of control 7 day chick embryo and control 10 day chick embryo *P*=0.000.

Also a high significant difference was seen between neural retina thickness of treated 10 day chick embryo and control 14 day chick embryo. *P* = 0.000

An examination of the cross sections of eye Retina (R) in 7 day chick embryos in treated group showed that the thickness of the Neural zone (NZ), Ganglion Cell layer (GCL), and Optic Fiber layers (OFL), increased in treated compared to the controls. See (Figure 4.21-B).

An examination of the cross sections for eye Retina in 10 day chick embryos in treated group showed that

The neural retina in the treated embryos seemed to be thicker compared to the control. Rods and Cones were larger. Henl's membrane seemed to be disintegrated.

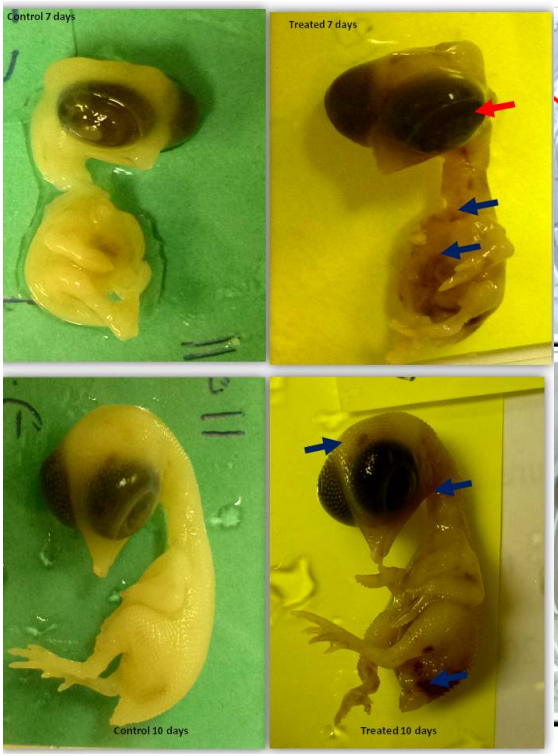

Figure 4: showing the sites of subcutaneous bleeding in 7 day (upper images) and 10 day (lower images) chick embryos (blue arrows), and the bigger eyes (red arrow)

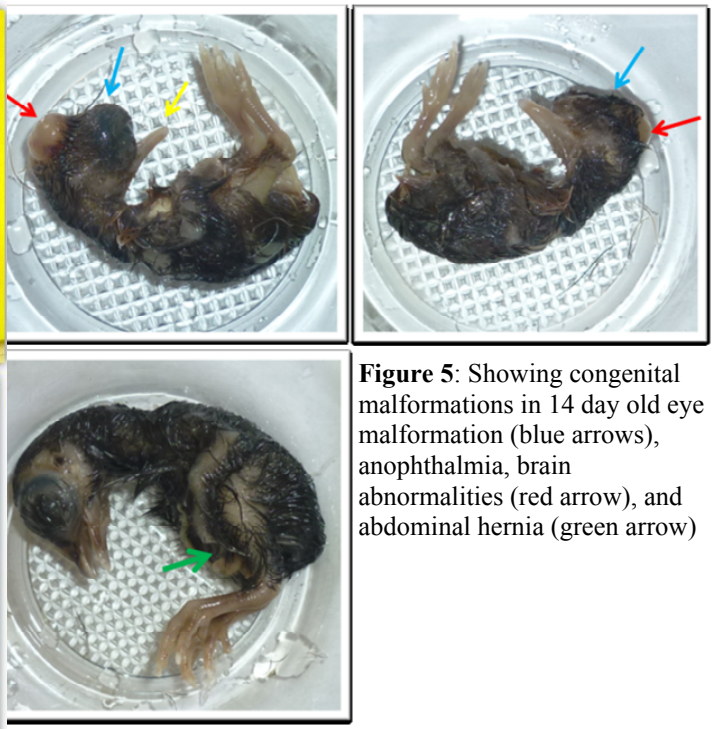

Figure 5: Showing congenital malformations in 14 day old eye malformation (blue arrows), anophthalmia, brain abnormalities (red arrow), and abdominal hernia (green arrow)

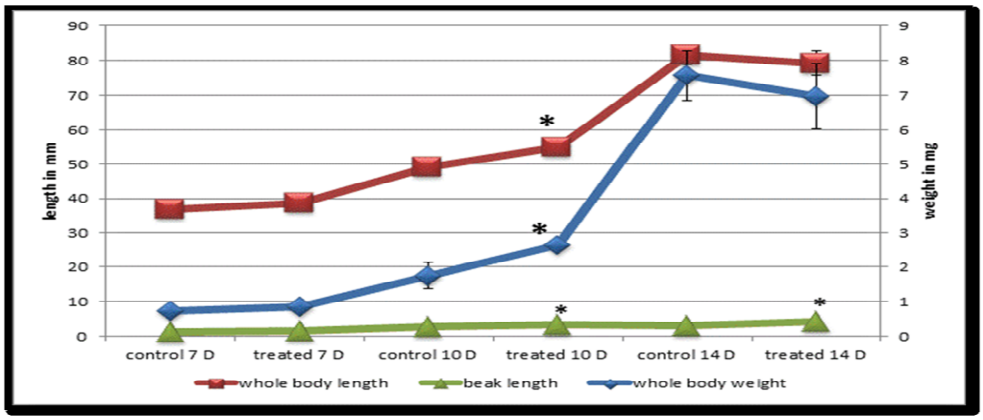

Figure 6: Graph showing the effect of mobile electromagnetic waves on chick embryo whole body length, whole body weight and beak length. Values are means ±SE, taken from 10 samples. For control and each treatment (*) $p< 0.05$

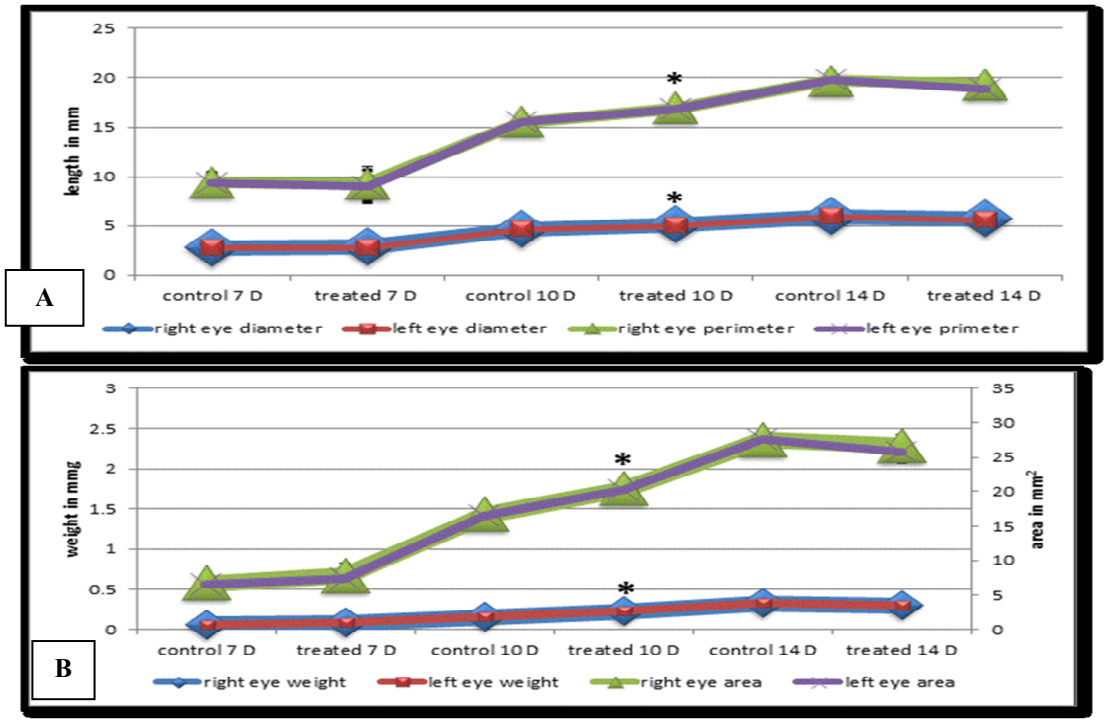

Figure 7: Graphs showing the effect of mobile electromagnetic waves on chick embryo right and left eye parameters. **(A)** Showing eye weight and eye area and **(B)** showing eye diameter and eye perimeter. Values are means ±SE, taken from 10 samples for control and each treatment (*) $p< 0.05$

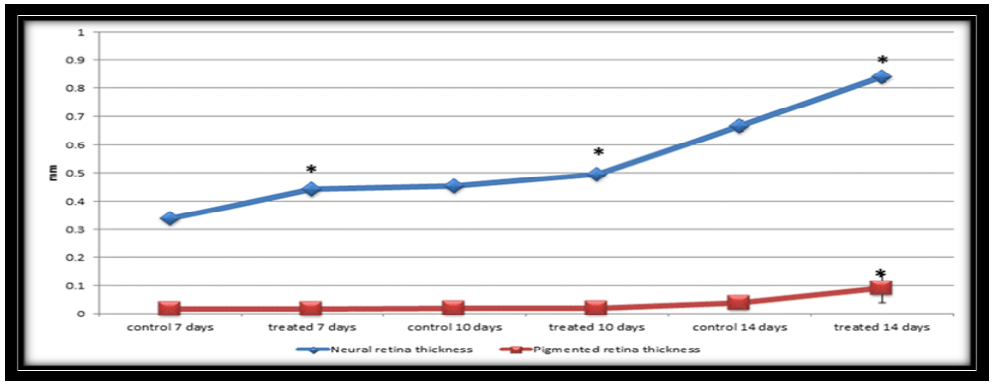

Figure 8: Graph showing the effect of mobile electromagnetic waves on chick embryo retina thickness. Values are mean ± SE (no of readings; 7 days 70, 10 days 52, 14 days 57(*) $p< 0.05$.

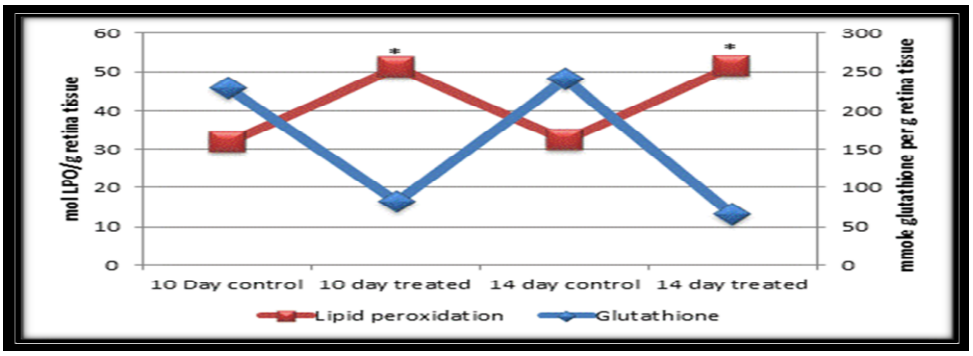

Figure 9: Graph showing the effect of mobile electromagnetic waves on chick embryo retina content of glutathione and lipid peroxidase. Values are mean ± SE (no of readings; 3 for each treatment taken from 5 embryos of each treatment. (*) $p < 0.05$

The Inner nuclear layer was less intensive. Inner Plexiform layer and ganglion cell layer seemed larger and the optic fiber layer seemed disintegrated compared to control See (Figure 10).

An examination of the cross sections of eye Retina (R) in 14 day chick embryos treated group showed that the neural retina in the treated seemed thicker compared to control. Also the rods and cones seemed more intensive compared to control. The inner nuclear layer seemed thicker and cells appeared disorganized. The Ganglion cell layer appeared also disorganized and thinner compared to control.

The optic fiber layer appeared disintegrated See (Figures 4.23-B; 4.24-B)

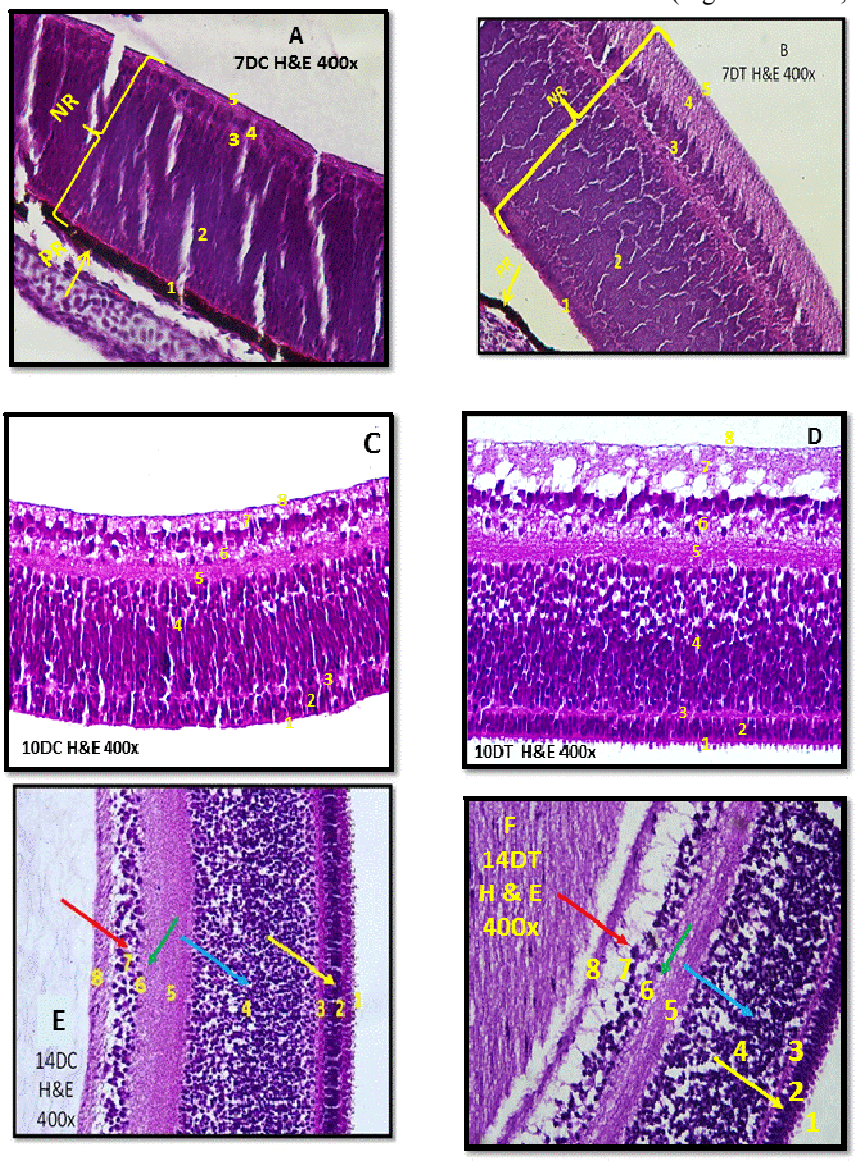

Figure (10): Histological sections of chick embryo retinas. (mag. 400X, H&E.)
(A) Control 7 day
(B) Treated 7 days, (PR) Pigmented Retina. (NR) Neural Retina. (1) External Limiting Membrane. (2) Neural Zone. (3) Ganglion Cell Layer. (4) Optic Fiber Layer. (5) Internal Limiting Membrane. Note that (NR) seems to be thicker and more differentiated on (B) compared to (A).
(C) Control 10 days
(D) treated 10 days
(E) control 14 days
(F) treated 14 days. (1)External Limiting Membrane. (2)Rods and Cones. (3) Henl's Membrane. (4) Inner Nuclear layer. (5)Inner Plexiform layer. (6)Ganglion Cell layer. (7) Optic Fiber layer. (8) Internal Limiting Layer. Note that rods and cones (2) are more differentiated in (D and F) compared to (C and E) (yellow arrow), layer (3) seems more distinct in (D and F) compared to (C and E) , layer (4) seems to have more spaces between cells in (D and F) compared to (C and E) (blue arrow), layers (5&6) seems to be thicker in (D and F) compared to (C and E) (green arrow), layer (7) in (D and F) seems to be disintegrated having many clear spaces compared to (C and E) (red arrow).

4. Discussion

The hazardous or beneficial biological effects of electromagnetic field on human and animals is the subject of many studies nowadays **(Lotifi *et al.*, 2012).** Mobile phone culture is spreading rapidly. The technology is quickly penetrating not only the lifestyles of adults, but is also becoming commonly used by children. **(Zareen *et al*., 2009).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900-1800 MHz during incubation period caused embryos to have different congenital malformation. Such as subcutaneous bleeding, anophthalmia, head abnormalities and abdominal hernia. Subcutaneous bleeding was reported in other studies. Several reports indicated harmful effects of 50-60 Hz electromagnetic waves on biological organs. Electromagnetic waves are able of causing hemorrhages, sinusoid denaturation, and an increase in lymphoidal tissues in white leghorn chick embryos exposed for 24 hour before incubation to 50-6- Hz. **(Lahijani *et al.*, 2011).**

Some of the abnormalities seen in this study were seen in other studies; **Lahijani *et al*., (2007)** studied the effect of electromagnetic waves with frequencies of 50 Hz on white leghorn chick embryo. Eggs were exposed for 24 hours before incubation. Results showed larger and abnormal brain structure, spina bifida, monophthalmia, microphthalmia, anophthalmia and growth retardation were observed in exposed embryos **(Lahijani *et al.*, 2007).** In this study anophthalmia was seen in 14 day embryos having abnormal brain tumors. Any malformation in the anterior part of brain may influence inductive activities of neural ectoderm **(Lahijani *et al.*, 2009).** Rapid cellular and molecular alterations in the rat brain were seen after exposure to mobile phone radiation **(Bonnefont *et al*., 2004).** Electromagnetic waves emitted by mobile phones are absorbed in the brain within range that could influence neuronal activity. Although the intensity of waves is very low, the oscillatory frequencies correspond to some of the oscillation frequencies recorded in neuronal tissue and could interfere with neuronal activity. **(Volkow *et al*., 2011).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 - 1800 MHz during incubation period caused increased growth in 7and 10 day embryos as seen through growth parameters (whole body weight, whole body length, beak length). Increased eye development was also seen (eye weight, eye diameter, eye area, and eye perimeter). This increase was significant on day 10 of incubation. Increase in growth usually indicates an increase in cell multiplication and, increase metabolism leading to an increase in glycogen consumption within the cell. Some studies showed greater embryonic growth and body mass in EMF exposed groups.

Parivar *et al*. (2006) reported a significant increase of cell division in the limb of mouse embryo due to electromagnetic waves 50Hz. **Volkow *et al*. (2011)** reported that electromagnetic waves of 0.901 W/kg emitted by mobile phones significantly increased brain glucose metabolism in the region closest to the antenna in human.

Ahijani and Ghafoori (2000) reported that there was no significant difference in measurement of body weight, length of crown to rump, length of tip of the beak to occipital bone, heart and liver weight to chick embryos exposed to 50Hz electromagnetic fields for 24hrs of before incubation. The difference between these results and the results of this study might be due to the difference in the electromagnetic field and the time of exposure, in the present study the electromagnetic field was higher and the time of exposure was more, which indicates that the field strength and exposure durance could be important factors determining the effect of electromagnetic radiation on chick embryos.

In the present study the extended exposure of chick embryo to electromagnetic waves 900-1800MHz till 14 days of incubation period caused a non-significant decrease in growth parameter. This might be due to different cellular responses to EMF during different embryological periods as cells might be trying to rebalance their growth and differentiation rate. **Batellier *et al*. (2008)** reported that chick embryo mortality of fertile eggs exposed to 900MHz was about 4.5% during the first 4 day of incubation, 1% from days 5 to 7, less than 1% from day 7 to 14, and 6.1% from day 18 to 21.

Zareen *et al.* (2009B) studied the effect of electromagnetic radiation emitted by mobile phones on chick embryos during incubation for 10 and 15 days. The eggs were exposed to different doses of mobile phone electromagnetic radiation. The exposure levels were as follows; Grade I: Low dose, short time duration exposure level, which resulted in significantly less weights and lengths. Grade II: High dose, short time duration exposure level, where weight increased. Grade III: Low dose, long time duration exposure level and Grade IV: High dose, long time duration exposure level.

Grade III and Grade IV resulted in significantly higher weight and length compared to the controls. This might be due to increased growth linked with higher dose of electromagnetic radiation. however it seems that The exposure grading might have been one of the methodological limitations of the study. As the amount of exposure was not mentioned. The study referred growth enhancement to several factors

such as EMF acting as soluble growth factors, or that EMF might be stimulating cell proliferation and differentiation and activating metabolic process, the study also mentioned that such growth could induce the probability of an uncontrolled neoplastic cellular proliferation induced **(Zareen, *et al.*, 2009).**

Lotifi *et al.* (2012) found that exposing chick embryos to 50 Hz electromagnetic field during the 21 day of incubation decreased significantly the amount of glucose in the blood serum of newly hatched chicks.

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 - 1800 MHz during incubation period caused a significant increase in embryonic retina lipid peroxidation and a significant decrease in glutathione level in days 10 and 14 of incubation compared to the controls. Electromagnetic waves of mobile phone may affect biological systems by increasing free radical (enhancing lipid peroxidation), and by changing the antioxidant defense system of tissue, thus leading to oxidative stress. Studies indicated that electromagnetic waves emitted by mobile phone of 900-MHz was associated with increased free radical production and lipid peroxidation levels and a decrease in glutathione level in blood and brain tissue in guinea pigs. **(Meral, *et al.*, 2007).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 and 1800 MHz during incubation period caused a significant increase in neural retina thickness in all ages of treated chick embryos compared to the controls. Many studies reported similar results under different exposures of electromagnetic radiation.

Zareen, *et al,* (2009A) reported that retinal development was delayed as a result of exposing chick embryos to 1800 MHz emitted by a mobile phone, however this delay was then transformed into a growth enhancement as the duration to EMF was expanded to 15 days of incubation. **Khaki *et al.* (2011)** studied the effect of electromagnetic waves with frequencies from 50 Hz to 60 Hz in rat. The experimental group was under electromagnetic waves for 4 weeks. Total retina thickness was significantly increased compared to the control.

In this study when statistically comparing the thickness of neural retina of treated 7 day chick embryo with that of control 10 day chick embryo no significant different was seen, on the other hand comparing control 7 day chick embryo with control 10 day chick embryo showed highly significant results P=0.000. This indicates that electromagnetic field EMF used in this study induced neural retina growth of 7 day treated chick embryo to reach that of control 10 day chick embryo.

This was not the case when comparing neural retina thickness of treated 10 day chick embryo and control 14 day chick embryo as there was a significant different, which indicates that between day10 and 14 embryo's neural retina did not response to EMF as did the neural retina of 7 day embryos. It might be that some factors were changed within the cells or cell environment.

This study showed that EMF seems to induce embryonic growth and differentiation during chick embryonic development till 10 days of incubation. The effect then turns to be depressive. More studies should be done on earlier development stages covering all aspects of developmental biology in order to understand the real effect of EMF on cellular behavior during early embryonic stages. There might be hope that EMF at might help regenerate loss body parts the induce cell dedifferentiation.

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