

# Histomorphometric Analysis of Chick Embryo Kidneys on Exposure to 1800 MHz and 2100 MHz Radiofrequency Radiation Emitted from Cell Phone

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## ABSTRACT

**Introduction:** With its sophisticated and multifunctional features, the cell phone has become an integral part of human life. Scientific reports are still inconclusive, regarding the possible ill effects of Radiofrequency Radiation (RFR) emitted from cell phones on biological tissues.

**Aim:** To evaluate the possible tissue damage in developing kidneys of chick embryos, following exposure to 1800 MHz and 2100 MHz radiofrequency radiation emitted from 2G and 3G cell phones.

**Materials and Methods:** This experimental study was conducted in Department of Anatomy at Mahatma Gandhi Medical College and Research Institute, Puducherry, India, from August 2011 to June 2015. Fertilised chick embryos (144±20 eggs) were divided into three groups with a sample size of 48 eggs per group. Group A was exposed to 2G radiation (1800 MHz), group B was exposed to 3G radiation (2100 MHz) and group C was a sham

exposed control group. The embryos were sacrificed from the 5<sup>th</sup>-12<sup>th</sup> day, and processed for routine histological procedures, to check structural and morphometric changes in the kidney. The standard epithelial height and nuclear diameter of both proximal convoluted tubule and distal convoluted tubule, karyorrhexis changes and diameter of urinary space were analysed using an ocular micrometer and square reticle. The results were statistically analysed using one-way Analysis of Variance (ANOVA).

**Results:** The results showed cytoplasmic changes (vacuolations) and nuclear changes (nucleomegaly, karyorrhexis) in proximal convoluted tubule and distal convoluted tubule, vascular changes (haemorrhage and infiltrations) in the interstitium and increased urinary space in the glomerulus of chick embryo kidneys.

**Conclusion:** Based on the study findings, it was concluded that RFR exposure from cell phones causes histopathological changes in the developing kidneys of chick embryos.

**Keywords:** Glomerulus, Interstitial oedema, Karyorrhexis, Oncosis, Standard epithelial height, Urinary space

## INTRODUCTION

Cell/mobile phones, a boon to the telecommunication industry, were first introduced in the form of analog cell phones in the 1980s. Since then, the cell phone industry has undergone tremendous growth with the introduction of 2G (900-1800 MHz), 3G (1900-2100 MHz), 4G (2300-2500 MHz) and recently rolled out 5G services (24 GHz-54 GHz) in as many as forty countries [1]. With the enforcement of various online activities like telework, online classes, online payment, online shopping, online gaming, to curb the spread of Coronavirus Disease-2019 (COVID-19) pandemic by countries, the number of cell phone users has increased exponentially in the past two years. At present, there are 7.26 billion cell phone users in the world, which translates to 91.69% of the world's population (current world population: 7.91 billion) [2].

Cell phones, while in use, emit non ionising Radiofrequency Radiations (RFR) which are absorbed into the user's body causing both thermal and non thermal stress [3,4]. Initially, RFRs considered being harmless, but with the increasing scientific evidence of the ill effects of RFRs on human health and various animal models, it has become a global concern in the past decades. Studies have shown an increased mortality rate and various congenital anomalies in chick embryos on RFR exposure in the frequency range of 900-1800 MHz emitted from Global System Mobile communication (GSM/2G) cell phones [5-7]. Exposure to a similar frequency also increased the rate of necrotic cells in blastocysts of mouse preimplantation embryos [8]. Various authors have reported histopathological changes in the liver, kidneys, eyes, and brain of different animal models on exposure to RFR emitted from both

second generation wireless telephony technology (2G) and third generation wireless telephony technology (3G) cell phones [9-14]. The role of RFR exposure ranging from 50-900 MHz causing altered antioxidant enzyme activities {Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX)} in biological tissues of different animal models resulting in oxidative stress is well established [15,16]. Various in-vitro and in-vivo studies have also shown increased Deoxyribonucleic Acid (DNA) damage in the form of DNA strand breaks and rearrangement of DNA segments on biological tissues of both animal models and human beings on exposure to RFR ranging from 900-1800 MHz [17,18].

Nevertheless, contradictory reports without any significant changes in the lens and retina of primate eyes on exposure to pulsed 60-1.25 GHz radiation and in the liver of mice models on exposure to 50 MHz electromagnetic fields, no effect on the level of antioxidants and nil DNA damage are also available [19-24].

The controversies in the medical literature regarding the possible negative impacts of RFR emitted from cell phones on both human beings and animal models coupled with growing concerns about RFR exposure on health among the general public and various ethical issues involved in human research, have prompted us to take up the present study. Furthermore, the existing literature shows no comparative studies on histopathological changes in kidneys to RFR exposure of different frequency ranges from cell phones. Hence, the present study was conducted, to determine the effect of RFR exposure from 2G (1800 MHz) and 3G (2100 MHz) cell phone on chick embryo kidneys and to compare and conclude which frequency range is more detrimental to developing kidneys.

## MATERIALS AND METHODS

This experimental study was conducted in Department of Anatomy at Mahatma Gandhi Medical College and Research Institute, Puducherry, India, from August 2011 to June 2015. The study followed all the procedures as per Ethical Guidelines for the care and use of experimental animals and was approved by Institutional Animal Ethical Committee (IAEC). Total (144±20) fresh fertile hen eggs *Gallus gallus domesticus* having similar weight (65-70±5 gms) were procured from Rajiv Gandhi College of Veterinary and Animal Sciences, Puducherry, India.

**Inclusion and Exclusion criteria:** Embryos that were healthy looking with a visible heartbeat and normal curvature were included in the study. Dead embryos, embryos showing congenital anomalies, and embryos without normal curvature were discarded.

The eggs were incubated in 12 batches of 12 eggs each, in a standard egg incubator (Standard digital incubator, Technico, India) at 37±0.5°C and 50-55% of humidity and ventilation. The fertilised hen eggs were grouped as A, B, and C with a sample size of 48 eggs per group.

- Group A was exposed to 2G cell phone radiation (900-1800 MHz)
- Group B was exposed to 3G cell phone radiation (1900-2100 MHz)
- Group C was treated as a sham exposed control group.

### Study Procedure

The incubation of the eggs was carried out in a chamber fitted with a popular brand of cell phone {Specific Absorption Rate (SAR) of 0.310 watts/kilogram, power-2 watts}. The cell phone was hung at a distance of 5 cm from the eggs. For group C, the cell phone was kept in switched-off mode, whereas, for group A and B, the cell phone was kept in silent operative mode with the headphone plugged in, for its automatic activation while receiving a call. The service provider network was the same for both 2G and 3G exposure and the intensity of radiofrequency waves was measured using a radiofrequency meter (RF meter, Less EMF Inc. USA) that was kept inside the incubator.

The RFR exposure was initiated at the 12<sup>th</sup> hour of incubation by ringing from another cell phone. This was repeated every half an hour with the duration of the call being three minutes each. Thus, the total exposure for 12 hours was 72 minutes followed by 12 hours of exposure free period. The embryos were thus exposed to RFR up to the 12<sup>th</sup> day of incubation and six embryos per day were terminated from the 5<sup>th</sup>-12<sup>th</sup> day [9].

The chick embryos were fixed in 10% formaline and processed for routine histological study. A 5 µm thick sections were cut using a rotary microtome (Radical Senior Precision Rotary microtome, model: RMT-30, India) from prepared paraffin blocks in the sagittal plane, coronal plane, and transverse plane and stained with Haematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) stain (Merck, Germany).

**Histomorphometric analysis:** The following indices were observed of both Proximal Convoluted Tubule (PCT) and the Distal Convoluted Tubule (DCT)

- Standard Epithelial Height (SEH): The standard epithelial height of both PCT and DCT was measured from the basement membrane to the tubular lumen under 400x magnifications using a Trinocular research microscope fitted with microphotographic attachment (Olympus, model CH20i, Japan).
- Nuclear diameter: For the measurement of the nuclear diameter, healthy looking nuclei showing prominent nucleoli were measured under oil immersion.
- Diameter of urinary space of glomerulus: The outer diameter (A) and inner diameter (B) of the glomerulus were measured and the urinary space was calculated as A-B. All the measurement was taken from every third section of each slide using a calibrated ocular micrometer (10 mm scale: Erma, Model OM-010, India). Twenty random fields were selected from each embryo.

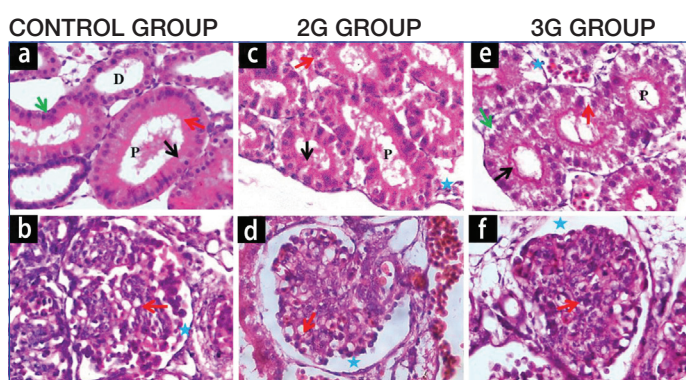
- Nuclear fragmentation (karyorrhexis): The number of nuclei showing karyorrhexis was counted in a randomly selected field using an eyepiece grid reticle of 0.5 mm squares containing 400 squares (Radical, India) under oil immersion from every third section of each slide [25]. Randomly selected 50 fields were analysed for each embryo in control and experimental groups.

## STATISTICAL ANALYSIS

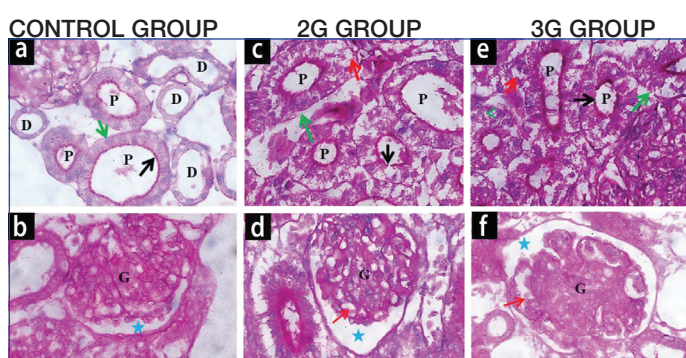
The observed data were subjected to one-way Analysis of Variance (ANOVA) and the significance was determined using a Turkey's post-hoc with p-value <0.05 for statistical significance. The statistical tests were done using the software Graph Pad Instat 3. All the data were expressed as Mean±SEM (Standard Error of the Mean).

## RESULTS

**Histopathological changes:** The kidneys of the control group embryos showed well-developed mesonephric tubules with normal structural features. The lining epithelial cells of both PCT and DCT showed intact basement membrane and brush border in PCT. The cells showed, fewer cytoplasmic vacuolations and relatively fewer nuclear changes in the form of nuclear fragmentation (karyorrhexis) and pyknosis [Table/Fig-1a,2a]. The glomerulus appeared normal with intact cells lining the parietal and visceral layer, with a well-developed glomerular capillary network [Table/Fig-1b,2b].



**[Table/Fig-1]:** Photomicrograph showing kidney of control, 2G and 3G group embryos (12<sup>th</sup> day) (H&E, 1000X). a): Shows control group embryos with normal cells lining PCT (red arrow) with intact brush border (black arrow) and basement membrane (green arrow). b): Shows the glomerulus of control group embryos with intact capillary plexus (red arrow) and normal urinary space (blue asterisk). c) and e) Shows vacuolations (red arrow) in PCT (P), disruption of brush border (black arrow), disruption of basement membrane (green arrow), and interstitial edema with infiltrations (blue asterisk) in both 2G and 3G group embryos. d and f): Shows a distorted glomerular capillary network with endothelium showing vacuolations (red arrow) and increased urinary space (blue asterisk) for 2G and 3G group embryos respectively.



**[Table/Fig-2]:** Photomicrograph showing PCT (P) and glomerulus (G) of control, 2G, and 3G group embryos (12<sup>th</sup> day) (PAS, 1000X). a): Shows normal looking PCT with intact brush border (black arrow) and basement membrane (green arrow) in control group embryos. b): Shows Glomerulus with intact capillary plexus with normal urinary space (blue asterisk); c and e): Both 2G and 3G group embryos showed vacuolations (red arrow) in PCT, disruption of brush border (black arrow), disruption of basement membrane (green arrow); d and f): distorted glomerular capillary network with endothelium showing vacuolations (red arrow) and increased urinary space (blue asterisk) (for 2G and 3G group embryos respectively).



However, 2G and 3G exposed embryos showed moderate to intense histopathological changes, in the form of increased vacuolations in the cytoplasm of lining cells, disruption of the luminal border and basement membrane of PCT. Cellular debris appeared in the lumen, due to the sloughing of lining cells [Table/Fig-1c,e,2c,e]. The nuclei showed increased pyknosis and karyorrhexis in comparison with the control. The intensity of these changes increased as the age advanced. The cuboidal cells lining the DCT and collecting ducts appeared normal during the initial days of incubation. However, similar histopathological changes as that of PCT were observed in DCT in advanced age groups. Moreover, inflammatory changes in the interstitial spaces, diffused interstitial oedema with lymphocytes, plasma cells, monocytes, macrophages and haemorrhagic changes were also observed [Table/Fig-1c,e,2c,e]. The glomerulus of both exposed groups showed degenerative changes with distorted glomerular capillary network and increased urinary space [Table/Fig-1d,f,2d,f].

**Histomorphometric changes:** The SEH of lining columnar cells of PCT showed a gradual increase as the age advanced in all three groups. The epithelial height was found to be significantly increased for both 2G group embryos (p-value <0.001) and 3G group embryos (p-value <0.001) when compared with control group embryos. On comparing 2G and 3G embryos, the 3G embryos showed increased height (p-value <0.01) [Table/Fig-3].

Age (days)	Group C (mm)	Group A (mm)	Group B (mm)
5	0.007±0.0000	0.007±0.0002	0.008±0.0000 <sup>b,c</sup>
6	0.007±0.0000	0.007±0.0001	0.009±0.0000 <sup>b,c</sup>
7	0.008±0.0000	0.010±0.0001 <sup>a</sup>	0.010±0.0001 <sup>b</sup>
8	0.009±0.0000	0.010±0.0001 <sup>a</sup>	0.011±0.0001 <sup>b</sup>
9	0.009±0.0001	0.011±0.0001 <sup>a</sup>	0.012±0.0001 <sup>b,c</sup>
10	0.011±0.0001	0.012±0.0001 <sup>a</sup>	0.013±0.0001 <sup>b</sup>
11	0.012±0.0001	0.012±0.0001	0.013±0.0001 <sup>b,c</sup>
12	0.0121±0.0000	0.013±0.0001 <sup>a</sup>	0.013±0.0001 <sup>b,c</sup>

**[Table/Fig-3]:** Mean height of lining cells of PCT in all the three groups of chick embryo.

Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>b</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>c</sup>significantly increased on comparing between Group A and Group B (p-value<0.01). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test

The SEH of lining cuboidal cells of DCT showed a gradual increase in height as the age advanced in all three groups. The epithelial height was found to be significantly increased in 2G (p-value <0.001) and 3G group embryos in comparison with the control group (p-value <0.001). On comparing 2G and 3G embryos, the 2G embryos showed increased height that was significant on the 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> day (p-value <0.05, p-value <0.05, and p-value <0.001 respectively) and the 3G group embryos showed significantly increased height on the 12<sup>th</sup> day of incubation (p-value <0.001) [Table/Fig-4].

Age (days)	Group C (mm)	Group A (mm)	Group B (mm)
5	0.003±0.00004	0.0049±0.00006 <sup>a,c</sup>	0.0045±0.00004 <sup>b</sup>
6	0.004±0.00005	0.0049±0.00006 <sup>a,c</sup>	0.0047±0.00005 <sup>b</sup>
7	0.0045±0.00007	0.0052±0.00007 <sup>a,c</sup>	0.0048±0.00008 <sup>b</sup>
8	0.0046±0.00007	0.0053±0.00005 <sup>a</sup>	0.0052±0.00004 <sup>b</sup>
9	0.0048±0.00003	0.0053±0.00009 <sup>a</sup>	0.0052±0.00004 <sup>b</sup>
10	0.0048±0.00005	0.0060±0.00007 <sup>a</sup>	0.0061±0.00006 <sup>b</sup>
11	0.0049±0.00006	0.0062±0.00006 <sup>a</sup>	0.0061±0.00005 <sup>b</sup>
12	0.0057±0.00004	0.0058±0.00006	0.0063±0.00007 <sup>b,c</sup>

**[Table/Fig-4]:** Mean height of lining cells of DCT in all the three groups of chick embryo.

Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>b</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>c</sup>significantly increased on comparing between Group A and Group B (p-value<0.01). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test

The nuclear diameter of PCT increased gradually in all three groups with advancing age. The diameter was significantly increased in both the 2G and 3G exposed groups than in the control group (p-value <0.001). In the 3G group, embryos showed an increased diameter that was more significant (p-value <0.05) than in the 2G group [Table/Fig-5].

Age (days)	Group C (mm)	Group A (mm)	Group B (mm)
5	0.0037±0.00004	0.0039±0.00005 <sup>a</sup>	0.0041±0.00004 <sup>b,c</sup>
6	0.0038±0.00003	0.004±0.00004 <sup>a</sup>	0.0042±0.00004 <sup>b,c</sup>
7	0.0038±0.00003	0.0041±0.00003 <sup>a</sup>	0.0042±0.00004 <sup>b</sup>
8	0.0039±0.00002	0.0041±0.00002 <sup>a</sup>	0.0042±0.00003 <sup>b,c</sup>
9	0.0040±0.00002	0.0041±0.00003 <sup>a</sup>	0.0043±0.00004 <sup>b,c</sup>
10	0.004±0.00001	0.0042±0.00006 <sup>a</sup>	0.0043±0.00004 <sup>b</sup>
11	0.0041±0.00002	0.0042±0.00004 <sup>a</sup>	0.0044±0.00005 <sup>b,c</sup>
12	0.0042±0.00004	0.0045±0.00004 <sup>a</sup>	0.0046±0.00004 <sup>b</sup>

**[Table/Fig-5]:** Mean nuclear diameter of lining cells of PCT in all the three groups of chick embryo.

Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>b</sup>significantly increased on comparing with Group C (p-value<0.001), <sup>c</sup>significantly increased on comparing between Group A and Group B. (p<0.05). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test.

The nuclear diameter of DCT of all three groups showed a gradual increase in diameter as the age advanced. The 2G embryos showed an increased diameter than the control group embryos (p-value <0.05). The 3G group also showed an increased nuclear diameter on comparing with the control group and the increase was significant only on the 5<sup>th</sup> and 6<sup>th</sup> days (p-value <0.05). On comparing 2G and 3G groups, the 2G group embryos, showed a significant increase in diameter (p-value <0.05) [Table/Fig-6].

Age (days)	Group C (mm)	Group A (mm)	Group B (mm)
5	0.0035±0.00004	0.0038±0.00003 <sup>a,c</sup>	0.0036±0.00004 <sup>b</sup>
6	0.0036±0.00004	0.0038±0.00003 <sup>a</sup>	0.0037±0.00004 <sup>b</sup>
7	0.0037±0.00004	0.0039±0.00004 <sup>a</sup>	0.0038±0.00005
8	0.0038±0.00003	0.0039±0.00002	0.0038±0.00003
9	0.0039±0.00002	0.0040±0.00002 <sup>a,c</sup>	0.0038±0.00004
10	0.0039±0.00001	0.0040±0.00004 <sup>c</sup>	0.0039±0.00002
11	0.004±0.00003	0.0041±0.00003 <sup>a,c</sup>	0.0040±0.00001
12	0.0041±0.00003	0.0041±0.00003	0.0041±0.00003

**[Table/Fig-6]:** Mean nuclear diameter of lining cells of DCT in all the three groups of chick embryo.

Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup>significantly increased on comparing with Group C (p-value<0.05), <sup>b</sup>significantly increased on comparing with Group C (p<0.05), <sup>c</sup>significantly increased on comparing between Group A and Group B (p-value<0.05). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test

The urinary space showed a gradual increase in diameter as age advanced in all three groups. Both the 2G and 3G groups showed a statistically significant increase in the urinary space on all days as compared with the control group embryos (p-value <0.001 and p-value <0.001 respectively). On comparing 2G and 3G groups, 3G group embryos showed an increased diameter of urinary space that was significant on all days (p-value <0.05) [Table/Fig-7].

In both 2G and 3G group, embryos showed increased karyorrhexis than the control group embryos (p-value <0.05 and p-value <0.01 respectively). On comparing between 2G and 3G group, the 3G group showed increased karyorrhexis than the 2G group, however, the increase was significant only on the 5<sup>th</sup>, 6<sup>th</sup>, and 12<sup>th</sup> day (p-value <0.001) [Table/Fig-8].

## DISCUSSION

Kidneys are moderately sensitive to radiation. The effects of radiations were observed in convoluted tubules and glomerulus in

Age (days)	Group C (mm)	Group A (mm)	Group B (mm)
5	0.007±0.0002	0.011±0.0003 <sup>a</sup>	0.013±0.0004 <sup>b,c</sup>
6	0.007±0.0002	0.011±0.0005 <sup>a</sup>	0.015±0.0005 <sup>b,c</sup>
7	0.008±0.0003	0.013±0.0004 <sup>a</sup>	0.016±0.0005 <sup>b,c</sup>
8	0.008±0.0002	0.015±0.0003 <sup>a</sup>	0.017±0.0003 <sup>b,c</sup>
9	0.009±0.0003	0.015±0.0006 <sup>a</sup>	0.018±0.0005 <sup>b,c</sup>
10	0.009±0.0003	0.015±0.0005 <sup>a</sup>	0.019±0.0005 <sup>b,c</sup>
11	0.010±0.0004	0.018±0.0005 <sup>a</sup>	0.020±0.0004 <sup>b,c</sup>
12	0.010±0.0004	0.018±0.0003 <sup>a</sup>	0.020±0.0008 <sup>b,c</sup>

**[Table/Fig-7]:** Mean diameter of glomerular space in all three groups of chick embryo. Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup> significantly increased on comparing with Group C (p-value <0.001), <sup>b</sup>significantly increased on comparing with Group C (p-value <0.001), <sup>c</sup>significantly increased on comparing between Group A and Group B (p-value <0.05). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test

Age (days)	Group C (number/sq.unit)	Group A (number/sq.unit)	Group B (number/sq.unit)
5	0.74±0.10	0.94±0.08	1.72±0.16 <sup>b,c</sup>
6	0.529±0.08	0.711±0.10	1.5±0.11 <sup>b,c</sup>
7	0.549±0.10	0.862±0.09	0.96±0.09 <sup>b</sup>
8	1.163±0.06	1.66±0.09 <sup>a</sup>	1.89±0.08 <sup>b</sup>
9	0.74±0.09	1.14±0.10 <sup>a</sup>	1.28±0.11 <sup>b</sup>
10	0.86±0.12	1.1±0.10	1.38±0.10 <sup>b</sup>
11	0.58±0.11	1.04±0.11 <sup>a</sup>	1.08±0.10 <sup>b</sup>
12	0.943±0.12	1.22±0.08	1.88±0.09 <sup>b,c</sup>

**[Table/Fig-8]:** Karyorrhexis in nuclei of kidney in all three groups of chick embryo. Values are means±SEM taken from six samples per day for Group C (control), Group A (2G) and Group B (3G) (n=48 chick embryos). <sup>a</sup>significantly increased on comparing with Group C (p-value <0.05), <sup>b</sup>significantly increased on comparing with Group C (p-value <0.01), <sup>c</sup>significantly increased on comparing between Group A and Group B (p-value <0.001). Significance determined by one-way ANOVA, followed by Tukey's post-hoc test

the form of cytoplasmic changes, nuclear changes, and vascular changes [26].

The histopathological changes observed in the PCT and DCT of the present study were similar to the observations made by researchers in mice exposed to RFR (900 MHz) from the mobile phone for variable exposure duration [27-29]. Al-Glaib B et al., (2008), reported glomerular atrophy, interstitial infiltrations, and vacuolations in renal tubules by exposing mice to 900 MHz RFR for a duration of one hour per day for 10 days [27]. A similar study by Hanafy LK et al., also reported glomerular atrophy and extravasated blood cells between renal tubules on RFR exposure of one hour per day for four weeks in rat models [28]. Khayyat LI exposed mice for a longer duration of eight hours for three days and 12 days and their findings showed atrophied glomeruli, cytoplasmic vacuolations and pyknosis of lining epithelial cells of the renal tubules, renal vein congestion and inflammatory changes between the renal tubules [29]. Ingole IV and Ghosh SK, reported similar changes in PCT with narrowed bowman's space of renal corpuscles in chick embryos on exposure to RFR of 900 MHz for four hours, five hours, and six hours for six, eight and 10 days [11].

In the present study, DCT showed isolated vacuolations; in PCT, the vacuolations were present in almost entire cells lining the tubules indicating necrotic changes. The vacuolations in the cytoplasm might be due to fatty metamorphosis or steatosis as they were PAS negative [Table/Fig-2c,e] [30].

The oedema and presence of RBCs in the interstitium of kidney tissue observed might be due to vascular damage caused by RFR. Exposure to RFR causes vascular dilatation, and vacuolations of the endothelium of peritubular capillaries with focal necrosis, resulting in haemorrhage and exudation of blood plasma containing variable infiltrates of lymphocytes, plasma cells, monocytes and macrophages. These changes were similar to the changes observed in acute interstitial nephritis [26,31]. Interstitial oedema in the kidney

tissue after RFR exposure was also reported by Al-Glaib B et al., Hanafy LK et al., and Khayyat LI [27-29].

The radiations are known to cause nuclear swelling (nucleomegaly), mild to moderate pyknosis, karyorrhexis (nuclear fragmentation), and karyolysis (dissolution of chromatin) [26]. In the present study, karyorrhexis and changes in nuclear diameter were significantly increased in 3G group embryos due to the lethality of 3G radiation.

Another observation was the increase in SEH of lining cells in PCT and DCT of both 2G and 3G group embryos as compared with control group embryos. RFR emitted from cell phones caused degeneration or weakening of plasma membrane due to non thermal effects [32,33]. This would have resulted in an increased influx of water (hydropic changes/oncosis) resulting in cellular swelling (cytomegaly) [26,30]. The 3G group embryos showed significantly increased SEH in PCT and on the 12<sup>th</sup> day in DCT. This probably might be due to the increased lethality of RFR emitted from the 3G cell phone.

The increased glomerular diameter observed, might be due to cytoplasmic changes caused by RFR. Exposure to RFR would have resulted in hydropic changes causing cytomegaly of cells, lining the parietal and visceral layer of glomerulus and endothelial cells lining the glomerular capillaries, which in turn resulted in an increase in glomerular diameter [26,30]. Moreover, the endothelial cells lining the blood vessels are highly sensitive to radiation effects and it manifests as vasodilatation and increased vascular permeability. In present study, these changes in glomerular capillaries, caused the plasma exudate to escape into the urinary space resulting in increased urinary space [26,31]. This observation was similar to the findings of Mugunthan N et al., who reported dilated glomeruli and urinary space in mice kidneys exposed to RFR of 900-1900 MHz for 48 minutes per day for a period of 30-180 days [12]. However, few studies reported glomerular atrophy on RFR exposure in rat and mice models as the duration of exposure was 3-12 days [27-29,34].

Hence, the effect of RFR exposure manifests differently, depending on the duration of exposure, frequency, and intensity of transmission, the number of exposures, the shape and size of the exposed organism, the water and mineral content of the organism, and the distance from the radiation source [35,36].

### Limitation(s)

Direct extrapolation of results of the present study on chick embryos to the human population may be limited, as the volume and size of kidneys, the architecture of renal tissue, tissue electromagnetic properties, penetration and interaction of RFR into the body might vary from one species to another. The present study was done only from 5<sup>th</sup>-12<sup>th</sup> day (organogenesis period) of incubation. Further study on prolonged exposure (growth and development period) is recommended.

### CONCLUSION(S)

Based on our experimental outcome, we conclude that the chronic exposure of chick embryo kidneys to RFR emitted from 2G and 3G cell phones resulted in histopathological and histomorphometric changes in kidney structures. Both the exposed groups showed increased cytoplasmic vacuolations, disrupted luminal border and the basement membrane, increased epithelial height and nuclear diameter, increased pyknosis and karyorrhexis in PCT and DCT as well as interstitial oedema with infiltrations, and increased urinary space of glomerulus. The 3G group embryos showed significantly increased changes because of the lethality of 3G radiations.

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