ORIGINAL ARTICLE

Effects of Cell Phone Radiations on the Metanephros Tubules in a Chick Embryo Model

Sabah Rehman[1], Shadab Ahmed Butt[2], Naureen Waseem[3], Hina Kundi[4], Abdul Rasool Qamar[5]

ABSTRACT

Objective: To determine the histomorphogical effects of radiations from conventional and advanced mobile phones on the developing kidney of chick embryo.

Study Design: Randomized Control Trial.

Place and Duration of Study: The study was conducted at Army Medical College, (NUST), Rawalpindi, Pakistan from January 30, 2012 to January 30, 2013.

Materials and Methods: Fifty fertilized, zero day, off white colored eggs of Fayoumi breed were selected according to inclusion criteria. Two groups II and IV were exposed to conventional mobile phone radiations, and two groups III and V were exposed to advanced mobile phone radiations for 15 and 30 minutes per 24 hours respectively. Group I was the control. The data was analyzed by SPSS 17 and, ANOVA test was applied to determine statistical significance.

Results: The mean proximal and distal tubular diameters were decreased in the experimental groups. The mean proximal tubular diameter decreased significantly when comparing group I with groups II, III, IV and V and no statistical significance was found when comparing the experimental groups.

The mean proximal luminal diameter decreased in experimental groups with statistically significant result between groups I and III and between I and V showing that the effects were more in advanced cell phone groups when compared with the control.

Regarding distal tubular diameter the results were statistically significant between I and III, II and III and II and IV. Mean distal luminal diameter decreased in the experimental groups with statistically significant result when comparing II and IV and, IV and V. The distal tubules responded to either the increase in the time of exposure from 15 to 30 minutes or when the chick embryo was exposed to advanced cell phone radiations as the results were more significant between the experimental groups, where $p<0.05$ was considered significant.

Conclusion: From this experimental study we can conclude that prolonged exposure to mobile phone radiations can lead to decrease in tubular as well as luminal diameter in the proximal and distal tubules of the developing kidneys of chick embryo.

Key Words: *Mobile Phones, Chick Embryo, Metanephros.*

Introduction

The electromagnetic radiations from the mobile phone are classified as 2B, by international agency for cancer research (IARC), which means it is a possible carcinogenic. There have been a number of epidemiological studies conducted, looking for a relationship between cell phone use and brain cancer development. One such interphone study was carried out by Cradis E et al, (2010) to observe the association of gliomas, schwanomma, parotid tumors and meningioma.[1] The results were unconvincing because of the faulty study design and methodology. An increased risk of glioma after ten years or more use of cell phones was reported in a study carried out in Germany[2] Moreover, another study showed that there was no risk of acoustic neuroma in the first ten years of cellular phone use.[3]

In some studies, the harmful effects of these radiations were seen. The reproductive capacity of drosophila melanogaster was observed to be decreased after mobile phone radiation exposure.[4] A study was conducted on 77 university students and deranged values of TSH and T4 were found.[5] In few studies the radiations were useful like in bone healing[6] and in some, there were found no increased risk of salivary gland tumors because of the mobile

[1,2,5]*Department of Anatomy*
Army Medical College, NUST, Islamabad
[3]*Department of Anatomy*
Al Nafees Medical College, ISRA University
Islamabad
[4]*Department of Anatomy*
Margalla Institute of Health Sciences
Islamabad

Correspondence:
Dr. Saba Rehman
Department of Anatomy
Army Medical College, NUST, Islamabad
E-mail: sabahrehman@live.com

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phone induced radiations.[7]

The effects of increased usage of these modern and advanced cell phones have been questioned by Agarwal et al.[8] Who studied the effects of cell phone radiation on male fertility. Prenatal exposure of 900MHz to rat kidney resulted in increase in kidney volume and decrease in number of glomeruli (Ulubay, 2015).[9]

The current study was conducted to observe and compare the effects of the radiations from the two types of cell phones. One using conventional mobile phone, will be exposed to the GSM radiations, whereas the advanced mobile phone user will be exposed to both type of radiations as it is used for multipurposes (voice and message communication, internet usage).

As cell phones are kept close to body, the kidneys are essentially exposed to the radiations. The developing tissue is exposed as well, and the pregnant women are prone to get exposure and can later have an effect on the developing fetal tissues. The study was conducted to observe how cellular radiations can effect the renal histomorphology of a developing renal tissue. The additional features in a cell phone expose the tissues to radiations of Wi-Fi and Bluetooth. Keeping in view, the study was conducted to observe the effects of the simple and conventional talk and text cell phones and, advanced and modern cell phones on the developing renal tissue.

Materials and Methods

The study was carried out in Army Medical College Rawalpindi in collaboration with Poultry Research Institute Rawalpindi for one year 30th January 2012 to 30th January 2013. The experiment was carried out with permission of ethical committee on animal experiments, of the Army Medical College, Rawalpindi. It was a randomized lab based control trial. Fifty fertilized eggs were divided into five groups, by simple random sampling.

A still-air incubator which was wooden and manual, with measurements of 24 inch (length) x 24 inch (width) x 12.5 inch (height) and having capacity of 100 eggs was used for the project. Zero day eggs were bought for experimental purpose. Fertile eggs of normal shape i.e., oval, off-white colored and medium sized of Fayoumi breed were selected and incubated at 37 ºC and humidity range of 50- 60%.[10] Unfertilized, refrigerated, abnormal shaped eggs (foot-ball shaped, pear shaped), eggs with color other than off-white and, tiny sized eggs were excluded.

Group I (control), Group II (Experimental, 15 minute GSM radiation exposure), Group III (Experimental, 15 minute GSM and Wi-Fi radiation exposure), Group IV (Experimental, 30 minute GSM radiation exposure), Group V (Experimental, 30 minute GSM and Wi-Fi radiation exposure). The temperature was monitored by mercury thermometer. The humidity was maintained by filling the plastic pans with water and monitored by hygrometer. The eggs were marked with 'X' on one side and 'O' on the other side with lead pencil so that egg turning is not missed. They were turned thrice a day manually, after every eight hours. The time of exposure to GSM radiations was 15 minutes (23 missed calls) and 30 minutes (45 missed calls), daily, in the II and IV groups In groups III and V, the GSM exposure was for 15 minutes (23 missed calls) daily. In group III, Wi-Fi radiations were induced by downloading files for 15 minutes. In group V, the time of exposure to GSM radiations was 30 minutes (45 missed calls) and to Wi-Fi radiations was also 30 minutes daily.

After 15 days incubation, the chick embryos were dissected by crack opening the shell at the broader end. The inner and outer shell membranes were removed and, the living embryos were decapitated and fixed in 10 % formalin for 48 hours and after fixation. They were dissected and the kidneys were exposed in the posterior abdominal wall of the chick embryo. Then they were processed and embedded. Tissues were cut into 5 microns thick sections. The sections were stained with Hematoxylin and Eosin (H&E) for routine histological study of kidney (Fig 1) and PAS stain to see the basement membranes and brush border of cells.

Micrometry was carried out for measurement of the diameters and epithelial height.

Maximum tubular diameter was taken from the basement membrane of the cells on one side to the basement membrane of the cells on the opposite side in rounded tubules. Three tubules in were selected randomly in three different fields (one tubule in one field) at X40 in one slide per specimen. The external diameter of tubule was measured by oculometer. Three readings per slide were taken and their average as final reading for that specimen.

Maximum luminal diameter was taken from the apical surface of one cell to the apical surface of the opposite cell in the rounded tubules and same procedure repeated.

The data was entered in data base using start package for social services (SPSS version 17). The results were expressed as mean and standard error of mean. The significance difference was determined using one way analysis of variance (ANOVA) for multiple comparisons. Results were considered significant at $p<0.05$.

Results

The (H&E) stained slides were observed for changes in the histomorphology of developing metanephros of chick embryo.

Mean tubular (external) and mean luminal (internal) diameters of proximal tubules in the control group, in metanephros were 40.53±.08µm and 12.51±1.00µm respectively.

The proximal tubular diameters were decreased in all the experimental groups. The mean proximal tubular diameter in the metanephric tissue of the experimental groups is mentioned in table Ia. The results of proximal tubular diameters were statistically significant when comparing control group I with the experimental groups and comparable between groups II and III, II and IV, and III and V.

The mean proximal luminal diameter in metanephric tissue of experimental groups are mentioned in table Ib. The results were statistically significant between groups I and III and between I and V (Table I).

Mean tubular (external) and mean luminal (internal) diameters in the distal tubules of the control group in the metanephric tissue were 25.84±1.04µm and 9.06±0.38µm respectively.

The mean distal tubular diameter of the metanephric tissue in experimental groups are mentioned in table IIa. The values of mean in experimental groups were lower than the control group.

The results of distal tubular diameter of metanephros were decreased in the experimental groups and statistically significant between groups I and III, groups I and V, groups II and III and between groups II and IV.

The mean distal luminal diameter in the distal tubules of metanephric tissue in experimental

Table Ia: Proximal tubular diameter in control and experimental groups II, III, IV and V (metanephros)

Groups	Proximal tubular diameter Mean ± SEM (µm)	Comparison	P-value
I	40.53±0.81 µm	II	0.008
		III	0.000
		IV	0.000
		V	0.000
II	37.07±0.54 µm	I	0.008
		III	0.000
		IV	0.005
III	30.30±0.41 µm	I	0.000
		II	0.000
		V	0.093
IV	33.33±0.77 µm	I	0.000
		II	0.005
		V	0.997
V	32.97±0.85 µm.	I	0.000
		III	0.093
		IV	0.997

Table Ib: Proximal luminal diameter in control and experimental groups II, III, IV and V (metanephros)

Groups	Proximal luminal diameter Mean ± SEM (µm)	Comparison	P-value
I	12.51±1.00 µm	II	0.48
		III	0.008
		IV	0.26
		V	0.003
II	11.16±0.14 µm	I	0.48
		III	0.34
		IV	0.99
III	9.55±0.38 µm	I	0.008
		II	0.34
		V	0.99
IV	10.82±0.52 µm	I	0.003
		II	0.99
		V	0.34
V	9.16±0.31 µm	I	0.003
		III	0.99
		IV	0.34

Table IIa: Distal tubular diameter in control and experimental groups II, III, IV and V (metanephros)

Groups	Distal tubular diameter Mean ± SEM (µm)	Comparison	P-value
I	25.84±1.04 µm	II	0.62
		III	0.000
		IV	0.045
		V	0.006
II	27.47±1.07 µm	I	0.62
		III	0.000
		IV	0.02
III	18.54±0.69 µm	I	0.000
		II	0.000
		V	0.15
IV	23.68±0.65 µm	I	0.045
		II	0.02
		V	0.36
V	21.42±0.29 µm	I	0.006
		III	0.15
		IV	0.36

Table IIb: Distal luminal diameter in control and experimental groups II, III, IV and V (metanephros)

Groups	Distal luminal diameter Mean ± SEM (µm)	Comparison	P-value
I	9.60±0.38 µm	II	0.75
		III	0.06
		IV	0.29
		V	0.76
II	9.06±0.45µm	I	0.75
		III	0.13
		IV	0.03
III	7.91±0.15µm	I	0.06
		II	0.13
		V	0.16
IV	10.51±0.15µm	I	0.29
		II	0.03
		V	0.03
V	9.04±0.38 µm	I	0.76
		III	0.16
		IV	0.03

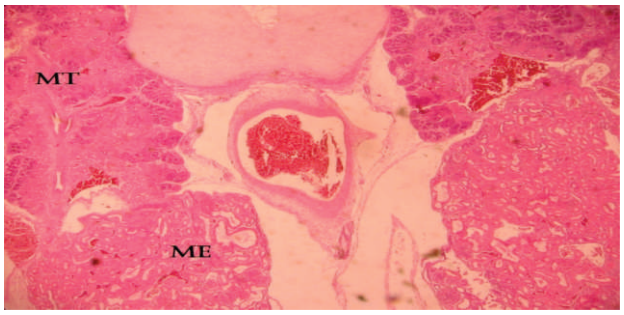

Fig 1: Photomicrograph showing mesonephros (ME) and metanephros (MT) in control group, lumen of tubules of mesonephros is more prominent (H&E, Approx. X 200)

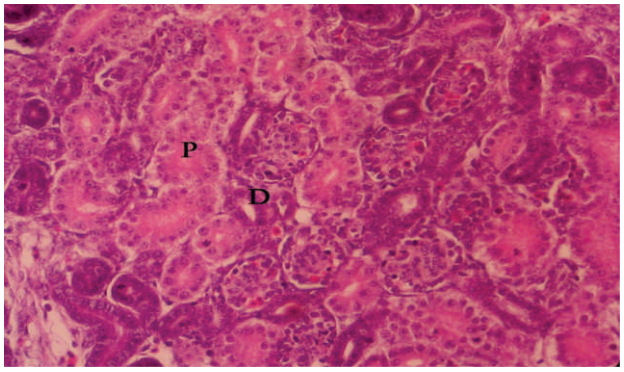

Fig 2: Metanephros in control group at 400X, H&E, P=Proximal, D=Distal Tubules

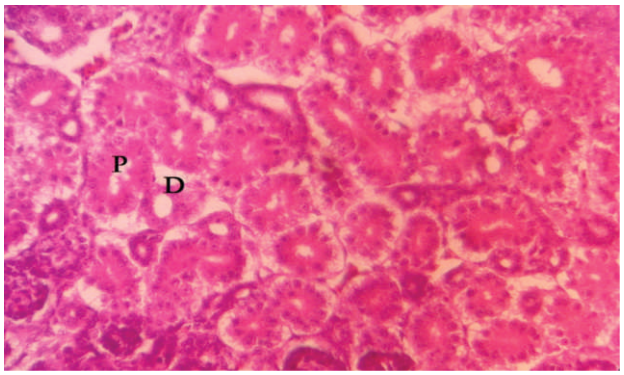

Fig 3: photomicrograph of metanephros in experimental group (iv), (H&E, Approx. X 400) P; proximal tubule, D; distal tubule

groups is mentioned in table IIb. The results were statistically significant between group II and IV and, group IV and V.

Discussion

The current study focused on the tubular and luminal diameter of the proximal and distal tubules of the developing metanephros. The tubular diameters were observed to be decreased in the experimental groups. The diameters decreased in all the experimental groups showing that there is effect of electromagnetic radiations on the diameters of developing tubules. In case of proximal tubular diameter the results showed that the diameter decreased statically in experimental groups than the control and proximal luminal diameter decreased also in the experimental groups with statistically significant result between I and III and I and V showing advanced cell phone radiations was affecting the internal diameters.

The mean distal tubular and luminal diameters decreased in the experimental groups but a trend of added decrease in mean diameter of tubules both externally and internally was noted in the group III.

That led to an aspect of study already seen in effects of GSM radiations on chick embryo growth in which EMFs exposure to them delayed the process of growth in the exposed embryos, there was a partial and then a trend of growth enhancement on progressively increasing the exposure levels was noticed.[11] The tubules appeared to recover from growth disruption after increase in the time of exposure as seen in the propensity of increase in the mean diameters in group IV and V in both proximal and distal tubular diameters. Hence, the advanced cell phone radiations after 15min/day exposure for 15 post-incubation days affected the development of the tubules of metanephros by decreasing their diameters hence, retarding the growth more as compared to conventional mobile phone radiations. Whereas, after 30min/day exposure for 15 post-incubation days, the tubular diameters showed improvement, depicting growth enhancement, that was more prominent after exposure to conventional mobile phone radiations than after advanced mobile phone radiations exposure.

Al-Glaib (2008)[12] observed increase in the tubular diameters in mice kidney because of GSM mobile phone radiations but Accini et al in 1988 in his study on effects of electromagnetic radiations on rabbits observed necrosis of the renal tubules.[13] Degeneration of renal tubules was noted in a study after exposure to electromagnetic radiations in mice for, 12 days.[14]

Moreover, study can be conducted further on other developing tissues in chick embryo or other animal model and effects can be correlated.

Conclusion

From this experimental study we can conclude that prolonged exposure to mobile phone radiations can lead to decrease in tubular as well as luminal diameter in the proximal and distal tubules of the developing kidneys of chick embryo.

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