EFFECT OF ELECTROMAGNETIC FIELD FROM 2G, 3G AND 4G MOBILE PHONES ON THE ORGANIZATION OF PURKINJE CELL LAYER OF RAT CEREBELLUM

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ABSTRACT

Objective: To study and compare the histomorphological changes induced by second generation (2G), third generation (3G) and fourth generation (4G) mobile phone electromagnetic fields on the organization of Purkinje cell layer of rat cerebellum.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: The study was carried out in the Anatomy department, Army Medical College Rawalpindi; in collaboration with animal house, National Institute of Health, Islamabad, from Nov 2014 to Nov 2015.

Material and Methods: Forty adult Sprague Dawley rats (20 male, 20 female), weighing 250-350grams,were taken and divided into 4 groups with 10 rats (5 male, 5 female) in each group. Group A served as control and was given normal diet and water *ad libitum*. Groups B, C and D were exposed to EMF from 2G, 3G and 4G mobile phones respectively, daily for 1 hour for 2 months. The animals were sacrificed on 60th day of the experiment, cerebellums were removed, fixed in 10% formalin, processed and stained with haematoxylin and eosin (H&E) for histological study.

Results: It was observed on microscopic examination that EMF from 2G, 3G and 4G mobile phones effected the organization of Purkinje cell layer of cerebellum; being double cell layer in group B and multiple cell layers in groups C and D.

Conclusion: It was concluded from current results that radiations from 2G, 3G and 4G mobile phones have deleterious effects on the organization of Purkinje cell layer of cerebellum with 3G and 4G causing more harm as compared to EMF from 2G mobile phones.

Keywords: Electromagnetic field (EMF), Fourth generation (4G) mobile phones, Purkinje cell layer, Rats, Second generation (2G), Third generation (3G) mobile phones.

INTRODUCTION

The mobile phones have become an obligatory part of the present era of sophisticated modes of communication[1]. It is not only used for its organizer tasks but also as means of entertainment as well as spending leisure time. However, the safety profile of mobile phones is yet to be established. Therefore mobile phones and their potential health hazards are the focus of extensive research over the past few years[2]. The extensive and unrestricted usage of mobile communications, mainly by children and adolescents, is raising alarm about the latent effects of electromagnetic radiations on the human organs, particularly the brain[3].

Persons living near mobile phone base stations and those who work in telecommunication manufacturing are more susceptible to mobile phone radiations[4]. High-frequency electromagnetic field (EMF) impairs the quality of sleep[5]. They also cause psychological disturbances and behavioral changes in youngsters[6]. It may cause headache in children and adults[7]. EMF can even affect the fetus if pregnant mothers are exposed to EMF, leading to low birth weight of neonates[8].

Second-generation (2G), or digital mobile phone technology was first launched in Finland

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in 1991. The only available technology in Pakistan until June 2014 was 2G. Its data transfer rate is 500 kilobit/sec (kbps). The key features of this technology include digitally encrypted phone conversations, bulk text messages, picture messages and multimedia messages.

Third generation (3G) of mobile telecommunications technology was first introduced in Japan in October 2001. It was launched in Pakistan in June, 2014. The average data transfer rate is 2000kbps and operational frequency is 2100 megahertz (MHz). It has advantages of wireless voice telephony, mobile internet access, fixed wireless internet access, video calls and mobile television.

Fourth generation (4G) of mobile telecommunications technology followed 3G, and was simultaneously launched in Pakistan i.e. in June, 2014. The average data transfer rate is 10000kbps and frequency is 1800 mega-hertz (MHz). The primary benefits are mobile broadband internet access, mobile web access, internet based telephony, high-definition mobile television, video conferencing, 3D television and cloud computing.

In rats, cerebellum is a part of brain that is located posterior to the cerebrum and is concerned with cognition and coordination of movements. It receives input from spinal cord and other parts of brain and integrates it to fine tune motor activity. Histologically, the cerebellum has distinct layers of superficial grey matter (cortex) and deep white matter (medulla). The cortex further has three layers-molecular, Purkinje cell layer and granule cell layer. The Purkinje cells are flask shaped cells arranged in a single layer in the cerebellar cortex. They form multiple synapses with granule cells and send inhibitory projections to deep cerebellar nuclei. The Purkinje cells form the sole output of all motor coordination in the cerebellar cortex[9].

The close proximity of mobile phones to users' head further increases the risk of absorption of EMF radiations. Previous studies have confirmed the nexus between extensive use of mobile phones and critical central nervous system (CNS) outcomes like Alzheimer's diseases, Amyotrophic lateral sclerosis, epilepsy and Parkinson's disease[10]. Acquisition of learned responses of male Wistar rats was found to be affected in a study, after exposing them to mobile phone radiations[11]. Disorders of hypothalamo-hypophyseal axis lead to disturbances in affective behavior, but also to disturbances of neurovegetative functions, which leads to behavioral changes and increased appetite and weight gain in EMF exposed animals[12]. An increased risk of glioma after ten years or more use of cell phones was reported in a study carried out in Germany[13].

The rationale of the present study was to identify the histomorphological changes induced by mobile phones electromagnetic fields on Purkinje cell layer of cerebellum.

MATERIAL AND METHODS

This lab based randomized control trial was conducted in the Department of Anatomy, Army Medical College, Rawalpindi; in collaboration with the Animal House, National Institute of Health, Islamabad: from Nov 2014 to Nov 2015.

The mobile phone used for 2G was Huawei Ascend G300. 3G signals were studied on Q Mobile X 35 whereas Samsung Galaxy S III was employed for 4G signals. The mobile network used for 2G and 3G was Ufone. 4G signals were provided through Zong long term evolution (LTE) microsim as it is the only network in Pakistan that provides 4G network till date.

Forty Sprague dawley rats, 3-4 months of age, grouped by using random number table method, weighing 250-350 grams, selected by nonprobability convenient sampling, were used in the experiment and were housed in controlled environment of animal house of NIH, Islamabad, The room was well ventilated and cycles of 12 hours light and 12 hours dark were maintained under a temperature range of 20-26°C with the help of central temperature regulating system[14]. Rats were fed with standard diet for two months. Water was provided ad libitum. Rats were

randomly divided into four groups (half male and half female, n=10 animals in each group).

Rats in group A served as control and were given normal diet and water *ad libitum*. Rats in group B were exposed to 2G radiations (900MHz) by placing a 2G mobile phone in a special shelf in customized cage. The EMF was generated by switching on the internet data connection for 60 minutes daily for 2 months. Rats in group C were exposed to 3G radiations (2100MHz) by placing a 3G mobile phone in a special shelf in customized cages. The EMF was generated by switching on the internet data connection and setting up an internet call through "Viber"/"Line" for 60 minutes daily for 2 months. Rats in group D were exposed to 4G radiations (2600MHz) by placing a 4G mobile phone in a special shelf in customized cages. The EMF was generated by switching on the internet data connection and setting up a video call through "Skype"/"We chat" for 60 minutes daily for 2 months.

All the animals were dissected and sacrificed under chloroform anesthesia. The brains were removed and cerebellums were separated and transversely sliced. The specimens were fixed in 10% formalin for 48 hours, dehydrated in rising grades of alcohol, washed in xylene and embedded in molten paraffin wax of melting point 56°C-58°C. Paraffin blocks were prepared, solidified, refrigerated and fixed in the chuk of rotator microtome. Five micrometer (µm) thick sections were obtained and stained with haematoxylin and eosin (H&E) and examined under light microscope.

The organization of Purkinje cell layer was observed and graded as single, double (2 layers of cells) and multiple (many layers).

Data were analyzed using statistical package for social sciences (SPSS) version 22. The qualitative parameters were expressed as frequency and percentages. Chi-square/ Fisher Exact test was applied for comparison of morphological features between the groups and a *p*-value <0.05 was considered significant.

RESULTS

Forty Sprague dawley rats with mean weight of 283.17 ± 14.51 grams were dissected at the end of experiment and their cerebellums were excised. The specimens were observed under light microscope and the histological features of cerebellum were noted. The specimen slides in control group A showed the normal three layers in the cerebellar cortex. The moleculer layer was present at the periphery, beneath which a single layer of uniformly arranged Purkinje cells was seen. The Purkinje cells showed regular and smooth margins.

In group B three rats showed doubled Purkinje cell layer and 7 rats showed single Purkinje cell layer like the control group. Frequency of double layer of cells was 30%.

Table-I: Frequency of organization of purkinje cell layer among the control and experimental groups.

Organization of purkinje cell layer	Control group A-control (n=10)	Experimental group B-2G (n=10)	Experimental group C-3G (n=10)	Experimental group D-4G (n=10)	*p*-value
Single	10 (100%)	7 (70%)			<0.001*
Double		3 (30%)	3(30%)		
Multiple			7 (70%)	10(100%)	

Table-II: Comparison of *p*-values of organization of purkinje cell layer among the control and experimental groups.

	Group A vs group B	Group A vs group C	Group A vs group D	Group B vs group C	Group B vs group D	Group C vs group D
Organization of purkinje cell layer	.21	.000*	<.001*	<.001*	.000*	.21

* =*p* value <0.05 statistically significant.

When compared with control group A it was found to be statistically insignificant (p>0.05) (table-I).

Three (30%) rats in group C (3G) showed doubled Purkinje cell layer and 7 (70%) rats showed multiple Purkinje cell layers. This frequency was significantly higher when compared to control group A and group B (p<0.001) (table-II).

All the rats in group D (4G) showed doubled multiple Purkinje cell layers (100%). This frequency was significantly higher when compared to control group A and group B (p<0.001).

In figure show the organization of purkinje cell layer in group A (Control), B (2G), C (3G) and D (4G). H&E 400X.

DISCUSSION

Effects of electromagnetic fields (EMF) on biological systems have been studied fairly extensively. However, due to the lack of conclusive results and unclear action mechanisms for these fields, investigation in this area continues to remain active[15].

In present study, all animals remained healthy and active throughout the experimental period. Organization of Purkinje cells was found to be highly altered following the mobile phone exposure. The Purkinje cells in group B (2G) were arranged in two layers but it was not found to be statistically significant when compared with group A (p=0.21). Successively in groups C (3G) and D (4G) the Purkinje cells were seen arranged in multiple layers. This frequency of organization of Purkinje cell layer was found to be significant when compared with group A and B (p<.000). This can be attributed to the fact that prolonged exposure to mobile phone radiations causes neuronal insult and induces some adjustive, changes in Purkinje cells leading to their overcrowding. Due to electromagnetic radiations' exposure over a period of two months, the Purkinje cells underwent some uncontrolled mitosis leading to their unrestrained multiplication. Exposure to EMF from mobile phones causes DNA damage and stimulates a cascade of pre-cancerous changes in the living tissue leading to impaired cell proliferation[16]. The current study is comparable with another study showing the same adaptive changes in Purkinje cells following stress[17].

Purkinje cells show adaptive changes by arranging themselves in multiple layers following the exposure of EMF. This change in organization of Purkinje cells layer causes the increase in thickness. The finding is in correlation to another study showing that prenatal stress increases the density of the Purkinje cell layer of the cerebellum[18]. In contrast, a study conducted on squirrel monkey cerebellum elaborating the effects of nonionizing radiations on Purkinje cells

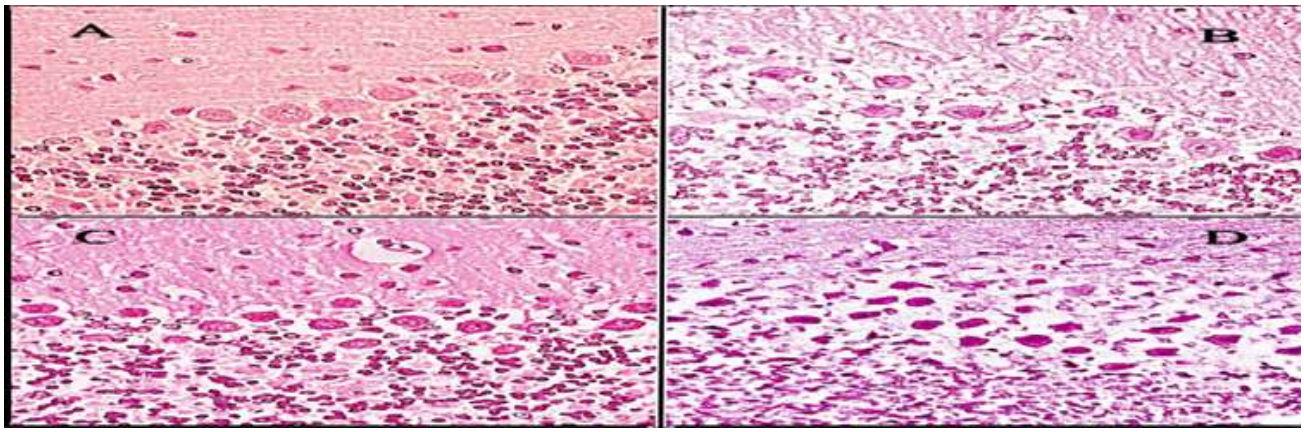

Figure: Organization of purkinje cell layer in group A (Control), B (2G), C (3G) and D (4G). H&E 400X.

layer showed no significant change in thickness and density[19].

It can be stated that radiations cause harmful effects on the cell genetic makeup, thus hindering the DNA repair capacity causing necrosis and cell death, particularly in brain cells[20].

This study is the first of its kind elaborating the comparison b/w the effects of 2G, 3G and 4G mobile phone radiations on the rat cerebellum. Previously, all the work that was done in this regard was focused on the effects of 2G and 3G radiations mainly.

From the discussion it can be clearly stated that mobile phone radiations have deleterious effects on the histomorphology of rat cerebellum with 4G radiations being the most hazardous.

CONCLUSION

It was concluded from current results that radiations from 2G, 3G and 4G mobile phones have deleterious effects on the organization of Purkinje cell layer of cerebellum with 3G and 4G causing more harm as compared to EMF from 2G mobile phones.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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