

# Effect of Electromagnetic Field (EMF) of Cellular phones on Purkinje Cell Margins and Perineuronal Space in the Cortex of Rat Cerebellum

MADIHA ALI<sup>1</sup>, MUHAMMAD IMRAN AFTAB<sup>2</sup>, MUHAMMAD ADEEL ALAM SHAH<sup>3</sup>, FAREEHA MUSHTAQ<sup>4</sup>, FAISAL MEHMOOD<sup>5</sup>, MOHAMMAD MOBUSHIR<sup>6</sup>, BUSHRA ANAM ALI<sup>7</sup>

<sup>1,3</sup>Assistant Professors of Anatomy <sup>2</sup>Associate Professor of Physiology, Independent Medical College, Faisalabad

<sup>4</sup>Assistant Professor, Department of Anatomy, CMH institute of Medical Sciences, Bahawalpur

<sup>5</sup>Senior Registrar, Department of Radiology, Holy Family Hospital, Rawalpindi

<sup>6</sup>Senior Registrar, Department of Orthopedic Surgery, Foundation University Medical College, Islamabad

<sup>7</sup>Demonstrator, Department of Pathology, Fazaia Medical College, Islamabad

Correspondence to Dr. Madiha Ali, Email: drmadiha86@gmail.com. Cell # 0315110254

## ABSTRACT

**Aim:** To observe and analyze the histomorphological changes prompted by second generation (2G), third generation (3G) and fourth generation (4G) mobile phones respectively, every day for 1 hour for 3 months. The rats were dissected on 90<sup>th</sup> day of the research. The cerebellums were taken out, fixed in 10% formalin, prepared and dyed with haematoxylin and eosin (H&E) for histological examination.

**Results:** It was noted on microscopic examination that radio waves from 2G, 3G and 4G cell phones caused harm to the margins of Purkinje cells of cerebellum. It was observed that the margins were 60% irregular in group B (2G) and 100% irregular in groups C (3G) and D (4G). Additionally, occurrence of perineuronal space was seen in around 60% of Purkinje cells in group B (2G) and 100% of Purkinje cells in groups C (3G) and D (4G).

**Conclusion:** The radio waves from 2G, 3G and 4G mobile phones have lethal results on the margins of Purkinje cells instigating the presence of perineuronal space around them in the cortex of cerebellum with 3G and 4G triggering further damage as equated to EMF from 2G mobile phones.

**Keywords:** Electromagnetic field (EMF), Fourth generation (4G) mobile phones, Purkinje cell layer, Rats

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## INTRODUCTION

We are living in an era of a variety of means of communication in which mobile phones have become a mandatory part of our contemporary life.<sup>1</sup> Mobile phones are not just important for their organizer works but also considered as source of enjoyment for spending free time. However, the health security specifications of mobile phones are still a reputable matter for debate. Therefore, extensive research has been carried out over the past few years regarding the use of mobile phones and the probable health menaces they might cause<sup>2</sup>. Cell phones connect to a remote base station (or mobile tower) through their antennas. They emit radio waves from these antennas which are absorbed by the parts of human body which are near to the phone. The broad and unhampered utility of mobile communications, is hovering apprehension about the concealed effects of electromagnetic emissions from the cell phones, on the human organs, principally the brain and other parts of nervous system<sup>3</sup>. The today's generations have started banking on mobile phones recklessly, while ignoring the cost they have to pay in shape of mental and physical losses<sup>1</sup>.

The researchers have been studying the effects of mobile phone EMFs on the health of the individuals since the last decade of 20<sup>th</sup> century<sup>2</sup>. Individuals who work in telecommunication manufacturing or those who live close to mobile phone base stations, towers or other radio emitting installations are more disposed to mobile phone radiations.<sup>4</sup> Mood disturbances, behavioural changes, social and developmental deficiencies continue to be a big

challenge to the new era of smartphone society<sup>3</sup>. A fair amount of data has been collected in this regard to warn such professionals of the ill effects of mobile tower radiations. The frequency spectra of mobile phones have been associated with disturbances in sleep wake cycles of the affected individuals. High-frequency electromagnetic field (EMF) blights the quality of sleep<sup>5</sup>. Numerous psychological disturbances and behavioral changes in youngsters have also been reported by the overt exposure to these radiations.<sup>6</sup> direct exposure to mobile phone screen for longer periods increases the eye strain and anxiety<sup>5</sup>. Mobile phone radiations are also the major cause of headache in children and adults.<sup>7</sup> It has been studied that fetal distress ensues if in case of over exposure of expecting mothers to EMF emissions which may result in intrauterine growth defects and low birth weight<sup>8</sup>.

The close proximity of cell phones to the head of the user further escalates the risk of infiltration of EMF emissions to affect the brain. Earlier researchers have identified a connection between uncontrolled usage of cell phones and grave central nervous system (CNS) consequences like epilepsy, Alzheimer's disease, Amyotrophic lateral sclerosis, and Parkinson's disease.<sup>9</sup> it is postulated that as mobile phones are used in close proximity to head, the EMF of mobile phones is converted into heat energy, thus causing damage to the core structures of the CNS. It was noted in a study that after exposure to mobile phone radiations, male Wistar rats were unable to elicit learned actions and motor activities.<sup>10</sup> Disorders of hypothalamo-hypophyseal axis in EMF exposed animals lead to instabilities in affective motor responses and neurovegetative functions causing behavioral deviations and augmented appetite and weight

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gain<sup>11</sup>. A research that went on in Germany proved that the exposure of 10 or more years to EMF caused increase in the incidence of glioma<sup>12</sup>. However some other studies failed to demonstrate a statistically significant correlation between impairment of central nervous system in young male Wistar rats and exposure to mobile phone waves at normal human usage frequencies<sup>13</sup>.

## MATERIALS AND METHODS

This research was a lab based randomized control trial. It was conducted in Army Medical College, Rawalpindi; in assistance with the Animal House, National Institute of Health, Islamabad from 1<sup>st</sup> May 2018 to 31<sup>st</sup> January 2019. 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 Zonglong term evolution (LTE) microsim.

Forty Sprague Dawley rats, 3-4 months of age, sorted by utilising random number table method, weighing 250-350grams, picked by nonprobability convenient sampling, were included in the research. At NIH, they were kept in contained and monitored settings of animal house. The experiment area was airy and periods of 12 hours illumination and 12 hours darkness were carried out under a temperature range of 20-26°C maintained with the help of central thermoregulatory system<sup>14</sup>. Rats were fed with standard diet for three months. Water was given ad libitum. Rats were sorted randomly into four groups (50% male and 50% female, n = 10 animals in each group).

Rats in group A served as control and were provided with normal food and water ad libitum. Rats in group B were revealed to the radio waves emitted by a 2G mobile phone. The cell phone was kept on a specific slab in specially designed cage for the rats. The electromagnetic radiations were created by putting on the internet data connection for 60 minutes everyday for 3 months. Likewise the rats in group C were put under the radiations emitted by a 3G mobile phone placed in the designed cage. This was done by switching on the internet data connection and setting up a call through "Viber" / "Line" for 60 minutes every day for 3 months. Lastly the rats of group D were kept under the radiations of a 4G mobile phone. Radiations were created by putting on the internet data and starting a video call via "Skype" / "We chat" for 60 minutes every day for 3 months.

After completion of 3 months of experimental time all the rats were exposed to chloroform in a big container and then they were dissected. The brains were taken out and cerebellums were detached and sliced in a transverse plane. The cerebellums were fixed in 10% formalin for 48 hours. After that they were dehydrated by using alcohol and bathed in xylene. Paraffin wax was used for making blocks and the cerebellums were embedded in molten wax of melting point 56°C-58°C. Paraffin blocks were solidified. After the refrigeration, fixing of tissues was done in the chuck of rotatory microtome. Five micrometer ( $\mu$ m) thick slices were made and haematoxylin and eosin (H& E) were employed for staining. After doing all the steps, the slides were examined under light microscope. The purkinje cell margins were observed and graded as regular and irregular. The perineuronal space was also recorded as present or absent.

Data was analyzed by means of SPSS version 24. The categorical variables were shown as frequency and percentages. Chi-square and Fisher Exact test were used for assessment of morphological structures among the groups and a  $p$  value <0.05 was declared significant.

## RESULTS

The mean weight of the rats was 283.17 $\pm$ 14.51grams. The rats 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 molecular 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 4 rats showed regular Purkinje cell margins with no perineuronal space and 6 rats showed irregular Purkinje cell margins with perineuronal space around them. Frequency of irregular Purkinje cell margin with presence of perineuronal space was found to be 60%. When equated with control group A, the difference was recognised to be statistically significant (i.e.  $p < 0.05$ ). All the rats in group C (3G) and D (4G) showed irregular Purkinje cell margins and perineuronal space (100%). This frequency was highly significant when compared to control group A and group B ( $p < 0.001$ ) [Tables 1-2, Fig. 1].

Table 1: Frequency of Purkinje cell margins and perineuronal space among the control and experimental groups

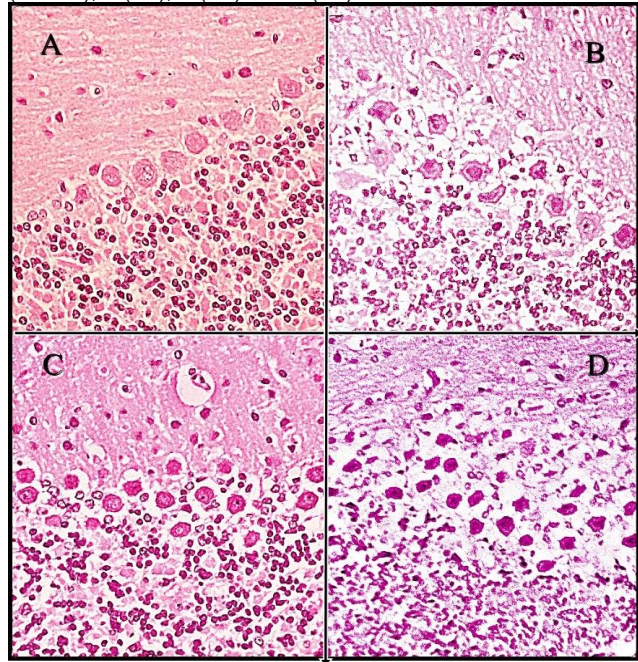
Purkinje cell margins	Control group A (n = 10)	Experimental group B-2G (n = 10)	Experimental group C-3G (n = 10)	Experimental group D-4G (n = 10)	P value
Regular	10 (100%)	4 (40%)			.000*
Irregular		6 (60%)	10(100%)		
Perineuronal space					
Present	0 (0%)	6 (60%)	10 (100%)	10 (100%)	.000*

Table 2: Comparison of  $p$  values of Purkinje cell margins and perineuronal space among the control and experimental groups

Parameter	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
Purkinje cell margins	.003*	.000*	.000*	.025*	.025*	>.05
Perineuronal space	.003*	.000*	.000*	.025*	.025*	>.05

(\* =  $p$  value < 0.05 statistically significant)

Fig. 1: Purkinje cell margins and perineuronal space in group A (Control), B (2G), C (3G) and D (4G)



## DISCUSSION

Effects of electromagnetic fields (EMF) on biological structures of living beings have been researched comprehensively. Nevertheless, owing mainly to the absence of irrefutable outcomes and vague mechanism for these fields, exploration in this area persists to be vigorously studied<sup>15</sup>.

The main aim of this study was to appraise the effects of EMF from different technology generations of cellular phones on the margins of Purkinje cells and the perineuronal space in the cortex of rat cerebellum. In current research, all rats stayed in good physical shape and dynamic during the course of the experimental time. Purkinje cell margins were found to be highly altered following the mobile phone exposure. The current study validated the findings of previous researchers who established the inverse effects of mobile phone data bands on the brain morphology<sup>16</sup>. When compared between groups it was noted that in spite of the normal single layer, the Purkinje cell margins were found to be irregular along with the presence of perineuronal space in group B (2G) in 6 animals. Successively in groups C (3G) and D (4G) all the animals showed irregular Purkinje cell margins along with the presence of perineuronal space. The frequency of Purkinje cell margins and perineuronal space was found to be significant in all groups when compared with each other ( $p < .000$ ). This can be attributed to the fact that prolonged exposure to mobile phone radiations causing neuronal insult induces some adjustive changes in Purkinje cells leading to their overcrowding. These results were also seen in a similar study on cerebellum by Azmy *et al*<sup>1</sup> showing that mobile phone exposure causes Purkinje cells' boundaries to become jagged and rough. The current study is comparable with another study showing the same

adaptive changes in Purkinje cells following stress<sup>16</sup>. The study outlines the ill effects of mobile phone radiations on the cerebellar morphology of the rats. Presence of perineuronal space around the Purkinje cells can be accredited to the shrinkage and atrophy of these cells<sup>17</sup>. Another study elucidated the detrimental effects of mobile phone radiations on the integrity of blood brain barrier and harming other CNS structures<sup>18</sup>. Damage to the perineuronal space also appears to be more apparent because of the presence of edema due to the radiation stress induced by the 2G, 3G and 4G cell phones. It was noteworthy that these changes worsened with the increasing strength of radiofrequency. This finding is in accordance with other studies in which marked changes were observed in basal ganglia of the animals treated with EMF from mobile phones in the form of white, round haloes of pericellular type around the cells<sup>19</sup>. In their study, Zymantiene *et al*<sup>20</sup> confirmed our findings by establishing a detrimental role of mobile phone waves and radiations on the morphology of brain.

## CONCLUSION

The radio waves from 2G, 3G and 4G cell phones induced significant changes in the histological and morphological orientation of Purkinje cell layer of cerebellum. Therefore, it is concluded that EMF generated by 2G, 3G and 4G cell phones has harmful results on cerebellum. It is further concluded that EMF from 4G and 3G cell phones is more precarious as compared to radio waves from 2G phones; with 4G mobile phone radiations being a little more hazardous than 3G mobile phone radiations. The ubiquitous exposure to mobile phone radiations is a potential threat to the mental health owing mainly to the histological changes in CNS structures.

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