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Effect of Exposure and Withdrawal of Cell Phone Radiations on *Swiss* Albino Mice

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ABSTRACT: The mobile phone now days, not only used for its basic applications, but also as means of entertainment as well as spending leisure time. Due to the Corona Virus Disease, 2019 (COVID-19) outbreak, the exposure to these radiations increased many folds. Unlike basic cell phones, the smart cell phone due to its multiple applications, varied frequency range, internet and wifi applications generates more electromagnetic radiations (EMRs) and hence the users are always surrounded by the hazardous electromagnetic fields (EMFs). The present study was intended to compare and evaluate the detrimental effect of RF-EMF radiations emitted from third generation (3G) and fourth generation (4G) cell phone onkidneys in adult male Swiss albino mice. The mice were exposed to 3G (SAR =0.406 W/Kg & 0.562W/Kg for body and head respectively), and 4G mobile phone (SAR = 0.458 W/Kg & 1.520W/Kg for body and head respectively) radiation during video call for a period of 4 hours/day for 120 days. The average power density at a distance of 6-8cm was 0.998 mW/cm^2 for 3G and 1.032 mW/cm^2 for 4G mobile phone. Following chronic exposure of 3G cell phone radiations, some of the glomerulus were atrophied and have increased urinary space. In 4G exposed groups, the tubule contains protein casts, indicating renal damage. No significant changes have been reported in blood urea and serum creatinine level in both the exposed groups. The changes were recovered to normal in on withdrawal of EMR.

Keywords: Renal damage, Exposure, Recovery, Cell Phone Generations, 3G/4G.

I. INTRODUCTION

The world of natural environment is now contaminated with electromagnetic fields (EMFs). Besides, electricity and radio communication, the mobile communication devices are the key sources of electromagnetic fields EMFs. While using these technological devices we expose ourselves to electromagnetic fields (EMFs) generated by these devices [1, 2]. It has been observed that EMFs create an impact on the health status of the living tissues and provides the reason for health problems; depending on variable factors like exposure conditions, dose, exposure duration, distance from the source, species and kind of tissues [3-7]. Generally, the cell phones are carried on the belts and radiation emitted by them may be mostly absorbed by the kidneys placed nearby it [8]. The mobile phones RFR may affect the organisms via different mechanisms. The first is EMFs specific effect, second is a heating or thermal effect and last one includes, a combination of both EMFs specific and thermal effect [9].

Earlier studies have reported detrimental effects of mobile phone RFR on various tissues of experimental animals [10-12]. The kidneys are very essential organs of the body. Every minute about 20% of the body blood is filtered by the kidneys, and are thereby at high risk of being affected by harmful substances [13]. In the previous studies involving the effects of cell phone on kidneys also showed deleterious effects. Radiofrequency waves emitted particularly by the third generation cell phones might have an effect on kidney [14, 15]. Due to exposure to these non ionizing radiations, swelling in epithehal cells of kidneys tubule followed by cell necrosis [16]. Even drinking of the EMFs exposed water causes the degeneration of renal tubules of kidney in adult *Charles foster* male rat [17]. Alteration in the kidney functions were also reported as a result of cell phone radiofrequency radiations exposure [12, 18].

However, [19], have reported that RFR of 900 MHz GSM (Global System for Mobile Communications) for 30 min/day (SAR=1 Watt/kg) for a month have not any histological change in the kidney of Balb/c mice. Similarly, rat's kidney exposed to wifi radiations [18] and mobile phone radiations did not revealed any histological alterations [20]. Despite large number of studies regarding the harmful effects of EMFs, the histopathological and morphological effects of cell phone radio frequency on kidney are still controversial.

Rapid proliferation of telecommunication with introduction of 3G & 4G mobile phones has incited to carry out the present study to assess the possible effects of chronic exposure to radiofrequency radiation emitted from 3G and 4G cell phone on tissue damage in the exposed animals. The main purpose of this research is to establish the relation between the chronic exposure on the living systems and recovery of the affected organs if exposure sources were withdrawn.The present

study was conducted to investigate the effects of EMR emitted by the cell phones (3G and 4G) exposure and withdrawal on renal tissues which further focused on biochemical and histological alterations in the *Swiss* albino male mice.

II. MATERIAL AND METHODS

Ethics statement: Experiments were conducted after taking the permission from institutional ethical committee (IEC) of Maharaja Agrasen University, Baddi, Solan, HP in its meeting held on dated 10.10.2019 (Approval No. MAU/SBAS/2019/206).

Animals: Adult *Swiss* albino mice of male sex, 6-8 weeks old were used for the experimentation. Experimental procedures were carried out as per accordance with institutional guidelines for animal care and use in the Maharaja Agrasen University. All the animals (control and experimental) were subjected to the similar environmental conditions (temperature 25 ± 3℃, relative humidity of 60± 10% and light/dark cycle of 12/12 hours), except the exposure field. Animals were fed with a standard pellet diet and water with ad-libitum [21].

Experimental design and exposure conditions: A total of thirty six (36) mice were randomly divided into three groups having twelve mice in each group. The Group I was acted as control. The Group II was exposed to 3G mobile phone (SAR =0.406 W/Kg & 0.562 W/Kg for body and head respectively), while Group III was exposed to 4G mobile phone (SAR = 0.458 W/Kg & 1.520 W/Kg for body and head respectively) during video call from a distance of 6-8 cm for 4 hours daily with exposure time of 2 hours each during morning and evening for 120 days. The average power density (PD) at a distance of 6-8 cm was measured as 0.998 mW/cm^2 for 3G and 1.032 mW/cm^2 for 4G mobile phone during video call with Electrosmog Meter (MECO- 2790; Mecon Pvt. Ltd.). After exposure of 120 days, six mice from each group were sacrificed, while remaining six mice in each group were kept unexposed for 30 days, to observe the recovery if any due to removal of radiation exposure and labeled as 3GR (3G recovery group) & 4GR (4G recovery group). Mobile phones were programmed in auto answer mode. Both the groups have been exposed with similar set of mobile phone as well as from same service provider (Fig. 1).

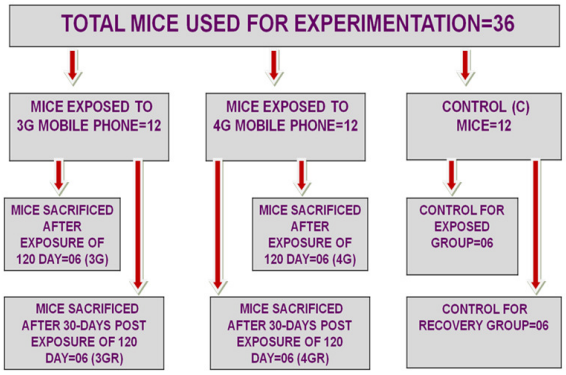

Fig. 1. Scheme of experimental design.

Evaluation of biochemical parameters: After the completion of experiment, six mice from each exposed and recovery group were sacrificed along with normal controls and blood samples were immediately processed for blood urea and serum creatinine.

Procedure: All the enzymatic assays were done by using commercially available kits and manufacturer's instructions were followed. About 1 ml of blood sample was taken in a plain centrifuge tube and was left for about 15-20 min to ensure proper coagulation of blood. The clear serum samples were obtained by centrifugation at 1000g for 20 min. Protein content was estimated according to the method of [22]. Creatinine was estimated by following standardized protocol given in diagnostic kit (Reckon Diagnostics) using Modified Jaffe's method [23] and Urea estimation was done by following standardized protocol given in diagnostic kit (Reckon Diagnostics) using modified Berthelot method [24].

Histological Studies: In order to evaluate and correlate biochemical and functional changes in the kidney, histopathological studies were carried out by performing haematoxylin and eosin staining [25]. Tissues were fixed in Bouin's fixative [26] for 24 hours and washed in water overnight. Then upgradation in 30 %(1h), 50% (1h), 70 %(1h) alcohol was done further followed by dehydration in 90% and absolute alcohol (1h) each. If needed three changes of absolute alcohol were given (30 min each) followed by clearing in absolute alcohol: Benzene mixture 3:1 (30 min), 2:1 (30 Min), 1:1 (30 min), 1:2 (30 min), 1:3 (30 min), in pure benzene (30 min) and finally in benzene: wax (1:1) for 1 hour. Then tissues were saturated with wax at 58-60℃ for overnight and embedded in paraffin wax with melting point of 58-60℃ [27]. Wax blocks were prepared and fixed in cork to make 5-7 µ thick section with microtome.

Staining Method: Delafield's Haematoxylin/Eosin staining technique (H/E) was used for histology [25]. The 5-7 µ thick sections were stretched smoothly on albumin coated slides in hot water. Dewaxing of Bouin's-fixed paraffin section was done in two changes of xylene. The sections were downgraded through different grades of alcohol to water – 100% (3 min), 90% (3 min), 70% (2 min), 50% (2 min), 30% (2 min), water (2 min) and stained in haematoxylin for 15-20 minutes. After removing the excess stain, the tissues were differentiated in acid water and ammonia water (one or two dips in each). Now the sections were upgraded in 90% alcohol by dehydrating the slides – 30% (4 min), 50% (4 min), 70% (4 min), 90% (4 min) and stained with eosin (1 min or 30 sec). After passing through 90 % (10 min), 100% (10 min), xylene (10 min) the sections were mounted in DPX under a cover slip (to maintain high refractive index) for microscopy and protect the sections during storage and examined under light microscope (Leica DC 100, PC I Interface Digital Camera).

III. STATISTICAL ANALYSIS

All data comparisons were tested for significance by using one-way ANOVA (analysis of variance) followed by post hoc Tukey's test; p values of <0.05 were considered significant. Results were expressed as

mean ±S.D. The variations in the values of these parameters were compared to the control and the variations observed in the exposed group. It helped us to understand the level of recovery if any as a result of removal of exposure source.

IV. RESULTS AND DISCUSSION

The present study was made to examine the effects of chronic exposure of 3G & 4G mobile phone radiation on kidney of *Swiss* albino mice at histological level. In order to evaluate and correlate histopathological changes in the kidney, biochemical tests for serum creatinine and blood urea were also performed. Creatinine is a chemical waste molecule produced during metabolism of muscle tissues. The normal range of creatinine in the blood is maintained by the kidneys. Creatinine is a very reliable indicator of kidney function. The elevated level of creatinine in the blood signifies kidney disease or impaired kidney function. Similarly blood urea is an indicator of kidney function. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. The elevated blood urea level means impaired kidney function. The kidneys maintain the blood urea and creatinine in a normal range. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys [28, 29].

The results of the present study revealed insignificant changes in the blood urea and serum creatinine levels in both the exposed groups ($p<0.05$) as compared to control. The blood urea showed minor decrease of 1.23% in 3G, while it is increased by 0.83% in 4G exposed groups. Upon removal of exposure for one month, the blood urea increased by 4.3% in 3GR group, while it is decreased by 2.83% in 4GR group (Fig. 2). Also, there was insignificant increase in the level of serum creatinine in 3G exposed group by 1.78% ($p<0.05$) and 11% in 4G exposed group ($p<0.05$). The serum creatinine level decreased by 2% in 3GR group and increased by 2.77% in 4GR group as compared to the control (Fig. 3). No stastistically considerable variations were observed among 3G & 4G exposed groups.

Our finding are similar to study of [18], who reported no significant change in the kidney functions in Wistar female rats exposed to 2.45 GHz radio frequency source (Averaged whole body specific absorption rate (SAR) 0.01 W/Kg, 24 hours daily for 40 successive days). Likewise, [12], reported significant increase of urea and creatinine level in male albino rats exposed to 900 MHz electromagnetic field radiation for 1 hour a day for 60 days when compared to control. However no significant change was reported in Balb/c mice exposed for four h/day to cell phone with a frequency of 915 MHz, for 60 days [20]. We have reported a non significant increase in the above parameters. The above parameters become normal in both the recovery groups, which are similar to the study of [12].

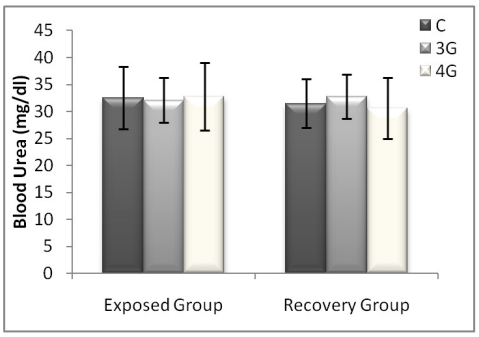

Fig. 2. Bar diagram displaying the comparative effect of exposure and withdrawal of 3G & 4G mobile phone radiofrequency radiation on blood urea in mice.

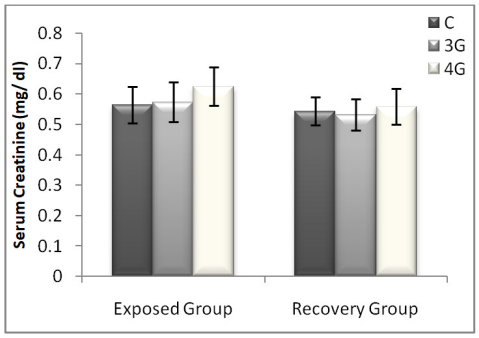

Fig. 3. Bar diagram displaying the comparative effect of exposure and withdrawal of 3G & 4G mobile phone radiofrequency radiation on serum creatinine in mice.

V. HISTOPATHOLOGY

Under the light microscopic studies, the TS of kidney of the control mice revealed normal architecture of renal tissue (Fig. 4 A & B). We have reported mild effect of these radiations on the kidney tissues of mice exposed to both 3G and 4G cell phone RFR. Following chronic exposure of 3G cell phone radiations, some of the glomeruli were atrophied and have increased urinary space (fig. 5 C&D). Earlier studies have reported atrophy of some glomeruli and extravasation of blood cells between kidney tubules in the kidney of rats exposed to 900 MHz radiations one hour per day for four week [30]. Likewise, atrophied glomerulus, infiltration of mononuclear leukocyte between the renal tubules, vacuolation and dilatation of some tubules was reported in mice exposed to mobile phone radiation for one hour/day for total ten days [31]. The atrophied glomeruli, cytoplasmic vacuolation with pyknotic nuclei in epithelial cells of the renal tubules, congested and dilated renal veins and intertubular inflammation were also observed by [32] in the mice exposed to EMF eight hours for three days and twelve days. The above changes were more pronounced in the animals exposed EMF for twelve days. 2G mobile phone 900-1900 MHz radiations leads to sclerotic glomerulus, hyalinised and vacuolated cytoplasm around renal tubules, lymphocytic infiltrations around interstitial tissues and congestion of renal blood vessel [33].

In our study we have reported few atrophied glomeruli and increased urinary space in 3G exposed groups, but

no mononuclear leukocytic infiltration and Cytoplasmic vacuolation were seen (Fig. 5 C & D).

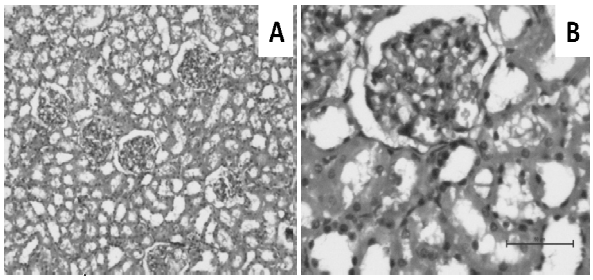

Fig. 4. Photomicrograph (H&E) of control mice kidney (A-100X; B-400X).

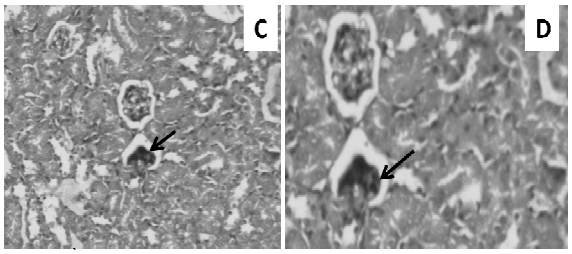

Fig. 5. Photomicrograph (H&E) of 3G exposed mice kidney (A-100X; B-400X).

Earlier studies reported deposition of **a**myloid protein within the kidneys glomerulus and convulated tubules of infant mice, which were exposed to cell phone radiations ¾ hour a day for one month, which may indicate the renal dysfunction [34]. Amyloidosis in the kidney showed symptoms of renal dysfunction [35]. Similarly, Obstruction of some convoluted tubules due to bleeding infiltrations within convoluted tubules and atrophied glomeruli were also reported in the kidney of infant mice exposed to mobile phone radiation for forty five minutes per day for one month [34]. We have also reported deposition of protein casts in the renal tubules of 4G exposed mice kidney; however no atrophied glomeruli were seen (Fig. 6 E&F). The kidney seems to be normal after removal of exposure for one month in the recovery group (Fig. 8 I & J). However, [34] reported increased amyloid protein deposits, even after one month of stopping exposure.

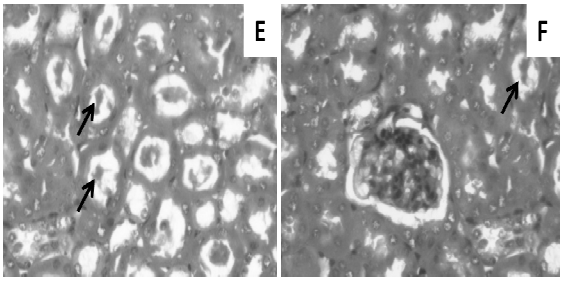

Fig. 6. Photomicrograph (H&E) of 4G exposed mice kidney (A-100X; B-400X).

The above changes recovered to normal in both the recovery groups, which are consistent to the findings of [12], where biochemicals changes in male albino rats due to exposure of mobile phone (900 MHz, 1 h/day for 60 days) were recovered after withdrawal of 30 days. Similarly, thirty days following magnetic exposure, the splenic tissues appeared almost normal and manifested a tendency towards recovery [36].

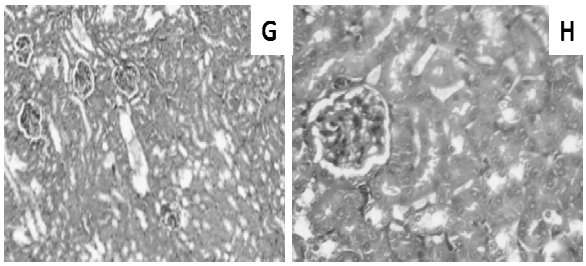

Fig. 7. Photomicrograph (H&E) of 3G recovery group mice kidney (A-100X; B-400X).

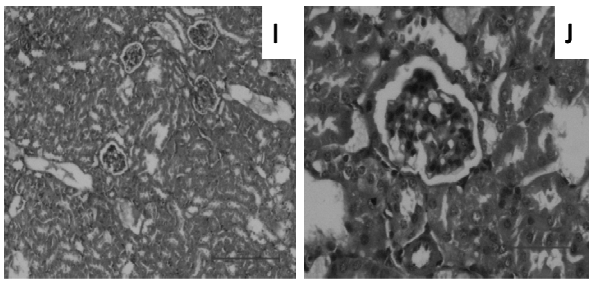

Fig. 8. Photomicrograph (H&E) of 4G recovery group mice kidney (A-100X; B-400X).

In present study, no well-founded explanation for the non-significant change in biochemical parameters after exposure was found which requires further investigations. Also, normalization of histological studies also requires more investigations to prove the exact reason for recovery.

VI. CONCLUSION

We have concluded that EMR emitted from mobile phone might produce impairments in exposed tissues. But complete withdrawal from EMR might overcome the deleterious effects of EMR exposure which brings it close to the normal state. So, people are advised not to use mobile phone for longer times which may help them in recovery from any impairment.

VII. FUTURE SCOPE

Still more experimentation is needed with the purpose of explaining the harmed intensity, frequency, duration and other parameters involving EMF to protect ourselves from these harmful effects.

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