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Review Article

Cell phone radiation exposure on brain and associated biological systems

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Wireless technologies are ubiquitous today and the mobile phones are one of the prodigious output of this technology. Although the familiarization and dependency of mobile phones is growing at an alarming pace, the biological effects due to the exposure of radiations have become a subject of intense debate. The present evidence on mobile phone radiation exposure is based on scientific research and public policy initiative to give an overview of what is known of biological effects that occur at radiofrequency (RF)/ electromagnetic fields (EMFs) exposure. The conflict in conclusions is mainly because of difficulty in controlling the affecting parameters. Biological effects are dependent not only on the distance and size of the object (with respect to the object) but also on the environmental parameters. Health endpoints reported to be associated with RF include childhood leukemia, brain tumors, genotoxic effects, neurological effects and neurodegenerative diseases, immune system deregulation, allergic and inflammatory responses, infertility and some cardiovascular effects. Most of the reports conclude a reasonable suspicion of mobile phone risk that exists based on clear evidence of bio-effects which with prolonged exposures may reasonably be presumed to result in health impacts. The present study summarizes the public issue based on mobile phone radiation exposure and their biological effects. This review concludes that the regular and long term use of microwave devices (mobile phone, microwave oven) at domestic level can have negative impact upon biological system especially on brain. It also suggests that increased reactive oxygen species (ROS) play an important role by enhancing the effect of microwave radiations which may cause neurodegenerative diseases.

Keywords: Cancer, Mobile phone, Reactive oxygen species, Tumor formation

Introduction

Since decades, the exponential growth of personal telecommunication devices like Global System for Mobile Communication (GSM) cell phone have become the issue of discussion. Cell phone frequencies vary according to the system used, ranging around 900 or 1800 MHz (GSM) and 2200 MHz [Universal Mobile Telecommunications System (UMTS)]. Mobile phone of radiofrequency radiation (RFR) raises concerns about possible implications to human health. Today one-third of the world's population relies on mobile phones for daily communication. Therefore increasing exposure to mobile phone and base station radiations, together with exposure to other sources of non-ionizing radiations (power lines, radar, etc.) are growing concern of possible adverse health effects. There are certain limits of radiation exposure and emissions where International Commission on Non Ionizing Radiation Protection (ICNIRP)[1] and Institute of Electrical and Electronics Engineers (IEEE)[2] have established to cover the limit of over-exposure to electromagnetic fields. The limit of mobile phone radiation exposure level set in United States and Europe is ~1.6 W/kg and 2.0 W/kg respectively. Generally people get exposed under these limits due to hand held mobile phones which are used as cordless phones in various positions with respect to the body. These positions intimate at the frequency range 40 MHz- 6 GHz. Such range of electromagnetic field penetrates deep into the tissue, causing an increase in the random molecular motion. This concern shows the possibility that exposure to radiofrequency (RF) radiations affects the genetic material (DNA) which is one of the subjects being highly debated in the current scientific fraternity.

The most fundamental molecule in the body, DNA itself, can act as a target for such radiation even when it is non-ionizing and at low-level[3]. Mashevich[4] concluded that radio frequency electromagnetic field

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(RF-EMF) from cell phone, at intensities similar to those emitted from contemporary cell phone, directly damages DNA. This is the same type of damage shown for UV and x-rays. Previous research with other types of EMF, not necessarily emitted by cell phone, indicated shape (conformation) changes in DNA[5]. Either strand breaks or conformational changes in DNA can result in the formation of damaged proteins in the body.

Any changes in DNA have a possible increase of cancer risk. Reason is that virtually the entire world is now being exposed with different sources and possible concern to DNA damage in somatic cells is often casually related to cancer. Great interest, therefore, was generated when a ubiquitous environmental exposure, power frequency electromagnetic radiation was first reported in 1979 to be a possible cause of brain cancer (and leukemia) in children[6]. Other studies have suggested that children are even more sensitive to electromagnetic radiations because the diameter of the head is smaller and Specific absorption rate (SAR) is greater when compared to adults[7,8]. Several other studies confirmed that exposure to electromagnetic fields may increase the incidence of cancer and DNA damage of sperm and brain cells[9-11].

Present review on mobile phone radiation exposure to human being has debated on brain system. The effects of EMFs emitted by mobile phones on the central nervous system (CNS) have become a particular focus of concern owing to the fact that mostly mobile phones are kept near head during talking mode and are in close proximity to the brain[12-14]. During these operations, the antenna of a cellular phone emits radio frequency electromagnetic fields that can penetrate 4-6 cm deep into the human brain[15,16]. Due to penetration of radiations, hippocampus and pineal gland may be affected by the decrease in their protein kinase C and melatonin activity respectively[17]. Experimental data from Hardell group[18,19] on health implications of long term mobile phone radiation exposure to brain resulting in tumor formation is more likely to occur on the side of the head that the cell handset is used. This group reported that one hour per day with continuous exposure to ten years or more causes brain tumor risk. Khurana *et al.*[20] concluded that there is an adequate epidemiologic evidence suggesting a link between prolonged cell phone usage and the development of an ipsilateral brain tumor. The microwaves at several frequencies are able to induce several changes at the level of brain DNA (single and double strand) and micronuclei (chromosomal aberration)[21-23,9]. Lai and Singh[24-26] also reported such effects due to exposure at microwave frequency (2450MHz). Several other studies reported significant changes in brain antioxidant enzymes at 2.45 GHz and 50 GHz[13, 27-29], alteration in calcium of rat brain[29,30], and an increase in brain glial cell[31].

Importance of dosimetry in biophysical parameters—At lower frequencies (<100 kHz), many biological effects are quantified in terms of current density in tissue and this parameter is most often used as a dosimetric quantity. At higher frequencies, many (but not all) interactions are due to the rate of energy deposition per unit mass. This is why the specific absorption rate (SAR) is used as the dosimetric measure at these frequencies. SAR is expressed as Wkg−1. The SAR is thus the absorbed power by the absorbing mass. The most obvious approach towards dosimetry analysis is to experimentally determine the SAR distribution in phantoms simulating animal and human bodies, as well as in real cadavers. One way of determining the local or whole-body SAR is by temperature measurements. The SAR is proportional to the temperature increase only when the effects of heat diffusion can be neglected. With these limitations in view, the SAR concept has proven to be a simple and useful tool in quantifying the interactions of RF/microwave radiation with living systems. This enables us to compare the experimentally observed biological effects in various species under various exposure conditions. The SAR is defined as the time derivative of the incremental energy (dW) absorbed by or dissipated in an incremental mass (dm) contained in a volume element (dV) of a given density (ρ)[32,33]. An equivalent method is to take a temperature measurement. The SAR is proportional to the temperature increase ΔT, when the effects of thermal conduction, convection and radiation are negligible, in the time interval Δt.

$$\text{SAR}= -\left(\frac{\mathrm{d}}{\mathrm{d}t}\right)\left(\frac{\mathrm{d}W}{\mathrm{d}m}\right)=\left(\frac{\mathrm{d}}{\mathrm{d}t}\right)\left(\frac{\mathrm{d}W}{(\mathrm{d}V)}\right)=C\frac{\Delta T}{\Delta t}$$

The same can also be evaluated, using the Poynting vector theorem for sinusoidal varying electromagnetic fields:

$$\text{SAR} = -\left(\frac{^{0}}{2}\right) - |E_{\text{i}}|^2 = \left(\frac{}{2}\right) - |E_{\text{i}}|^2$$

where $|E\text{i}|^2$ is the peak value of the internal electric field (in Vm−1). SAR is also dependent upon the wave type, that is, square, sine or triangular. The power of the square is larger than the other two. The average SAR is defined as the ratio of the total power absorbed in the exposed body to the mass in which it is absorbed, which is not necessarily that of the total body. The possible mechanism shows that radio frequency EMW transmit signals from the cellular phone to the base stations and antennas. The frequency of such waves is low and ranges from 800-2200 MHz. However there is still risk to the user, because the human body can act as antennas that absorb these waves and convert them into eddy currents[34].

Recently Hamada *et al.*[35] discussed the mechanisms of cell phones operation and explained that the sound wave produced from the speaker goes through a transmitter that converts the sound into a sine wave. This sine wave then travels to the antenna, which then projects the wave out into space. Average power usage of the transmitter is about 0.75 - 1 Watt, with a maximum of 2 W. The propulsion of the electric sine wave running through the transmitter circuit also yields an electromagnetic field. As the electric current oscillates back and forth, these electro-magnetic fields continue to build up and collapse, resulting in electromagnetic radiation[36]. Lastly, the actual SAR value arising from a wireless device may differ from the reported maximum. This is due to multiple factors such as proximity of the cell phone to the body, usage mode (talk versus standby mode), and the use of hands-free (Bluetooth) devices[37].

Induction of oxidative stress—Exposure from different type of environmental factors such as chronic disease state and aging or on gonadal injury can induce a state of oxidative stress associated with an increased rate of cellular damage that results in genotoxicity. Oxidative stress is a condition induced by oxygen and oxygen derived free radicals commonly known as reactive oxygen species (ROS)[38]. Normally adequate level of cellular antioxidants, mainly superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reductase maintain the free radicals scavenging potential in brain. Decrease in SOD, GPx and increase in CAT activity[17,21,23] have been reported. Oxidative stress is the result of an imbalance between ROS generation and intrinsic ROS scavenging activities. A situation in which there is a shift in this balance towards pro-oxidants, because of either generation of excessive ROS or diminished antioxidant capacity is referred to as oxidative stress status (OSS). Its assessment may play a critical role in monitoring neurodegenerative diseases. However, it is more important to measure the ROS level in brain. Nitric oxide and superoxide radicals combine to form highly reactive peroxynitrite radicals that induce endothelial cell injury[39,40]. This may result in altered blood flow to the blood brain barrier (BBB). Generally, free radical results in DNA strand break through oxidation of critical sulfhydryl (-SH) group and alter the cellular integrity and function with increased susceptibility to attack by toxicants. From the above studies, it may be concluded that RF-EMF are responsible for the formation of ROS due to which such changes may occur[23,28,41-43].

Mechanism of free radicals formation and cell function—Reactive oxygen species are free radicals, which play a major role in mechanisms of the biological effects induced by electromagnetic radiation. In aerobic cells, reactive oxygen species (ROS) are generated as a by-product of normal mitochondrial activity. If not properly controlled, ROS can cause severe damage to cellular macromolecules, especially DNA. There is a linear correlation between the overproduction of ROS and DNA damage induced by electromagnetic radiation. There is a building bridge of a possible mechanism between free radicals formation and cell function. It defines that electromagnetic fields induce changes during apoptotic process in cells due to oxidative stress of reactive oxygen species. The radio frequency field, acting especially on Ca^{2+} ions, induces variations in ionic homeostasis as described in the models. Perturbation of the Ca^{2+} through its uptake by mitochondria initiates (release from the endoplasmic reticulum) the apoptotic cascade. This change in Ca^{2+} results in the release of cytochrome c from mitochondria, activation of caspase 9 and, consequently, of the effector caspases 3, 6 and 7, and, finally, cell death through apoptosis. This is possible indication of programmed cell death (apoptosis) or DNA fragmentation. Moreover, the overproduction of free radicals in leydig cells of semniferous tubule and brain (hippocampus, pineal gland) may decrease the level of protein kinase C, melatonin and antioxidant

enzymes. The present discussion could be helpful to establish the possible mechanism between microwaves induced free radicals formation and biological effects.

Another possible mechanism through which exposure to 900-MHz microwaves might affect the CNS could involve the phosphorylation status of subunits of neurotransmitters through the alteration of intracellular enzymes such as kinases and phosphatases. This hypothesis is supported by few studies which suggest that exposure to radiofrequencies emitted by mobile phones can modify the expression of different enzymes in a variety of cells and tissues[44,45]. It has been suggested that EMR directly affects neurons by reducing the neuronal reactivity, increasing the neural membrane conductivity and prolonging their refractory period[41,46-49]. Other studies have shown that the electrophysiological changes may occur due to alteration of Ca^{2+} homeostasis[48,49].

Several recent studies on RE-EMF as discussed here have been summarised in Table 1.

Table 1—Recent cell phone studies on brain system

Animal Model	Frequency/ SAR	Observations	Reference
Wistar rat brain	9.9 GHz / 1.0 W/kg (2 h /day for 35 days)	Significant increase in calcium ion efflux and ornithine decarboxylase. A significant decrease in PKC activity was also recorded	Paulraj and Behari[49]
Wistar Albino adult male rats	900 MHz (RF) emitted from mobile phone. [2 h/day (7 days a week) for 10 months]	A significant increase in protein carbonyl was recorded in exposed group	Dasdag *et al.*[46]
Wistar rat brain	2.45 GHz/ 0.14 W/Kg (2 h/day for 45 days)	Reduced melatonin and increased caspase-3 and calcium ion concentration	Kesari *et al.*[29]
Wistar rat brain	900MHz/ 0.9W/Kg (2 h/day for 45 days)	Biochemical changes induce oxidative stress. A significant increase in apoptotic cells and decrease protein kinase C activity in hippocampus	Kesari *et al.*[17]
Wistar rat brain	2.45 GHz/ 0.11 W/Kg (2 h/day for 35 days)	Induced oxidative stress, leads to DNA damages in brain	Kesari *et al.*[28]
Neonatal mice	900MHz/ 4W/kg (for 60 min)	RF fields don't produce microglial activation	Finnie *et al.*[47]
Sprague Dawley rats	900 MHz/ 1.5 W/kg (15 min/day for 8 weeks	GFAP shows by increased adverse effect on rat brain	Ammari *et al.*[107]
Wistar rat	10GHz/ 0.014 W/kg 2 h/day for 45 days)	EMF increased ROS production and induce micronuclei formation in blood cells	Kumar *et al.*[42]
Wistar rat brain	50GHz/ 8.0 x 10^{-4} W/kg (2 h/day for 45 days)	EMF induced oxidative stress, leads to DNA damage and reduction in PKC activity	Kesari *et al.*[21]
Wistar rats	900MHz/ 2 W/kg (2 h/day for 4 weeks)	Induces Pyramidal cell loss in hippocampus	Bas *et al.*[108]
Wistar Albino rat brain	900 MHz/ 2 W/kg (60 min/day for 5 weeks)	Effects of prenatal exposure to EMF on the number of granule cells Inhibit granule cells neurogenesis in the dendrite gyrus in the hippocampus	Odaci *et al.*[14]
Fisher rat brain	900 MHz/ 0.6 W/kg (2 h/week for 55 weeks)	Reduced the memory function by albumin leakage	Nittby *et al.*[109]
Wistar rat brain	900 MHz/ 1.5 W/kg (45 min/day for 7 days) 900 MHz/ 6 W/kg (15 min/day for 7 days)	Affect brain activity by decreasing cytochrome oxidase activity	Ammari *et al.*[110]
Young rats	900 MHz/ 0.3-3 W/kg (2 h/day for 5 weeks)	No evidence of breakdown in BBB in young rats	Kumulin *et al.*[111]
Human endothelial cells EA hy 926, EA hy 926V1	900 MHz GSM 2.8 W/kg (for 1 h of exposure)	Changes in gene and protein expression. Mobile phone radiation exposure disturb the cytoskeleton component F-actin fibers by phosphorus of HSP27	Leszczynski *et al.*[112]

(Contd.)

Table 1—Recent cell phone studies on brain system – *(Contd.)*

Human Lymphoblustomy cell line (TK6)	1.9 GHz/ 1 W/kg (1 hour)	Significantly elevated the expression of HSP27, HSP70, FOS and JUM showed stress response	Chauhan *et al.*[84]
Human derived immune cell lines HL-60, MM6	1.9 GHz/ 10 W/kg (6 h, 5 min on 10 min off)	No changes in stress related gene expressions	Chauhan *et al.*[85]
Wistar rat brain	2.45 GHz/ 0.11 W/kg (2 h/day for 35 days)	Decrease PKC activity in hippocampus and increase glial cell population	Paulraj *et al.*[31]
Human epidermaid cancer KB cells	1.95 GHz/ 3.6 mW/kg (exposure duration 1,2,3 h)	Affect the refolding kinetics of eukaryotic proteins. MW-EMF induces apoptosis through the inactivation of ras-erk survival signaling due to enhanced degradation of ras and Raf-1	Caraglia *et al.*[93]
Human endothelial cell line EA hy 926 cells	900 MHz/ SAR-? (1 h)	Changes in protein expression involved in the structure of cell	Nylund and Leszczynsk[86]
Female fischer rat brain	GSM 915 MHz/ 2.20 W/kg (2 hrs)	Blood brain barrier permeability: albumin leakage and nerve cell damage	Salford *et al.*[66]
Zebra finches (Taenopygia guttata)	900 MHz/ 0.05 W/kg (for 30 min)	Changes in the amount of neurons activity by more than half of brain cells	Beasond and Semm[113]
Neonatal mice (slices of rat hippocampus)	700 MHz/ 1.6-4.4 mW/kg (for 5-15 min)	Alter electrical activity in hippocampus slices of brain	Tattersal *et al.*[114]
Rat astrocytes	GSM 1800 MHz/ 0.3-0.46 W/kg (4 day exposure)	EMF increased Blood brain barrier permeability for C^{14} sucrose	Schirmacher *et al.*[115]

Mobile phone exposure effects on protein kinase C (PKC) activity—PKC, an isozyme differs in its structure, biochemical properties, tissue distribution, subcellular localization, and substrate specificity. It has been classified as conventional (α, β, γ) isozymes which is Ca^{2+}-dependent. Remaining PKC isozymes are activated by diacylglycerol (DAG). All PKC isoforms show different distribution among various cells. The α isoform is found in all cells, γ is found in neuronal cells and β isoform is found in various tissues. Binding of hormone or other effectors molecule to the membrane receptor results in activation of phospholipase C (PLC) via a G-protein-dependent phenomenon. The activated PLC hydrolyzes phosphatidylinositol-4, 5-bisphosphate (PIP_2) to produce DAG and inositol- 1, 4, 5-trisphosphate (IP_3). The IP_3 causes the release of endogenous Ca^{2+} that binds to the cytosolic PKC and exposes the phospholipid binding site. The binding of Ca^{2+} translocates PKC to the membrane, where it interacts with DAG and is transformed into a fully active enzyme (Fig. 1).

Mobile phone radiation exposure resulting in altered PKC activity has been linked with various types of malignancies[48]. Different levels of PKC and activation of various isozymes have resulted in brain tumor[17,21,31]. The working mechanism behind this is tumor promoting phorbol esters such as 12, O-tetradecanoyl phorbol-13- acetate (TPA) which have a structure, very similar to diacylglycerol and activate protein kinase C directly, both *in vitro* and *in vivo*[50]. Several laboratory studies reported that the protein kinase C is the receptor of tumor promoters[51]. Since several decades, investigators[52,53] indicated that acute and chronic, continuous or pulsed wave irradiation to animals could produce morphological alterations in biological cells and tissue. Studies have suggested that PKC may involve in regulation of a variety of cellular events including modulation of receptor functions for major hormones and certain enzymes such as adenylate cyclase and ornithine decarboxylase in brain. Therefore, low level EMF targeted PKC in the membrane can lead sequentially to a variety of altered intracellular events in the cells[54]. PKC is a key regulatory enzyme in signal transduction mechanism governing cellular responses[55].

PKC is present in rat hippocampus, semniferous tubules and leydig cell[48,56-57]. Beside this, PKC plays a key role in a variety of pathologic states including oncogenesis[58] and in mediating cellular responses to extracellular stimuli involved in proliferation, differentiation and apoptosis in a number of non-neuronal cells[27,59]. Evidences suggest that PKC modulates ion conductance by phosphorylating membrane proteins such as channels, pumps, and ion exchange proteins, besides its role in extrusion of Ca^{2+}, immediately after its mobilization into the cytosol. The activation of this enzyme is thought to be biochemically dependent on Ca^{2+}. Recently, Kesari

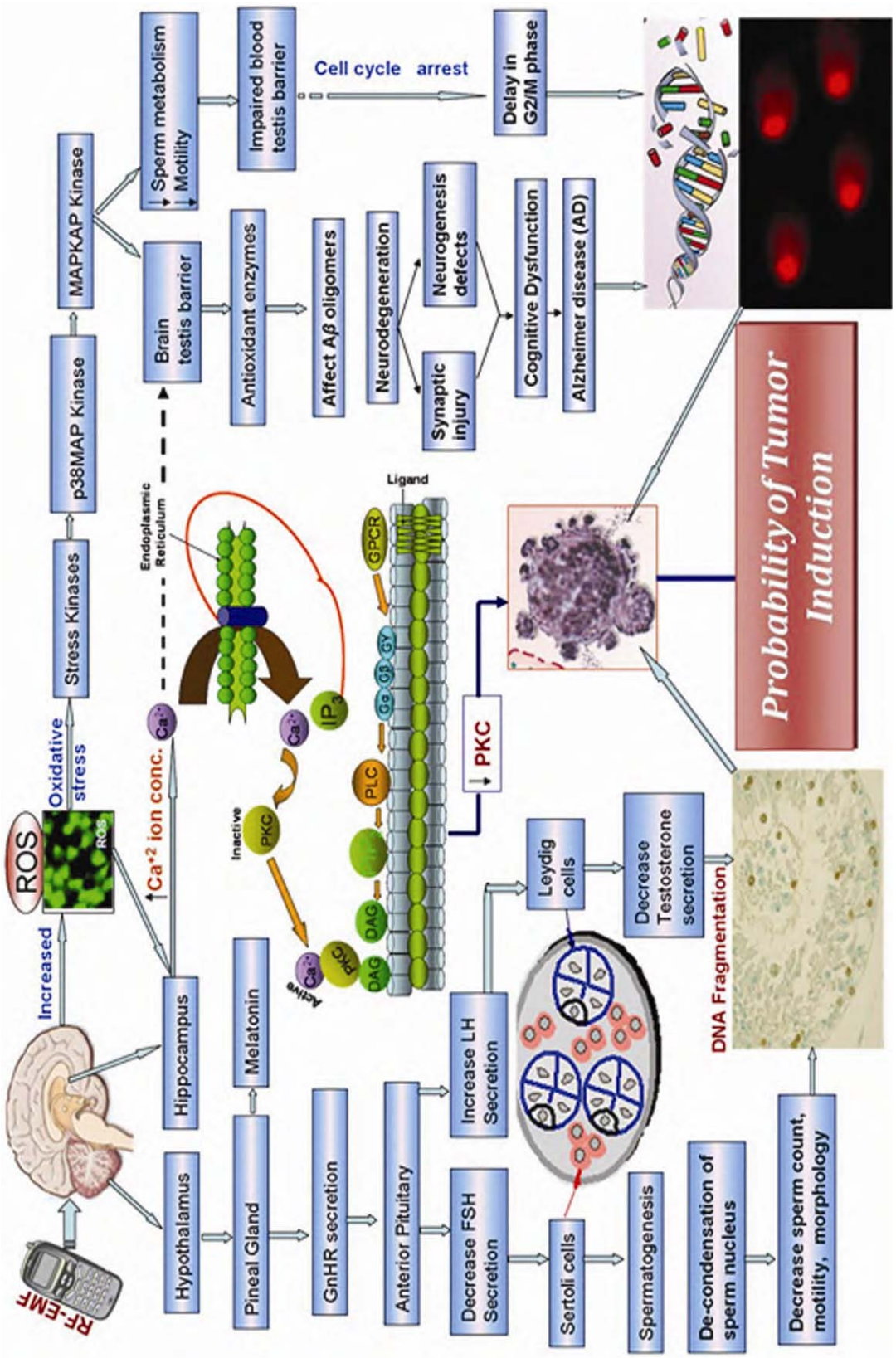

Fig. 1—Showing the exposure to RF-EMF can increase the permeability of the blood brain barrier allowing the influx of large plasma proteins like albumin into the brain causing structural damage to some enzymes of the plasma membrane like NADH oxidase which in turn can accelerate reactive oxygen species (ROS) formation. Increase in ROS can stimulate mitogen-activated protein kinase (MAPK). MAPK pathway plays a dual role in promoting tumor as well as inducing infertility. Another enzyme which is adversely affected is the PKC which shows abundance in the Hippocampus of brain. The ROS decelerates its pathway which severely increases the Ca^{2+} concentration thereby inhibiting melatonin production and adversely affecting the varied processes governed by it.

et al.[17] reported that the PKC activity (in whole brain of Wistar rat) is reduced significantly (*P*= 0.0483) in exposed group (3242 ± 679.10 p32 counts/mg protein), as compared to sham exposed (4609 ± 1325.51 p32 counts/mg protein). Moreover in brain hippocampus of exposed group (4122 ± 1283.73 p32 counts/mg protein), showed a significant decline (*P*=0.0047) as compared to sham exposed group (6491 ± 963.53 p32 counts/mg protein). Several other studies on brain suggested that mobile phone radiation alter the activity of PKC on neurological system, when exposed with 147 MHz amplitude and 2.45GHz of microwave frequency. A significant decrease in the activity of PKC in developing rat brain, was recorded more in hippocampus as compared with whole brain data[17,31]. Butler *et al.*[60] suggested that cells might be functionally depleted of PKC by prolonged radio frequency exposure to biologically active phorbol esters.

Generally, it has been accepted that the maximum quantity of PKC is found in hippocampus. Hippocampus is an integral part of the brain's limbic system and its glucocorticoid and mineralocorticoid receptors are involved in behaviour regulation as well as regulation of the hypothalamo-pituitary adrenal (HPA) axis[61-65]. It is also a site of long-term potentiation (LTP)—the cellular mechanism believed to underlie learning and memory. Damage to neurons in the hippocampus may therefore lead to impaired learning and memory, behavioural disturbances, as well as negatively impacting the functioning of the HPA axis. Salford *et al.*[66] found that rats which were exposed to GSM 900 EMR, resulted in structural damage to the brain. The hippocampus in particular showed the presence of darker, shrunken neurons in the pyramidal cell layer. The authors postulated that the damage was caused by albumin leakage from the blood brain barrier into the brain.

Blood brain barrier (BBB)—The blood-brain barrier has a vital role in the body to excrete toxins from the blood stream from reaching sensitive brain tissues. It is a selectively permeable, hydrophobic barrier that is readily crossed by small, lipid-soluble molecules. It serves, not only to restrict entry of toxic polar molecules into the brain, but also as a regulatory system that stabilizes and optimizes the fluid environment of the brain's intracellular compartment. A dysfunctioning BBB allows influx of normally excluded hydrophilic molecules into the brain tissue. This might lead to cerebral oedema, increased intracranial pressure and, in the worst case, irreversible brain damage. Opening of the BBB may subject the central nervous system (CNS) to assault from extraneous micro-organisms. It has a dual role in preventing the brain from damage, while stabilizing and optimizing the fluids surrounding the brain. The BBB has thus been a subject of investigation because of its vicinity to the radiation source and its central function in the human brain. Because of its penetration, exposure of an electromagnetic field could cause significant alteration in BBB behaviour. The possibility of RF exposure on blood brain barrier and positive indication in leakage or decrease in level could be the issue which can be explored upon. In addition to this Persson *et al.*[67] reported that pathological leakage of the blood-brain barrier occurs with exposure to 915 MHz cell phone frequency. Salford *et al.*[68] showed leakage through the blood-brain barrier (or increased permeability) is caused by 915 MHz RFR. Radio frequency waves have the ability to open up the blood-brain barrier to leakage.

Mobile phone induced genotoxicity—Mobile phone induced genotoxicity is effectively studied by using comet assay and micronucleus assay. Chromosomal abnormalities are a direct consequence of DNA damage such as double strand breaks and misrepair of strand breaks in DNA, resulting in chromosome rearrangement. At present, the micronucleus test is the most popular short-term assay for evaluation of clastogenicity[69-71,23,41]. Micronuclei arise from chromosomal fragments or chromosomes that are not incorporated into daughter nuclei at the time of cell division. The peripheral blood micronucleus test is used to evaluate acute clastogenic effects and assess chronic damage of chromosomes, induced by radiofrequency microwave radiations[23,71].

The basic phenomenon of micronuclei induction is shown during RBC formation, erythroblasts expel their nucleus and may also damage the chromosome in the cytoplasm of young erythrocyte (in the form of micronuclei). Due to their relatively small size, the radiofrequency-induced MN is likely to change via a clastogenic effect. Therefore, during proliferation, the cells continue to divide and cause chromosomal damage such as breaks and exchanges, which eventually lead to formation of micronuclei. This may also participate in cell fragmentation. MN has small, nucleus-like structures present in the cell, especially relevant in assessing genotoxicity effect. The induction of MN in bone marrow or peripheral red blood cells (RBC) is widely accepted as a sensitive predictor of the

clastogenic potential of chemical and radiation exposure[72]. Kesari *et al.*[23] recently showed a significant increase in micronuclei induction by decreasing the ratio of polychromatic erythrocyte (PCE) and normochromatic erythrocyte (NCE) in blood cells of mobile phone radiation (900 MHz) exposed group (0.67 ± 0.15) as compared to control group (1.36 ± 0.07). It was observed that a significant increase of MN induction in cultures irradiated at mobile phone frequency for 35 days at the SAR of 0.9 W/kg. Kumar *et al.*[42,71] also showed the causative effect by lowering the percentage of PCE/NCE ratio and higher level of ROS at different frequencies (10 GHz, 50 GHz). Garaj-Vrhovac *et al.*[72] also reported significant increase in micronuclei frequency following treatment of human lymphocytes with radiation in the microwave range (900-1800MHz). The significant changes in the frequency of micronucleated PCE in the experimental group is an indication of mobile phone radiation induced chromosomal damage. MN formation occurred with the loss of chromosome fragments due to microwave radiation[23,71].

In *in vivo* study on human subjects Gandhi and Anita[73] found a correlation between mobile phone usage and genetic damage as measured in the *in vivo* capillary blood micronucleus test. The micronucleus test was performed on 20 mobile phone users and 8 controls. The authors found 0.25% cells with micronuclei in mobile phone users *vs*. 0.05% in controls. Only 4% of the control subjects presented cells with micronuclei whereas this was more than 83% in the mobile phone users. Micronucleated cells were especially found in subjects with higher exposure levels, e.g., in a person using a mobile phone for 8–9 h per day for 2 years.

Cytogenetic studies of microwave radiation were conducted *in vitro* and *in vivo*, and have yielded contradictory and often intriguing experimental results[74,75]. Some reports suggest that exposure of human cells to radiofrequency radiation does not result in increased cytogenetic damage[76-78]. On the other hand there is a range of studies showing that radiofrequency radiation can indeed induce genetic alternation after exposure to electric field[79,80].

The comet assay is now a well-established genotoxicity test for the estimation of DNA damage at the individual cell level both *in vivo* and *in vitro*. The comet assay has widely been used to detect primary biological effects on the level of DNA molecule in human and animal cells exposed to several environmental or occupational substances[81-83]. Over the past decade, the comet assay has become one of the standard methods for assessing genome damage with a variety of applications in genotoxicity testing as well as in the fundamental research of DNA damage and repair. The comet assay detects primary DNA damage and makes it possible to study the repair kinetics at the level of a single cell. There is a variety of possible modifications of the assay, which facilitates the detection of single-strand DNA breaks, alkali-labile sites, double-strand DNA breaks, incomplete excision repair sites, and interstrand crosslinks. The comet assay can also be used to assess DNA fragmentation associated with cell death, especially apoptosis[81,82].

Molecular mechanism of cytotoxicity—An imbalance between free radical pro-oxidants and anti-oxidants has important implications for both physiological and biochemical processes in the biological system. From the molecular point of view, Hsp70s are an important part of the cell's machinery for protein folding and help to protect cells from stress. An induction of the increased heat shock protein activation by the RF-EMW exposure might also lead to inhibition of the apoptotic pathway that involves apoptosome and caspase 3 (ref. 60). One of the best-documented responses is the induction of transcription, which can be induced by short exposures to low-frequency electric and magnetic fields[61]. Similar to the above systems, the higher frequency (approx. 900 MHz) electromagnetic irradiation emitted from mobile phones also induces expression of proteins in various cells[84-88]. Among the proteins whose expression is induced by mobile phone irradiation are transcription factors[84,89], and HSPs (heat-shock proteins)[90], such as HSP27[91] and HSP70[92,93]. The elevated expression of these proteins may participate in the induction of various cellular processes that appear to be affected by mobile phones radiations[90], which include replication[86], cell-cycle progression[94] and apoptosis[95,93] A major mechanism that regulates transcriptional activity in response to extracellular stimuli is the activation of the mitogen-activated protein kinase (MAPK) cascades. These cascades are a group of signal transduction pathways which mediate the effects of various stimuli to regulate essentially all stimulated processes, including proliferation, differentiation, metabolism and the stress response[96,97]. Indeed, it has been shown that lengthy exposure to mobile phone irradiation on

human endothelial cells can activate the p^{38} MAPK, c-Jun-N-terminal kinases (JNK) and extracellular-signal-regulated kinases (ERK) cascades[98,86], although reduction in p^{38}MAPK levels has also been reported in human epidermoid cancer cells[93]. This activation is mediated by ROS that are produced upon irradiation by membranal NADH oxidase[41].

Caspase: An activator of apoptosis—The activation of caspase-3, a member of the cysteine aspartic acid specific protease family, plays a key role in the early phases of apoptosis in mammalian cells. Genotoxic stress induced by electromagnetic field exposure on biological system is indicated by caspase 3. After activation of caspases, cells undergo apoptotic death through a number of substrates. Therefore, apoptosis is also considered to be involved in the impairment of the spermatogenesis and seminiferous tubules. It was investigated whether EMF radiation would alter the biology of cells and act as a tumor-promoting agent. Liu *et al.*[99] reported that exposure to the EMF of 1950-MHz TD-SCDMA may not promote the tumor formation, but continuous exposure damaged the mitochondria of astrocytes and induce apoptosis through a caspase-3-dependent pathway with the involvement of bax and bcl-2.

Apoptosis is induced by ROS through cytochrome c and caspases 3 and 9 which in turn leads to a high rate of single and double strand DNA break. Kesari and Behari[22] have reported an increased apoptosis in Wistar rat model of testicular organ due to microwave exposure at 2.45 GHz and 0.11 W/kg of SAR on 35 days of exposure. The study was confirmed by terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay and it was found that DNA fragmentation and apoptosis is induced by mobile phone and microwave frequencies[22,27]. Such regulation of apoptosis is based on the intracellular dominance of various proteins that induce or inhibit the apoptotic process, such as BAX, Bcl, caspase-3 and several key enzymes[100]. The initiator caspases 8 and 9 with effectors caspase 3 considered as the main executors of apoptosis[101]. The effectors caspase-3 share both pathways-mitochondrial pathway through caspase 9 and death-receptor pathway through initiator caspase 8[102]. Usually caspase 9 and 3 are activated to execute apoptosis. Lee *et al.*[103] also reported that EMF may induce cell death (apoptosis) in several *in vivo* studies mostly on mice and rats. However, an induction of the increased Hsp27 activation by the RF-EMW exposure might also lead to inhibition of the apoptotic pathway that involves apoptosome and caspase 3 (ref. 41). They showed that such effects may be caused due to overproduction of ROS. Study of Karinen *et al.*[104] and Blank and Goodman[105] indicated an induction of heat-shock proteins and apoptosis that induces stress on living cells. Another important study of Sokolovic *et al.*[106] indicated a reduction of melatonin synthesis and activation of Fenton's reaction which increase the concentration of free radicals and peroxides having the ability to damage the DNA. Caspases activated by apoptotic signals cleave various cellular substrates such as actin, poly (ADP-ribose) polymerase, fodrin and lamin, which may be responsible for the morphological changes that occur in the cells. Such changes lead to cell damage and induction of apoptosis. Recently, an increased number of apoptotic cells were recorded in mobile phone radiation exposed (2 h/day for 35 days) group of Wistar rat sperm ($P< 0.005$) with a significant decrease ($P=0.022$) in G2/M phase[20]. Moreover a significant increase ($P=0.008$) in brain caspase-3 activity was recorded in mobile phone exposed group (47.35±1.92) as compared to the sham exposed (44.52±0.87). An increased caspase-3 activity shows the possibility of tumor promotion due to increased apoptotic cells.

Retrieval effect of pulsed magnetic field in response to EMF—The present studies are apparent to find out the mechanism of such causes which may be controlled by the application of pulsed electromagnetic field (PEMF). An application of PEMF (50 and 100 Hz, magnetic component) is its capability of eliminating ROS effect by providing a paired electron, thereby eliminating the stressor effect (antioxidant effect). The major difference between electromagnetic field (EMF) generated from various sources (mobile phone, microwave oven) and the pulsed electromagnetic fields (100 Hz) is that the later induces circulating electric current in the tissues because of its constantly changing magnetic flux[116]. It is suggested that microwave field penetrates inside the biological body, thereby inducing fields and perturbing the endogenous physiological processes. It has been estimated that the maximum size of the electric fields produced in head area by the antenna of the mobile phone is around 100 V/m, although the fields inside the brain would be appreciably less. For fields of this size the following mechanisms are most likely to produce non thermal biological effects:

(a) through the movement of large cells, and (b) through the attraction between neighboring cells, which become dipoles under the effect of EMR[117].

In an interesting study Kumar *et al.*[43] exposed the animals with PEMF at 100Hz of frequency. Experiment was carried out by treated rats that had previously been exposed to 2.45 GHz radiation with PEMF. They demonstrated that PEMF is being used as healing agent which activates the normal physiology of the body. Result concludes that the impact of PEMF may decrease the radiation effect and get the biological system normalized. The de-stressor effect comes into play because of the antioxidant role of magnetic field of the applied pulsed field at low frequency. PEMF activates normal metabolic processes, indirectly acts through the endocrine system, and controls the main cause of stress in the exposed system. Authors conclude the studies reveal that oxidative stress is a major mechanism affecting health, and microwave fields cause chronic stress *via* ROS overproduction. But, PEMF therapy provides significant protection by controlling ROS production. Several other studies showed that the PEMF exposure promotes the restoration of the bone loss in osteoporotic bones[118] and also bone formation and bone healing by the confirmation of no DNA strand break[119]. PEMF also has a variety of biological impacts such as bone healing[120], balancing of the neuroendocrine system including hormone production and melatonin levels[121].

Conclusion

Present review explores the possible pathways between EMF effect and the function of central nervous system (CNS). The possible mechanism behind it has been found due to free radicals formation, which involved in the RFR-induced DNA strand breaks, significant changes in antioxidant enzymes (SOD, GPx, CAT, MDA) and hormonal disbalance (melatonin) in brain. As a consequence of increase in free radicals or reactive oxygen species, various cellular and physiological processes can be affected including gene expression, release of calcium from intracellular storage sites, cell growth, micronuclei and apoptosis. Apoptosis is well known indicator of tumor promoter. Any tumor promoting effects of RF-EMF might be due to the effect it has on PKC, histone kinase, pineal melatonin which may accelerates neuronal cell death and promotes neurodegenerative processes as well as promote brain carcinogenesis. All of these studies reveal that oxidative stress is a major mechanism affecting health, and the microwave fields cause chronic stress via ROS overproduction. It is concluded that radiofrequency electromagnetic wave from commercially available cell phones might damage to whole brain. However, more studies are necessary to provide absolute evidence against microwave radiations emitted from cell phone and its towers, which can be provided by *in vitro* and *in vivo* studies in combination with physical biomodeling.

Future Studies

Radio Frequency/microwaves emitted from cell phones and their towers are responsible to cause biological damage to different organs i.e. brain, sperm, kidney etc. Several studies have reported causative effect of radio frequency electromagnetic field on different organs[17,28,29,46,47,110]. But in the present scenario, very less or almost none has found a solution against protection to these radiations. There are several known herbal plants available with known compounds which can act as antioxidants, anti cancerous leads[122,123]. Radioprotective potential of caffeic acid (green tea) and its extracts are rich source of polyphenolic compounds[124]. Polyphenolic compounds found in fruits and vegetables exhibit strong antioxidant activities. The polyphenols present in green tea (*Camellia sinensis*) have numerous health benefits due to their anti-inflammatory and anti-oxidant activities which may also be useful agents against microwave exposure. It will be interesting to see the positive effect of phytochemicals from green tea when tested against cancer cell lines and to see their possible inhibition of cell proliferation either by inducing cell cycle arrest or inducing apoptosis finally leading to cell death of the microwave exposed cell populations. There are many more herbal plants i.e. Ginkgo biloba, Aloe-vera, which will be useful for further studies where it could be ensured the possible changes which may occur in microwave induced subjects. The shielding against these radiations emitted from cell phone and their towers need to be restricting by using chemo-protective medicinal plant.

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