Introduction
There are sufficient evidences of increasing trends in a number of human health problems. Over the last few decades, the progressive changes in environmental, lifestyle, dietary or occupational factors may play an important role behind these issues. Among lifestyle factors such as use of illicit drugs, tobacco smoking or chewing, alcohol, caffeine etc. have a profound negative impact on general health. Various health problems like high blood pressure, high blood cholesterol, diabetes mellitus, obesity, infertility, developmental defects, cardiovascular disease, and even some types of cancer are related to nutritional as well as lifestyle factors to some extent. Exposures to environmental pollution remain a major source of health risk throughout the world, though risks are generally higher in those countries where poverty and weak environmental legislation combine to cause high pollution levels. Urbanization and industrialization along with economic development have led to increase in energy consumption and waste discharges. Since last two decades, apart from the conventional sources of pollution, electromagnetic pollution has emerged as a new threat to human health.

Environmental levels of radiofrequency (RF) energy are routinely encountered by general public. Every day, we are swimming in a sea of electromagnetic radiation (EMR) produced by electric lines, electrical appliances, wiring in buildings, and other technologies that are part of modern life. From the dishwasher to microwave oven in the kitchen and the clock radio next to our bed, to the cellular phone we hold to our ear, sometimes for hours each day exposure to EMR is growing and becoming a serious health threat. We encounter the invisible electromagnetic radiation which surrounds us each day emanating from diverse sources.

Exposure to electromagnetic fields (EMF) is not a new phenomenon. However, during the last century, due to growing electricity demand, advancement in technologies and changes in social behavior, environmental exposure to man-made electromagnetic fields has been steadily increasing. The entire population is exposed to a complex mix of weak electric and magnetic fields, both at living and working place, from the generation and transmission of electricity, domestic appliances and industrial equipment to telecommunications and broadcasting. Wireless communication, electric devices and infrastructure are the hallmarks of modern life.

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The proliferation of these technologies in recent years has dramatically increased our exposure to electromagnetic radiation (EMR) or electromagnetic fields (EMF).

Tiny electrical currents exist in the human body due to the chemical reactions that occur as part of the normal body functions. For example, neurons relay signals by transmitting electric impulses even the heart is electrically active. Low-frequency electric fields influence the human body just as they influence any other material made up of charged particles. Electric fields influence the distribution of electric charges at their surface of conductive materials causing the current to flow through the body to the ground. Low-frequency magnetic and electric fields induce circulating currents within the human body. The strength of these currents depends on the intensity of the outside magnetic field. If sufficiently large, these currents could cause stimulation of nerves and muscles or affect other biological processes. Thus, exposure to EMR or EMF can alter the normal bodily functions.

Radiations can be classified as ionizing or non-ionizing radiation. X-rays and Gamma rays are two form of ionizing radiation. These radiations contain sufficient electromagnetic energy to strip atoms and molecules from the tissue and alter chemical reaction in the body. Human beings are constantly exposed to low level of ionizing radiation from natural sources which are referred to as natural background radiation. Its main sources are radioactive materials on earth surface contained in coal, radioactive gases leaking from earth and cosmic waves etc.

Fig. 1: Electromagnetic spectrum

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Non-ionizing radiation consists of lower part of spectrum. Examples of non-ionizing electromagnetic radiation are static electromagnetic fields from direct current (0 Hz), low frequency waves from electric power (50-60 Hz), extreme low frequency (ELF) and very low frequency (VLF) fields (30 kHz), radio frequency (RF) and microwave (MW-30 kHz to 300GHz), ultraviolet (UV), visible light, infrared (IR-above 300GHz).

Radio frequency radiation is of great interest because it is emitted from the instruments of common use e.g. mobile telephony, broadcasting and TV, microwave oven, radar, portable and stationary radio transceivers, personal mobile radio etc. In general, the exposure to EMR from different sources is divided into two categories: “continued” and “pulsed” according to the characteristics of the emitted waves. The biological effects of pulsed wave exposure are even more harmful than that of continuous variety from other sources.

<table>
<thead>
<tr>
<th>Frequency range</th>
<th>Frequencies</th>
<th>Some examples of exposure sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static</td>
<td>0 Hz</td>
<td>VDU (video displays); MRI and other diagnostic/scientific instrumentation; Industrial electrolysis; Welding devices</td>
</tr>
<tr>
<td>ELF</td>
<td>0-300 Hz</td>
<td>Power lines; Domestic distribution lines, Domestic appliances; Electric engines in cars, train and tramway; Welding devices</td>
</tr>
<tr>
<td>IF</td>
<td>300 Hz-100 kHz</td>
<td>VDU; anti theft devices in shops, hands free access control systems, card readers and metal detectors; MRI; Welding devices</td>
</tr>
<tr>
<td>RF</td>
<td>100 kHz-300 GHz</td>
<td>Mobile telephony; Broadcasting and TV; Microwave oven; Radar, portable and stationary radio transceivers, personal mobile radio.</td>
</tr>
</tbody>
</table>

Table 1: Typical sources of electromagnetic fields

Mobile telephony has become cosmopolitan source of emitting RF radiations. Use of cellular phones has increased exponentially and become an important part of everyday life throughout the world. Worldwide use of mobile phones had been increased up to 7.3 billion by 2014. Most cellular and cordless telephones have either

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A small antenna attached or the antenna is integrated into the body of the telephone. Because the antenna of the cellular phone remains close to the user's head, while using it and near the reproductive organs, while keeping it in the pocket at standby mode in males specially, such telephones create greater RF exposure than other types of RF system. Exposure of RF energy depends on the frequency of the cellular phone used. More commonly cellular phones operate at a frequency of 850-1800 MHz. The specific absorption rate (SAR) defines the amount of RF energy absorbed into local tissues and represents a measure for evaluating the emission of transmitters located nearby the body. For cellular phones, the permissible limit of SAR varies from 0.12 to 1.6 watts/kg of body weight.

Mobile (cellular) telephony is based on two-way radio communication between a portable handset and the nearest base-station. Every base-station serves a cell, varying from kilometers in rural areas to hundreds of meters in extent in densely populated areas, and is connected to the conventional land-line telephone network and to neighboring stations. As the user of a mobile phone moves from cell to cell, the call is transferred between base-stations without interruption.

It is generally accepted that RF fields do not directly damage DNA because the photon energy of radiation from mobile phones is much lower than the energy necessary to break chemical bonds. However, it is possible that radiation exposure can alter the cellular constituents, such as free radicals indirectly affect DNA. Heating of biological tissue is a consequence of microwave energy absorption by the tissue’s water content. The amount of heat produced in the body of a living organism depends primarily on the power intensity of the radiation once it has penetrated the system, on some electrical properties of the biomatter and on the efficiency of body’s thermoregulation mechanism. Above a certain intensity of microwaves temperature, homeostasis is not maintained and affects on health.

No exposure occurs from a mobile phone being switched off. Phones operated in the standby mode cause typically much lower exposure compared to mobile phones operated with maximum power, but an exact figure for this lower exposure depends...
on the details of the transmission path to base stations and on the traffic requested by the communication protocol and by incoming/outgoing SMS.

Two areas of the body, the eyes and testes, are known to be particularly vulnerable to heating by RF energy because of the relative lack of available blood flow to dissipate the excessive heat load (blood circulation is one of body’s major mechanism for coping with excessive heat), cataract formation and reduced sperm counts are well-documented in acute exposure hazards. There is a growing body of evidence that exposure to high density microwaves can cause detrimental effects to the testis and eyes which induce significant biologic changes involving the reproductive system, cardiovascular system, hematopoietic system and through thermal action. Meanwhile, the non-thermal effects of 915MHz microwaves used in mobile phones has been controversial.

Biological effects are measurable responses to a stimulus or to a change in the environmental condition. These changes are not necessarily harmful to your health. For example, driving a car, reading a book, playing football, eating an apple or listening to music will produce a range of biological effects. But none of these activities is expected to cause health effects. Our body has sophisticated mechanisms to adjust to many varied influences we encounter in our environment. Ongoing change forms a normal part of our lives. However, the body does not possess adequate compensation mechanisms for all biological effects. Those changes which are irreversible and cause stress to the system for long periods of time may constitute a health hazard. A harmful effect causes detectable impairment of the health of the exposed individual or of his or her offspring; a biological effect, on the other hand, may or may not result in an adverse health effect.

It is not disputed that electromagnetic fields above certain levels can trigger biological effects. Experimental studies indicate that short-term exposure at the levels present in the environment or in the home do not cause any apparent detrimental effects. National and international guidelines have restricted the exposures to higher levels that might be harmful. The current debate is focused on whether long-term low level exposure can evoke biological responses and influence mobile phone users' well being.

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People are concerned about how long-term exposure to excessive EMR may impact human health and nature, while the science on the health impacts of this form of radiation is still inconclusive. It is recommended to the pregnant women to avoid excessive use of mobile phones because prolonged exposure to electromagnetic radiation might be associated to the complications related to developmental defects, congenital malformations, spontaneous abortions, birth weight, and gender ratio. Reduction of melatonin level due to exposure of electromagnetic radiation threatens our health and can result in psychiatric disorders like depression, shortened attention span and inability to sleep. It causes digestive problems, lack of concentration, and loss of memory. Apart from health risks, the study also indicates that mobile phone radiation creates environment hazards like disappearance of sparrows and insects like butterflies and bees from big cities.

The biological effects of RF energy are proportional to the rate of energy absorption and the level of absorption varies little with frequency. RF energy has the ability to heat human tissue, much like the way that the microwave ovens heat food and can be hazardous if the exposure is sufficiently intense or prolonged. Damage to tissue may be caused by exposure to high level of RF energy because the body is not equipped to dissipate the excessive amount of heat generated. Commercial AM/FM radio and TV broadcast stations transmit very high levels of RF energy. Some of their antennas radiate power levels of several megawatts but, fortunately these antennas are placed on high towers or buildings where no humans are nearby.

A growing concern for possible adverse effects of mobile phones on human health evokes a flurry of scientific activity to evaluate this dilemma. There is no epidemiologic evidence today showing that occupational or daily life exposures to microwaves do any harm to human reproductive processes, but experimental animal studies have suggested that microwaves can produce intra-uterine effects including teratogenic effects. There is a general concern that children or adolescents may be more sensitive to RF field exposure than adults. In the area of biological effects and medical applications of non-ionizing radiation lot of articles have been published over the past 30 years. Some researchers have attributed a collection of symptoms to low

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levels of exposure to electromagnetic fields at home including depression, anxiety, nausea, headaches, suicide, loss of libido, and fatigue.

Despite the feeling of some people that more research needs to be done, scientific knowledge in this field is now more extensive than for most chemicals. General eye irritation and cataracts have sometimes been reported in workers exposed to high levels of radiofrequency and microwave radiation, but studies on animal do not support the idea that such forms of eye damage can be produced at levels that are not thermally hazardous. A number of epidemiological studies suggest small increases in risk of childhood leukemia with exposure to low frequency magnetic fields in the home.

Fig. 2: Effects of mobile phone on human body

Many different sources and exposures to electromagnetic fields in the living and working environment, including microwave oven, computer screens, radiofrequency welding machines, electric blankets, radar, have been evaluated by the WHO and other organizations. The list of evidences is showing that exposure to fields at typical environmental levels does not increase the risk of any adverse outcome such Studies on the genotoxic impacts of electromagnetic radiations on male reproductive organ of swiss albino rat
as congenital diseases, spontaneous abortions, low birth weight, and malformations.
There have been some reports of associations between health problems and presumed
exposure to electromagnetic fields, such as reports of prematurity and low birth
weight in children of workers in the electronics industry, but the scientific community
has not regarded these incidences as being necessarily caused by the field exposures.

Some individuals report "hypersensitivity" to electric or magnetic fields. The
victims have reported depression, lethargy, sleeping disorders, aches and pains,
headaches, and even convulsions and epileptic seizures could be associated with
electromagnetic field exposure. There is little scientific evidence to support the idea
of electromagnetic hypersensitivity yet any accepted biological mechanism to explain
hypersensitivity has not been proposed. In recent studies, it was found that individuals
do not show consistent reactions under properly controlled conditions of
electromagnetic field exposure. Research on this subject is difficult because many
other subjective responses may be involved, apart from direct effects of fields
themselves. More studies are continuing in this field.

As part of its mission to protect public health and in response to public
concern over health effects of EMF exposure, WHO established the International
EMF Project 16 in 1996 to assess the scientific evidence of possible health effects of
EMF in the frequency range from 0 to 300 GHz. The EMF Project encourages
focused research to fill important gaps in knowledge and to facilitate the development
of internationally acceptable standards limiting EMF exposure.

A genotoxic action of radiofrequency electromagnetic fields (RF-EMF) has
been demonstrated in vivo and in vitro by a large number of investigators and using
various genetic endpoints and a wide variety of cells and tissues. The literature
contains controversial reports on the effects of RF-EMF on oxidative stress,
mitochondria, heat shock proteins, DNA damage, free radical metabolism, apoptosis
pathway, cell differentiation, and the degenerative changes in plasma membrane.
Among the effect of RF-EMF on various body organs, effect of RF-EMF on brain and
testicular tissue is the most researched area. Recent studies suggest that RF-EMF
emitted from cell phones can reduce the fertilizing potential of men. The majority of

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these studies have been conducted using exposure to Global System for Mobile Communication (GSM) signals.

The human body acts as parasitic antennae that receive the EMF from external sources. Specifically both electrical and magnetic fields can induce electrical current inside living tissue. However, the generated internal currents are of much lower strength and different directions from the external ones. These alterations in strength and directions reflect the electrical properties of human body, such as permittivity and conductivity. To understand these properties, human tissue is best described as a medium dielectric property due to high content of water in addition to other molecules and ions. When living tissue is exposed to EMF, the dielectric molecules will be polarized, the extent of such polarization is called permittivity. Conductivity, on the other hand, describes the conduction current density produced by an applied electric field.

There has been growing public concern on the effect of electromagnetic radiation on human reproductive system. Reproduction is a complex, stepwise series of processes that begins with gametogenesis, continue through fusion of gametes, placenta formation, embryonic development, parturition, growth, and postnatal adaptation, and completed with development of sexual maturation of the newly formed organism. Gametogenesis (oogenesis, spermatogenesis) in all animals is a biological process more sensitive to environmental stress than other developmental-biological processes that take place at later stages of animal development. These reproductive processes do not take place in the polluted environment. Electromagnetic pollution is known to be carcinogenic, mutagenic, or toxic to the reproductive system, but has not been adequately tested for reproductive toxicity.

The differences between male and female in terms of reproductive anatomy and biological mechanisms are important factors in reproductive toxicology. The structure and processes that are involved in male reproductive function and that might be influenced directly or indirectly by radiation include the hypothalamic-pituitary-gonadal axis, testes, efferent ducts, epididymes and male accessory organs, formation

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and composition of semen, sexual behavior. Because of the easier accessibility of gametes and gonads in male, these are easily affected by the radiations.

A number of recent reports have suggested a possible link between cell phone use and male infertility. Male reproductive system is highly compartmentalized and sensitive biological system that requires the integration of intrinsic and extrinsic factors to properly function. The testes are very important organs situated externally to the body and enclosed by the scrotum. The testicular parenchyma is the site of an intense proliferation and differentiation of the germinal cells that will become the sperm cells. The Leydig cells present in the interstitial space are responsible for synthesis of male reproductive hormone-testosterone which is needed in very high quantities for maintenance of reproductive tract and spermatogenesis. The testes are very sensitive to temperature variation and for this reason the scrotum, which contains the testicular parenchyma, has a contractile specialized structure. Mammalian spermatozoa leaving the testes have to undergo distinct morphological and biochemical changes during the epididymal transit before being capable to fertilize the ovum.

Standby communication signals did not significantly affect the sperm quality, but daily usage of mobile phone for prolonged time might have negative effect on testicular tissue. The radiation may alter the hormonal milieu and microenvironment in testicular tissue, necessary for healthy sperm production. Additionally sperms are electrically cells and their exposure to cell phone electromagnetic radiation and current may affect their normal morphology, count and even their motility. Defective plasma membranes, oxidative stress and calcium depletion are the expected cellular mechanisms, mediating the harmful effects of cell phone radiation on sperm and male fertility potential.

An initial study suggested that the use of cell phones adversely affects semen quality by decreasing sperm morphology, motility, count, and viability which might contribute to male infertility. Leydig cells, seminiferous tubules and spermatozoa are the main targets of the damage caused by the mobile phones on the male reproductive system. Temporary sterility is possible after exposure of the testes to high level RF
radiation or to other forms of energy that produce comparable increases in temperature. Male reproductive functions can possibly be affected by electromagnetic field radiation emitted by mobile phones via three mechanisms: an EMF specific, a thermal molecular effect or a combination of both effects. Scrotal hyperthermia and oxidative stress are the main mechanism by which the damage is generated. Testicular temperature is 2-3°C lower than rectal temperature and the optimal temperature for spermatogenesis is considered to be 35°C. From this point of view, the habit of keeping mobile phone in the trouser pocket or the duration of its use may have a major impact on possible generation of hyperthermia and oxidative stress as well.

Fig. 3: Effect of RF-EMR on various aspects of testicular functions.

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Fig. 4: Effect of RF-EMR on sub-cellular molecular pathways.

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Exposure to RF-EMF can induce alterations in many sub-cellular mechanisms. The plasma membrane may be a target of RF-EMW. Human spermatozoa are highly susceptible to oxidative stress (OS) due to high contents of poly unsaturated fatty acids (PUFA) in their cell membrane. Changed plasma membrane potential and calcium efflux with resultant calcium depletion leads to decrease in the activity of protein kinase C (PKC). This decrease leads to alteration in many membrane bound and cytoplasmic enzymes, ion channels and proteins as well as inducing apoptosis.

RF-EMW mediated OS in semen might be responsible for decline in motility and viability of spermatozoa. RF-EMW might trigger uncontrolled cell proliferation by its action on various plasma membrane enzymes and receptors. Short-term exposure to RF-EMW can lead to increase in the activity of plasma membrane NADH oxidase enzyme, which increases ROS formation. ROS has impact on PKC, histone kinase, heat shock protein, DNA and apoptosis.

Studies have reported declines in sperm motility with decreases in PKC activity. Recently, decreases in the activity of PKC in developing rat brains that were exposed to 2.45 GHz of microwaves have been reported. Thus, RF-EMW exposure decreases PKC activity and the PKC enzyme is important in sperm motility. However, DNA damage might depend on experimental set up, duration of exposure and SAR. Many studies have indicated that EMW decreases the size of the testicular organs.

Heat shock protein (hsp) increases the response to electromagnetic radiation and ROS. This protein slows down the metabolism of the sperm and impairs the blood testicular barrier and interferes with apoptosis of damaged and transformed sperms. Genotoxic effect of RF-EMF on sperm is either through the production of ROS or through direct clastogenic chromatin breaking effect. RF-EMW may stimulate extracellular superoxide production in semen by its action on plasma membrane enzyme, which can lead to decrease in sperm motility and viability.

A decrease in the diameter of the seminiferous tubules has been reported after exposure to radiofrequency radiations. Similarly, the duration of possession and the daily transmission time of cell phones correlated negatively with the proportion of rapidly progressive motile spermatozoa, suggesting that prolonged use of cell phones...
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might have negative effects on sperm motility. Recent epidemiological studies have highlighted a reduction in the fertilizing potential of exposed males and the role of cell phone exposure on sperm morphology, motility, and viability. However, the significance of these studies is low due to a lack of a control population (men who do not use cell phones), which would be extremely difficult to maintain.

Additionally, an in vivo human exposure study to investigate the effects of cell phone radiation on semen parameters is not feasible due to ethical issues. Despite the increasing number of reports on the effects of electromagnetic radiation (EMR) in various biological systems, no satisfactory mechanism has been proposed to explain the effects of this radiation. Further, the previous studies have been conducted following the short term exposure duration either in vivo or in vitro. So more work is needed on the use of cell phone and fertility with better study designed by incorporating confounding factors associated with fertility.

Hence keeping all the issues in mind, discussed above, the present study is undertaken to study the genotoxic effects of chronic exposure of mobile phone radiation on rat sperm cells, its effect on hormone secretion from the male gonad, to assess associated histological and ultrastructural effects on the fine structure of testicular tissue of rat, if any, in exposed animals and to compare the results with the corresponding control group. The model for the current study was selected as rat because of its easy availability, its susceptibility and widespread use in pharmacological and toxicological studies and its short life span. A commercially available Reliance CDMA ZTE S160 mobile handset was used as the source of radiation working on 2G network at the frequency 800MHz.

OBJECTIVES

- Effect of EMR on histomorphology of testis of rat in vitro.
- EMR induced changes in fine structure of various stages of germ cells and variation in topography of different types of somatic cells in rat testis.

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- Electron microscopic analysis of histological slides of rat testis after exposure to EMR.
- Influence of EMR on steroidogenic output of rat testis.
- Neutral Microgel Electrophoresis of rat sperms.
- Statistical analysis of the data to derive any conclusions.