International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijamr Volume 11, Issue 2 -2024

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2024.11.02.004

A Comparative study on the biochemical changes in nail of smart phone and normal phone users

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Keywords

Electromagnetic fields (EMF), Mobile phones, Nail samples, Oxidative stress, Antioxidant enzymes, Nutritional status.

Abstract

Electromagnetic fields (EMF) are omnipresent in modern society, stemming from both natural phenomena and human-made devices, with over 3 billion individuals worldwide exposed daily. Scientific inquiry into the potential long-term effects of EMF on biological systems is escalating due to its capacity to induce significant changes and adverse effects. The biological impacts of EMF are broadly categorized as thermal and non-thermal, where thermal effects result from heat generated by EMFs, particularly from radiofrequency (RF) fields, potentially elevating tissue temperatures. Conversely, non-thermal effects stem from energy absorption and subsequent tissue alterations not directly linked to temperature changes. While the biophysical mechanisms of RF-EMF interactions with living cells remain incompletely understood, studies increasingly emphasize the significance of non-thermal effects.

This study aims to investigate the effects of EMF exposure, particularly from mobile phones, on nail samples, assessing various parameters to gauge physiological responses. Major objectives include determining protein levels, evaluating antioxidant enzyme activity (superoxide dismutase and catalase), assessing nutritional status (vitamin C, vitamin E), quantifying free fatty acids, and measuring calcium and iron levels in nail samples. A comparative analysis between smart phone and normal phone users aims to elucidate potential differences in EMF impacts.

Findings indicate that EMR exposure from smart phones induces oxidative stress evidenced by decreased levels of antioxidant enzymes and non-enzymatic antioxidants. Moreover, EMF exposure correlates with alterations in protein, iron, and calcium levels in nail samples, suggesting potential cellular damage and

inflammatory responses. The observed increase in calcium levels in smart phone users may indicate a protective response against EMF-induced damage.In conclusion, this study sheds light on the complex interplay between EMF exposure and physiological responses, highlighting potential implications for public health.

1. Introduction

Mobile phones have become our time's most popular communication devices with the help of the addition of new functions. Mobile phones generate an electromagnetic field (EMF) around themselves while they operate by transmitting radio frequency signals. The fact that these gadgets are so widely used has raised worries about the possible biological harm that the electromagnetic fields they produce may cause. Furthermore, it was noted that EMF exposure decreased blood serum element levels, including those of magnesium, iron, and copper4.One of the serum elements that is essential to the active sites of many different proteins involved in respiration, energy metabolism, and DNA synthesis is iron. Although haemoglobin, which carries oxygen, contains 65% of the iron in humans, the remaining 10% is contained in the structures of myoglobin, cytochrome, and ion-containing enzymes. 20-30% of the iron in the body is non-toxically stored as bound to ferritin and hemosiderin molecules. The transportation of the iron is achieved by the transferrin iron.

Health issues about mobile phone radiation have been brought up, particularly in light of the significant global rise in the usage of wireless mobile phones. Low-level radiofrequency (RF) electromagnetic fields in the microwave region are released by mobile phone antennae, with wavelengths beginning at roughly 900 MHz. The human body produces circulating currents when exposed to low-frequency magnetic fields.

The intensity of the magnetic field outside determines how strong these currents are. These currents may stimulate muscles and nerves or have an impact on other biological processes if they are large enough. In comparison to other parts of the body, the human brain is exposed to comparatively high specific absorption rates (SARs) because of the near proximity of mobile phones to the head. It has been suggested that an electro magnetic field affects the biological functioning of nerve cells and causes changes in neurotransmitter contents, but that different brain regions may react to radiofrequency radiation (RFR) in different ways. The efflux of calcium ions from brain tissue is an important neurochemical effect of RFR as calcium ion plays an important role in the functions of the nervous system such as the release of neurotransmitters, Many hazardous effects on the nervous system have been described due to electromagnetic field(EMF) of digital mobile phone.

EMF emitted from mobile phone could affect sleep, learning and memory, attention, cognitive performance, headache and disturbances in blood brain barrier permeability. Recently, disturbances in the hypothalamic, thalamic and striatal amino acid neurotransmitters after short-and long-term exposure to electromagnetic radiation (EMR) were reported. However, the influence of mobile phones on heart rate and blood pressure is still problematic. Additionally, Balik et al. reported no effects on redness and disturbance of vision; nonetheless, certain statistical evidence suggested that using a mobile phone could result in secretion, inflammation, and lacrimation of the eyes in addition to blurred vision. Because they offer so many innovative features, mobile phones have emerged as the most widely used communication tool in the modern world. As mobile phones become more commonplace, worries about the potentially dangerous health effects of the electromagnetic field (EMF) they produce have grown. Mobile phones operate by transmitting radio frequency signals, which creates an electromagnetic field (EMF) surrounding them.

The 21st century has seen the rise in popularity of smart phones, a new breed of mobile device.

Smart phones have surpassed feature phones in popularity. A basic mobile phone is referred to as a feature phone. And now because it's a need for survival, many individuals would feel empty without this device. These days, we use our mobile phones for a variety of tasks in our daily lives, such as arranging and keeping appointments, communicating with clients, and acting as a voice recorder and watch. For many people, mobile phones are essential for managing their daily schedules. Mobile phone usage is hard to categorize in one way because mobile phones are more than just technical devices. As technology progresses, cell phones are starting to become a need. In addition to being tools for communication, mobile phones are also thought of as gadgets with robust communication networks in addition to their other features.

1.1 EMR Associated disease

Male infertility: The reproduction is a lifelike experience for couples. Nevertheless, it is very hard to contrive for the child. Basically the infertility is a wider problem in the universe due to different causes. The male infertility problem creates the sperm production (sperm production in low number) or the transfer process of sperm. The infertility problems are sperm motility, sperm count, sperm morphology, functions of sperm, impaired Leydig cell, Sertoli cell, abnormality of sperms, to stop delivery of sperm, a chronic health problem. The causal agent of male infertility is because of testicular impairment resulting in the testicles not producing the sperm cells, the infertility of man includes the imbalance of hormone, behavioural problem and psychological troubles. The negative impacts on male fertility are smoking, use of drugs, alcohol abuse, tight underwear, radiation exposure, pesticides, paint, lead, and radioactive.

Brain tumour: : It is a mass of tissue which is abnormal and in this condition the body cells die and replaced by the cells and other tumours. The abnormal cells grow and they produce a flock which is named as the main neoplasm. These come out of the several cells, which constitute the brain, CNS (Central Nervous System). There are several cases of brain tumours such as astrocytic and the gliomas tumours.

There are two types of BRAIN TUMOUR: Malignant Tumour (Cancerous Tumour): The malignant tumour is a cancerous tumour that is either primary or secondary. The malignant tumour can be elementary or secondary brain tumours. It starts in the mind. The primary malignant brain tumour is fast growing tumour than the secondary malignant brain tumour where immediate treatment is important for primary malignant tumours because it can open rapidly and damages the spinal cord and other regions of the head.

The Benign tumour is noncancerous tumour means that stay in one spot and do not attack the other region of the head or body. It is a very slow growing brain tumour. It can be removed by the treatment and not come back. The brain tumour can occur at any age and the cause of brain tumours has been still not solved. In that respect are various symptoms of brain neoplasms that are: numbered, headache, seizures, mood changes, memory trouble, trouble walking, hearing, visual sensation, vomiting and nausea. The brain tumour is divided into two grading which are:

Low grade tumours: The low grade brain tumour's grade 1 or 2 which are slow in growing and facing pages. They include malignant cells that are free to proliferate, which implies they are contained in one area and do not spread to other parts of the body or the brain. The level 2 is glamorous that will arise after the discussion.

High grade tumours: The high grade brain tumour's grade 3 or 4 that is either primary tumours or secondary tumours. There are several cases of primary high grade tumour, according to the case of the brain cells. The Glioma is also the case of primary high grade brain tumour that can be either grade 3 or tier 4. The more immediate treatment for the high grade brain tumours is important, it can propagate rapidly and damages the spinal cord and other regions of the head. The discussion of secondary brain tumours will vary

according to the type of tumour like (breast, lung, etc.).

Ear hearing function: Age is a major factor in the development of hearing problems, which can also result from loud noises. There are various instances of hearing issues that lead to difficulties hearing, such as people not being able to listen well and having to ask the same questions over and over. Listening to loud music is one of the causes of hearing loss. There are four types of hearing loss classifications: sensory, conductive, neural, and mixed. One-sided hearing loss (single spike) or bilateral hearing loss (both ears) are possible. At that moment, there are certain losses to some extent, including residual hearing, hard of hearing, hearing impairment, and deafness.

Sensory: This is the type of loss range from minor to deep, that affects the frequencies than others.

Conduct: Diseases or barriers in the outer or center ear that frequently interfere with all hearing frequencies are the cause of conductive loss of hearing.

Neural: Damage to the central nervous system (CNS) is the cause of this kind of hearing loss.

1.2 Nail

A nail is a horn-like envelope covering the tips of the fingers and toes in most primates and a few other mammals. Nails are similar to claws in other animals. Alpha keratin, a strong, protective protein, makes up finger and toenails. Several animals' horns and hooves contain this protein as well.

The six components of the nail structure are the root, nail bed, nail plate eponychium, paronychium, and hyponychium. Every one of these six parts has a particular purpose, and if any of these parts of the nail structure are compromised, the nail may take on an unusual appearance

Nail Root: The germinal matrix is another name for the nail's root. The lunula, a white crescent that resembles its edge, is visible. This nail's root section extends several millimeters into the finger below the skin and beneath the nail. The majority of the nail's and the nail bed's volume is produced by it.

Nail Bed: The sterile matrix is another name for the nail bed. It reaches the hyponychium from the lunula, or border of the nail root. Blood arteries, nerves, and melanocytes—which make melanin—are found in the nail bed. The nail grows outward from the root along the nail bed, adding material to the bottom of the nail to give it more thickness. The nail bed is smooth when the nail grows properly; if the nail grows incorrectly, it may split or acquire unsightly ridges at the surface.

Nail Plate: The real fingernail, or nail plate, is composed of transparent keratin. The blood vessels underneath the nail are what give it its reddish hue. To assist secure the nail to the nail bed, the bottom of the nail plate features grooves that run the length of the nail.

Eponychium: The cuticle is another name for the eponychium. The cuticle is located in the space between the nail plate and the finger's skin. It creates a watertight barrier by joining these components.

Perionychium: Also referred to as the paronychial edge, the paronychium is the skin that extends over the sides of the nail plate.The space between the skin of the fingertip and the free edge of the nail plate is known as the hyponychium. It offers a watertight barrier as well.

The tissue (or germinal matrix) that the nail covers is known as the matrix, sometimes known as the matrix unguis, keratogenous membrane, nail matrix, or onychostroma. It is the area of the nail bed that has blood arteries, lymph, and nerves under the nail. The nail plate is made of cells that are produced by the matrix. The size, length, and thickness of the matrix determines the width and thickness of the nail plate; the fingertip's shape indicates whether the nail plate is flat, arched, or hooked.

As long as it is nourished and kept in good health, the matrix will keep making new cells. Older nail plate cells become squeezed, flat, and translucent as newer nail plate cells push them forward during the process of nail plate cell formation. This gives the nail bed underneath a pink hue by highlighting its capillaries.

The lunula ("small moon") is the visible part of the matrix, the whitish crescent-shaped base of the visible nail. The lunula can best be seen in the thumb and may not be visible in the little finger.
The nail bed is the skin beneath the nail plate. Like all skin, it is made of two types of tissues: the deeper dermis, the living tissue which includes capillaries and glands, and the epidermis, the layer just beneath the nail plate, which moves toward the finger tip with the plate. The epidermis is attached to the dermis by tiny longitudinal "grooves" called matrix crests (cristae matricis unguis). In old age, the nail plate becomes thinner, and these grooves become more visible.

The anterior border of the nail plate that corresponds to the cutting or abrasive edge of the nail is known as the free margin (margo liber), sometimes known as the distal edge. The epithelium beneath the nail plate where the free edge and fingertip skin meet is called the hyponychium (sometimes called the "quick"). It creates a barrier to keep the nail bed safe. The seal that separates the hyponychium from the nail plate is called the onychodermal band. It is located in the area of the nail where the nail bed stops, right under the free edge. Fair-skinned individuals can identify it by its glassy, greyish color. Some people have it completely hidden, while others have it quite noticeable.

The cutaneous fold that covers the sides and proximal end of the nail is known as the nail wall (vallum unguis). The lateral margin, also known as the margolateralis, is located on the sides of the nail beneath the nail wall. The cutaneous slits into which the lateral edges are implanted are known as the nail groove or fold, also known as the sulcus matricis unguis. The soft tissue border surrounding the nail is called the paronychium, and an infection in this region is known as paronychia.

1.3 Function

The purpose of a healthy fingernail is to prevent damage to the fingertip, the distal phalanx, and the surrounding soft tissues. It also serves to enhance precise delicate movements of the distal digits through counter-pressure exerted on the pulp of the finger. Even though the nail lacks nerve endings, it functions as a counterforce when the tip of the finger touches an object, increasing the sensitivity of the fingertip. Lastly, the nail serves as a tool that makes it possible to do specific cutting or scraping tasks as well as what is known as a "extended precision grip" (such as extracting a splinter from one's finger).

1.4 Growth

The growing part of the nail is under the skin at the nail's proximal end under the epidermis, which is the only living part of a nail.

In animals, the length of the terminal phalenges, or outermost finger bones, is correlated with the nail growth rate. Therefore, in humans, the nail on the index finger develops more quickly than the nail on the little finger, and the growth rate of fingernails can reach four times that of toenails.

The average monthly growth of human nails is 3 mm (0.12 in). Toenails take twelve to eighteen months to fully regrow, whereas fingernails take three to six months. Season, age, sex, amount of exercise, food, and genetics all affect actual growth rate.

The longest female nails known ever to have existed measured a total of 601.9 cm, an average of 60.19 cm (23.7 inches) for each fingernail. Despite what is commonly believed, nails do not grow after death; rather, the appearance of growing nails and hair is caused by the skin's dehydration and tightening.

1.5 Permeability

It is a common misconception that nails are impermeable barriers. In fact, it is much more permeable than the skin, and the composition of the nail includes 7–12% water. This permeability has implications for penetration by harmful and medicinal substances; in particular cosmetics applied to the nails can pose a risk.

Water and a variety of other substances can pass through the nail, such as paraquat, a human-toxic herbicide that acts quickly, urea, which is frequently found in hand and finger creams and lotions, and a number of fungicidal agents, including salicylic acid, miconazole (marketed under the name Monistat), and sodium hypohlorite, the active ingredient in regular household bleach (typically only in a 2-3% concentration).

1.6 Effect of nutrition

Vitamin A is an essential micro-nutrient for vision, reproduction, cell and tissue differentiation, and immune function. Together, calcium and vitamin D help to maintain homeostasis, contract muscles, transmit nerve impulses, coagulate blood, and maintain membrane structure. Dryness and brittleness might result from low calcium, vitamin D, or vitamin A levels.

Excessive dryness, discolored nails, and rounded or curled nail ends can all be symptoms of low vitamin B12. Fragile nails with vertical and horizontal ridges are the result of inadequate consumption of both vitamins A and B. Strong nail development may be aided by certain over-the-counter vitamin supplements, such as biotin and some multivitamins, though this is highly subjective.

Protein is a building material for new nails; therefore, low dietary protein intake may cause anaemia and the resultant reduced haemoglobin in the blood filling the capillaries of the nail bed reflects varying amounts of light incident on the nail matrix resulting in lighter shades of pink ultimately resulting in white nail beds when the haemoglobin is very low. The majority of the light spectrum is absorbed when hemoglobin levels are near 15 or 16 grams; only the pink color is reflected back, giving the appearance of pink nails.

Essential **free fatty acids** play a large role in healthy skin as well as nails. Splitting and flaking of nails may be due to a lack of linoleic acid

1.7 Protein and nails

A Nail is a horn like envelope covering the tips of the fingers and toes most primates and a few other mammals. Finger nails and toe nails are made up of a tough protective protein called alpha-keratin. Keratin is recalcitrant, highly di sulfide bonded and is generally inaccessible to common proteases. The peptide linkages that make up the keratin structure can only be broken by specific kinds of proteases known as keratinases. Select proteases that exhibited keratinolytic activity were applied to hair samples that underwent mechanical, biochemical, and microscopic examinations to assess degradation. The inclusion of reducing chemicals, primarily sodium thioglycolate, significantly increased the amount of keratin breakdown, which reached 90% after 16 hours of enzymatic treatment.

1.8 Impact of mobile phone on human health

Collagen tissue grew in cells exposed to mobile radiation, according to earlier research. After an hour, skin morphological changes and enhanced fibroblast activity are brought on by mobile phone radiation. According to another study, skin cells undergo exocytosis when exposed to 900 MHz mobile phone radiation.

Exposure of finger- and toe-nails to ionizing radiation generates an Electron Para magnetic Resonance (EPR) signal whose intensity is dose dependent and stable at room temperature for several days. The dependency of the radiation-induced signal (RIS) on the received dose may be used as the basis for retrospective dosimetry of an individual's fortuitous exposure to ionizing

radiation. In nails irradiated up to a dose of 50 Gy, two radiation-induced signals have been identified: a quasi-stable (RIS2) and stable signal (RIS5). Both RIS signals show up as a singlet line shape using X-band EPR, with an apparent g-value of 2.0044 and a line width of about 1.0 mT. In this effort, we aim to determine the precise chemical makeup of the free radicals generated by radiation that underlie the signal.

Mobile phones communicate by transmitting radio waves through a network of fixed antennas called base stations. Unlike ionizing radiation like gamma or X-rays, which may break chemical bonds and induce ionization in the human body, radio frequency waves are electromagnetic fields. These DOPA amino acids are likely formed from the exogenous oxidation of tyrosine in keratin by the oxygen from the air prior to irradiation. We show that these DOPA amino acids can work as radical traps, capturing the highly reactive and unstable sulfur-based radicals and/or alkyl radicals generated during the radiation event and are converted to the more stable o-semi quinone anion-radicals. With treatment of nail clippings, it might be possible to rebuild the unstable RIS2 after its degradation, based on our understanding of the oxidation-reduction features of the RIS. But in addition to recovering the RIS2, the procedure can also recover a mechanically-induced signal (MIS) that interferes with normal function and is created when a nail is clipped. Thus, in order to improve the RIS measure's detection limits and precision, employ the recovered (regenerated) RIS2.

1.9 Nail and Tetramethylammoniumhydroxide (TMAOH)

Nail is composed of keratin proteins. The sulphur in cysteine molecules in adjacent keratin proteins link together in disulphide chemical bonds. These di sulphide bonds are very strong and very difficult to break apart. These di sulphide chemical bonds linking the keratins together are the key factor in the durability and resistance of nail fiber. They are largely resistant to the action of acids but the di sulphide bonds can be broken apart by alkali solutions, making the nail weak.

Tetra methyl ammonium hydroxide (TMAH), an alkaline solution, has been utilized in the past as a viable substitute for microwave-assisted acid digestion of biological materials for the determination of trace elements by various atomic spectrometry techniques. Moreton and Delves used TMAH in one of their diluents for the determination of total Hg in whole blood by ICP-MS, and TMAH was used to dissolve biological samples for the determination of Tl, Pb, Ag, Hg, and Cd by electro thermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS).

2.0 Physical properties of TMAOH

CAS	: 75-59-2
Class	: Corrosive and toxic
Physical state	: Liquid
Colour	: Colourless to straw coloured
Solubility	: 100% in water
pH	: Very strong base, >13@20°c
Boiling point	: 60-65°C

2.1 Potential health effects

Inhalation : May be harmful if inhaled. Material is extremely destructive to the tissue of the mucous membranes and upper respiratory tract.

Skin : May be fatal if absorbed through skin. Causes skin burns.
Eyes : Causes severe eye burns.
Ingestion : May be fatal if swallowed.

Aggravation of pre-existing conditions: Persons with pre-existing skin disorders or eye problems or impaired liver or kidney function may be more susceptible to the effects of the substance

2.2 EMR and human health

A long- term expose to EMR would overcome problems that were discussed in relation to existing epidemiological studies, including the Interphone study. These problems include recall

bias and other aspects of exposure assessment, selection bias due to high proportions of on-responders, too short induction period, and restriction to intracranial tumours. Health effects of RF exposure in children. To date no study on children exists. Studies on young animals can also be used to address this problem. The possibility that a child's dosimetry will differ from an adult's must be considered in this study.

Distribution of exposure within the population. The introduction of personal dosimeters has allowed for the description of population-wide individual exposure as well as the evaluation of the relative contributions of various sources to the overall exposure. For this kind of project, it would be necessary to choose groups of individuals with various traits and have them wear dosimeters for a certain amount of time.Replication of a number of experimental research is required. Studies involving sleep quality measures and cognition in relation to genotoxicity serve as examples. The effect on human health must be taken into account in biomarker research. A reliable exposure assessment that takes into account all pertinent exposure sources is crucial. One general observation is that high-quality dosimetry must be used in all research.

2.3 EMR and antioxidants

Free radicals are reactive molecules produced during the con-version of foods into energy through oxygen. The formation of free radicals is an oxidation reaction that occurs on an oxygen basis. Since oxygen is essential for survival, the formation of free radicals cannot be avoided. The Fenton reaction is a catalytic process that converts hydrogen peroxide, a product of mitochondrial oxidative respiration, into a highly toxic hydroxyl free radical. Some studies have suggested that EMF is another mechanism through the Fenton reaction, suggesting that it promotes free radical activity in cells. Even though some researchers have found that ROS have positive effects, excessive ROS production can harm cells and lead to a variety of illnesses. The energy of free radicals is insufficient, which is why they operate like thieves that take energy from other cells and rob a person to fulfill themselves. These radicals react with many biomolecules, including DNA. Numerous investigations have revealed that EMF exposure may cause exposed cells to produce reactive oxygen species both in vitro and in vivo. The NADPH oxidase enzyme, which is found in the plasma membrane, regulates the first phase of ROS generation in the presence of RF. As a result, ROS cause matrix metallo proteases to become active, which starts intracellular signaling cascades that warn of the existence of external stimuli. These changes transcription and protein expression are observed axposure. (Kazemi et al.,) investigated the effect of exposure to 900-MHz on the induction of oxidative stress and the level of intracellular ROS in human mononuclear cells.

One major factor contributing to oxidative damage in lipids, proteins, and nucleic acids is an overabundance of raised ROS levels. As a result, it alters gene expression and enzyme activity, which ultimately results in a number of illnesses, such as rheumatoid arthritis, diabetes, arthrosclerosis, lack of appetite, sleep disorders, dizziness, and stroke. Lipid peroxidation may also arise from the breakdown of the prooxidant-antioxidant balance brought on by an unchecked rise in ROS. The process known as "lipid peroxidation" occurs when unsaturated fatty acid-containing phospholipid components oxidize, quickly destroying cell membranes. Lipid peroxides build up in the membrane as a result of this process, converting polyunsaturated fatty acids into chemicals that have biological activity. Lipid peroxidation thus causes substantial cellular damage, including disruptions to membrane transport, modifications to the structure, fluidity of the cell membrane, harm to protein receptors inside membrane structures, and alterations in the function of cell membrane enzymes. (Hoyto et al.,)

Additionally, oxidative damage to lipids in blood vessel walls may have a major role in the development of atherosclerosis, according to epidemiological research. Since cell phones are held close to the head while being used, studies usually concentrate on the brain. There is ample evidence that electromagnetic fields (EMF) can

impact neuronal functioning within the human brain. The heat shock response explains the relationship between electromagnetic fields (EMF) and neurological diseases. Heat shock, exposure to heavy metals, and environmental insults including electromagnetic fields (EMFs) are the main concerns of the heat shock protein (HSP) response. HSP is typically a hallmark in stressed cells. Stress proteins are produced by living things to help them withstand environmental stresses. It is believed that the heat shock response serves as a general reaction to a broad range of stimuli, including oxidative damage. Numerous environmental stimuli that induce UV, ionizing, and laser radiation in humans and other mammals also cause cellular stresses that change the amounts of Hsp90 and 70. HSP alterations are also brought on by non-ionizing radiation in a number of tissues, such as the skin, testicles, brain, and heart. According to studies, these results represent a cellular stress adaption or readjustment.

2. Materials and Methods

Collection and extraction

Right hand nails were clipped with a stainless steel cutting instrument and preserved in metal free plastic free plastic tubes at room temperature. The sample was washed by using ethanol afterwards , nails were weighed 10-20mg and incubated into 15ml conical tubes with 1ml of 25% Tetra methyl ammonium hydroxide at room temperature over night.Following this the volume was made up to 10ml with a solution containing 1% Nitric acid . And this mixture was further used for different analysis.

Estimation of protein by Lowry's method

Principle:

The protein reacts with Folin's phenol reagent is the presence of alkaline copper solution to give an

intensity of blue colour complex which can be estimated colorimetrically at 680nm.

Reagents:

1. 2% Sodium Carbonate in 0.1N Sodium Hydroxide (Solution A).
2. 0.5% Copper sulphate (CuSO4.5H2O) IN Potassium sodium tartrate (Solution B).
3. Alkaline Copper solution: Mix solution A and B in the ratio of 50:1
4. Folin-phenol Reagent
5. Protein solution: (Stock standard): Weigh accurately 50mg of bovine serum albumin(Fraction V) and dissolved in distilled water and make up to 50ml in a standard flask.
6. Working Standard: Dilute 5 ml of the stock solution to 50ml with distilled water in a standard flask.

Specimen: Nail

Test procedure:

Estimation of protein:

1. Pipette out 0.2,0.4,0.6,0.8,1.0 and 1ml of the working standard into a series of test tubes.
2. Pipette out 0.8 ml of test solution in "T" test tube.
3. The volume of all the test tubes were made up to 2ml. A tube with 2ml of water serves as the blank.
4. 5ml of reagent C to each tube including the blank was added and mixed well and allowed tost and for 10minutes.
5. Then 0.5ml of folin,s phenol reagent was added and mixed well and incubated at room temperature in the dark for 30min. Blue colour was developed.
6. The reading was taken at 680nm.
7. A standard graph was drawn and calculate the amount of protein in the extract sample.

Estimation of ascorbic acid (Vitamin C)

Principle:

Ascorbic acid in plasma is oxidized by to form dehydro ascorbic acid. Which react with acidic 2,4-dinitrophenyl hydrazine to form bishydrozone which is measured at 540nm.

Reagent

1. Stock standard ascorbic acid solution
2. Working standard ascorbic acid solution
3. 5% trichloro acetic acid
4. Sulphuric acid 4.5M
5. 2% 2,4-dinitrophenyl hydrzine(dissolved in sulphuric acid 4.5M)
6. 5%Thiourea solution
7. 0.6% Copper sulphate solution
8. Dinitrophenyl hydrazine-Thiourea-Copper sulphate reagent (DTCS): 5ml of thiourea,5ml of copper sulphate and 100ml of 2,4 dinitro phenyl Hydrazine reagent were combined.

Specimen: Nail

Procedure

1. Pipette out 0.1-0.5ml of the working standard into a series of test tubes with Concentration ranging 10 to 50µg
2. Pipette out 1ml of the sample extract in "T" test tubes
3. The volume to 1ml in all the test tubes was made with 5% trichloroacetic acid. A tube with 1ml of trichloroacetic acid serves as the blank.
4.Then 0.2ml of dinirophenylhydrazine - Thiourea-Copper sulphate reagent was added and the tubes were kept in boiling water bath for 10 minutes. Then cooled at room temperature.
5.The orange red colour were formed.
6.And it was dissolved by adding 3ml of 85% sulphuric acid with strirring and the colour developed was read at 520nm.
7. A standard graph was drawn and calculated the amount of protein in extract sample

Estimation of Tocopherol (Vitamin E)

Principle:

The tocopherol reduces ferric to ferrous ion which forms a pink coloured complexn with more sensitive reagent such bathophenanthroline. Ortho-phosphoric acid added as a chelating agent to reduce carotene interference by preventing its oxidation and stabilization of colour by binding excess ferric ions and thus preventing their photochemical reaction.Absorbance of the stable chromophore is measured at 540nm.

Reagents

1. Standard vitamin E solution
2. Working vitamin E solution
3. Absolute Ethanol
4. Hexane
5. 0.02% Bathophenanthroline
6. 0.001M Ferric chloride
7. Orthophosphoric acid

Specimen: Nail

Procedure:

1. Pipette out 0.2,0.4,0.6,0.8 and 1ml of the working standard into series of test tubes with concentration ranging 20-100µg.
2. Pipette out 4ml of nail in test tube labelled as T tube.
3. The volume to 4ml in all the test tubes was made with ethanol. A tube with 4ml of ethanol serves as the blank.
4. 0.4ml of bathophenanthroline reagent was added to all the tubes.
5. After 1 minute 0.4ml of ortho phosphoric acid was added to all the tubes.
6. Care was taken to avoid unnecessary exposure to direct light.
7. The pale pink colour developed was read at 540nm.
8. A Standard graph was drawn and calculate the amount of vitamin E in the sample extract.

Antioxidants

Assay of superoxide dismutase (sod) by method of Kakkar et al., (1984)

The O_2 substrate for superoxide dismutase generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O_2 builds in the solution formation of adeno chrome accelerates because O_2 also reacts with O_2 Formed during oxidation epinephrine to form adenochrome. Superoxide dismutaase reacts with O_2 formed during oxidation and therefore slowdown the rate of formation of adenochrome. The ability of superoxide dismutase to inhibit auto oxidation of epinephrine is measured by observing the increase in absorbance at 480nm in spectrophotometer.

Reagent

1. 50mM Carbonate-bicarbonate buffer:pH-9.8
2. Epinephrine: 1.8mM(Freshly prepared)

Specimen : Nail

Procedure

1. The tubes labelled blank, control, and test are taken. To the control and test tube 0.8 and 0.75 ml of water was added. Then 1.8ml of carbonate and bicarbonate buffer was added.
2. 1.2ml of water and 1.8ml of carbonate and bicarbonate buffer was taken in blank tube.
3. By using the blank tubes, for zero adjustment, 0.4ml of epinephrine was added to the control tube and auto oxidation of epinephrine to the test tube and the reaction was observed by measuring the change in the optical density at 480nm for 3minutes in a colorimeter.
4. One unit of superoxide dismutase activity is defined as the amount of enzyme required to produce 50% inhibition in epinephrine auto oxidation.

Assay of catalase(CAT) (Hydrogen peroxide oxidoreductase

The activity of CAT was determined in nail by the method of Sinha(1972).CAT catlyzes the decomposition of hydrogen peroxide (H_2O_2) and the rate of decomposition of H_2O_2 can be measured as the activity of enzyme. The reaction is allowed to take place in presence of H_2O_2 as substrates and the reaction arrested at different time intervals. The unutilized H_2O_2 at various time intervals can be quantified at 620nm by using dichromate -acetic acid reagent

H_2O_2 produced in several reaction in the cells is highly toxic and must bescavenged promptly to avoid injury to metabolic machinery of the tissues. The enzymecatalase decomposes H_2O_2 into $2H_2O_2$ and o_2 molecules.

Reagents

1. Stock Dichromate acetic acid reagent.
2. Working dichromate acetic acid reagent.
3. 5% potassium dichromate.
4. Phosphate buffer (0.01M) (ph7.4).
5. 0.2M Hydrogen peroxide solution.

Sample: Nail

Procedure

1. A series of three test tubes labelled as T1, T2, T3 and control tube as C was taken.
2. 1ml of phosphate buffer was taken followed by the addition of 0.1ml sample.
3. Then 0.5ml of hydrogen peroxide was added to the tubes T2, T3.
4. The reaction in the T2 and T3 tubes were stopped at 30 & 60 seconds by the addition of 2ml dichromate acetic acid reagent.
5. T1 tube is considered as 0 second tube. In this tube dichromate acetic acid reagents was added before the addition of 0.5ml of peroxide.
6. In a control tube, 1.0ml of buffer and 2ml of dichromate acetic acid reagent were taken.
7. The tubes were kept in boiling water bath for 10minutes and the colour developed was read at 620nm.

8. Catalase activity was expressed as µmoles of hydrogen peroxide consumed min/mg of protein.

Estimation of calcium

Calcium is precipitate as calcium as calcium oxalate and is titrated with standard potassium permanganate which also act as self indicator.

Reagents required

1. Standard oxalic acid (0.01N).
2. Potassium permanganate.
3. 10% acetic acid solution.
4. 1N H2SO4 (sulphuric acid).
5. 4% oxalic acid.
6. 4% ammonium oxalate.

Procedure

Titration 1

Standardisation of potassium permanganate

Standard oxalic acid vs potassium permanganate

10 ml of standard oxalic acid and 10ml of 1N H_2SO_4 (sulphuric acid) was added. Then the solution was heated to bearable warm. The hot solution was titrated against potassium permanganate taken in the burette.
The end point was the appearance of pale permanent pink colour.

Titration 2

Estimation of calcium

Test: 2ml of solubilized calcium and 2ml of 1N H_2SO_4 (sulphuric acid) was added. Then the solution was heated to bearable warm. The hot solution was titrated against potassium permanganate taken in the burette.

The end point was the appearance of pale permanent pink colour.

Control: The blank titration was done by taking 2ml of 1N(H_2SO_4) sulphuric acid and was heated to bearable warmth and titrated against standardised potassium permanganate the difference between test and blank value was calculated. From the value the amount of calcium present in nail sample was calculated.

Estimation of Iron

Ferrous iron gives pink colour with 2,2 dipyridyl reagent which can be colorimetrically estimated sodium sulphite is used as the reducing agent to convert ferric iron.The intensity of colour developed was read at 540nm using green filter.

Reagents required

1. Stock standard ferrous sulphate Solution
2. working standard ferrous sulphate solution
3.2,2 dipyridyl reagent
4. Sodium sulphite (0.1M)
5.Sodium acetate buffer

Procedure

1. Pipette out 0.5,1.0,1.5,2.0,2.5 of the working standard into series of test tubes with concentration ranging 2-10µg.
2. Pipette out 2.0 ml of nail in test tube labelled as T tube.
3. The volume to 3ml in all the test tubes was made with distilled water . A tube with 3ml of distilled water serves as the blank.
4. 1.5ml of Sodium sulphate reagent was added to all the tubes.
5. 2,2 Dipyridyl reagent was added to all the tubes.
6. The pink colour developed was read at 520nm.
7. A Standard graph was drawn and calculate the amount of Iron in the sample extract.

Statistical analyses

Data were analysed by using a commercially available statistics software package (statistical package for social sciences for windows; version 10; SPSS). This statistical significance of mean values between different groups was determined by applying one-way ANOVA followed by *post hoc* Bonferroni's test; and the value of $p < 0.05$ was considered significant.

3. Results and Discussion

A specialized keratinous epidermal appendage, the nail grows 2 to 3 mm per month and replaces itself completely in 6 to 9 months. Approximately 10% of all dermatologic illnesses are nail abnormalities, despite the fact that this structure is easily missed. In order to distinguish between anatomic components that are not a part of the nail unit (such as lateral nail folds, nail plates, and eponychium) and the nail unit (such as hyponychium, nail bed, proximal nail fold, and matrix), this contribution first gives an overview of the basic anatomy of the nail. There will also be a presentation of each nail structure's purpose. The keratin content (hair type vs. epithelial type), sulfur content, and mineral composition—including magnesium, calcium, iron, zinc, sodium, and copper—of the typical nail plate are discussed along with its chemical profile. The rest will concentrate on nail symptoms associated with malnutrition. Almost all nutritional deficiencies have some sort of impact on nail development. Lastly, a brief review of biotin and its potential use in treating nail diseases will be covered, along with anecdotal evidence of its usage in the context of brittle nail condition when it comes to nutritional and dietary supplements.

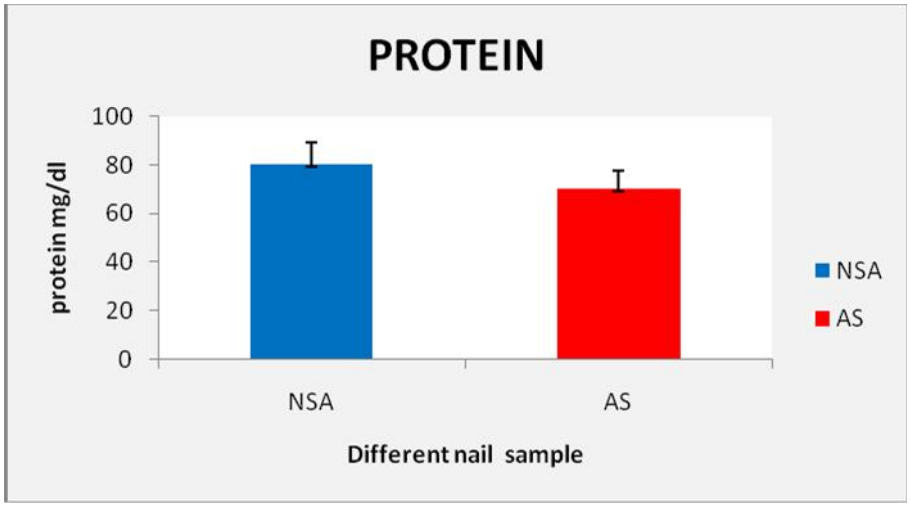
Table: 1 Quantitative analysis of protein in nail of normal phone (control) users by Lowry's method

Sample no.	Gender	Age	Amount of protein present in NP Users(mg/dl)
1.	Female	19	80
2.	Male	19	77
3.	Female	20	81
4.	Male	20	76
5.	Male	20	82
6.	Male	19	69
7.	Female	19	73
8.	Male	18	65
9.	Female	18	59
10.	Female	19	82

Table:2 Quantitative analysis of protein in nail of smart phone (test) users by Lowry's method

Sample no.	Gender	Age	Amount of protein present in SP Users(mg/dl)
1.	Female	22	74
2.	Male	21	70
3.	Male	21	66
4.	Male	22	78
5.	Male	22	55
6.	Female	21	61
7.	Female	22	70
8.	Male	23	59
9.	Male	21	74
10.	Male	22	70

Levels of protein in nail sample of normal (control) and smart phone (test) users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p<0.05$ *for* control sample vs test sample.

Protein:

Protein are the essential nutrient for the human body. They are the building blocks of the body tissue and can also serve as a fuel source .It is made up of amino acids that are attached to one another long chains. There are 20 different kinds of amino acids and the sequence in which the different amino acids are arranged helps to determine the role of that particular protein. And it plays an important role in transporting molecules throughout the body, helping repair cells and make new ones, Protecting the body from viruses and bacteria. And in nails is made up of tough protective protein called **alpha –keratin**. This protein is also found in the hooves and horns of the different animals. (Harvard T.H 2018).

The result showed the level of protein that is significant decreased in smart phone users due to the emission of radiofrequency electromagnetic field leading to the absorption of radiation by the brain in users of handheld mobile phones has raised concerns regarding potential effects on health. The adult cerebellum's levels of both excitatory and inhibitory amino acids significantly decreased, according to the current research. This effect was also noticeable in the adult only midbrain.

Therefore, the increase in the permeability of blood brain barrier due to exposure to EMR may increase the influx of glucose to the brain. It is well known that glucose represents the main source of glutamate, aspartate and glycine. Accordingly, the significant increase in most of the amino acids in the midbrain and cerebellum recorded after EMR exposure, in the smartphone users. Both glutamic and aspartic acid levels recorded an early significant decrease after EMR exposure.

Mean while in case general decrease in both excitatory amino acid levels was observed being significant after EMR exposure.The present data showed a rapid significant decrease in amino acids in the nail adult after 1 hour of EMR exposure.(N.A.NOOR et.al.,)

Blood Brain Barrier(BBB) has been a favorable subject 0f investigation due to EMF exposure , for even a slight variation in its permeability can lead to tissue damage. Non thermal effects are identified by **leakage of albumin** through the BBB. Two hours exposure to the radiation from a global system for mobile communications phone at 915 MHz, at non thermal SAR values of 12mw/kg and 120mw/kg give rise to focal albumin extravasation and albumin uptake into neurons after 14 days exposure.(jitendaribehari 2010)

Calcium

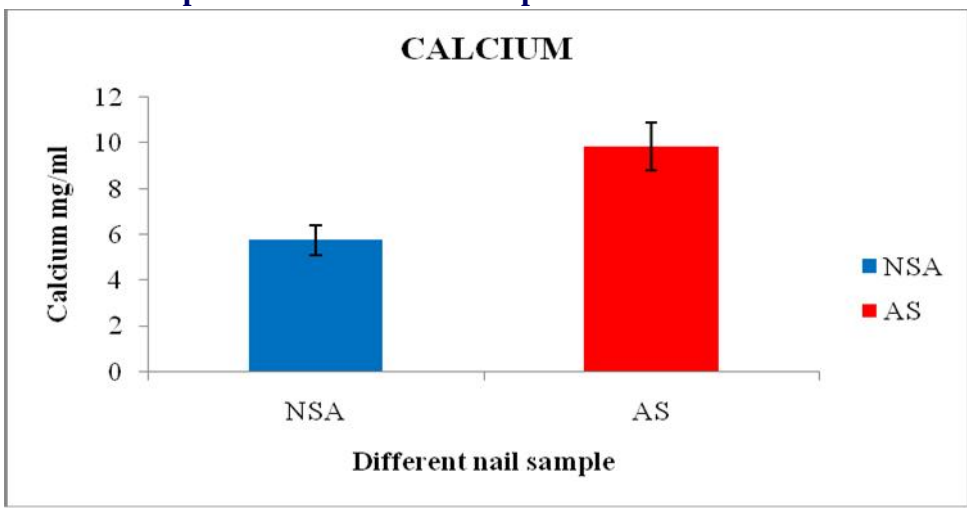
Table: 3 Quantitative analysis of calcium in nail of normal phone (NP) users

Sample no.	Gender	Age	Amount of Calcium present in NP Users(mg/dl)
1.	Female	19	10
2.	Male	19	8
3.	Female	20	5
4.	Male	20	9
5.	Male	20	4.6
6.	Male	19	9
7.	Female	19	4
8.	Male	18	5
9.	Female	18	5
10.	Female	19	8

Table: 4 Quantitative analysis of calcium in nail of smart phone (SP) users

Sample no.	Gender	Age	Amount of Calcium present in SP Users(mg/dl)
1.	Female	22	7
2.	Male	21	12
3.	Male	21	13
4.	Male	22	16
5.	Male	22	10.5
6.	Female	21	12
7.	Female	22	15
8.	Male	23	12
9.	Male	21	10
10.	Male	22	13

Levels of calcium in nail sample of normal and smart phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p=0.000$ *for* control sample Vs test sample.

Calcium is the fifth most abundant substance in our body behind nitrogen, hydrogen, oxygen, and carbon. Calcium is needed in our bodies to keep us healthy and strong. Ninety nine per cent of our body is calcium is located in our bone. Calcium is an important ubiquitous second messenger regarding the maintenance of cellular homeostasis, and is therefore tightly regulated. Calcium induces cell death when internalised into cancer cells after permeabilization of the cell membrane by electroporation. It is established that radiation causes damage to the lipids and proteins of the cell membrane; and ionizing radiation also causes permeabilization of the cell membrane by peroxidation of the phosphor-lipid layer.

The result is observed in calcium that is significantly increased in smart phone users than in normal phone users. A mass effect from calcium can interfere with absorption of the other alkaline earth metals and compete for bone deposition, Coronary calcium scoring has equivalent radiation exposure to smartphone, and similar to the level of background radiation exposure experienced over 3–4 months in most cities. **radiation** , and some other medications increase the risk for **osteoporosis** In these cases, the onset of Ca dependent hyper polarization induced by the magnetic field would be expected to be more efficient and the above mechanisms difficult to dissociate.

The inhibition of differentiation exerted at particular field intensities could be compensated by the ability to mobilize Ca ions. On this basis, Ca signaling may be seen as an agent protecting against damages produced by exposure to electromagnetic fields. In the presence of even silent alterations in mechanisms acting on intracellular Ca or KCa channels, chronic exposure to magnetic fields might induce pathological conditions. (Reipert et al., 1997; Lacy-Hulbert et al., 1998; Hatch et al.,1998).

This latter point implies that BT2-cAMP modulates the Ca influx without using voltage-gated Ca channels in our system by interfering with the surface potential and indirectly raising KCa activity. It was previously mentioned that rather than Ca being released from the stores, extracellular Ca mediated the process of depolarization-repolarization. (Ar-cangeli et al., 1987). In this instance, the shift in the L-type Ca channel activation curve that BT2-cAMP promotes is merely an indirect result of surface charge variations and has no bearing on the processes that underlie the hyperpolarization that BT2-cAMP induces. The primary mechanism by which developing cells are shielded from EMF shocks by Ca is the L-type voltage-gated Ca channel. Through alterations in intracellular calcium concentration, the magnetic field may disrupt processes related to cell division, differentiation, and membrane voltage variations. In this work, we show that two competing mechanisms govern the interaction between a 50/60-Hz magnetic field and cell differentiation.

The surface charge potential shift that distinguishing agents induce can be stopped by ELF-EMF. Simultaneously, it stimulates the increase in intracellular Cain a dose-dependent manner. The increase in cytoplasmic divalent ions, by opening the K Ca, acts as a rescue agent re-establishing cell’s commitment to differentiation.. The simultaneous onset of both mechanisms prevents alterations in differentiation. We propose that cells are normally protected against electromagnetic insult. The scenario just de-scribed might be very different in cells with an efficient Ca permeability system (Brown and Higashida, 1988a, 1988b).

Free fatty acids

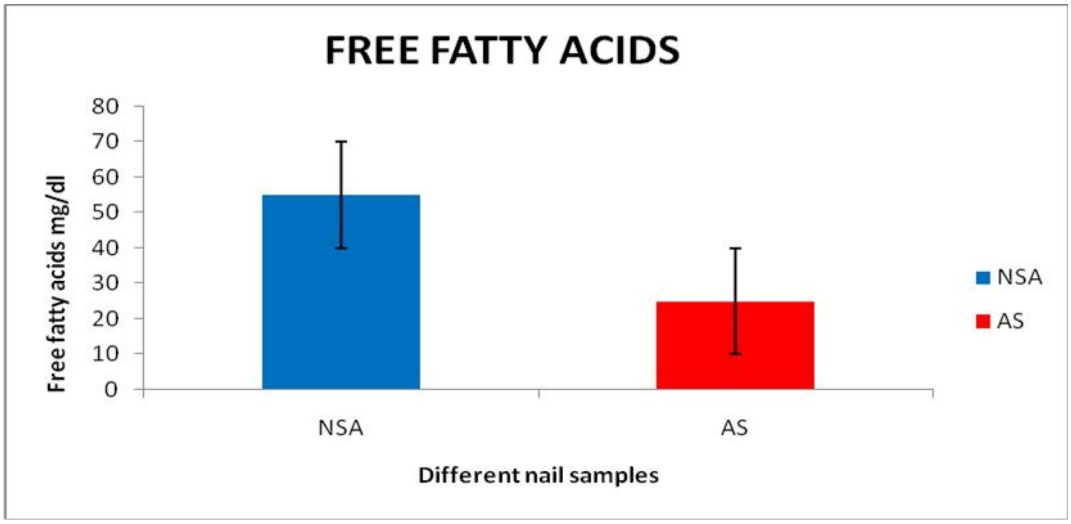
Table: 5 Quantitative analysis of free fatty acids in nail of normal phone (np) users by Fathof's et.al., method

Sample no.	Gender	Age	Amount of Free fatty acid present in NP Users(mg/dl)
1.	Female	19	50
2.	Male	19	52
3.	Female	20	46
4.	Male	20	48
5.	Male	20	46
6.	Male	19	47
7.	Female	19	50
8.	Male	18	51.5
9.	Female	18	51
10.	Female	19	46

Table: 6 Quantitative analysis of calcium in nail of smart phone (SP) users by Lowry's method

Sample no.	Gender	Age	Amount of Free fatty acid present in SP Users(mg/dl)
1.	Female	22	25
2.	Male	21	27
3.	Male	21	30
4.	Male	22	28.5
5.	Male	22	20
6.	Female	21	21
7.	Female	22	20.5
8.	Male	23	26
9.	Male	21	31
10.	Male	22	30

Level of free fatty acids in nail sample of normal and smart phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

Fatty acids are straight-chain carboxylic acids (either saturated or unsaturated). They are derived from the hydrolysis of fats or can be synthesized from two carbon units (acetyl- or malonyl-CoA) in the liver, mammary gland and, to some extent adipose tissue. Nearly all have an even number of carbon atoms. The result observed that the level of free fatty acid is significant in mobile phone users especially in the smart phone users Individual fatty acids, free fatty acids (FFA), or the non-esterified fatty acids (NEFA), circulate primarily in association with albumin. They are an important metabolic fuel. Extremely low frequency electromagnetic fields interact with human by inducing electric fields inside the body. These induced fields represent the internal exposure or “dose". In living things, a variety of natural endogenous electric fields also exist internally. These fields arise from normal physiological activity, and extend into adjacent tissues throughout the body.

The endogenous fields High density lipoprotein cholesterol after ELF-EMF exposure. Values significantly different in comparison with control and test group by ANOVA. A significant difference was observed at in comparison with smart phone and normal phone users by any field induced by external exposure to electromagnetic fields. Some studies about biological effects due to 50–60 Hz electromagnetic fields exposure have been performed with rodents .Assessment of possible human health effects has been to a certain extent supports on these studies. Extremely low-frequency electromagnetic fields exposure is generally believed to be innocuous for human health due to their low-level energy exposition, which is of a magnitude well below that required to affect the metabolic rate of the human body However, an increasing number of studies have reported that ELF-EMF exposure is capable to eliciting in vivo and in vitro bio effects. The increased oxidative-stress involves oxidative DNA damage, **lipid peroxidation** and may cause a number of systemic disturbances.

Superoxide dismutase

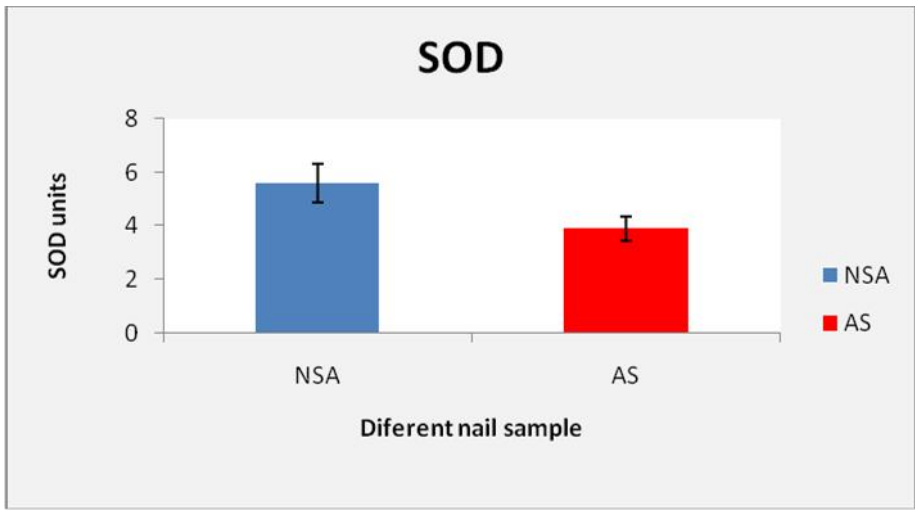
Table: 7 Assay of superoxide dismutase in nail sample of normal phone (control) users by Kakkar et. al.,(1984) method

Sample no.	Gender	Age	Amount of SOD present in NP Users(Units/mg Protein)
1.	Female	19	5.6
2.	Male	19	4.8
3.	Female	20	5.4
4.	Male	20	4.9
5.	Male	20	5.6
6.	Male	19	4.5
7.	Female	19	5.2
8.	Male	18	4.8
9.	Female	18	5.6
10.	Female	19	5.4

Table: 8 Assay of superoxode dismutase in nail sample of smart phone (control)users by Kakkar et al., method

Sample no.	Gender	Age	Amount of SOD present in SP Users (Units/mg of protein)
1.	Female	22	3.9
2.	Male	21	4.2
3.	Male	21	3.2
4.	Male	22	3.8
5.	Male	22	4.2
6.	Female	21	.9
7.	Female	22	3.7
8.	Male	23	5.0
9.	Male	21	3.8
10.	Male	22	3.9

Activity level of antioxidant enzyme in the nail sample of smartphone users and normal phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

Superoxide dismutase

Cells under stress or not both create reactive oxygen species, or ROS. Plants possess highly evolved defense mechanisms against reactive oxygen species (ROS), which include inhibiting ROS generation and initiating its elimination. O2 production and elimination are balanced in an unstressed state. On the other hand, the defense system may get overwhelmed in situations where there is a high production of ROS. Plants use more enzymatic or non-enzymatic antioxidant activities in response to an increase in ROS that their defense mechanism is unable to eliminate. (Alscher and Hess, 1993)

The level of activity of antioxidant enzyme (SOD) shows significant decrease in nail sample of smart phone users than normal phone users. Radio frequency electromagnetic radiation from mobile phones induces oxidative stress in smart phone users. Thus the increased activity of SOD are effective in quenching and clearing the toxic free radicals from cells (Kusano C and Feerari B,

2008). Rats exposed to EMR (group 2) exhibited a significant decrease in their SOD, CAT, and GSH-Rx activities concurrently, suggesting that the animals were under oxidative stress.According to (Ahmadpoor et al. 2009), there is an imbalance between the body's antioxidant capacity and reactive oxygen species (ROS), which leads to oxidative stress.

Thus, a weakened antioxidant defense system, an increase in ROS production, or a combination of the two could be the cause of this phenomenon. Enzymes that the mammalian body naturally produces as part of its endogenous antioxidant system to deal with reactive oxygen intermediates are SOD, CAT, and GSH-Rx. The superoxide anion radicals are changed by SOD into H2O2 and H2O (Lawler & Song, 2002). H2O2 is broken down by CAT into H2O and oxygen (Spolaries& Wu, 1997). The well-known antioxidant glutathione (GSH-Rx) converts H2O2 to H2O. Glutathione undergoes oxidation during this process, forming oxidized glutathione.

The oxidized glutathione is reduced back to glutathione in the presence of the enzyme called glutathione reductase (Bayse, Baker, &Ortwine, 2005; Russel,1998).However, it becomes more effective when the oxidative stress is extreme, ROS scavenging enzyme such as SOD and CAT are degraded as noted in the exposed group in this Study. In order to confront and overcome the oxidative stress, the aforementioned enzymes in the sera of groups 3 and 4 in the current investigation tended to rise in a dose-dependent manner to be greater than that of the control when EMR exposed animals were given SO. The findings of Snakar and Rao (2006), who observed a clear increase in SOD and CAT activities along with a decrease in lipid peroxidation as indicated by an increase in thiobarbituric acid reactive substances (TBARS) after 45 days of giving SO as the only edible oil to be consumed during that time to a group of hypertensive patients, were supported by these results.

All of these results show that SO is an exceptionally rich antioxidant oil because, in addition to being rich in fat-soluble vitamins like tocopherol, it also contains other protective compounds like sesamin, sesaminol, and sesamolin (Fukuda, 1990). Overall, our findings supported the findings published by Chaudrase Karan et al. (2007). They reported that in adults with acute liver injury induced by acetaminophen (APAP), sesame oil preserved intracellular glutathione levels and decreased ROS levels.

Catalase

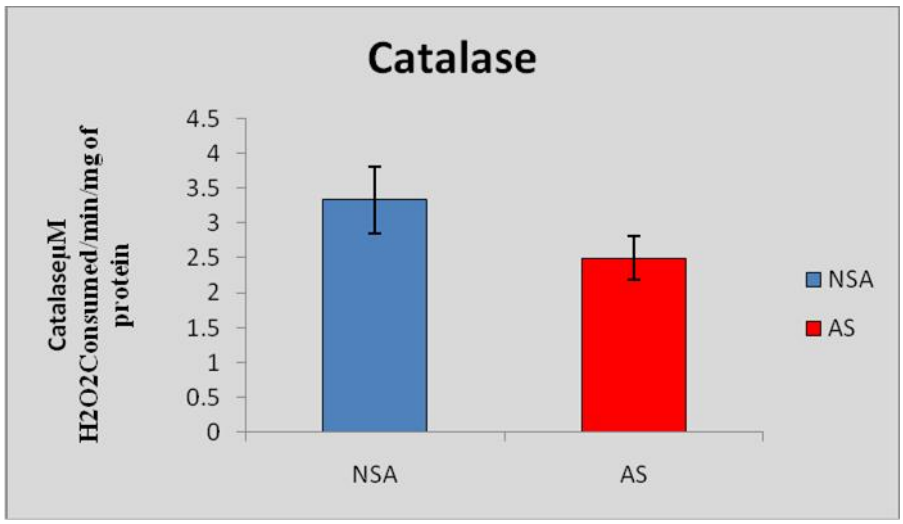
Table: 9 Assay of Catalase in nail sample of normal phone (control) users by Sinha's method

Sample no.	Gender	Age	Amount of Catalase present in SP Users(μMH_2O_2 Consumed/min/ mg of protein)
1.	Female	19	3.33
2.	Male	19	4.1
3.	Female	20	3.50
4.	Male	20	3.23
5.	Male	20	4.11
6.	Male	19	3.33
7.	Female	19	3.25
8.	Male	18	4.39
9.	Female	18	5.22
10.	Female	19	3.33

Table: 10 Assay of catalase in nail sample of normal phone (control)users by Sinha's method

Sample no.	Gender	Age	Amount of catalase present in SP Users(μMH_2O_2 Consumed/min/ mg of protein)
1.	Female	22	2.50
2.	Male	21	2.88
3.	Male	21	2.50
4.	Male	22	3.22
5.	Male	22	2.48
6.	Female	21	2.66
7.	Female	22	3.22
8.	Male	23	4.20
9.	Male	21	2.55
10.	Male	22	2.53

Activity level of antioxidant enzyme in the nail sample of smartphone users and normal phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

Catalase is a common enzyme, found in nearly all living organisms. It catalyses hydrogen peroxide into water and oxygen and protects organisms from free radicals It also has industrial uses to prevent certain contaminants in food and as a disinfectant for contact lenses and a cleansing agent in some other products. catalase is constantly in battle against the effect of free radicals to the body. It transforms harmful superoxide radicals into hydrogen peroxide which later breaks down into water and oxygen. Found extensively in organisms that live in the presence of oxygen, catalase prevents the accumulation of and protects cellular organelles and tissues from damage by peroxide, which is continuously produced by numerous metabolic reactions. In mammals, catalase is found predominantly in the liver.

The level of activity of antioxidant enzyme catalase shows significant decrease in the smart phone users indicates the oxidative stress due to exposure of electromagnetic radiation from the mobile phone. Acute exposure to RF fields of cell phones could modulate the oxidative stress and free radical generation by production of extracellular superoxide, enhancing lipid peroxidation and reducing the activation of SOD and GSH-Px, free radical scavengers.(Mohamed et al.,) concluded that adults exposed to cell phone EMF for long term exposure being carried out it showed that the plasma total anti-oxidant capacity was significantly decreased in all exposed groups, while the MDA level in the cardiac tissue was only significantly elevated in the test group compared to the matched control group. So the decrease in plasma total antioxidant capacity might be the result of its exhaustion in defending the free radical believed to be generated with RF-EMF. The significantly increased cardiac MDA content encountered in the present study in the smart phone and normal phone users, with the longest duration of exposure, points to the limits of the cardiac antioxidants to cope with the excessive MDA generation due to RFEMF exposure. It has been suggested that increased total oxidant status levels due to RF radiation emitted from GSM cell phones might play a role in inducing oxidative damage by increasing lipid peroxidation and oxidative stress.

It is well recognized that EMF affects biological systems by raising ROS, which changes the tissue's CAT levels and results in oxidative stress. (Odaci et al.) observed a decrease in CAT levels in an EMF-exposed adults. Exposure to EMF during the prenatal period also caused oxidative stress in developing embryos. This oxidative stress persisted through postnatal day 21.(Vuokkoet al.) reported that EMF exposure led depression of antioxidant systems because of raised lipid peroxidation and generation of free radicals. By raising the levels of xanthine oxidase and carbonyl group activity and decreasing CAT activity, mobile phones caused oxidative damage in live cells.(Özgüneret al.)

Vitamin C and Vitamin E

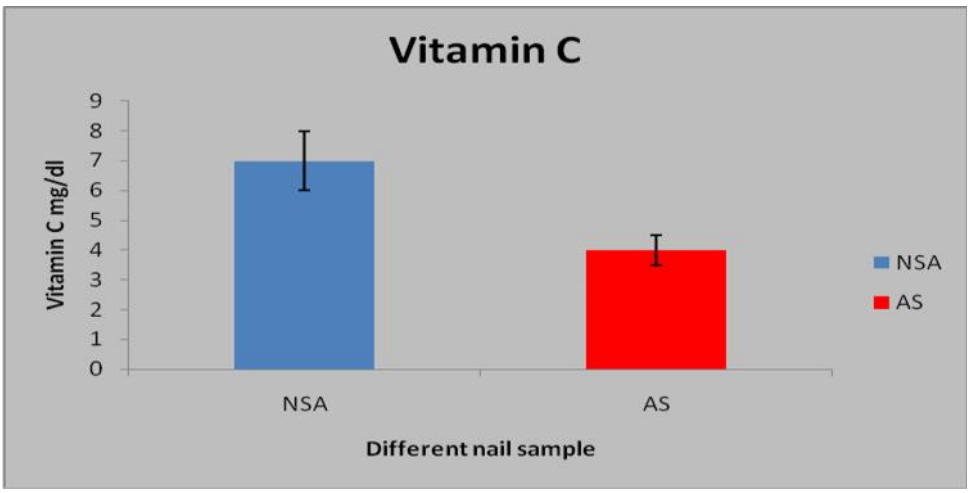
Table: 11 Quantitative analysis of vitamin c in nail of normal phone (NP) users

Sample no.	Gender	Age	Amount of Vitamin C present in NP Users(mg/dl)	Amount vitamin E Present in NP Users(mg/dl)
1.	Female	19	7	7
2.	Male	19	6	5
3.	Female	20	8	7
4.	Male	20	7	8
5.	Male	20	8	6
6.	Male	19	7	7
7.	Female	19	8	6
8.	Male	18	9	7.5
9.	Female	18	8	8.2
10.	Female	19	6.5	7

Table: 12 Quantitative analysis of vitamin e in nail of smart phone (SP) users

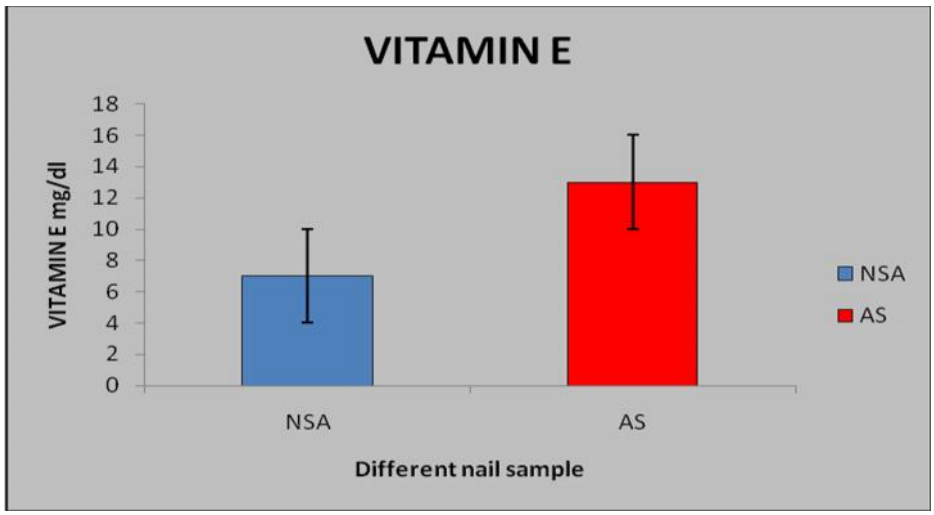
Sample no.	Gender	Age	Amount of Vitamin C present in NP Users(mg/dl)	Amount of Vitamin E present in SP Users
1.	Female	22	4	13
2.	Male	21	6	11
3.	Male	21	5	13
4.	Male	22	5	12
5.	Male	22	4	11.6
6.	Female	21	6	12.3
7.	Female	22	7	13.2
8.	Male	23	8	13
9.	Male	21	4.2	11.8
10.	Male	22	5	12.5

Level of Vitamin C in nail sample of normal and smart phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

Level of vitamin E in nail sample of normal and smart phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

Vitamins C and E are powerful antioxidants Vitamin C, also known as L-**ascorbic acid**, is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. ... Cells accumulate vitamin C via a second specific transport protein. **Vitamin E** is a group of eight fat soluble compounds that include four tocopherols and four tocotrienol. Vitamin E deficiency, which is rare and usually due to an underlying problem with digesting dietary fat rather than from a diet low in vitamin E, can cause nerve problems. The crucial function played by Vitamin E that makes it a vitamin is poorly understood, but may involve antioxidantfunctions in cell membranes. Other theories hold that vitamin E – specifically the RRR stereoisomer of alpha-tocopherol – act by controlling gene expression and cell signal transduction.. The magnitude of these effects in EMR-test is significantly higher than that of EMR-control, except **vitamin A**.

The level of activity of Vitamin C and Vitamin E Shows significant difference ($p>0.05$) between the smart phone and normal phone users, indicating that exposure to acute doses of EMR is more effective in induction of **oxidative stress** and in reducing the anti oxidative capacity than fractionated doses of the same type of radiation. These include enzymatic and non enzymatic antioxidants that lower the steady-state concentrations of **free radical** species, oppose sources that generate cellular oxidants and limit the likelihood that oxidative damage will occur. Cellular antioxidant defence mechanisms include low-molecular weight molecules such as reduced glutathione and vitamins C, E and A and antioxidant enzymes such as SOD, glutathione peroxidase (GSH-Px) and CAT (Atilla *et al*., 2004; Lowry *et al*., 1951; Sundram *et al*., 1996; Piacentini *et al*., 2001; Nahed *et al*., 2004; Goh and Barlow, 2002).

Studies have demonstrated that antioxidants, such as melatonin, caffeic acid phenyl ester, vitamin C and vitamin E, prevent the oxidative stress and apoptosis caused by EMR in tissues. This prophylactic treatment could thus be used to counteract the observations recorded by(Mailankot et al.,) who found that adults exposed to mobile phone emissions for long term had impaired because the RF-EMR exposure resulted in a significant increase in lipid peroxidation and a low GSH content in the test is and epididymis. Notably, pulsed electro magnetic field(PEMF is generated by many medical device used. Therefore, the issue of whether vitamins C and E could be used along with PEMF to relieve the adverse effects of EMR remains to be elucidated. In summary, EMR has a negative effect on testicular function through the induction of oxidative stress and the concomitant disruption of the testicular antioxidant status. Given the importance of vitamins C and E in this defensive strategy, the ability of antioxidants, such as vitamins C and E, to ameliorate this pathology confirms their importance in overcoming oxidative stress in this context. Vitamins C and E ameliorate the EMR-induced oxidative stress in the testes, thus facilitating the restoration of testicular tissue morphology and function by suppressing testicular lipid peroxidation and restoring the levels of GST and GSH to normal physiological levels.

Iron

Iron is an important component of haemoglobin, the substance in red blood cells that carries **oxygen** from your lungs to transport it throughout your body. **Haemoglobin** represents about two-thirds of the body's iron. If you don't have enough iron, your body can'**t** make enough healthy **oxygen-carrying** red blood cells.

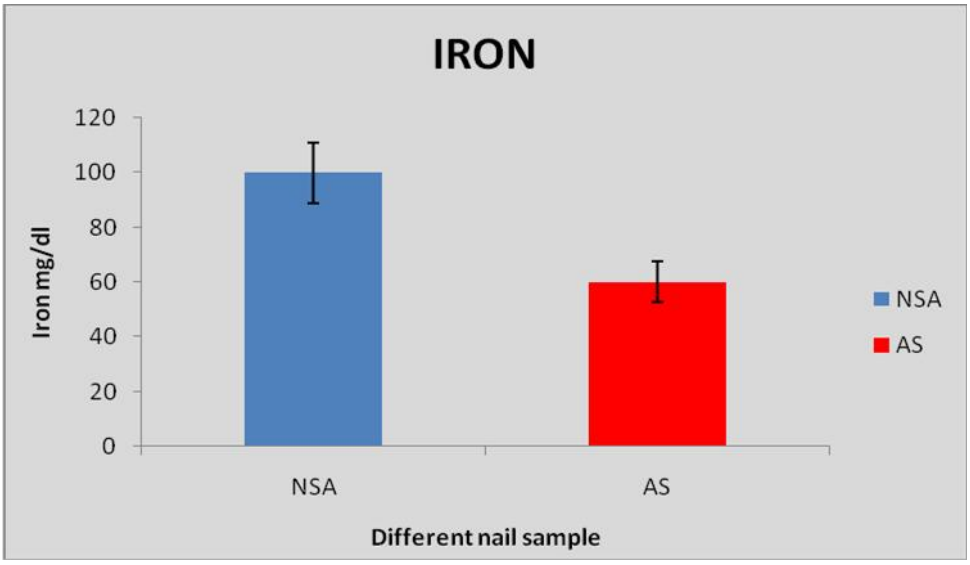
Table: 13 Quantitative analysis of iron in nail of normal phone (NP) users

Sample no.	Gender	Age	Amount of Iron present in NP Users(mg/dl)
1.	Female	19	60
2.	Male	19	58
3.	Female	20	45
4.	Male	20	32
5.	Male	20	66
6.	Male	19	75
7.	Female	19	64
8.	Male	18	63
9.	Female	18	58.5
10.	Female	19	66

Table: 14 Quantitative analysis of iron in nail of smart phone (SP) users

Sample no.	Gender	Age	Amount of Iron present in SP Users(mg/dl)
1.	Female	22	100
2.	Male	21	75
3.	Male	21	80
4.	Male	22	100
5.	Male	22	85.5
6.	Female	21	90.2
7.	Female	22	94.8
8.	Male	23	98
9.	Male	21	88
10.	Male	22	100

Level of iron in nail sample of normal and smart phone users



Values are expressed as mean ± SD for 10 sample in each group. Data were analysed by one-way ANOVA followed by *post hoc* Bonferroni's test. $^{*}p>0.05$ *for* control sample Vs test sample.

The result observed in iron parameter shows the significant difference between the smart phone and normal phone users, which the nail sample is induced by the electromagnetic radiation, Deficiency or surplus of iron in the body leads to various diseases. Iron deficiency negatively affects the immune system, thyroid metabolism and cognitive functions. On the other hand, iron surplus is related to various pathological conditions such as cancer, neurodegenerative diseases, and damage of liver and testicles. Therefore, while keeping the iron levels and other iron parameters within a certain interval is important for body function, various complicated mechanisms take part in the establishment of this balance. There are studies in the literature which show that EMF in different doses affect iron parameters. The implementation durations of EMF exposure and total experiment durations vary in these studies.(Elferchichietal.,) observed that a exposure to a static magnetic field (SMF) of 128 mT negatively affected the iron levels in smart phone and normal phone users. Fat at minutes to EMFs created by mobile phones decreased the levels of ferritin. They explained this decrease with the oxidative stress occurring because of EMF. In addition to the studies where EMF is applied for short durations, there are also other studies where applications are made for longer durations. It was shown that ferritin and iron values were affected negatively by long exposure to EMF. On the other hand, contrary to knowledge on the negative effects of. Transferrin is primarily formed by the bonding of apotransferrin synthesized in the liver with (Fe3d. Like wise,there are studies which reported that short (5 to 15 days) exposure to 128 mT SMF increased the transferrin levels, while these studies found a negative relationship between iron levels of nail .. As a conclusion, mobile phone usage is becoming more and more widespread in the society. It is a necessity to investigate the potentially harmful effects of EMF created by mobile phones on different systems. In this study, it was determined that the EMF created mobile phone son speech and standby did not affect iron and ferritin levels.

4. Conclusion

Mobile or cell phones are nowadays an integral part of modern telecommunications in every individual life. In many countries, over half of the population use mobile phones and the mobile phone market is growing rapidly. As billions of people use mobile phones globally, a small increase in the incidence of adverse effects on health could have major public health implications on long term basis. Besides the number of cell phone calls per day, the length of each call and the amount of time people use cell phones are important factors which enhance the health related risk. Mobile phones emit radiofrequency energy, a form of non-ionizing electromagnetic radiation, which can be absorbed by tissues close to the phone. The amount of radiofrequency energy a mobile phone user is exposed depend on many factors as the technology of the phone, the distance between the phone and the user, the extent and type of mobile phone use and the user’s distance from cell phone towers.

The role of reactive oxygen species on the nail sample was evidenced in subjects exposed to EMR from smart phones. The level of oxidative stress was measured in terms of SOD, was found to be significantly decreased in test subjects.

The antioxidants level observed in mobile phone users with/ without electromagnetic radiation showed the influence of oxidative stress on smart phone users. The test subjects were found to have decreased levels of enzymatic antioxidant CAT, giving evidence for the overproduction of free radicals in the smart phone users.

EMR emission from smart phone was found to suppress the level of non- enzymatic antioxidants such as vitamin C and vitamin E, showing the inflammatory conditions in the nail sample having EMR exposure from mobile phone.

The levels of protein was found to be decreased in nail sample of subjects who used smart phone. This might be due to the emission of radiofrequency electromagnetic field from the smart phones which fragment the protein and damages it.

The level of iron was found to be decreased in the smart phone users as the nail sample is induced by the electromagnetic radiation from smart phone. EMFs created by mobile phones decreased the levels of iron. The iron level decreased with the oxidative stress occurring because of EMF.

In contrast the level of calcium was found to be increased in subjects who are using smart phones. Ca might play protecting role against damages produced by exposure to electromagnetic fields. In the presence of even silent alterations in mechanisms acting on intracellular Ca, chronic exposure to EMR might induce pathological conditions. Hence increased level of calcium shows inflammatory condition existed in the subjects who were using smart phones.

In conclusion, prolonged exposure to electromagnetic radiation (EMR) from smart phones leads to oxidative stress evidenced by decreased levels of antioxidant enzymes (SOD and CAT), vitamins C and E, and proteins in nail samples. Additionally, EMR exposure correlates with alterations in iron and calcium levels, suggesting potential cellular damage and inflammatory responses.

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DOI:10.22192/ijamr.2024.11.02.004

How to cite this article:
R. Aruna, P. Saranya Devi, S. Jayakumar and M. Hemalatha. (2024). A Comparative study on the biochemical changes in nail of smart phone and normal phone users. Int. J. Adv. Multidiscip. Res. 11(2): 53-83.
DOI: http://dx.doi.org/10.22192/ijamr.2024.12.02.004