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In Vitro Study Of Effect Of Radiation Emitted By Mobile Phone On Osmotic Fragility And Other Blood Parameters.

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ABSTRACT

Mobile phones are considered one of the most important inventions of 21st century. With growing number of cellular telephone users and ground stations, the effect of electro-magnetic fields (EMF) emitted by them on human beings is an interesting issue. Researchers have shown that microwave radiation from mobile phone causes harmful effects on blood cells in human body. There is increase in red blood cells (RBCs) count, decrease in white blood cell (WBCs) count and lymphocytes count after prolonged exposure to microwave radiation. The effect of RF-EMF (Radiofrequency electromagnetic fields) by mobile phone was studied on following blood parameters in vitro: Red blood cell (RBC) count, White blood cell (WBC) count, Platelet count and Osmotic fragility. Two venous blood samples were collected from healthy, young volunteers more than 18 years. One sample was exposed to the radiation from mobile phone (Dual band EGSM 900/1800 MHz) kept at a distance of one cm from the sample for one hour in talk mode inside the wooden box. Another sample was used as a control. The control sample was kept under identical condition but without RF-EMW exposure. Immediately after exposure to cell phone radiation, both specimen (control and exposed) were analyzed for osmotic fragility, RBCs count, WBCs count and platelet count. Significant difference was seen in RBC count and WBC count between exposed and unexposed sample with $p < 0.001$. Wilcoxon sign rank test gave $p < 0.05$ for the platelet counts which was also significant. There was no significant difference in osmotic fragility $p = 0.083$. The radio frequency electromagnetic field created by mobile phones and many electronic devices affects red blood cell count, white blood cell count and platelet count. Osmotic fragility is not affected by RF-EMF emitted by mobile phone in vitro.

Keywords: ELF- EMFs- Extremely Low Frequency Electromagnetic field, EMF electromagnetic Field, RBC- Red Blood Cell, RF/MW- Radiofrequency/Microwave, WBC- White Blood Cell, GSM- Global System for Mobile Communication

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INTRODUCTION

The positive aspect of technologic innovation makes life easier. It may also involve components that impair the quality of life via its certain negative effects. Electromagnetic wave generated by many natural and human made sources can travel long distances and play a very important role in daily life. Resulting from the technological innovations, the use of electromagnetic fields have gradually increased and thus people are exposed to electromagnetic waves at level much higher than those present in nature. The main sources of electromagnetic field are electromagnetic devices such as mobile phones, electrical and wireless devices. Mobile phones are used in position very close to the human body and require a large number of base station antennas. The resulting health issues have repeatedly been raised by public and scientists [1].

Electromagnetic waves create imbalance in the cell function. It is known from the magneto-hydrodynamics that when a stationary, transverse magnetic field is applied externally to a moving electrically conducting fluid, electrical currents are induced in the fluid. The interaction induced currents and the applied magnetic field produces a body forces (Lorentz force) which tends to retard the movement of blood [2].

Many studies have reported that radiation from mobile phones are absorbed by the human body and turn inside to heat. This biological effect can damage vital organs. The report of the Australian Radiation Protection in 2005 have confirmed that 70% of the waves emitted by mobile phones are absorbed in the user's head which leads to increase the speed of nerve impulses, blood pressure and heart rate. Also, exposure to electromagnetic waves leads to an imbalance in the circulatory system, increase in blood flow, and disruption in blood pressure [3,4], decrease in the hemoglobin [5]

Aims and objectives:

- To estimate RBC count in control and exposed blood sample
- To estimate WBC count in control and exposed sample
- To estimate platelet count in control and exposed sample
- To estimate osmotic fragility in control and exposed blood sample
- To compare the values of RBC count, WBC count, platelet count, and osmotic fragility in control and exposed blood samples.

Review of literature

Radiation is the emission or transmission of energy in the form of waves or particles through space or through material medium [6]. Radiation is often categorized as either ionizing radiation or non-ionizing radiation depending on the energy of the radiated particles.

Ionizing radiation is produced by unstable atoms. Unstable atoms differ from stable atom because they have an excess of energy or mass or both. Unstable atoms are said to be radioactive. In order to reach stability, these atoms give off or emit the excess energy or mass. These emissions are called radiation.

Radiation has the property of ionizing matter through which it passes; either directly or indirectly. In addition to the ability to ionize matter, its other important general characteristic is that it will pass through material substances. These two properties results in certain biological, physical and chemical changes in the material through which radiation passes.

Life has evolved in the continuous presence of natural background radiation like cosmic rays and terrestrial radiation. In addition to natural background radiation, human being of the modern world is exposed to radiation from various artificial sources as well. The largest such source is the use of radiographs (X-rays) in medical diagnosis. Lesser sources include radioactive minerals in building material, phosphate fertilizers, and radiation emitting components of television sets, smoke detectors, video display terminals and radionuclides released in the production of nuclear power. Additional dose of radiation is received by workers in various occupations, depending on their particular work assignments and working conditions.

Ionizing radiations include:

- Electromagnetic radiations of short wavelength and high energy (x-rays and gamma rays)
- Particulate radiation, which vary in mass and charge (electrons, protons, neutrons, alpha particles, and other atomic particles).

The term non-ionizing radiation refers to several form of electromagnetic radiation of wavelengths longer than those of ionizing radiation. As wavelength lengthens, the energy value of electromagnetic radiation decreases, and all non-ionizing forms of radiation have less energy than cosmic rays, gamma rays and x-rays. In order of increasing wavelength, non-ionizing radiation includes ultraviolet (UV) radiation, visible light, infrared radiation, microwave radiation, and radiofrequency radiation. All forms of electromagnetic radiation have the same velocity of 3×10^{10} cm/s in a vacuum [7].

The most public health issue related to non-ionizing electromagnetic radiation is the widespread use of mobile phones. More than two billion people around the world are now regularly user of these hand held devices. Whether or not there is any deleterious effect of long term exposure to microwave radiation remains an open question.

The International Agency for Research on Cancer (IARC) is coordinating the INTERPHONE PROJECT, in which epidemiologist from 13 countries are investigating possible mobile phone cancer risk. Each country is running its own case control study. The combined data projections point to a total of more than 5100 cases of benign and malignant brain tumours, more than 1100 cases of acoustic neuromas and more than 100 cases of malignant parotid gland tumours. These effects are to be analyzed together [8].

Five northern countries have pooled their data regarding the effects of electromagnetic radiation emitted from mobile phones. They observed a statistically significant increase (39%) in gliomas on the side of head the phone was used among those who had used cell phones for at least ten years [9]. A similar ipsilateral, long term risk has been seen for acoustic neuroma, a benign tumour of the acoustic nerve by the Swedish group [10].

Lai and Singh, who have shown that ELF-EMFs (Extremely Low Frequency Electromagnetic Field) can cause DNA breaks, had previously found similar effect at RF/MW (Radio frequency/Microwave) frequencies [11].

These findings have revoked major controversy with clear implication for the safety of mobile phone. More recently, others have found that mobile phone signals at low intensities can damage DNA both in live animals and in cell cultures [12, 13].

Leif Salford and Bertil Persson of Sweden's University of Lund have pointed to changes in the blood brain barrier following low-level microwave exposure. More recently, they have observed cellular damage in the brains of exposed rats after only a 2 hour exposure to very low intensity mobile phone radiation [14].

A Swiss group led by Alexander Borbely and Peter Achermann at the University of Zurich has shown that a single 30-minute peak exposure to a 1W/Kg (in the head) microwave signal simulating that from a GSM mobile phone had an immediate effect on the brain's electrical activity which lasted through most of the night's sleep. This group has found that "pulse modulation is crucial for RF-EMF (radiofrequency electromagnetic field) induced alteration in brain physiology" [15].

A number of research team has seen improvements in cognitive function and reaction times in psychological test by small but significant amounts following exposure to mobile radiation [16,17].

Agarwal A et al. evaluated effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. They observed no significant difference in sperm concentration between exposed and unexposed sample. Sperm motility was significantly lower in exposed samples compared with unexposed samples. They concluded that cell phone radiation causes oxidative stress in neat semen and leads to decrease in spermatozoa motility and viability [18].

Hasan HR and Issmer AH have shown that the long exposure to emitted radiations from base stations and heavy using of mobile phone at domestic level has negative impact on human health due to increase the concentration of peroxy nitrite, which can in turn cause many diseases like cancer [19].

Mariam S. Alghamdi et al. investigated the effects of electromagnetic fields on some hematological parameters of white male mice. They found a decline in hemoglobin, hematocrit and red blood cell count in addition to the platelet count after short and long exposure to two types of mobile phone (Alcatel, Nokia). It was observed that the average number of white cells and lymphocytes increased significantly, indicating the increase to body's immune response to radiation [20].

The harmful effect of electromagnetic radiations emitted from video display unit (VDU) on red blood cells of Swiss albino mice kept 20 cm away, at power density of $0.295 \mu\text{w}/\text{cm}^2$ was investigated at the interval of 7, 14, 28, 42 and 56 days. The scanning electron micrograph of RBCs showed altered morphology. Red blood cell count, hemoglobin concentration was reduced up to day 42 of irradiations. The exposed mice consumed less feed and water and have reduced body weight. All the changes normalised in late effect group. Collectively, these findings indicate that EMF issuing from VDU is harmful and adversely affects biological system [21].

However, due to the thermal and other physical effects of exposure to radiofrequency radiation (RFR) from GSM on human hematological parameters [22,23] there exists a possibility of difference in hematological parameters of blood having been inadvertently exposed to RFR during making or receiving calls by laboratory worker while holding the blood samples for transfusion or analyses [24]. Thus, in this study, our objective was to analyse the effect of mobile phone radiation emitted from mobile phone in active (talk) mode on hematological parameters.

MATERIAL AND METHOD

Thirty seven healthy volunteers participated in this study to analyse the effect of radio frequency electromagnetic field created by mobile phone on various blood parameters.

Study design: Experimental study

Study sample: Healthy young volunteers more than 18 years were included in this study. It was conducted in the department of Physiology, Kasturba Medical College (KMC), Manipal.

Inclusion and exclusion criteria

Healthy young volunteers more than 18 years and less than 35 years, not taking any medicine that can affect complete blood count, or osmotic fragility.

Devices Used in the Experiments (Figure 1a, 1b)

Mobile Phone (Dual band EGSM 900/1800 MHz)

Wooden box for controlled environment.

Neubauer's chamber, RBC pipette, WBC pipettes, test tubes, Pasteur pipette.

Dacie's fluid for RBC count, Turk's fluid for WBC count, ammonium oxalate for platelet count, 1% sodium chloride and distilled water for (different tonicity) osmotic fragility.

Procedure

Collection of blood samples

After taking Institutional Ethics Committee clearance, this study was conducted.

Two venous blood samples were collected from healthy, young volunteers after taking informed, written consent in EDTA tubes. These samples were used in assessment of RBC count, WBC count, platelet count and osmotic fragility as per the standard protocol described by Barbara A. Brown [25].

Both samples were used at room temperature to avoid the effect of temperature on blood parameters. One sample was exposed to the radiation from mobile phone (Dual band EGSM 900/1800 MHz) kept at a distance of one cm from the sample for one hour in talk mode inside the wooden box. Another sample was used as a control. The control sample was kept under identical condition but without RF-EMW exposure (figure 1a, 1b).

Immediately after exposure to cell phone radiation, both specimen (control and exposed) were analyzed for osmotic fragility, RBCs count, WBCs count and platelet count.



Figure 1a: Test sample kept at a distance of one cm from mobile phone (Dual band EGSM 900/1800 MHz). Control sample kept under identical condition outside the box.

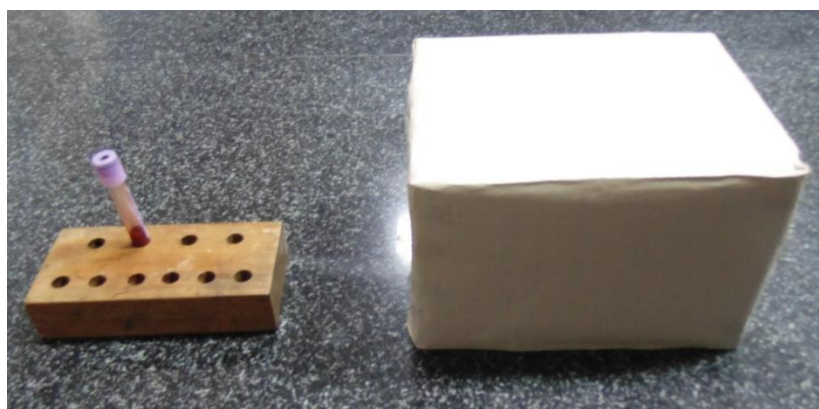


Figure 1b: Test sample exposed to RF-EMW from mobile phone for one hour in talk mode inside the wooden box. Control sample kept under identical condition but without RF-EMW exposure.

Red Blood Cell count

Blood was sucked in RBC pipette up to 0.5 mark and diluting fluid (Dacie’s fluid) in pipette up to 101 mark above the bulb by using constant suction. The content of bulb mixed thoroughly for 2 minutes by rotating the pipette with its tip pressing against the palm of the left hand. Neubauer’s chamber was used for counting RBC. Five medium RBC squares were counted. The dilution used in this method was 200 times. Only uniformly distributed cells on slides were considered. Finally, the number of cells in undiluted blood was calculated and reported as the number of red cells per mm³ of whole blood.

White Blood Cell count

Blood was drawn exactly up to the 0.5 mark and WBC diluting fluid (Turk's fluid) exactly up to the 11 mark in WBC pipette. The content of bulb mixed gently for few seconds. Glacial acetic acid in Turk's fluid leads to destruction of red blood cells by hemolysis and gentian violet stains the nuclei of white blood cells. In four corner squares under the low power objective, white blood cells were counted. Dilution in this method was 20 times.

Platelet count

Platelets were calculated by Brecher-Cronkite method. In this method, ammonium oxalate solution makes the platelets refractile and lyses the red blood cells. Platelets were then counted using haemocytometer.

One percent ammonium oxalate solution was drawn into WBC pipette up to 0.5 mark to avoid adhesion of platelets onto the capillary stem if blood was taken first. Then blood was drawn in pipette till mark 11. After charging, the counting chamber was kept on moist blotting paper and covered by a petri-dish for 20 minutes. Then platelets were counted in 80 small squares of RBC ruling area.

All precaution was taken to minimize the error involved in manual method.

Osmotic fragility

Hypotonic solutions were prepared of increasing hypotonicity by mixing the required number of drops of 1% sodium chloride solution to distilled water in the test tubes serially from 1 to 10. A drop of venous blood was added to each of the test tubes containing graded sodium chloride solutions. Blood was mixed in the solution by tilting test-tube gently. Tubes were kept in order 1 to 10 in the rack for 30 minutes without disturbing. After 30 minutes, findings regarding osmotic fragility were analysed.

Statistical Analysis

Analysis of data was performed using SPSS 20 (Statistical Package for the Social Sciences) for Windows. Comparison of data was done by Wilcoxon Sign Rank test. P-value less than or equal to 0.001 was considered significant.

RESULTS

Thirty seven young healthy volunteers participated in this study. Thirty two (86.5%) were male and 5 (13.5%) were female.

The mean age of the volunteers was 26.16 years (19 years- 34 years).

Wilcoxon sign rank test was performed to determine the significant difference between the average RBC count, WBC count and platelet count of the blood samples not exposed to mobile phone radiation and those exposed to mobile phone radiation. P-values of ≤ 0.001 were considered to be significant

Red Blood Cell count

The median red blood cell count in the control sample was 4.76 million/ cu.mm of blood. The median red blood cell count in the exposed sample was 4.70 million/ cu.mm of blood. All the studied cell counts were in the normal range. The results indicated that the average blood RBC count is significantly different between the exposed and control samples (Table 1). . Wilcoxon Signed Ranks Test showed p value of 0.001.

White Blood Cell count

The median white blood cell count in unexposed blood was 7600 cells/ cu.mm of blood. The median white blood cell count in exposed blood was 7500 cells/ cu.mm of blood. This change in white blood cell count was statistically significant and p value was 0.001 (Table 1).

Platelet count

Median platelet count was 2.50 lakhs/ cu.mm of blood in control blood sample. Median platelet count was 2.50 lakhs/ cu.mm of blood in exposed blood sample. Wilcoxon Signed Ranks Test showed p value of 0.003(Table 1).

Significant difference was seen in RBC count and WBC count between exposed and control sample with $p < 0.001$. Wilcoxon sign rank test gave $p < 0.05$ for the platelet counts which was also significant.

Osmotic fragility

There was no significant difference in osmotic fragility after one hour of continuous exposure of mobile phone radiation. Starting point of hemolysis was taken in consideration for comparing osmotic fragility in exposed and control samples. Wilcoxon sign rank test gave $p = 0.083$ (Table 1).

Variable	MEDIAN(Q1, Q3)		P -value
	Control	Exposed	
Red Blood Cell count	4.76 (4.45, 4.51)	4.70 (4.36, 5.00)	0.001 **
White Blood Cell count	7600(5750, 9138)	7500 (5700, 9130)	0.001 **
Platelet count	2.50(1.85, 3.12)	2.50(1.80, 3.00)	0.003*
Osmotic fragility	0.45(0.45,0.40)	0.45(0.45,0.45)	0.083

Table 1 **= highly significant, *= significant, p value of 0.001 considered as significant value

DISCUSSION

Technology, the lifeline of modern day society has its own problems. Every electronic equipment used in day to day life produce electromagnetic field.

Mobile phones have become basic need of the present day society. Omni presence of these gadgets cannot be ruled out. This gadget has converted the world as a small place where people are connected in every moment of life. But, these phones emit electromagnetic radiation which poses various effects on human cells. Health care providers and laboratory workers of blood bank use mobile phones while handling blood or blood product like platelets intended for transfusion. This study was conducted to analyze the effect of mobile phone radiation on selected blood parameters in vitro.

Blood from 37 young healthy volunteers were used in this study. The aging process, addictive substances like nicotine and alcohol, prevalent disease affect the health and quality of blood cells. Young healthy participants with mean age of 26.16 years without any addiction were selected to nullify these effects.

Red blood cell count

There was significant decrease in RBC count after exposure to one hour of continuous mobile phone generated EMW radiation.

Electromagnetic radiation can cause deformation of red blood cells. The functions of red blood cells are related to their mechanical shapes. This abnormal morphology can change the superficial energy and mechanical characteristics of red blood cells. The decrease in superficial charge density of red blood cells may increase their aggregation and density leading to rouleaux formation and cell lysis [21].

Junguang Yong et al studied radiation Injury of Red Blood Cells to microwave radiation with different power density. They found that the effects of low-power density of EM irradiation on the RBCs were dependent not only on the irradiation dose, but also on the irradiation time. After 60 minutes of EM radiation, the red cells changed in shape, size and superficial charge density in their study [26]. Abu Bakr El-Bediwi AB et al found decreased red blood cells in their study [27].

In the study conducted by Singh H et al on Swiss albino mice showed decrease in RBC count along with their hypochromia. The structure of cell membrane was found altered in their study. The RBC count and hemoglobin concentration reached to their normal values after removal of exposure [21].

Hasan HR and Issmer AH found increased concentration of peroxynitrite after long exposure to emitted radiations from base stations and heavy using of mobile phone at domestic level. This compound can damage the cell by interfering with proper functions of different biomolecules. They found significant increase in Superoxide dismutase activity in volunteers staying near base station antennas. Erythrocytes are vulnerable to oxidative damage due to continuous exposure to high oxygen tension and presence of large amount of iron, a potent catalyst for oxygen free polyunsaturated fatty acids which are major targets for peroxidation [19].

White blood cell count

Statistically significant change in white blood cell count was found after one hour radiation exposure to electromagnetic waves generated by mobile phone.

Aly AA et al studied the effect of RF fields on leucocytes. There was a significant change in the leucocyte behavior upon application of RF fields, including more rapid changes in shape (cell shrinking, expanding and rolling). They also found that RF radiation caused damage to the leukocytes cells from specific person's blood; the cells expanded and lost its ability to move [28]. El-Bediwi AB et al have shown reduction in white blood cells and cell lysis in rats after exposure to electromagnetic radiation in their study [27].

Alghamdi MS have reported decline in hemoglobin, hematocrit and red blood cells count, in addition to the platelets count after short and long exposure to both types of mobile phone (Alcatel, Nokia). It was observed that the average number of white cells and lymphocytes increased significantly, indicating the increase to the body's immune response to radiation. These effects of radiation were observed in vivo studies [20].

Limitation

Thirty seven young healthy volunteers participated in this in vitro study. Response of human immune system is nullified and additional environmental factors interfere with the response of in vitro studies. So, it is essential to conduct similar in vivo study with a larger sample size.

The limitations of this study are:

- This is an in vitro study
- Small sample size
- Limited hour of exposure

CONCLUSION

Mobile phones and many electronic devices have become an integral part of human life. However, the radio frequency electromagnetic field created by these devices affects red blood cell count, white blood cell count and platelet count. The integrity of cell membrane of blood cells as measured by osmotic fragility is not affected by RF-EMF emitted by mobile phone in vitro.

In vivo studies with larger sample size is required to analyse the effects of mobile phones and similar electronic devices on human body.

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