I*n-vitro* cytogenetic assay measuring chromosomal aberration frequencies induced as effect of cell phone radiation as active source in human peripheral blood lymphocytes

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Abstract: **Recent increase of genetic disorders in human may be correlated to the increased exposure to electromagnetic fields produced by various instruments, including cell phones. Because of its radiation, difference or abnormality in number of chromosomes is one which is most likely to be affected to cell phone radiation. As well, the normal cells tend to turn toxic and may induce more cell death too. Chromosome preparations can be initiated from any dividing cell population and can even be prepared from no dividing cells, such as peripheral blood lymphocytes, that can be stimulated by mitogens to divide for a brief period of time. Toxicant-induced exchanges between sister chromatids of each chromosome can be quantitated by differentially staining the chromatids using giemsa in chromosomal aberration studies. Thereby, the aim of this study is to assess the adverse effects of cell phone radiation and evaluate the mutagenic and cytotoxic potential in living cells and test on the effect on cell radiation on normal cells can also be identified using cytotoxicity tests.**

Keywords: **mobile phones, chromosomal aberration, chromatids, toxicity**

Introduction

Cell phones have been around for several decades, it really did not become popular ,Until 1983 (Federal Communications Commission), and finally were considered common in the 1990s (Crittenden, 2009). Mobile phones were one of the greatest findings of the humans. They have become as a routine thing which is inseparable from our lives. The first mobile phone call was made 40 years ago ,on April 3, 1973, by Motorola employee Martin Cooper.

The mobile phone must be kept on the ears in the head for communication. The head is the most sensitive organ in the human body. This triggered many scientists to study for the effects on user health and explore mechanisms of interaction responsible for reported biological sequel onhumans, animals and organic cells from acute and chronic exposures from mobile phone frequencies. Generally, the higher the frequency the less able electromagnetic radiation is to penetrate materials. The maximum powers that GSM mobile phones are permitted to transmit by the present ICNIRP standards are 2 W and 1 W at 900 Hz and 1800 Hz, respectively. Radio frequencies induce RF electric fields in tissue a part of the radiated energy will be absorbed in tissues [1].

Some of the recent research found that the effects of cell phone radiation increases the amount of glucose in the brain, aprocess that “occurs with infections and other inflammatory processes, and leads to theproduction of potentially damaging reactive oxygen radicals that can alter the ways that cells andgenes work” (Davis, 2011, Para. 4).

When there is some disturbance in the nervous system or the brain, e.g., by RFR, morphological, electrophysiological, and chemical changes can occur. A significant change in these functions will inevitably lead to a change in behaviour. Indeed, neurological effects of RFR reported in the literature include changes in blood-brain-barrier, morphology, electrophysiology, neurotransmitter functions, cellular metabolism, calcium efflux, responses to drugs that affect the nervous system, and behaviour.[2],[3].

The problems associated with the RF radiation which was studied in literature are RF sickness, electroencephalographic changes, cell proliferation and blood pressure changes, blood-brain barrier leakage,[4]altered EEG patterns[5] and decreased fertility in mice.[6] Cancer risks and genotoxicity from exposure to RF fields *in vivo* and *in vitro* have rather been points of cynosure since equivocal evidences exist.[7],[8],[9],[10], Apparently no studies have documented genotoxicity in mobile phone users.

Chromosomal Aberration

Chromosomal aberrations (CA) in peripheral blood lymphocytes is a reliable biomarker of genotoxic exposure to both physical and chemical agents , and an increase in CA frequency indicates the risk of exposure to mutagenic agents. The purpose of the in vitro chromosome aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells. [11]

There are two types of structural aberration, chromosome or chromatid. Chemical mutagens mostly induce chromatid type of aberration, but chromosome-type aberrations also occur.. The potential of a chemical to induce numerical aberrations is identified by the increase in polyploidy.

The invitro chromosome aberration test may employ cultures of cell lines or primary cell cultures. The criteria for selecting cells are growth ability in culture, stability of the karyotype, chromosome number, chromosome diversity and spontaneous frequency of chromosome aberrations. At the present time, the available data suggest that it is important to consider the p53 status, genetic (karyotype) stability, DNA repair capacity and origin (rodent versus human) of the cells chosen for testing (Pfuhler*et al*., 2011)..For demonstration of chemical safety in human these characteristics above are considered.

Although problems exist in the extrapolation from in vitro results to the in vivo situation, the lymphocyte offers several advantages as a test system.Since the availability of the human cells is more and the required amount of the blood is only in ml which can be obtained daily, lymphocytes are convenient. The lymphocytes are found in most of the places in the body and some are long-lived. Virtually all the peripheral blood lymphocytes are synchronized cell populations in the same Go or Gl stage of mitotic interphase, and, in healthy individuals, these cells are only infrequently involved in mitotic proliferation in vivo. Invitro technique use mitogens to undergo mitotic division in lymphocytes and thus provide a ready source of dividing cells for the scoring of chromosome aberrations. These are excellent techniques available for making chromosome preparations from lymphocytes and the cells have a low spontaneous chromosome aberration frequency. [12]

Many researches are done for several decades on chromosome aberrations in peripheral blood lymphocytes has been successfully used to examine people exposed to radiation during various accidents [13-21]. The results of cytogenetic analysis serve as a basis for estimating absorbed radiation doses and for predicting possible negative effects of irradiation.

Sample Preparation

Solid test substances should be dissolved or suspended in appropriate solvents or vehicles and diluted if appropriate prior to treatment of the bacteria. Liquid test substances may be added directly to the test system and /or diluted prior to treatment. Fresh preparations should be employed unless stability data demonstrate the acceptability of storage. Cell cultures are exposed to the test substance without metabolic activation. At predetermined intervals after exposure of cell cultures to the test substance, they are treated with a metaphase arresting substance (colchicines), harvested, stained and metaphase cells are analyzed. Dose formulation concentrations of 1.25, 2.5 and 5.0 mg/ml of samples were prepared by serial dilution for the study. [22]

Materials and Methodology

Human lymphocytes

Human peripheral blood Lymphocytes obtained from a healthy adult non-smoking female donor without any recent history of illness and under no medication was used for this study.

Vehicle Controls

Distilled water/ Phosphate Buffered Saline was used as vehicle and the same at the volume of 0.1ml /culture were used in the assay in duplicates.

Positive Controls

Mitomycin C (Sigma, CAS no.50-07-07) in distilled water at the concentration of 1 µg/ml in the assay.

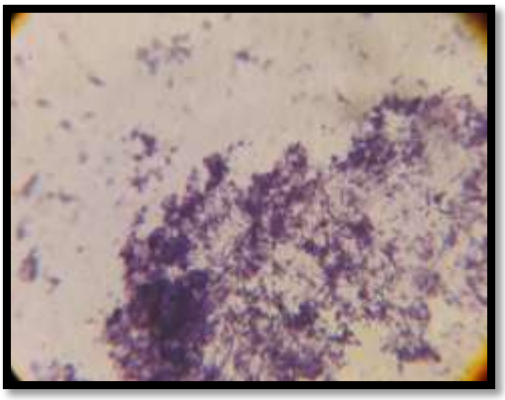
Methods

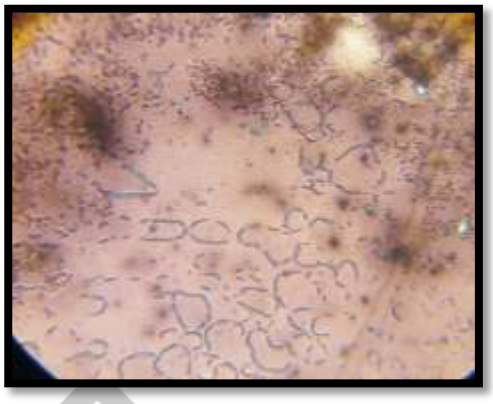
Lymphocyte culture and slide preparation

The venous blood was collected in sterile test-tubes with sodium heparin. Lymphocyte enriched plasma was obtained. After that, 0.5 ml of the blood plasma was mixed with 2 ml of RPMI-164 medium containing 15% fetal calf serum. 2.5% of phytohaemagglutinin, 2 mM glutamine and antibiotics. The cultures were incubated at 37^0 C for 48 hours. Chromosome preparations were obtained according to standard procedures [22]. Slides used for analysis of unstable chromosome aberrations were kept for 10mins at room temperature and then subjected to Giemsa stain, and after which it was immersed in distilled water and then air dried as required. Then the slides are viewed under the microscope.

FIGURES

POSITIVE CONTROL *NORMAL CONTROL*





5 mg *2.5mg*

1.25 mg

Table no. 1 CA scoring

Dose (mg/ml culture)	Total number of cells scored	Percent numerical aberration	Mean of structural aberration	Total number of aberration	Total no. of cells with aberration	No. of aberration per cell	Aberration frequency (%)
1.25	100	7	3	10	1	10	3
	100	9	0	9	2	5	
2.5	100	16	1	17	4	4	11
	100	17	5	22	7	3	
5	100	24	2	26	15	2	36
	100	26	1	27	21	1	
Positive control	50	0	0	0	30	0	65
Mitomycin C	50	0	0	0	35	0	

From the above results it is concluded that the test sample can cause partially mutagenicity.

Result and Discussion

Result

From the table values we can see that at 5mg/ml concentration of the sample out of 140 cells almost 50% (67 cells) got aberrated. In 2.5mg/ml concentration of the sample 28 % (40 cells) of the cells got aberrated. In 1.25mg/ml concentration of the sample 17 % (21 cells) of the cells got aberrated.

Discussion

Chromosome aberrations are the cause of many human genetic diseases and there is substantial evidence that chromosome mutations and related events causing alterations in oncogenes and tumour suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals. From the above values, we can see that the number of cells which got aberrated decreases according to the concentration of the sample used. So it can be reported that the mobile phone causes radiation which aberrates the human cells. Mobile phone causes mutagenicity at certain level. So prolong use of the mobile phones will show some health effects on human body. It can be said that Chromosomal Aberrations are microscopically visible changes in the single DNA molecules of chromosomes and chromatids. From the results it is concluded that the test sample used is partially mutagenic.

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