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CYTOGENETIC DAMAGES INDUCED BY CHRONIC EXPOSURE TO MICROWAVE NON-IONIZING RADIOFREQUENCY FIELDS

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Non-ionizing radiation has a significant and positive impact on modern society through a number of uses. There is increasing public concern regarding the health risks of radio-frequency (RF) radiation, particularly that produced by mobile phones. Concern regarding the potential risks of exposure to EMFs has led to many epidemiological investigations, but the effects of EMF exposure on human and other mammalian cells are still unclear. One of the most frequently asked questions about the effects of microwave radiation on biological systems is whether they produce genotoxic effects and could be there a possible link with oncogenic processes. It is most difficult to get accurate and reproducible results for the studies that tell us most about the effects of EMF on humans. Based on some "weak" evidence suggesting an association between exposure to radiofrequency fields (RF) emitted from mobile phones and two types of brain cancer, glioma and acoustic neuroma, the International Agency for Research on Cancer has classified RF as 'possibly carcinogenic to humans' in group 2B. Literature results suggest that pulsed microwaves from working environment can be the cause of genetic and cell alterations. Taken together, the increased frequency of DNA damages, increased intensity of oxydative stress and production of reactive oxygen species as well as prolonged disruption in DNA repair mechanisms could be possible mechanisms for microwave induced cytogenetic damages even at low-level electromagnetic fields. Although there were contradictory results about harmful effects of electromagnetic fields we recommend that the mobile phone should be kept as far as possible from the body during conversations and also during usual daily activities to reduce the absorption of radiation by cells. In addition, the appropriate intake of antioxidant-rich food or drugs may be helpful for preventing the genotoxic effects that could be caused by mobile phone use. *Acta Medica Medianae 2013;52(4):48-52.*

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Introduction

The character of the natural electromagnetic field has altered significantly due to technological progress and numerous man-made sources such as those used in industry, traffic, medicine, radio and television communications as well as in everyday life. The most frequent sources of NIR are mobile phones and cell towers which emit microwave radiation (MWR). They emit powerful electromagnetic field (EMF), the effects of which have been unknown yet. These sources of non-ionizing radiation cause atmospheric pollution similar to the pollution from various industrial sources (1).

Radio frequency (RF) and microwave radiation are considered as a type of non-ionizing electromagnetic radiations present in the environment and are perceived as health risk. Increased exposition of RF electromagnetic field (EMF) produced by the appliances used in the telecommunications, industry and medicine may lead to biological effects in more individuals. Non-ionizing radiation has a significant and positive impact on modern society through a number of uses. There has been a growing concern among the public regarding the potential human health hazard of exposure to these frequencies by these appliances (2).

In the recent years, MW has attracted a great deal of attention due to its increased usage in occupational environment, which lead to a

large number of publications regarding health hazards of MW (3).

Microwave radiation produces biological effects in the form of physically and chemically changed tissue, molecules and changes of the whole living organism which may further cause a change in the functions of separate organs or organic systems. With regard to a specific absorbed dose (SAR), biological effects are either thermal, if above 1 W/kg, or non-thermal, if below 0.1 W/kg. Recent investigations of cell proliferation and DNA synthesis after microwave exposure indicate that biological effects occur at a power density that is sufficient to induce thermal cell damage. No values for threshold dose intensity or a dose-effect relationship can be determined positively so far for any biological system (4).

Meta-analyses have noted that there may be a causal relationship between mobile phone use and the incidence of brain tumors.5 Although evidences in humans are limited, RF-EMR has recently been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC), and any pragmatic measures to reduce exposure, such as hands-free devices, should be strongly recommended (6).

There is increasing public concern regarding the health risks of radio-frequency (RF) radiation, particularly that produced by mobile phones. Concern regarding the potential risks of exposure to EMFs has led to many epidemiological investigations, but the effects of EMF exposure on human and other mammalian cells are still unclear. One of the most prominent questions about effects of microwave radiation on biological systems is that they produce genotoxic effects and if there could be a possible link with oncogenic processes.

Evaluation of genotoxic effects

Studies to evaluate RF EMF effects in cells, animals, and humans have used many approaches (7).

In vitro studies are usually divided into genotoxicity and nongenotoxicity studies. The examined genotoxic effects include micronucleus (MN) formation, chromosomal aberration, primary DNA damage assessed using alkaline and neutral comet assays, sister chromatid exchange, and mutation, whereas nongenotoxic studies have examined cell proliferation and cell cycle distribution, gene expression (mRNA and protein), the immune system, transcriptomics (microarray analysis), apoptosis, and reactive oxygen species (ROS). The general focus of studies on EMF has been on the relation between EMF carcinogenicity, where all studies are considered equally important irrespective of whether the results were obtained in humans, animals, or cells. However, for evaluation of effects that may occur in humans, the results are weighted as such that the epidemiological study is more significant than the experimental animal study, which in turn is more significant than the cellular study. When looking at the accuracy and reproducibility of the study, the results of cellular studies have greater accuracy and reproducibility than experimental animal studies, which in turn have greater accuracy and reproducibility than epidemiological studies (8).

Therefore, it is most difficult to get accurate and reproducible results for the studies that tell us most about the effects of EMF on humans. Based on the some "weak" evidence suggesting an association between exposure to radiofrequency fields (RF) emitted from mobile phones and two types of brain cancer, glioma and acoustic neuroma, the International Agency for Research on Cancer has classified RF as 'possibly carcinogenic to humans' in group 2B (6).

It must be mentioned that consensus about genotoxic effects of RF EMF has not been attained. There was an opposite thinking about this problem and a few meta-analyses which did not show the connection between RF effects and tumors development. For example, in a more recent meta-analysis and systematic review, Repacholi et al.(9) have concluded that the data from not only genotoxicity studies but also from in vivo oncogenicity, tumor promotion, brain and other head tumors do not support a causal relationship between RF exposure emitted from mobile phones and the incidence of brain cancer or other tumors of the head.

Genotoxic effects of MW

In vivo evidence of non-thermal influences, mainly under exposure to actual GSM phone radiation showed increases in DNA single and double strand breaks in rats, micronuclei formation in the liver and brain tissue in rats (4).

There have also been some results that indicate reduced efficiency of lymphocyte cytoxicity and increase of chromosome aberrations and micronuclei in human blood lymphocytes (10).

Results showed that there was a higher frequency of specific chromosome lesions in cells that had been irradiated and that microwave radiation causes changes in the synthesis as well as in the structure of DNA molecules. The exposed cultured Chinese hamster cells showed increased frequency of structural chromosome damage related to time of exposing. The chromosomal damage included chromatid and chromosome breaks and a dicentric chromosome appearance. After 15 min of exposure, the total percentage of damage was 4.8%, that is 0.049 aberrations per cell analyzed. After 30 min of exposure, this was 6.3% or 0.064 aberrations per cell analyzed and after 60 min of exposure it amounted to 15% that is 0.151 aberrations per cell, compared to 1.7% in control group. Most of the damages were breaks, acentric fragments and dicentrics. In this group, ring chromosomes, symmetric exchanges and tetraploids

were also noted (11). Results of this study lead to the assumption that exposure to microwave radiation should be assessed at two levels: as a transitory, probably thermally caused imbalance of enzymes activity, and as a permanent change in the genome of proliferated cells which can be associated with neoplastic transformation. This also suggests an interesting dose-response relationship.

The other authors who investigated genotoxic effects of microwave radiation describe the existence of interaction mechanisms without accompanying heating. Interaction at molecular level can bring about perturbations in the complex macromolecular biological systems - cell membranes and subcellular structures. The authors concluded that oxidative stress after exposure to microwaves may be the reason for many adverse changes (12). The obvious effects of microwaves exposure on biological systems (animal models) is presented by increasing free oxygen radicals, which may enhance lipid peroxidation levels thus leading to oxidative damage. In rats there was an increase in malondialdechide (MDA) level even when MW was below current exposure limits. Increasing of MDA level is accompanied by decreasing of GSH concentration. Additionally, there was a less significant increase in SOD activity demonstrating induction of oxidative stress (13).

It is also showed that DNA damage and intracellular ROS increase in cultured human lens epithelial cells induced by acute exposure to MW. Cells exhibited significant intracellular ROS increase in addition to the significant DNA damage examined by the comet assay. Authors concluded that DNA damage induced may be associated with the increased ROS production (14).

This is in line with our previously published results where significant increase in lipid peroxidation as a direct indicator of the hepatocytes and brain cells' injury under a long-term (90 days) mobile phone microwave exposure were shown. The effects of chronic microwave exposure were also manifested by hyperkalemia that could be the possible systemic marker of impaired cells membrane fluidity and increased permeability (15).

RF in combination with mitomycin C, a DNA alkylating agent, was shown to increase DNA strand breaks more than with exposure to RF alone (16). However, we noticed that there were opposite results and many papers claim that RF exposure does not break DNA bonds. Therefore, some studies have concluded that RF exposure does not cause DNA strand breaks and chromosomal aberations (17). Similar findings present other studies where no detectable DNA strand breakage was observed by the alkaline comet assay. But, taken together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells (18).

The cytogenetic damage effects of MW have been shown in many human cell lines especially in human lymphocytes. In the study with in vitro micronucleus, a test on human lymphocytes was used as an indicator of cytogenetic damage resulting from exposure to non-ionizing radiation. The authors showed that mobile phones' MW with frequency of 1800 MHz are able to induce micronucleus formation (MN) in short-time exposures to medium power density fields, but with great interindividual variations (19).

Evaluation of double-strand DNA breaks by the foci technique, which appears to be more sensitive than the neutral comet assay, showed that the latter RF frequency had induced a long-lasting inhibition of DNA repair (20). Also, several reviewers have presented the evidence that increased incidence of MN predicts enhanced cancer risk in humans (21,22). It is well documented in the literature that MN arise as a result of clastogenic (chromosome breakage) and/or aneugenic (lagging and/or unequal segregation of whole chromosomes caused by spindle disturbances during cell division) action of genotoxic agent. Hence, a positive correlation is known to exist between MN and chromosomal aberrations (CA) end-points. However, it must be mentioned that large meta-analysis data between MN and CA end-points did not indicate such positive correction and no significant differences were observed between Rf exposed and control cells in all 19 tests for MN (23).

Oxidative stress and MW- pathophysiological link with single strand DNA breaks

One of the most aggressive oxygen free radicals is peroxynitrite which is produced from nitric oxide, in the state of hyperoxydative stress after exposure of cells to MWs. In some studies, the rise in oxidative stress markers parallels the rise in nitric oxide, suggesting a peroxynitrite-mediated mechanism.

Peroxynitrite elevation is usually measured through a marker of peroxynitrite-mediated protein nitration, 3-nitrotyrosine (3-NT). There are some studies where 3-NT levels were raised after EMF exposure (24). Although these cannot be taken as definitive, when considered along with the evidence on oxidative stress and elevated nitric oxide production in response to EMF exposure, they strongly suggest a peroxynitrite-mediated mechanism of oxidative stress in response to EMFs.

Such a peroxynitrite-mediated mechanism may explain numerous studies showing the single-stranded breaks in DNA, as shown by alkaline comet assays or the similar microgel electrophoresis assay, following EMF exposures in most such studies (25). We must mention that there were some studies which had not confirm this finding (26).

Oxidative stress after MW exposure and reducing of antioxidants protection have been shown to greatly increase the generation of DNA single-strand breaks following EMF exposure as has also been shown for peroxynitrite-mediated

DNA breaks produced under other conditions. It has also been shown that one can block the generation of DNA single-strand breaks with a nitric oxide synthase inhibitors (27):

Opening of voltage-gated calcium channel leads to increased intracellular Ca, which stimulates nitric oxide synthases and increase nitric oxide which in turns is converted in peroxynitrite in state of hyperoksidative stress after MW exposure. It can be concluded that the data on generation of single-strand DNA breaks, although quite limited, support a mechanism involving nitric oxide/peroxynitrite/free radical (oxidative stress).

Conclusion

Results suggest that pulsed microwaves from working environment can be the cause of genetic and cell alterations. Taken together, the increased frequency of DNA damages, increased intensity of oxydative stress and production of reactive oxygen species as well as prolonged disruption in DNA repair mechanisms could be possible mechanisms for microwave induced cytogenetic damages even at low-level electromagnetic fields. Although there were contradictory results about harmful effects of electromagnetic fields, we recommend that the mobile phone should be kept as far as possible from the body during conversations and also during usual daily activities to reduce the absorption of radiation by cells. In addition, the appropriate intake of antioxidant-rich food or drugs may be helpful for preventing the genotoxic effects that could be caused by mobile phone use.

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CITOGENETSKA OŠTEĆENJA UZROKOVANA HRONIČNIM IZLAGANJEM MIKROTALASNOM NEJONIZUJUĆEM ZRAČENJU

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Nejonizujuće zračenje ima značajan pozitivan uticaj na razvoj i funkcionisanje savremenog društva. Međutim, postoji sve veća zabrinutost zbog štetnog uticaja ove tehnologije na zdravlje ljudi, posebno one koje može ispoljiti mobilna telekomunikacija. Da bi se analizirali ovi efekti, sprovedene su brojne epidemiološke i eksperimentalne studije, ali su efekti ovih zračenja na ljude i druge sisare još uvek nedovoljno poznati. Jedno od najčešće postavljanih pitanja jeste da li mikrotalasna zračenja imaju genotoksični potencijal kod ljudi i da li postoji njihova povezanost sa razvojem tumora. Mora se napomenuti da je vrlo teško dobiti precizne i pouzdane podatke o efektima elektromagnetnih radijacija na humanu populaciju. Na osnovu nekih, "nedovoljno jakih" dokaza, koji ukazuju na moguću povezanost elektromagnetih zračenja poreklom od mobilne telefonije i dve vrste tumora mozga, glioma i akustičkog neurinoma, Internacionalna agencija za istraživanje kancera klasifikovala je radiofrekventna zračenja kao moguće kancerogene za ljudsku populaciju u grupi 2B. Podaci iz literature govore o tome da mikrotalasna zračenja u ljudskoj okolini mogu prouzrokovati genetske i ćelijske alteracije. Ovo se najverovatnije odvija posredstvom nekoliko mehanizama, od kojih su najvažniji direktno oštećenje DNK, porast intenziteta oksidativnog stresa i produkcije reaktivnih kiseoničnih radikala, kao i prolongirani poremećaji reparacije DNK, koji se dešavaju čak i nakon izlaganja mikrotalasnom zračenju slabog intenziteta. Iako postoje kontradiktorni podaci o štetnosti mikrotalasnih zračenja, trebalo bi preporučiti preventivni pristup kod upotrebe i korišćenja novih tehnologija, koji podrazumevaju skraćenje razgovora, držanje aparata što dalje od tela tokom konverzacije i dnevnih aktivnosti, kako bi se redukovala količina apsorbovane energije. Ujedno, pravilna ishrana bogata antioksidansima može imati preventivne efekte u prevenciji genotoksičnih efekata koje može izazavati korišćenje mobilnih telefona. *Acta Medica Medianae 2013;52(4):48-52.*

Ključne reči: *citogenetika, oštećenja, mikrotalasi, nejonizirajuće zračenje, polja radio-frekvencije, mobilni telefoni*

