Cytogenetic Monitoring of Personnel Occupationally Exposed to Microwave Radiation of GEM Radar

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Abstract. In the present study we analyzed and followed-up on the DNA damaging effects of microwave radiation of GEM radar equipment within microwave field of 10 μW/cm^2 to 10 mW/cm^2 in personnel occupationally exposed to frequency range of 1.5 GHz to 10.9 GHz. The single cell gel electrophoresis (SCGE)/comet assay as a tool for the biomonitoring of individuals accidentally, environmentally or occupationally exposed to physical or chemical agents was used to evaluate possible genotoxic effect on peripheral human blood lymphocytes. The comet assay is a method that allows efficient determination of single-strand breaks (SSB) and double-strand breaks (DSB), as well as alkali-labile sites in the DNA of single cells. The comet assay was carried out under alkaline conditions. We measured the baseline comet assay effect in whole blood samples. Parameter of the comet assay was studied in workers occupationally exposed to microwave radiation of GEM radar and in corresponding unexposed control subjects. It was found that in the subjects who were occupationally exposed to microwave radiation, the levels of DNA damage increased compare to control group and showed interindividual variations. As a measure of DNA damage tail length was used, calculated from the centre of the head and presented in micrometers (μm). Mean value of exposed group was 13.54±1.44 as opposed to control mean value that was 13.15±1.39. Differences between mean tail lengths were statistically significant (P<0.05, ANOVA). The results of this study indicate that individuals occupationally exposed to microwave frequency of GEM radar equipment may experience an increased genotoxic risk, emphasizing the importance of individual biomonitoring, limiting exposure and radiation safety programs.

KEYWORDS: GEM radar; microwave radiation; DNA damage; peripheral human blood lymphocytes; comet assay.

1. Introduction

During last few decades of health-related electromagnetic fields (EMF) research a large number of studies has been accumulated in this area. [1-2] The nature of basic interactions depending on the field frequency between electromagnetic fields and cells, tissues and organisms is now well understood. Nevertheless, there is still an open controversy in science and in the public on the health impact of high voltage power lines, cordless phones, mobile phones and their base stations, wireless local area networks and Bluetooth. In recent years, microwave (MW) radiation has attracted a great deal of attention due to its increased usage in professional environment, witch lead to a large number of publications regarding health hazards of microwave radiation. Number of studies constantly disagrees and yield contradictory results about real effect of microwave radiation on human health and especially on genetic material of the cell. Due to the growing application of cellular phones the proportion of population exposed to this type of radiation is increasing and if we add up proportion of occupationally exposed personnel to this number there is necessary need to study possible health risk of this type of radiation. [3]

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Cytogenetic studies of microwave radiation were conducted *in vitro* as well as *in vivo* and yield contradictory and often intriguing results. [4-7] Some of the published reports suggested that exposure of human cells and animals to radiofrequency radiation do not result in increased cyto/genetic damage. [8-12] In another hand there is a range of studies with positive results stating that radiofrequency radiation can indeed induce genetic alternation after exposure to electric field. [13-17]

Due to ever increasing usage of microwave radiation over the past few decades and because the data about microwaves often yield contradictory results, the aim of this study was assessment of DNA damage induced by microwave radiation of GEM radar equipment within microwave field of 10 $\mu W/cm^2$ to 10 mW/cm^2 in personnel occupationally exposed to frequency range of 1.5 GHz to 10.9 GHz. For that purpose the single cell gel electrophoresis (SCGE)/comet assay as a tool for the biomonitoring of individuals accidentally, environmentally or occupationally exposed to physical or chemical agents was used to evaluate possible genotoxic effect on peripheral human blood lymphocytes.

2. Materials and Methods

2.1 Subjects Characteristics

The study population of workers comprised of 14 blood donors (1 female and 13 male; average age, 43.5 years, age range, 35-57 years) and the same number of corresponding control subjects. The present study was performed in accordance with high standards of ethics. Blood donors were informed about the aim and the experimental details of the study. All of them gave their informed consent and volunteered to donate blood for sampling. They were healthy at the moment of blood sampling and interviews. Every volunteer completed a standardized questionnaire, designed to obtain relevant details about the current health status, history and lifestyle. No subject reported medicinal treatment over 6 months before blood sampling. None of them had a history of occupational exposure to known genotoxic chemicals.

2.2 Blood Sampling

Peripheral blood samples, taken from a workers occupationally exposed to microwave radiation of GEM radar were collected in heparinised tubes (BD vacutainer, Becton-Dickinson, NJ, USA) under sterile conditions by venipuncture. After collection, all blood samples were randomly coded, cooled to 4 °C, transported to the laboratory and processed as quickly as possible.

2.3 The Alkaline Comet Assay

The comet assay was carried out under alkaline conditions, basically as described by Singh et al. [18] Fully-frosted microscopic slides were prepared. Each slide was covered with 1% normal melting point (NMP) agarose (Sigma). After solidification, the gel was scraped off the slide. The slides were then coated with 0.6% NMP agarose. When this layer had solidified a second layer containing a whole blood sample (4 μL) mixed with 0.5% low melting point (LMP) agarose (Sigma) was placed on the slides. After 10 min of solidification on ice, slides were covered with 0.5% LMP agarose. Afterwards the slides were immersed for at least 1 h in ice-cold freshly prepared lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris/HCl, 1% Na-sarcosinate (Sigma), pH 10, with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Kemika) added fresh to lyse cells and allow DNA unfolding. The slides were then randomly placed side by side in a horizontal gel-electrophoresis tank. The unit was filled with freshly prepared electrophoretic buffer (300 mM NaOH, 1 mM Na_2EDTA, pH 13.0) and the slides were set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali labile sites. Denaturation and electrophoresis were performed at 4°C under dim light. Electrophoresis was carried out for the next 20 min at 25 V (300 mA). After electrophoresis the slides were washed gently three times at 5-min intervals with a neutralization buffer (0.4 M Tris/HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 μg/mL) and covered

with a coverslip. Slides were stored at 4°C in humidified sealed containers until analysis. To prevent additional DNA damage, handling of blood samples and all steps included in the preparation of slides for the comet analysis were conducted under yellow light or in the dark. Furthermore, to avoid possible position effects during electrophoresis, each replicate was processed in a different electrophoretic run.

Slides were examined at 250× magnification with a fluorescence microscope (Zeiss, Germany), equipped with an excitation filter of 515-560 nm and barrier filter of 590 nm. A total of 100 comets per culture were scored. Comets were randomly captured at a constant depth of gel, avoiding the edges of the gel, occasional dead cells and superimposed comets. The microscope was connected to a black and white camera with a computer-based image analysis system (Comet Assay II, Perceptive Instruments Ltd., UK). This image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components, and then evaluates the range of derived parameters. As a measure of DNA damage in this study tail length was used. Tail length was calculated from the centre of the head and presented in micrometers.

2.4 Statistical Analyses

Each experimental set contained duplicated slides .The various parameters (tail length, tail intensity and tail moment) measured in the exposed and control groups were evaluated using Statistica 5.0 package (StaSoft, Tulsa, USA). Each sample was characterized for the extent of DNA damage by considering the mean ± SD (standard deviation of the mean), median and range of the comet parameters. In order to normalize distribution and to equalize the variances, a logarithmic transformation of data was applied. Multiple comparisons between groups were done by means of ANOVA on log-transformed data. Post-hoc analysis of differences was done by Scheffé test. The level of statistical significance was set at $P<0.05$.

3. Results

Mean values for the tail length of the comet assay for exposed and control group are summarized in the Table 1. Figure 1 is showing distribution of tail lengths measured in peripheral blood lymphocytes of the group occupationally exposed to microwave radiation and the corresponding control group.

Mean values (± standard deviation of the mean) of comet lengths were 13.15±1.39 μm for the control group and 13.54±1.44 μm for the group occupationally exposed to microwave radiation. Differences between mean tail lengths were statistically significant ($P<0.05$, ANOVA). The range of tail lengths in the exposed group was 10.90-21.79 μm and 9.62-17.95 μm in the corresponding control group.

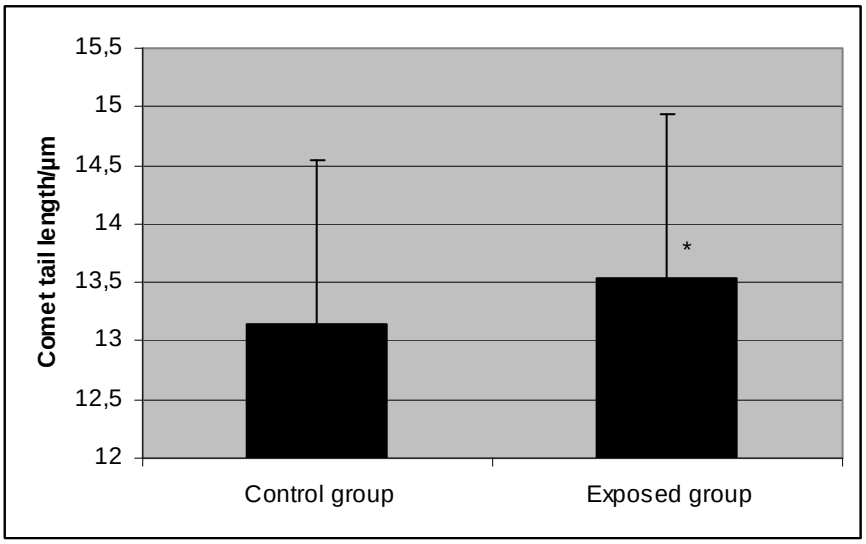
In general, exposure to microwaves of GEM radar caused primary DNA damage, as opposed to control group lymphocytes.

Table 1: Results for the mean values of tail length parameter of comet assay in peripheral blood samples of workers occupationally exposed to microwave radiation of GEM radar and corresponding control group.

Group	Tail length/μm			
	Min	Mean±SD	Max	Median
Exposed group	10.90	13.54±1.44*	21.79	13.50
Control group	9.26	13.15±1.39	17.95	13.09

*statistically significant in compare to the corresponding control ($P<0.05$, ANOVA)

Figure 1: Mean comet tail length in peripheral blood samples of workers occupationally exposed to microwave radiation of GEM radar and corresponding control group.



*statistically significant in compare to the corresponding control (P<0.05, ANOVA)

4. Discussion

In our study the comet assay was used as the sensitive method in detection of DNA damage after exposure to radioactive source. Over the past decade comet assay has become one of the standard methods for assessing genome damage with variety applications in genotoxicity testing as well as fundamental research in DNA damage and repair. [19-20] The comet assay permits the detection of primary DNA damage and the study of repair kinetics at the level of single cells. [21-22] There is a variety of possible modifications of the assay, facilitating the detection of single-strand DNA breaks, alkali-labile sites, double-strand DNA breaks, incomplete excision repair sites and interstrand crosslinks. The comet assay can also be used to assess DNA fragmentation associated with cell death or related to apoptosis. [23-24] Based on the results gained by using the alkaline comet assay it is to presume that microwave radiation had impact in basal DNA damage in peripheral blood lymphocytes of workers occupationally exposed to what was shown in significant DNA migration after microwave exposure. Extent of DNA measured in micrometers in comet tail of the exposed group is pointing to damaging effect of microwaves to the cells DNA structure.

There are number of studies confirming our results gained after measuring DNA alterations caused by GEM radar exposure of professionally exposed workers. [25-28] In one of the previous work done by Garaj-Vrhovac [25], the effects of radiofrequency electromagnetic radiation on the cell kinetics and genome damages in peripheral blood lymphocytes were determined in lymphocytes of subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.

Another study done on workers exposed to microwave radiation showed that microwaves possess some mutagenic characteristics typical of chemical mutagens. In that study the micronucleus assay with a new mathematical approach to separate clastogenic from aneugenic activity was used. The comparisons of frequencies of size distribution of micronuclei in the lymphocytes of humans exposed to microwaves were preferentially clastogens. [26] In addition, Lalić et al. [27] in their study reported a higher frequency of chromatid breaks and acentric and dicentric fragments in peripheral blood lymphocytes of individuals working at radio-relay stations (presumed to be exposed to high levels of RFE) than in controls. In another study, Estecio and Silva [28] reported significantly higher frequencies of anomalous metaphases in blood lymphocytes from persons presumably exposed to radio-frequency energy (RFE) from video display monitors.

5. Conclusion

Number of evidence supports the concept that DNA is the crucial target of most carcinogens and those mutations in tumor suppressor genes and oncogenes may lead to cancers. As a result, individuals with increased DNA damage and defective DNA repair are at a higher risk for development of cancer. [29-30] In this context, the application of this assay could also bee extended to the possible diagnostic use in cancer prediction [31-32] especially for those who are occupationally exposed to microwave radiation.

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REFERENCES

[1] BRECKENKAMP, J., BERG, G., BLETTNER, M., Biological Effects on Human Health due to Radiofrequency/Microwave Exposure: a Synopsis of Cohort Studies. Radiat. Environ. Biophys. 42(3) 141 (2003).

[2] JAUCHEM, J.R., Effects of Low-level Radio-frequency (3kHz to 300GHz) Energy on Human Cardiovascular, Reproductive, Immune, and Other Systems: a review of the recent literature. Int. J. Hyg. Environ. Health. 211(1-2) 1 (2008).

[3] OTTO, M., VON MÜHLENDAHL, K.E., Electromagnetic Fields (EMF): Do They Play a Role in Children's Environmental Health (CEH)? Int. J. Hyg. Environ. Health. 210(5) 635 (2007).

[4] BRUSICK, D., et al., Genotoxicity of Radiofrequency Radiation. Environ. Mol. Mutagen. 32 1 (1998).

[5] VERSCHAEVE, L., MAES, A., Genetic, Carcinogenic and Teratogenic Effects of Radiofrequency Fields. Mutat. Res. 410 141 (1998).

[6] VIJAYALAXMI, OBE, G., Controversial Cytogenetic Observations in Mammalian Somatic Cells Exposed to Radiofrequency radiation. Radiat. Res. 162 481 (2004).

[7] VERSCHAEVE, L., Genetic Effects of Radiofrequency Radiation (RFR). Toxicol. Appl. Pharmacol. 207(2) 336 (2005).

[8] MAES, A., et al., Cytogenetic Effects of Microwaves From Mobile Communication Frequencies (954 MHz). Electro-Magnetobiol. 14 91 (1995).

[9] MALYAPA, R.S., Measurement of DNA Damage After Exposure to 2450 MHz Electromagnetic Radiation. Radiat. Res. 148 608 (1997).

[10] GARSON, O.M., et al., A Chromosomal Study of Workers with Long-term Exposure to Radio-frequency Radiation. Med. J. Aust. 155 289 (1999).

[11] LI, L., et al., Measurement of DNA Damage in Mammalian Cells Exposed In Vitro to Radiofrequency Fields at SARs of 3–5 W/kg. Radiat. Res. 156 328 (2001).

[12] McNAMEE, J.P., et al., No Evidence for Genotoxic Effects from 24 h Exposure of Human Leukocytes to 1.9 GHz Radiofrequency Fields. Radiat. Res. 159 693 (2003).

[13] GARAJ-VRHOVAC, V., FUČIĆ, A., HORVAT, D., Comparison of Chromosome Aberration and Micronucleus Induction in Human Lymphocytes After Occupational Exposure to Vinyl Chloride Monomer and Microwave Radiation. Period. Biol. 92 411 (1990).

[14] GARAJ-VRHOVAC, V., HORVAT, D., KOREN, Z., The Relationship Between Colony-forming Ability, Chromosome Aberrations and Incidence of Micronuclei in V79 Chinese Hamster Cells Exposed to Microwave Radiation. Mutat. Res. 263 143 (1991).

[15] MAES, A., COLLIER, M., VERSCHAEVE, L., Cytogenetic Investigations on Microwaves Emitted by a 455.7 MHz Car Phone. Folia. Biol. 46 175 (2000).

[16] TICE, R.R., et al., Genotoxicity of radiofrequency signals: I. Investigation of DNA Damage and Micronuclei Induction in Cultured Human Blood Cells. Bioelectromagnetics**.** 23 113 (2002).

[17] PAULRAJ, R., BEHARI, J., Single Strand DNA Breaks in Rat Brain Cells Exposed to Microwave Radiation. Mutat. Res. 596(1-2) 76 (2006).

[18] SINGH, N.P., et al., A Simple Technique for Quantitation of Low Levels of DNA Damage in Individual Cells. Exp. Cell. Res. 175 184 (1988).
[19] COLLINS, A.R., The Comet Assay for DNA Damage and Repair: Principles, Applications, and Limitations. Mol. Biotechnol., 26(3) 249 (2004).
[20] KUMARAVEL, T.S., et al., Comet Assay Measurements: A Perspective. Cell Biol Toxicol. (2008) [Electronic publication ahead of print]
[21] OLIVE, P.L., DNA Damage and Repair in Individual Cells: Applications of The Comet Assay in Radiobiology. Int. J. Radiat. Biol. 75 395 (1999).
[22] PIPERAKIS, S.M., VISVARDIS, E.E., TASSIOU, A.M., Comet Assay for Nuclear DNA Damage. Methods Enzymol. 300 184 (1999).
[23] OLIVE, P., BANATH, J.P., DURAND, R.E., Heterogeneity in Radiation-induced DNA Damage and Repair in Tumor and Normal Cells Using The Comet Assay. Radiat. Res. 122 86 (1990).
[24] HELLMAN, B., VAGHEF, H., BOSTRÖM, B., The Concepts of Tail Moment and Tail Inertia in The Single Cell Gel Electrophoresis Assay. Mutat. Res. 336 123 (1995).
[25] FUČIĆ, A., et al., X-rays, Microwaves and Vinyl Chloride Monomer: Their Clastogenic and Aneugenic Activity, Using the Micronucleus Assay on Human Lymphocytes. Mutat. Res. 282 265 (1992).
[26] GARAJ-VRHOVAC, V., Micronucleus Assay and Lymphocyte Mitotic Activity in Risk Assessment of Occupational Exposure to Microwave Radiation. Chemosphere. 39(13) 2301 (1999).
[27] LALIĆ, H., LEKIĆ, A., RADOŠEVIĆ-STAŠIĆ, B., Comparison of Chromosome Aberrations in Peripheral Blood Lymphocytes from People Occupationally Exposed to Ionizing and Radiofrequency Radiation, Acta. Med.. Okayama. 55 117 (2001).
[28] ESTECIO, M.R., SILVA, A.E., Chromosome Abnormalities Caused by Computer Video Display Monitors' Radiation, Rev. Saude. Publ. 36 330 (2002).
[29] SASAKI, Y.F., et al., The Comet Assay With Multiple Mouse Organs: Comparison of Comet Assay Results and Carcinogenicity With 208 Chemicals Selected from The IARC Monographs and U.S. NTP Carcinogenicity Database. Crit. Rev. Toxicol. 6 629 (2000).
[30] WANG, A.S., et al., Comet Assay with Nuclear Extract Incubation. Anal. Biochem. 337(1) 70 (2005).
[31] KUMARAVEL, T.S., BRISTOW, R.G., Detection of Genetic Instability at HER-2/neu and p53 Loci in Breast Cancer Cells Using Comet-FISH. Breast. Cancer. Res. Treat. 91(1) 89 (2005).
[32] WATERS, D.J., et al., Noninvasive Prediction of Prostatic DNA Damage by Oxidative Stress Challenge of Peripheral Blood Lymphocytes. Cancer. Epidemiol. Biomarkers. Prev. 16(9) 1906 (2007).