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Does radio frequency radiation induce micronuclei frequency in exfoliated bladder cells of diabetic rats?

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Objective. For many years there has been a discussion among both experts and the general public regarding the effects of radio frequency (RF) radiation on the human organism. The purpose of the present study was to evaluate the relationship of micronucleui (MN) frequency and RF radiation in exfoliated bladder cells of non-diabetic and diabetic rats.

Methods. Three groups were used in the experiment: Group I (n=6): diabetic group without RF exposure; Group II (n=6): diabetic group exposed 2100 MHz RF radiation and Group III (n=6): control animals (non-diabetic group, no RF exposure). RF exposure in the experiment resulted in a whole body average SAR of 0.24 W/kg with an ERMS field of 17.5 V/m in non-thermal levels.

Result. Results showed that there was no statistically important differences between non-RF exposed diabetes group and control group; Group I and Group III ($p>0.05$). There was no statistically important differences between diabetes group and diabetes+RF exposed group (Group I and Group II) ($p>0.05$). RF exposure did not result in increased MN frequencies in exfoliated bladder cells of diabetic rats with respect to control animals (Group II and Group III), either and this result found no statistically important ($p>0.05$).

Conclusions. This study suggested no possible genotoxic effects of RF radiation among human beings especially with chronic disorders, such as diabetes.

Key words: micronuclei (MN), genotoxicity, diabetes, radio frequency (RF) radiation

For many years, there is a discussion between both the experts and the general public regarding the impact of radio frequency (RF) radiation on the human organism. In general population, normal and sick people are exposed to RF radiation due to increasing demand of mobile phones and related base stations and on the other hand, diabetes is very common illness in modern life. Diabetes is a serious and growing health care problem worldwide associated with severe acute and chronic complications. Excessive production of oxygen-free radicals through glucose auto-oxidation and non-enzymatic glycation, especially in diabetic patients with poor glycemic control, can accelerate the oxidative damage to the macromolecules, including DNA damage. Type 1 insulin-dependent results from an absolute deficiency of insulin caused by the destruction of insulin-secreting pancreatic *b*-cells. Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and relative insulin deficiency. In both type 1 and 2, chronic hyperglycemia is the primary cause of the clinical complications of the disease (Andreassi et al. 2011). Diabetic complications in target organs arise from the chronic elevations of

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glucose via increased production of reactive oxygen species (ROS) and reactive nitrogen species and subsequent oxidative stress. Excessive production of oxygen-free radicals through glucose auto-oxidation and non-enzymatic glycation, especially in diabetic patients with poor glycemic control, can accelerate oxidative damage to the macromolecules, including DNA damage (Andreassi et al. 2011). Streptozotocin (STZ) has drawn attention as a potential source of oxidative stress, which induces genotoxicity. A single intraperitoneal (i.p.) injection of STZ (150 mg/kg) may cause DNA damage in the liver and kidney, which recovers slowly with time (Imaeda et al. 2002). STZ has broad spectrum antibiotic activities and antineoplastic properties and is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic beta cells. STZ is a potent alkylating agent known to directly methylate DNA and is highly genotoxic, producing DNA strand breaks, alkali-labile sites, unscheduled DNA synthesis, DNA adducts, chromosomal aberrations, micronuclei, sister chromatid exchanges, and cell death (Bolzan and Bianchi 2002). In recent years, many of us have grown increasingly aware of the possible dangers posed by RF radiation. As electrical and wireless applications continue to become more ubiquitous in society, so human beings exposure to RF radiation continues to climb. Although low levels of natural electromagnetic radiation have existed throughout the history in our world, the current degree of RF exposure experienced is unprecedented in the history of the human race. Whether from cell phones, computers, or wiring in the home, electromagnetic fields (EMFs) are all around us, and they may significantly contribute to the development of many of today's diseases and a notable example is diabetes. Havas (2008) has studied interesting case of involving of Type 1 and Type 2 diabetics. The goal of the study was to compare and evaluate the plasma glucose levels and insulin needs in both the inside and outside of EMF environments. In all cases, exposure to "dirty electricity" (EMFs or RF radiation) caused substantial increases in blood sugar and/or an increased need for insulin. EMF emitting devices or environments produced negative metabolic changes that were evident within minutes, yet began to resolve just as quickly once the individual entered an electromagnetically clean environment. It is not completely clear how these effects are mediated. It is possible that the stimulation of stress proteins by EMFs could account for the increases in glucose, though there are likely multiple ways in which EMFs undermine our health that currently remain unexplored (Havas 2008).

In 2014, our group has studied the frequency of MN in non-diabetic animals (Gurbuz et al. 2014). In that study, we have found no effect of 1800 and 2100 MHz RF radiation on the number of MN in exfoliated bladder cells of rat. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups ($p>0.05$). In our other study with 1800 MHz RF radiation, exposure period was changed as 20 min/day, 5 days/week during a month and again we did not find any changes in micronucleus frequency in rat bladder cells with respect to the control group ($p>0.05$) (Gurbuz et al. 2010). Diabetes may also be a risk factor for bladder cancer, but findings from epidemiological studies are inconsistent. Zhu et al. (2013) findings support the hypothesis that men with diabetes have a modest risk for the bladder cancer developing. Up to date, there is no study available investigating MN frequency in bladder exfoliated epithelial cells of diabetic rats under the exposure of RF radiation; therefore, the objective of this study was to evaluate MN frequency in exfoliated bladder cells of diabetic rats under the exposure of 2100 MHz RF radiation.

Material and methods

Animals. Eighteen adult male Wistar albino rats with body weights between 220-270 g were used in the experiment. The rats were randomly separated into 3 equal groups: Group I (n=6): diabetic group with no RF exposure, Group II (n=6): diabetic group exposed 2100 MHz RF radiation, and Group III (n=6): control animals (normal group without RF exposure). All rats were housed in Plexiglas cages in a room with controlled temperature (22°C), humidity (50-55%), and a 12-h light-dark. All activities within the scope of the study were performed with the approval of Gazi University Experimental Animal Ethics Committee under the supervision of a veterinarian and in compliance with the provisions of the Strasbourg Universal Declaration of Animal Rights of 1986. The rats were fed by laboratory pellet chow and water was given *ad libitum*. The experiment was performed after a stabilization period in the laboratory for several days. None of the animals died during the experiment. RF animals were exposed to 2100 MHz RF radiation for 30 min/day, 5 days per week for one month. Rats of the control group were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation.

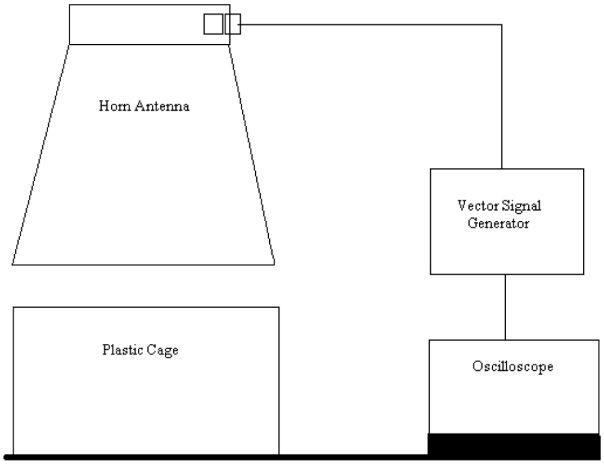

Fig. 1. Radio Frequency exposure system.

Diabetes was induced by a single i.p. injection of STZ (Sigma-Aldrich, St. Louis, MO) at the dose of 65 mg/kg body weight (b.w.) in 0.1 M citrate buffer (pH 4.5). Seventy-two h after the injection, blood glucose was measured using a One Touch Ultra Glucose meter (Life Scan Inc. USA) and rats with blood glucose levels over 250 mg/dl were considered diabetic. Diabetic RF group exposed RF radiation after diabetic situation started. One month after exposure, the animals were anesthetized with ketamine (45 mg/kg) and xylazine (5 mg/kg) by intramuscular (i.m.) injection prior to the capitation.

Exposure system. The vector signal generator (Rohde & Schwartz, SMBV100A, Germany) was used to create a RF radiation in the experimental setup (Fig. 1). Emitted power of the generator was fixed during the whole exposure durations. ETS Lindgren horn antenna (ETS Lindgren, Model 3164-03, USA) was used to emit the RF radiation from the generator. Polymethyl methacrylate plastic cage (15x20x20 cm), which the rats were housed in, was placed symmetrically along the axis which is perpendicular and 5 cm below the mid-line of the horn antenna. The cage was constantly aerated to avoid the possibility of any increase in temperature inside the cage. A cage was placed in the near field of the antenna to obtain sufficient field intensity. Applied RF fields' electric field was measured with EMR 300 (Narda, Germany) via the electric field probe type 8.3. The root mean square value of electric field (E_{RMS}) was found as 17.5 V/m. The RF environmental background level was 0.1-0.21 V/m. Average power density was measured at a reference point which was the mid-point of the bottom of the cage wall facing the horn antenna. The maximum power density was observed along the axis of the antenna and it decreased uniformly with the distance from the antenna's axis.

The fundamental dosimetric quantity of RF radiation is specific absorption rate (SAR), which is the rate of absorption of RF energy in the body, whose unit of measure is watts/kilogram (W/kg). This provides basic restrictions on exposure, specified in terms of whole body and localized SAR, to prevent adverse health effects related to whole-body heat stress and excessive localized tissue heating (Hansson et al. 2011). For practical purposes, SAR indicates absorbed electromagnetic power (W) per kilogram (kg) of tissue. The value of 4 W/kg is accepted worldwide as the threshold for the inducement of biological thermal effects (Bernardi et al. 2003). SAR was calculated using the following equation (Dasdag et al. 2008; Esmekaya et al. 2010): $SAR = \sigma/\rho[E_{RMS}^2][W/kg]$, where E_{RMS} is the root mean square value of the electric field (V/m), σ is the mean electrical conductivity of the tissues in Siemens/meter (S/m) and ρ is the mass density (kg/m^3) (ICNIRP 1998). The rat body was assumed an equivalent tissue based on the average of the dielectric properties of the 36 tissues in the rat segmented at Brook Air Force Base. Conductivity (0.87 S/m) and mass density (1105 kg/m^3) were derived for the equivalent tissue by using dielectric properties and mass densities of these tissues. The RF exposure in the experiment resulted in a whole body average SAR of 0.24 W/kg with an E_{RMS} field of 17.5 V/m.

Body temperature of rats was recorded by rectal measurements prior and after exposure session. RF radiation exposure did not result in any rectal temperature increase.

Exfoliated bladder cell MN analysis and scoring. The rats were killed and the bladders isolated. Exfoliated cells were scraped from the internal walls. The scrapings were smeared on microscope slides and air-dried.

Table1

Micronuclei frequencies in exfoliated bladder cells of three groups

Group	n	MN frequencies (‰) Mean ± S.D.
Diabetes	6	3.00 ± 1.41*#
Diabetes+RF	6	2.33 ± 1.21*
Control	6	1.50 ± 1.04

*p>0.05 compared with control group
#p>0.05 compared with Diabetes + RF group

Slides were stained by the Feulgen reaction (Stich et al. 1982) with modification (Konopacka et al. 1988). Micronuclei were scored in 1000 epithelial cells per animal. Criteria for identifying MN were based on those given by Countryman and Heddle (1976) and (Belien et al. 1995). All the slides were scored by the same observer. Observer did not know the group identity. The rat samples were selected and evaluated randomly, with the same observer. Whole experiment was running under a blind condition.

Statistical analysis. Data were expressed as mean ± standard deviation (SD) for each group. Mann Whitney U Test was used to assess significance and $p<0.05$ was considered to be statistically significant.

Results

Micronuclei frequencies in exfoliated bladder cells of all groups are given in Table 1. There is no statistically important differences in MN frequencies of exfoliated bladder cells of rats in non-exposed diabetic group, with respect to control animals and diabetic RF-exposed groups ($p>0.05$). No statistically important differences were observed in MN frequencies of exfoliated bladder cells of diabetes+RF group with respect to control animals, either ($p>0.05$).

Discussion

Streptozotocin is a potent alkylating agent known to directly methylate DNA and is highly genotoxic, producing micronuclei, other DNA damage, and cell death (Bolzan and Bianchi 2002). STZ has drawn attention as a potential source of oxidative stress, which induces genotoxicity (Imaeda et al. 2002). In present study, STZ was used to create a diabetes model. It has been shown that RF radiation exposure did not increase MN frequency in diabetic animals with respect to control animals. There was no statistically important difference observed between only diabetic groups (without RF exposure) with respect to controls. Imaeda et al. (2002) have shown the effects of STZ on DNA damage in the liver and kidney. STZ induced DNA damage that might contribute to the development of hepatic or renal disease. Shaik et al. (2010) have researched that T2DM patients under long term of treatment with pioglitazone and glimepiride in combination showed increased frequency of MN from exfoliated oral mucosa cells as compared to controls. Shettigar et al. (2012) have shown that the induction of MN due to increased glycosylation in T2DM. They have found that the increased glycosylation seems to induce oxidative damage in the DNA of the diabetic patients, which manifests as an increased in blood culture MN frequency.

Martinez-Perez et al. (2007) have studied that 15 Mexican patients (40-56 years old) with T2DM developing five years being treated with oral hypoglycemic drugs (sulfonylurea and/or metformin), significantly high levels of MN frequency were found in binucleated lymphocytes. Zuniga-Gonzalez et al. (2007) have indicated that diabetes results in elevated frequencies of MN polychromatic erythrocyte and that at least in humans, folic acid can protect against the elevation. Diabetes is associated with a high risk of health complications, mainly due to excessive free radical production that could result in an increased frequency of MN. This cytogenetic damage also indicates an enhanced risk of cancer according to Martinez-Perez et al. (2007). Hanna-Mitchell et al. (2013) have studied that impact of diabetes on bladder uroepithelial cells. Diabetic bladder dysfunction (DBD), a prevalent complication of diabetes, is characterized by a broad spectrum of symptoms including urinary urgency, frequency, and incontinence. Diabetes impacts urothelial homeostasis.

The purpose of the present study was to evaluate the association of MN frequency with diabetes and RF exposure in exfoliated bladder cells of rats. It has been found that there is no statistic difference in MN frequencies in exfoliated bladder cells between the diabetic group and control groups of animals ($p>0.05$). Micronuclei frequencies did not increase in exfoliated bladder cells of rat in RF-exposed diabetes group with respect to control group. There are no statistically important differences in MN frequencies in exfoliated bladder cells between the diabetic and RF-exposed diabetic animals ($p>0.05$).

Radio frequency exposure may not induce MN formation in diabetic animals. However, diabetic causes alone may induce some oxidative damages. Due to the ethic principles of Gazi University, the project included only 18 animals, 6 rats per group. The relationship between MN frequency and RF exposure should be investigated again with high number of animals. So, there is a need to study diabetic cases under the exposure of RF radiation. Diabetic people could be more vulnerable to RF radiation with respect to the normal population.

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