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Research article

INFLUENCE OF HIGH FREQUENCY ELECTROMAGNETIC FIELDS PRODUCED BY ANTENNAS FOR MOBILE COMMUNICATION ON THE STRUCTURE OF THE PANCREAS IN RATS: HISTOLOGICAL AND UNBIASED STEREOLOGICAL ANALYSIS

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The emission of high frequency electromagnetic fields (HF EMF) produced by antennas for mobile communications has been controversially alleged to have adverse health effects. The aim of our work was to examine whether there are effects on living organisms from HF EMF produced by mobile communication antennas. In this experiment Wistar strain rats were exposed to HF EMF with the following characteristics: 1.9 GHz frequency, 0.24 A/m intensity, electric field strength of 4.79 V/m, and SAR (specific absorption rate) value of 2.0 W/m^2. Exposure time was 7 hours per day, 5 days per week, over the course of sixty days. This experiment was conducted on a total of 30 male rats divided randomly into two equal groups: one group of animals was exposed to GSM fields (Global System of antennas for Mobile Communications) as described above whereas the other group of animals was not exposed to any GSM fields. In our study, results show that the quantity, diameter and numerical density of the islets of Langerhans in the pancreatic tissue increased in rats exposed to HF EMF compared to the unexposed group. The volume density, number and numerical density of pancreatic cells also changed in rats that were exposed to the HF EMF compared to the unexposed group. Our study shows a change in the stereological and histological parameters of rat pancreatic tissue due to the effects of HF EM fields produced by antennas for mobile communication.

Key words: HF EMF, islets of Langerhans, connective cells, endothelial cells, exocrine cells

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INTRODUCTION

This study was created in order to check if HF EMF emitted by antennas for mobile communication produced effects on the pancreatic tissue and consequently on the whole organism of the rat. To do so, these fields were applied in an *in vivo* model for a duration of two months. There are many studies which indicate that HF EMF negatively affect many biological systems: cardiovascular [1], nervous [2-4], endocrine [5-7] and reproductive [8]. Also, research has shown that these fields increase the secretion of sex hormones [5], decrease the secretion of TSH hormone [6], affect nerve conduction, cognitive function, and the blood-brain barrier permeability [9], change the concentration of glucose in the blood [10,11], decrease the effectiveness of the immune system response [12,13] and change the speed of cell division [14] and apoptosis [15].

Morphologically, the pancreas of rats is divided into exocrine and endocrine sections. The exocrine part of the pancreas includes acinar and duct cells with connective tissue, blood vessels, and nerves and it comprises more than 95% of the pancreatic mass. The endocrine part of the pancreas consists of isolated islands called islets of Langerhans. The endocrine part of the pancreas produces hormones and secretes them into the blood and comprises 1-2% of the pancreatic mass [16,17]. Based on research done by Han-Hung et al. (2011), the rat pancreas shows differences in size distribution, number and volume of the islets of Langerhans. Histological studies of the pancreas show that, on average there are three times as many larger islets than smaller islets. However, the smaller islets release two times more insulin when stimulated than the larger islets [18].

For our experiment we have applied HF EMF from base stations of mobile telephone networks (GSM network). The emission of HF EMF caused by GSM networks has been controversially alleged to have adverse health effects. However, the effects of artificial electromagnetic fields on biological systems were examined a few decades ago and it was stated that there was no evidence of negative effects [19]. There are many studies related to this issue, mainly to the type of the acting fields, either low or high frequency, as well as the type of exposed tissue [20]. It is important to note that the frequency of the field utilized in this study has low photon energy and is not powerful enough to break atomic bonds [21]. It is also important to note that mobile phones utilize artificial HF EMF [22].

Prior to this study, there were only a few studies and published papers about the effect of HF EMF emitted by antennas for Mobile Communication on the structure of the pancreas in rats. These studies indicate that HF EMF negatively affect the morphological characteristics of the pancreatic tissue. They induce an increase in the number and diameter of the islets of Langerhans, as well as changes in the number of endocrine and exocrine cells.

The subject of the here reported study is important because it is estimated that the number of mobile subscribers is 5 billion people worldwide. Therefore, any possible adverse health effects of HF EMF through the use of mobile phones could affect more than half of the world's population [21]. Thus, this experiment was designed to examine the morphological changes caused by exposure to HF EMF within the endocrine and exocrine cells of the pancreas, in Wistar rats. Both stereological and morphometrical analyses have been used to identify changes caused by HF EMF produced by mobile phone antennas on the structure of the pancreatic tissue.

Bearing in mind the omnipresence of HF EMF in our environment and their different effects on male Wistar strain rats, the hypothesis and the specific aims of this study were: to document changes in the morphology of pancreatic tissue in the number, diameter and percentage distribution of the islets of Langerhans; to analyze changes in the morphology of the islets of Langerhans by using an unbiased, design-based stereological approach and immunohistochemical methods; and to demonstrate changes in the morphology of the endocrine and exocrine cells, as well as changes in the connective tissue and lumen of blood vessels using an unbiased, design-based stereological approach.

MATERIAL AND METHODS

Animals and experimental design

All animal procedures were in compliance with Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee on Animal Experiments at the Faculty of Sciences, University of Banja Luka, No. 01-9-192.2/15, Bosnia and Herzegovina. All surgical procedures were performed under anesthesia, and efforts were made to minimize the suffering of the animals. The experiment was performed on 30, sexually mature, four months old, Wistar strain male rats divided randomly into two groups at the vivarium at the Faculty of Sciences in Banja Luka. One group (Group A) of 15 rats was exposed to HF EM fields (the exposed group) and the other group (Group B) of 15 rats was the control group and was not exposed to any measurable HF EMF. All animals lived in laboratories with constant temperature conditions (22±2 ^{0}C) and were exposed to a natural photoperiod. Both groups had unlimited access to tap water and food pellets. Group A was exposed to HF EMF of GSM, 7 hours a day (from 08:00 to 15:00), 5 days a week, for 60 days. There were three individuals in each Plexiglass cage for laboratory animals. They were exposed to HF EMF GSM in one area of the laboratory in their cages, this exposure was the same over the cage surface, after which they were brought to the same room as the control group. The source of HF EMF was an antenna for Mobile Communication. The control group, Group B, was always kept in a separate area free of any mobile devices or other appliances that generate HF EMF. The strength of the HF electromagnetic fields that were used in our experiment was measured by "PROTECT" Ltd, (Report number 066/09, report date September

14, 2015, report time 11:00, air temperature at the moment of HF EMF intensity measurement was 20^0C). The equipment used to measure the strength of the fields was HF 6080 Rev2 No. 01099, Hyper LOG 6080, manufactured by AARONIA Germany. The instrument frequency and measurement range was 1 MHz – 7 MHz, 10-90 dBm, antenna 680 MHz–6 GHz, manufactured in 2005. The strength of the electromagnetic fields used in our experiment was 1.9 GHz frequency, 0.24 A/m intensity, electric field strength of 4.79 V/m, and specific absorption rate value of 2.0 W/m^2.

Tissue preparation for histological analyses

After sixty days of exposure, the rats were decapitated, all pancreatic tissue was removed and fixed in Bouin solution and processed using a standard procedure for paraffin embedding. Samples were cut in a frontal plane on a Leica rotary Microtome RM 2165, Leica Mycrosystems, Wetzlar, Germany, in 4 μm thick serial sections. For the histological analysis, paraffin slices were stained with hematoxylin-eosin (H&E) (both stains by Merck, Darmstadt, Germany). Histological analyses of the control and exposed pancreatic sections were done using the following methods: Malory Azan and Masson's trichrome staining, in order visualize to blood vessels i.e. endothelial cells, and Victoria blue 8GX-floxin light green method was used in order to visualize the connective tissue i.e. connective cells (all stains by Merck, Darmstadt, Germany).

Morphometrical analyses

The counted parameters were: number, numerical density, diameter and distribution of the islets of Langerhans by size. The number of islets of Langerhans was determined by counting all islets that were present in each cross section of the pancreatic tissue. The numerical density of the islets of Langerhans per unit area (No) was determined by the formula No = N/AT, where N is the number of islets in each section and AT is the area of the section. Conventional morphometrical analysis was used to calculate the islet diameter. By using a calibrated linear scale, the major axes (a) and minor axes (b) of the islets were measured and the mean islet diameter was calculated. The distribution of the islets of Langerhans by size was analyzed on the basis of the percentage frequency of islets of a certain size in relation to the total number of islets per animal.

Stereological analyses

Stereological analyses were performed on every 5th stained section per all 30 animals using a multipurpose stereological grid M42, a Weibel grid [23], under a light microscope at a magnification of x400. We used MBF System software for the stereological counting with P2 grid and Cavalieri's principle. For measuring stereological parameters and imaging of the pancreatic cells we used a Leica 8000D microscope with a MEGA VIEW camera and a digital transfer as well as photo analysis software system. The

numerical density (Nvi) of the islets of Langerhans in the pancreatic tissue was determined based on the following formula:

$$Nvi = 0.63 \times \sqrt{\frac{No^3}{Vv}} \quad (mm^{-3});$$

where: No=number of islets of Langerhans and Vv=volume density of islets of Langerhans [23]. Researchers also measured: volume density of blood capillaries and connective tissue, and exocrine and endocrine cells in the pancreatic tissue. The volume density (Vv) of each rat's pancreas: AC (acinar, exocrine cells), EC (endocrine cells), CC (connective cells) and BV (endothelial cells) was determined based on the following formula [23]: $Vv=Pf/Pt$ (mm^0), Pf= number of hits: AC, EC, CC and BV in each section of the pancreatic tissue. Pt= total number of hits. All sections of pancreatic tissue were used to count the exocrine and endocrine cells, connective cells and endothelial cells of blood vessels. The number of these cells enabled the determination of their numerical densities (Nv). The numerical density, or the number of cells per volume unit, was estimated using a physical dissector design. The nuclei of exocrine, endocrine, connective and endothelial blood vessels' cells were designated as the reference points, and the cells were counted according to standard counting rules: (a) if their nuclei appeared within the unbiased counting frame in the reference section; (b) if they did not touch the border lines of the frame or its extensions, and (c) if they did not appear on the look-up section. The numerical density of exocrine, endocrine, connective and endothelial blood vessels' cells (Nv) is the number of counted cells (Q) contained in an analyzed tissue volume (Vo). The volume of the analyzed tissue is estimated as the product of the number of counting frames (ΣPi), the area of the counting frame ($a=2500^2$) and the dissector height (h), which was equal to section thickness (3 μm). These relations are presented in the following formula:

$$Nv = \frac{Q}{Vo} = \frac{Q}{\Sigma_{i=1}^{n} Pi \times a \times b} \quad (mm^{-3}) \qquad [24].$$

Immunohistochemistry analyses

Also pancreatic cross sections were colored using immunohistochemical methods, DAKO $LSAB^+$/HRP technique in order to visualize the endocrine pancreatic cells: alpha and beta and determine their location in the islets of Langerhans. The principle of $LSAB^+$/HRP is as follows: after blocking endogenous peroxidase activity, pancreatic tissue sections are incubated with the appropriate primary antibody followed by incubation with biotin antibody binding. Polyclonal rabbit anti-human antibody raised against hormones glucagon and insulin were used for the immunohistochemical staining of pancreatic tissue. Streptavidin was marked with peroxidase, using the streptavidin-of a homogenizd substrate: H_2O_2 and DAB/3-amino-9-acyl-Korbazol, AEC (Dako Cytomation, catalog No: AO566, AO564, AO619 and AO115).

Negative control staining slides were incubated in the absence of primary antibodies. Immunohistochemical methods enabled us to check the change in the percentage distribution of beta and alpha cells in the islets of Langerhans in the pancreatic tissue of animals exposed to HF EMF compared to the unexposed. Alpha and beta cells was counted on each islet of Langerhans and afterwards their percentage representation was counted in relation to the total number of cells in the whole islet.

Statistical analysis

All results were expressed as means for fifteen animals per group ± standard deviation (SD). The data were tested for normality of distribution by the Fisher's test. One-way analysis of variance followed by the Wilcoxon sign-rank tests were used in order to compare differences between the groups. A probability value of 5% or less was considered as statistically significant. The data were statistically analyzed by ANOVA, SSPS 2010. All the parameters were expressed as means and in intervals of high and low values.

RESULTS

Histological and Morphometrical analysis of the islets of Langerhans

Changes in structure of pancreatic tissue in rats after being exposed to HF EMF (fields of 1.9 GHz frequency, 0.24 A/m intensity, electric field strength of 4.79 V/m, and SAR value of 2.0 W/m^2) were determined by histological and morphometrical analysis. The main histological and morphometrical analysis of the islets of Langerhans included measurements of the following parameters: number, numerical density, diameter and distribution of islets of Langerhans in relation to their size (Fig. 1 and Fig. 2).

All analyzed islets of Langerhans of pancreatic tissue in the control group show a characteristically round, biconvex or oval shape (Fig. 2 a, c, e and g, pointing arrows). However, in the exposed group a significant number of islets of Langerhans show uncharacteristic bean-shaped, polygonal or three-cornered shapes (Fig. 2 b, d, f and h, pointing arrows).

The number of islets of Langerhans in the pancreatic tissue of the exposed group of animals (No=10.09±1.741) increased by 12% compared to the control group (No=8.82±1.203) (Fig. 1A; Fig. 2). This increase in the number of islets of Langerhans, however was not statistically significant ($p=0.267$ for trend $p<0.05$).

The numerical density of the islets of Langerhans in the pancreatic tissue was 16.6% greater in the exposed group of animals (0.7942±0.1014 mm^{-3}) compared to the control group (0.6621±0.0926 mm^{-3}) (Fig. 1B). This increase was statistically significant ($p=0.021$ for trend $p<0.05$).

In addition, the mean diameter value of the islets of Langerhans in the exposed group of animals was 192.016±71.693 μm compared to the control group mean diameter

value of 124.791±49.032 μm. This increase of 35% in the exposed group compared to the control group (Fig 1C; Fig. 2 b, d, f, h) was statistically significant (p=0.0081 for trend p<0.05).

All analyses of the distribution of the islets of Langerhans in the pancreatic tissue, according to their diameter size, show that results varied between the two groups (Fig. 1D). Pancreatic tissue from the control group of rats had a distribution of islets of Langerhans with a diameter range from 40 μm to 380 μm. The mean diameter within this range was 125 μm; that is 31% of the analyzed islets had this diameter. Large islets of Langerhans in the control group, as defined by having a diameter of 300 μm make up 12% of the analyzed islets. On the other hand, the exposed group of rats had a distribution of islets of Langerhans with a diameter range of 40 μm to 560 μm. Within this range, 25% of the islets of Langerhans had a diameter of 125 μm. The islets of Langerhans with a diameter of 300 μm make up over 23% (Fig. 1D). 11% of the control group had a diameter in the range from 300 to 380 μm but 31% in the exposed group had a diameter from 300 to 380 μm. A notable finding is that the pancreas of animals exposed to HF EM fields contain islets of Langerhans with a diameter of

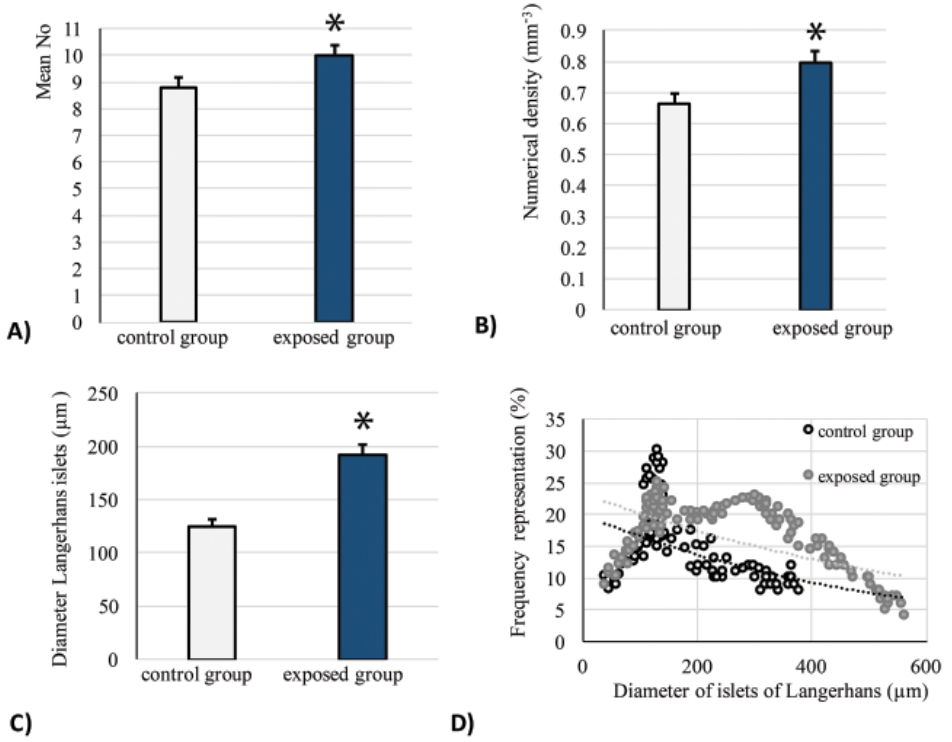

Figure 1. Charts which show stereological parameters of islets of Langerhans in pancreas of control and exposed rats. **A**-number of islets of Langerhans (No); **B**-numerical density of islets of Langerhans (mm^{-3}); **C**-diameter islets of Langerhans (μm); **D**-distribution islets of Langerhans by size (%). All values are provided as the mean±SD; n = 15, p<0.05. (*statistical significant difference).

381 μm to 560 μm, which did not occur in the endocrine tissue of the control group of animals (Fig. 1D; Fig. 2 b, d and f, pointing arrows). Pancreas of animals that were exposed to HF EMF have larger diameters of the islets of Langerhans which did not occur in the control group, and have a higher percentage of islets of Langerhans with larger diameters than in the control group of animals.

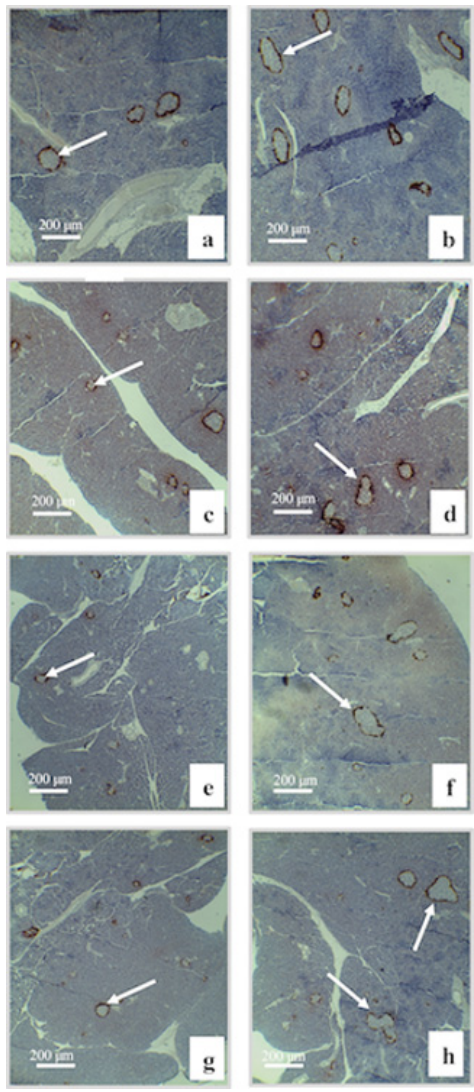

Figure 2. Histological representation of islets of Langerhans in pancreas of rats, control (section **a**, **c**, **e** and **g**) and exposed (section **b**, **d**, **f** and **h**) groups. Immunohistochemically staining endocrine cells (DAKO LSAB$^+$/HRP technique) in islets of Langerhans, were magnified at x20. These micrographs clearly show changes in the shape and increase number of islets of Langerhans in pancreatic tissue in the exposed group (section b, d, f and h) when compared with the control group (section a, c, e and g). Rats' pancreatic tissue shows an increase in the diameter of islets of Langerhans (pointing arrows) in the exposed group (section b, d, f and h) in comparison with control group (section a, c, e and g).

Stereological analysis of pancreatic cells

Changes in stereological parameters of pancreatic tissue in rats after exposure to HF EMF were determined by analysis of pancreatic cells. Basic stereological studies of pancreatic cells included measurements of the following parameters: volume density, number and numerical density of following pancreatic cells: exocrine cells (AC), endocrine cells (EC), connective cells (CC) and endothelial cells of blood vessels (BV) (Fig. 3).

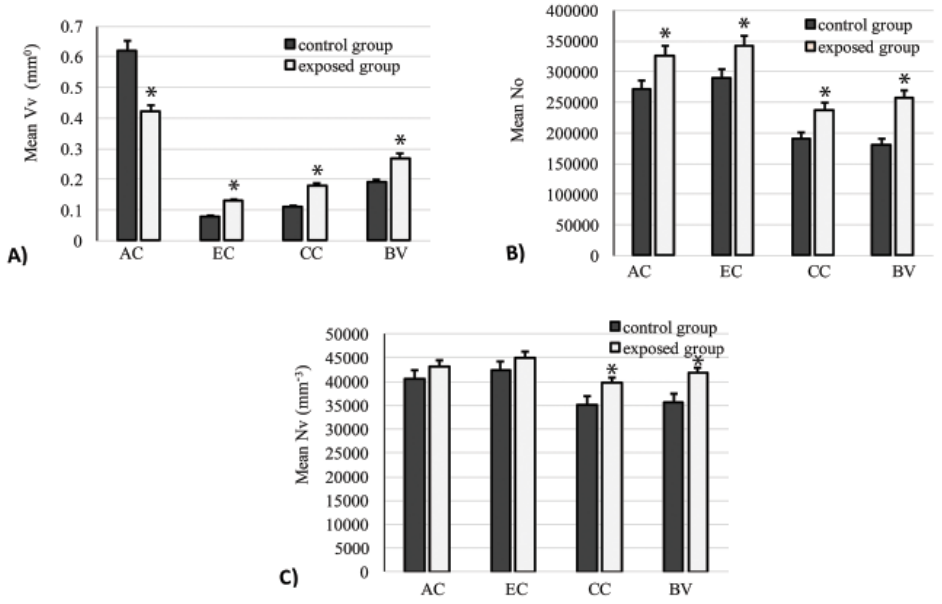

Figure 3. Charts which show stereological parameters of pancreatic cells in control and exposed rats, include: acinar cells (AC), endocrine cells (EC), connective tissue (CC) and blood vessels tissue (BC). A-volume density cell of pancreas (mm^0); B-cell number (No); C-numerical density (mm^{-3}). All values are provided as the mean±SD; n = 15, $p<0.05$. (*statistical significant difference).

Stereological analysis in the exposed group of rats shows an increases in the volume density of examined pancreatic cells: endocrine cells (from 0.08±0.02 mm^0 to 0.13±0.02 mm^0), connective cells (from 0.11±0.02mm^0 to 0.18±0.02 mm^0) (Fig. 4 a and b) and endothelial cells of blood vessels (from 0.19±0.02mm^0 to 0.27±0.03 mm^0) (Fig. 4 c and d) as compared to the control group (Fig. 3A). Only the volume density of exocrine cells in the pancreatic tissue decreased (from 0.62±0.02mm^0 to 0.42 ±0.02 mm^0) in the exposed group of animals compared to the control group (Fig. 3A). All of the above listed changes were statistically significant (endocrine cells: $p=0.034$ for trend $p<0.05$; exocrine cells: $p=0.016$ for trend $p<0.05$; connective cells: $p=0.027$ for trend $p<0.05$; and endothelial cells of blood vessels: $p=0.021$ for trend $p<0.05$).

Figure 2 shows increased volume density (Fig. 2 b, d, f and h, pointing arrows) in endocrine cells namely islets of Langerhans; Figure 3A shows a lower volume density

in exocrine cells in the pancreatic tissue of the exposed group compared to the control group of rats. Micrograph 4 shows an increased volume density in connective cells (Fig. 4 b, pointing arrows) and endothelial cells of blood vessels (Fig. 4 d, pointing arrows) in the pancreatic tissue of the exposed group compared to the control group.

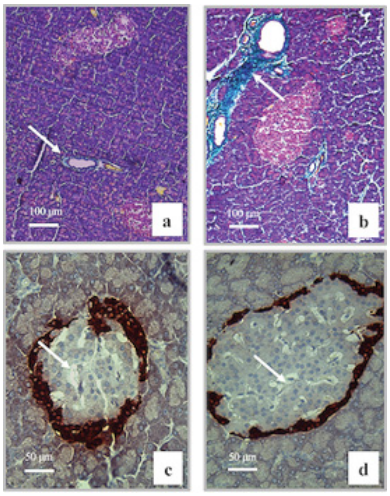

Figure 4. Histological representation of pancreatic cells in pancreas of rats, control (section **a** and **c**) and exposed (section **b** and **d**) groups. Victoria blue 8GX-floxin light green typical for examining connective cells (section a and b) and immunohistochemically staining, DAKO LSAB$^+$/HRP technique, for examining alpha and epithelial blood vessels' cells (section c and d), x40 and x100 magnification for optimal viewing of connective and alpha cells. The micrographs show increases in volume density of connective cells (pointing arrows), epithelial cells of blood vessels (pointing arrows) and alpha cells (brown cells) in the pancreatic tissue of the control group (section a and c) in comparison with exposed group (section b and d).

Another significant stereological parameter in our experiment was the number of cells in the pancreatic tissue in the exposed group as compared to the control group. There was an increase of all type of pancreatic cells in the exposed group (Fig. 3B) compared to the control group: exocrine cells by 16.9% (from No=271219±82419 to No=326415±94475) (Fig. 5 b), endocrine cells by 15.2% (from No=290054±85981 to No=341902±89945) (Fig. 5 d), connective cells by 19.8% (from No=190546±69338 to No=237490±763211) and endothelial cells of blood vessels by 29.3% (from No=181327±61890 to No=256501±75903). All of these increases in the number of all types of pancreatic cells were statistically significant (endocrine cells: $p=0.025$ for trend $p<0.05$; exocrine cells: $p=0.029$ for trend $p<0.05$; connective cells: $p=0.021$ for trend $p<0.05$; and endothelial cells of blood vessels: $p=0.011$ for trend $p<0.05$).

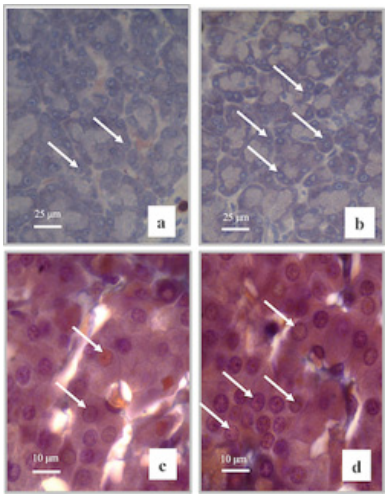

Figure 5. Histological representation of pancreatic tissue of rats in the control (section **a** and **c**) and exposed (section **b** and **d**) groups was conducted by using Immunohistochemical staining, DAKO LSAB$^+$/HRP technique of exocrine cells (section a and b), which were viewed at x200 magnification and Malory-Asan staining of endocrine cells (section c and d), which were viewed at x400 magnification. These micrographs show increases in the number (pointing arrows) of exocrine (section b) and endocrine cells (section d) in the pancreatic tissue of the control group in comparison with exposed group.

Figure 5 shows the increased number of exocrine (Fig. 5 b, pointing arrows) and endocrine (Fig. 5 d, pointing arrows) cells in the pancreatic tissue of the exposed group compared to the control group.

In our work, the numerical density of cells is the stereological parameter used for analyzing pancreatic cells. The value of this parameter increased in the pancreatic tissue of HF EMF exposed rats compared to the control group (Fig. 3C). The mean value of numerical cell density in the exposed group increased in all types of pancreatic cells: exocrine cells by 5.9% (from 40679±7514 No/mm^3 to 43212±8133 No/mm^3), endocrine cells by 6.2% (from 42316±7912 No/mm^3 to 45116±80423 No/mm^3), connective cells by 11.4% (from 35132±7478 No/mm^3 to 39658±7609 No/mm^3) (Fig. 6 b) and endothelial cells of blood vessels by 14.9% (from 35588±7491 No/mm^3 to 41799±7694 No/mm^3) (Fig. 6 d) in animals that were exposed to high-frequency

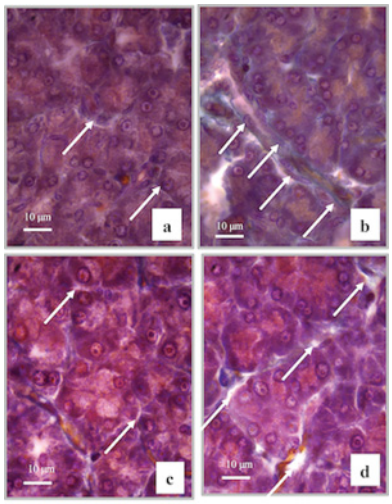

Figure 6. Histological representation of pancreatic tissue of rats, control (section **a** and **c**) and exposed (section **b** and **d**) groups. Malory-Asan staining of pancreatic cells for visualizing connective cells (section a and b) x400 magnification, and Masson staining of pancreatic cells for visualizing epithelial cells of blood vessels (section c and d), x400 magnification. The following micrographs show increases in the numerical density (pointing arrows) of connective cells (section b) and epithelial cells of blood vessels (section d) in the pancreatic tissue of the control group in comparison with exposed group.

EM fields compared to the control group (Fig. 3C). The increase in numerical density value of exocrine cells ($p=0.072$ for trend $p<0.05$) and endocrine cells ($p=0.095$ for trend $p<0.05$) of the pancreatic tissue of the exposed group compared to the control group was not statistically significant. Whereas the increase of the numerical density of connective cells ($p=0.034$ for trend $p<0.05$) and endothelial cells of blood vessel ($p=0.016$ for trend $p<0.05$) in the pancreatic tissue of the exposed group was statistically significant.

Immunohistochemistry analysis of the islets of Langerhans

The mean value of the relative percentage of alpha and beta cells in the islets of Langerhans in the exposed group increased as follows: alpha cells from 14.51±3.71% to 19.83±4.16% (Fig. 4 d) and beta cells from 64.98±9.15% to 70.34±10.11%. The increase in percentage of alpha cells ($p=0.063$ for trend $p<0.05$) and beta cells ($p=0.115$ for trend $p<0.05$) in islets of Langerhans of the exposed group compared to the control group was not statistically significant.

DISCUSSION

This research was undertaken because the effect of HF EMF effect on biological systems, especially the pancreas, has not been sufficiently studied. In our research, changes in pancreatic tissue were based on the determination of the changes in histological parameters of both exocrine and endocrine components of the pancreas and changes in stereological parameters of pancreatic cells. Our results show that rats exposed to HF EMF developed changes in the distribution of the islets of Langerhans according to their diameter size, number of islets of Langerhans per section, and their numerical density in the pancreatic tissue. The number and numerical density of the islets of Langerhans were increased in the exposed group of animals in comparison with the unexposed. The results showed that there is a greater quantity of small islets of Langerhans in unexposed animals and a greater quantity of large islets of Langerhans in the exposed group of animals. In the pancreatic tissue of animals exposed to HF EMF there are islets of Langerhans with diameter from 381 μm to 560 μm, which are not present in the control group.

High-frequency electromagnetic fields cause the islets of Langerhans to grow excessively but secrete less hormones [25]. According to Gholampour et al. (2011), there are mechanisms responsible for the increase of the diameter of the islets of Langerhans, an increase in the number of endocrine cells and a greater accumulation of the amount of hormones in endocrine cells [26]. This is due to the fact that the ratio between the two endocrine cell hormones is important for the endocrine cell proliferation *in vivo* [27].

Pancreatic islets of Langerhans grow in different ways if the rats are treated with corticosteroids [28], are in normal postnatal development [29], pregnant [30], obese or have a hormonal imbalance [31]. Endocrine cells then proliferate, become dominant in order to meet the organism's demands for increased hormone synthesis. Alexandrescu et al. concluded that the islets of Langerhans grow at the cost of reduction of the exocrine part of the pancreas and that growth is influenced by environmental factors. This research confirmed their findings because rats exposed to HF EMF showed a similar increase in size, density and dimension of islets of Langerhans. These authors further note that each islet grows independently from other islets through a combination of processes based on a decreased apoptotic index and endocrine cell chromatin replication [32].

Our results indicate that exposure to HF EMF caused changes in the pancreatic tissue architecture, i.e. volume density, number, and numerical density of exocrine and endocrine cells, connective cells and endothelial cells of blood vessels. In this research, the decrease in volume density of exocrine cells in the exocrine part of the pancreas occurred due to an increase in volume density of endocrine cells. That increase is reflected in increased percentages of alpha and beta cells. Lardon et al. (2004) published similar findings, stating that the decrease in the volume density of exocrine cells happened due to an increase in the volume density of endocrine cells,

because exocrine cells have cell replacement strategies with endocrine cells on the rat pancreatic tissues [33,34]. Other research data has documented the presence of individual endocrine cells in the exocrine part of the pancreas; those cells look like exocrine cells but synthesize hormones that synthesize endocrine cells [32]. After examining, analyzing, and documenting an increase in the number and numerical density of the endocrine, connective cells and endothelial cells of blood vessels in the pancreatic tissue of rats exposed to HF EMF, we concluded that these increases were due to the exposure to HF EMF. The unexposed rats had no such increases. This is probably, because HF EMF increases the mitotic activity of the cells as indicated by Teta et al. [35].

In addition, the reduction of the exocrine part of the pancreas compared to the endocrine part is largely influenced by hormones synthesized and secreted. Namely, endocrine cells synthesize and secrete hormones much faster, are quicker to react to external stimuli, and their hormones also influence the mitosis of new endocrine cells [36]. In addition, in other cases, such as a diseased animal [37], high levels of ethanol in the blood of animal [38] or stress [39] lead to a decrease in volume density exocrine cells, combined with an increase in the number of endocrine cells in the pancreatic rat tissue.

Our research results revealed an increased number of cells, as well as an increased volume density and numerical density of endocrine cells in the pancreatic tissue of animals exposed to HF-EMF compared to the control group. In this research, another parameter for analyzing the effect of HF EMF on pancreatic tissue was also the change in pancreatic connective tissue: volume density, number and numerical density of connective cells. The increase of connective tissue, disturbed collagen fiber architecture due to exposure to HF EMF, was significant, as also noted in the work of Tzaphlidou et al. (2006) [40]. Also, other authors state that HF EMF affect the formation of chondrocytes in the connective tissue, osteosynthesis [41] and the regeneration of connective tissue in rats [42,43].

The pancreatic tissue is densely vascularized owing to the rich capillary net, while the islets of Langerhans are more vascularized than the exocrine part. Endothelial cells are involved in the transport of oxygen, nutrients, angiogenic substances, and growth factor molecules to all pancreatic cells [36]. In this experiment, the treatment of rats with HF-EMF resulted in increased parameters of blood vessels' endothelial cells compared to the control group: volume density, numerical density and capillary cell number. The work of McKai et al. shows that HF-EMF stimulate hormonal angiogenic growth [44], induce vascular endothelial cells growth and affect the vascular endothelial growth factor (VEGF) [45], as well as endothelial cell permeability [46]. Jaroslavet al. (2004) revealed that magnetic fields increase angiogenesis *in vitro* and *in vivo* through releasing a mitogenic growth factor- fibroblast growth factor [47]. This also corresponds with research on HF-EMF published by Chuhua et al. (2013) that indicate that the increase in volume density of blood vessel's cells is a consequence of

adaptation to the increase in size of the islets of Langerhans in the exocrine part of the pancreas, as well as angiogenesis [48,49].

With the rapid increase in the number of mobile phone users, the potential adverse effects of HF EMF radiation emitted by antennas for Mobile Communications have become a serious concern. Fifty years ago, this type of HF EMF did not exist; however, today there is not a single place on our planet that is not covered by the HF EMF signal [50]. The effects of HF EMF on biological systems generally depend on their strength and frequency [51,52]. This work is intended to provide some basic information about several aspects regarding the effect of HF EMF on pancreatic tissue of rats.

CONCLUSION

To conclude, the results of our research show that the exposure of rats to HF EM fields by antennas of Mobile Communication networks causes changes in the morphology of pancreatic tissue as well as increasing the number, numerical density, diameter and percentage distribution of the islets of Langerhans as well as the number, volume density and numerical density of endocrine, connective and blood vessels' endothelial cells of the pancreatic tissue. The results of this research show that these high-frequency fields affect the alpha and beta cells within the islets of Langerhans in the pancreas of rats. Further research is needed on the effects of HF EFM on other biological systems within rats.

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Authors' contributions:

SP, RG, MM and ZR contributed equally to this work. SP and MM contributed to the experimental design. RG and SP contributed in the stereological counting. MM and ZR conducted the part of the experiment with the animals. SP and ZR drafted the manuscript. All authors critically reviewed the content and approved the final version for publication.

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UTICAJ VISOKOFREKVENTNIH ELEKTROMAGNETNIH POLJA KOJA STVARAJU ANTENE MOBILNE KOMUNIKACIJE NA STRUKTURU PANKRESA PACOVA: HISTOLOŠKA I NEPRISTRASNA STEREOLOŠKA ANALIZA

PARAŠ D. Smiljana, GAJANIN B. Radoslav, MANOJLOVIĆ LJ. Maja, RUŽIĆ NJ. Zoran

Kontraverzni su navodi o štetnom zdravstvenom efektu visokofrekventnih elektromagnetnih polja (VF EMP) koja proizvode antene mobilne komunikacije. Cilj našeg rada bio je ispitati da li postoje uticaji VF EMPa proizvedenih od strane antena mobilne komunikacije na žive organizme. U našem eksperimentu pacovi Wistar soja bili su izlagani VF EMP-a sledećih karakteristika: frekvencije 1.9 GHz, intenziteta 0.24 A/m, jačine električnog polja 4.79 V/m i SAR (specifična stopa apsorpcije) vrednosti od 2.0 W/m^2. Pacovi su izlagani VF EMP-a sedam sati dnevno, pet dana u nedelji, ukupno šezdeset dana. Ukupno je za eksperiment korišteno 30 mužjaka pacova Wistar soja podeljenih u dve jednake eksperimentalne grupe: u prvoj grupi pacovi su bili izlagani poljima VF EMP - GMS (Globalni sistem antena mobilne komunikacije) mreže, spomenutih karakteristika, dok u drugoj grupi pacovi nisu bili izlagani GMS poljima. U našoj studiji rezultati pokazuju da se broj, prečnik i numerička gustina Langerhansovih ostrva u tkivu pankreasa povećala kod pacova koji su bili izloženi VF EMPa u poređenju sa grupom koja nije bila izlagana. Volumenska gustina, broj i numerička gustina ćelija pankreasa, takođe se promenila kod pacova koji su bili izlagani VF EMPa u poređenju sa onima koji nisu. Naša studija pokazuje promenu stereoloških i histoloških parametara tkiva pankreasa pacova usled efekata VF EMPa proizvedenih od strane antena mobilne komunikacije.