A Study of the Effects of Electromagnetic Field on Islets of Langerhans and Insulin Release in Rats

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Abstract
Objective: Nowadays, modern technologies are increasingly used in domestic industries, home appliances, and cell phones. This has highlighted the necessity of protecting human beings from the impacts of electromagnetic fields (EMFs) as a new challenge. Therefore, the present study aimed to find out what impacts EMFs have on the structure of Langerhans Islets and the insulin secretion levels in rats.

Materials and Methods: Fourteen 12-week-old male rats were selected randomly: 5 as a control group and 9 as an experimental group. The experimental group was exposed to an EMF produced by an electromagnetic device, with a frequency of 50 Hz and intensity of 3 mT 4 h a day for 6 weeks. At the end of the 6 weeks, blood and pancreas tissue samples were taken for enzyme linked immunosorbent assay (ELISA) test and preparation for microscopic studies.

Results: Results showed that in EMF exposed group insulin level was decreased (P < 0.05), which was associated with decreased area and perimeter of pancreatic islets (P < 0.05).

Conclusion: Exposure to EMF impacts insulin secretion by influencing the size of pancreatic islets.

Keywords: Electromagnetic Field, Langerhans Islets, Insulin, Rats

Introduction
Studies show that electromagnetic fields (EMFs) have a range of complicated effects on the vital molecules (DNA, ionic channels, and other body proteins) and activities of the nervous system and other organs (1). EMFs are classified according to their frequency and long wave. EMF frequency differs due to the sources. Among these sources, we can name: printers, vacuum cleaners, cellular phones, television sets, hair dryers, microwaves, etc. (2). As the use of computer technology grows all over the world, we must anticipate more reports on disorders of normal life due to exposure to EMF (3). Many studies have been conducted by the World Health Organization to assess the effect of electromagnetic waves on human life. This shows that international organizations pay attention to this issue. The studies showed that the waves have a detrimental impact even if they are low intensity (4).

Pancreas is one of the body organs that play a key role in metabolism and adjustment of blood glucose. The insulin secreted by the Langerhans Islets plays many vital roles (5). The effects of insulin on overall body metabolism are as follows: controlling the cellular absorption of special materials particularly glucose; increasing DNA replication and protein synthesis; changing the activity of many enzymes; increasing the glycogen synthesis; increasing the synthesis of fats; increasing the esterification of fatty acids; decreasing the proteolysis; decreasing gluconeogenesis; reducing autophagia; increasing the absorption of amino acids; affecting the muscular tone and relaxation of arteries; enhancing alertness and learning ability (6). The identification of the factors that disturb insulin release is a priority in pathophysiology of diabetes. Diabetes results from a
disorder in carbohydrate metabolism. It is caused by deficiency in the release of insulin and insufficient insulin function or both (7,8). It seems that EMF can affect insulin release and glucose level regulation. Glucose blood concentration is the main stimulus of insulin secretion from Langerhans Islets (6). Fundamental researches are still required to investigate the influence of EMFs on the cell, tissue, and organs. It is reported that ELF-EMFs, lead to decrease in glucose concentration in the serum of healthy volunteers and diabetics (9). However, it has been reported that exposure, to alternating magnetic field, has no effect on serum concentration of insulin and glucose of experimental animals (10). Besides, there is other studies point to suppressing effect of exposure to EMF on insulin secretion with a secondary increase in serum glucose concentration (11). In addition, was presented evidence that long-term exposure to EMF (10 Hz, 8 mT and 50 Hz, 20-50 mT) led to decrease in glucose concentration in the serum of experimental animals (12,13). A hypoglycemic effect of EMF (50 Hz and 3.4 mT) has been confirmed by clinical studies on healthy volunteers and diabetics (14). Glucose has a potent effect on β-cell mass growth causes to both β-cell hyperplasia and hypertrophy (15). The endocrine pancreas is a plastic organ especially because of the high ability of the β-cell mass to change according to the insulin demand (16). This property has been demonstrated in physiological as well pathophysiological conditions such as pregnancy and obesity (17). Since, there are scarce reports in the published works concerning the effect of exposure to EMF on morphometric features of rat pancreatic islets accompanied with insulin release changes, the evidence for effects of EMF on insulin release and structure of islets remains controversial issue yet. Thus, the current study was undertaken to evaluate the influence of exposure to EMF effect on insulin release and structure of pancreatic islets in rats.

Materials and Methods
Fourteen 12-week-old male Wistar rat that weighed 130 ± 10 g were selected from animal house of the Tabriz University of Medical Sciences, Iran. They were kept under standard conditions and had access to food and water. Animals were fed on compact food in the form of granules and water. This food was consisted of all essential ingredients including vitamins and minerals. Environmental conditions (temperature and humidity) in all animal holding areas were continuously monitored. Temperature was maintained in the range of 20°C-30°C, and relative humidity was monitored at 35-60%. The lighting was turned off or on under a 12 h cycle. The rats were divided into two groups after 1 week of adaptation to the new environment: the rats of the control group and experimental group.

The rats of the experimental group were exposed to waves produced by the electromagnetic generator of Anatomy Department at a frequency of 50 Hz and intensity of 3 mT for 4 h a day for 6 weeks. The equipment was based on Helmholtz coil that works following Fleming's right hand rule. It produced and alternate current of 50 Hz frequency. The intensity of EMF could be controlled by a transformer. The equipment had two main parts. In the first, there were two copper coils placed one above the other separated by a distance of 50 cm between the coils (the exposure area) there was a cylindrical wooden vessel the interior of which had a chamber for keeping the cages of experimental animals. The second part was the transformer which checked the input and output voltage with a voltmeter and current with an amperemeter. The equipment was calibrated by gausssmeter. To prevent an increase of temperature inside the chamber a fan was fitted at the top, and the temperature was checked. After the test, the rats were made unconscious using ketamine-xylazine. Later, an incision was made in the chest of each rat and 2 cc of blood was taken from the heart for measuring the serum levels of insulin. The samples of pancreas tissue were placed in Bouin's fixative.

A Swedish-made insulin kit was used to measure the serum levels of insulin. The blood samples were kept under lab conditions between 45 and 60 min until they clotted completely. The serum was then separated using a centrifuge at low speed for 10 min. The serums were kept frozen (−20°C) before the test. The serum insulin of the samples was measured using enzyme linked immunosorbent assay (ELISA) method. At the end, 100 ml of conjugated enzyme 1 × solution was added to the samples which were incubated at room temperature (18-25°C) for 2 h on a shaker at 700-900 RPM. Six stages rinsing was done with 350 ml of wash buffer. After the final rinsing, the plates were turned over the absorbent paper. 200 ml of TBM substrate was added to the samples, and then they were incubated at room temperature for 15 min. Then, 50 ml of stop solution was added to the samples, and they were placed on the shaker to mix the acids well. The reaction was stopped by adding acids. Finally, the result of colorimetry was read by the ELISA system at the wavelength of 450 nm.

The pancreas samples of each rat were fixed in Bouin’s fixative solution for histological purposes. After the tissue samples had been fixed completely, they were dehydrated by rising concentration gradient of ethanol, clarified by xylol and molded in paraffin. Four-micrometer sections were made using microtome. Gomori’s chrome alum hematoxylin phloxine staining was used for staining the pancreas tissue and marking the Langerhans Islets. The samples were studied using optic microscope. 20 slides were selected from nearly 150 slides in each block. The area and perimeter of the islets of both the control and experimental groups were measured using the Motic Image software program.

Statistical analysis
After calculating the mean of the serum levels of insulin, as well as the area and perimeter of
Langerhans Islets of the control and experimental groups, they were analyzed with the SPSS statistical software (version 16.0, SPSS Inc., Chicago, IL, USA) and t-test. P < 0.05 was used as the minimum value of significant difference between the means.

**Results**

This study investigated the effects of EMF on insulin release and morphometric changes in pancreatic islets. Results showed that EMF with 50 Hz and intensity of 3 mT over 6 weeks changed the serum levels of insulin and morphometric features of the islets. According to the results of this study, at the end of the exposure time decrease in blood concentration of insulin was observed in the EMF exposed rats (Table 1). In the morphometric study of the islets of Langerhans, a meaningful decrease in the area was observed in the experimental rats compared to the control group (Table 1). The quantitative parameters for mean perimeter of Langerhans islets showed a significant decrease in perimeter of EMF exposed group (Table 1). A comparison of photomicrographs of islets in control and experimental groups showed a significant decrease in area and perimeter in EMF exposed rats (Figure 1).

**Table 1.** Mean plasma concentration of insulin, islets area and perimeter of control and experimental rats [Mean ± standard deviation (SD)]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Insulin (pmol/l)</th>
<th>Islets area (sqµm)</th>
<th>Islets perimeter (µm)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.8657 ± 0.32</td>
<td>362754 ± 19580</td>
<td>9247 ± 319</td>
</tr>
<tr>
<td>Experimental (EMF exposed)</td>
<td>0.5511 ± 0.15*</td>
<td>114746 ± 91370</td>
<td>4456 ± 192*</td>
</tr>
</tbody>
</table>

P < 0.05 as compared to the control; EMF: Electromagnetic field

**Figure 1.** Photomicrographs from Langerhans Islets, obtained from light microscope. (Gomori’s staining) Control group (A and B), experimental group (C and D). (a) Longitudinal section from control group (×800). (b) Transversal section from control group (×800). (c) Longitudinal section from experimental group (×800). (d) Transversal section from experimental group (×800). The comparison of A with C and B with D show decrease in islets size of experimental group.
Discussion
Since, the main stimulus of insulin secretion is the increase in glucose blood concentration, decreased concentration of glucose circulating in blood can suppress insulin release from islets. Catecholamines may be as important as glucose in the control of insulin secretion. It’s shown that both epinephrine secretion by the adrenal medulla and norepinephrine secretion by sympathetic nerve terminals increase glucose level (18). Previous investigations have shown that EMF stimulation alters catecholamine metabolism. It’s shown that dopamine levels in PC12 cells were significantly reduced within 10-15 min under EMF (19). Besides, is observed decrease in catecholamine levels in the hypothalamus of mice after exposure to EMF from the 1st day of gestation throughout the pregnancy (20). In other studies, no significant influence (21) or increasing effect (22) was seen on glucose level in the blood of experimental animals after exposure to EMF. Also, decrease of plasma glucose levels in male mice exposed to EMF has been observed (23). In agreement to our results, is reported that EMF attenuated insulin secretion (24). Thus, it seems likely that exposure to EMF prevents from increase of glucose level via decreasing catecholamine levels that probably led to decrease of insulin release from islets in EMF exposed animals. Also others found that EMF decreased glucose-stimulated insulin secretion by increase in cellular adenosine 5’-triphosphate, adenosine 5’-diphosphate, membrane depolarization, and cytosolic free calcium ion concentration (25). In contradiction to our results, other studies reported that EMF increased insulin secretion (10-26). It must be added that there are contradictory findings indicating that EMF increase in islet size (27). Furthermore, inconsistent to our results, in other study reported an increase in islet area and perimeter, as well as the absolute mass and relative density of the islets in the pancreas of dexamethasone-received rats (28). There is scarce information concerning the effect of EMF on morphometric changes of Langerhans Islets. Therefore, this possibility is to be considered: Since the pancreatic β-cells are responsible for the maintenance of the glucose levels within a very narrow range, their population undergoes compensatory changes that led to decrease in islets area and perimeter. As it’s postulated that effects of EMF is critically dependent on the duration of exposure and strength of the field (29).

Conclusion
The results of this study showed exposure to EMF led to the decrease of insulin blood concentration accompanied to the reduction of size of the pancreatic islets. It’s concluded that exposure to 50 Hz EMF induces adaptive changes in insulin release and structure of pancreatic islets.

Ethical issues
This research was approved by the Ethics Committee of Tabriz University of Medical Sciences.

Conflict of interests
We declare that we have no conflict of interests.

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References