Original article

The Effects of Cellular Phone Towers Radio Frequency Electromagnetic Radiation (RF EMR) on the Thyroid Gland Viability of Young and Adult Wistar Rat Males

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Abstract

The aim of the present study was to evaluate the security of living nearby cell phone towers, which emit Radio Frequency Electromagnetic Radiation (RF EMR). Two experiments were used to monitor that radiation affection *in vivo,* to study the alteration of TSH and thyroid hormones (T4, T3) in the articulture structures well as histopathological changes of thyroid gland in young and adult males Wistar rats for long term exposure. 28 days old male rats of weight ranging between 35 - 54.2 g. were used. Rats were exposed to radio frequency (RF) electromagnetic radiation emitted by cell tower base station in Khartoum State. The rats were divided into 4 groups. Control animals were kept without exposure to any RF EMR. The rats were exposed for 8 weeks to RF EMR (57nw/m^2), (37nw/m $^{2)}$ and (10nw/m^2). The exposure to radiation was on daily basis for 10 hours at temperature 30 –33ºC. The experiment was repeated using adult male rats with 101.8 to 127.2 g BW (body weights) for 4 weeks. Thyroid functions test (TSH, T4, T3) were estimated. Thyroid glands were removed for histological changes. Mean serum levels of TSH in all young exposed to radiation showed significance increase levels (P<0.05=0.004). No significant difference in TSH level was detected between control adult and adult rats exposed to RF EMR (P>0.05=0.099). Significant decrease of levels of T4 were recorded in young male rats P=0.000. No significant difference in the levels of T4 of groups of adult male and control (P=0.834). However, the levels of T3 recorded a significant increase in adult-radiated only (P>0.05=0.046). Histopathological examination of the thyroid glands among groups of young and adult males rats exposed to RF EMR showed various changes. Most of thyroid follicles were enlarged indicating hypothyroidism especially in young rats. In both young and adults, even exposure to the lowest radiation exhibited hyperplasia and vaculation of some follicular cells. A compact tumor was detected in a young male exposed to 57 nw/m^2 RF EMR. Dilated blood vessels were observed in an adult male exposed to 10 nw/m^2 RF EMR. Hyperplasia, vaculation, disruption of some follicles walls with fusion of neighboring follicles and blood vessels congestion were observed in an adult male exposed to37nw/m^2 RF EMR.

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Introduction

Sudan telecommunication sector has witnessed fast growth in the number of mobile telephone users. There are many base stations (antennae) distributed all over the country. There is increased public concern over possible adverse health effects due to electromagnetic field (EMF) radiated by these equipments (Hamadan, 2010). Recent increase of hormonal disorders in human may be correlated to the increased to EMF produced by various instruments including base stations (antenna). Because of its position near the buildings, human systems are some of the most likely to be exposed to EMF (Behari and Rajamani, 2012). Although EMF frequently stimulates glandular activity in the short term, long-term exposure is often harmful in that the gland ceases to work properly. An example of this is the thyroid gland, which is in an exposed position in the front of the neck (Eskander *et al*., 2012). The thyroid gland is one of the most exposed vital organs to EMR. Production of thyroid hormones is essential for metabolism, temperature regulation and many other vital processes in the body (Sinha, 2008). So small changes in thyroid hormones in the blood are sufficient to alter the brain function of subjects (Bernal 2007, Mortavazi *et al.*, 2009 and Lauer *et al.*,

2013). Eskander *et al.,* (2012) found that people who lived for six years within 100 meters of cell phone base stations showed a highly significant loss in their ability to produce thyroid hormones. Single exposure to microwaves increased plasma TSH levels and resulted in a higher activity of the rats' thyroid gland (Saddiki *et al.*, 1986). Another study on the thyroid in two months old rats exposed to 50 HZ electric field for 8 hours a day for 4 weeks showed no effect on plasma TSH, T4 and thyroid structure, but the level of T3 decreased (Portet and Cabanes, 1988). Matavulj *et al.*, (1999), Matavulj *et al.*, (2000), and Rajkovic *et al.*, (2003) investigated the influence of 50 HZ EMF (50-500 μT) for 2-4 months on male rats exposed from 1-day old. Results of these studies showed an alteration on thyroid follicular epithelium, follicular colloid content and inter-follicular connective tissue, these changes pointed to a decrease thyroid activity. However, repeated irradiation of rats with microwaves decreased the functional activity of the thyroid gland (Navakitikian *et al.*, 1990). Koyu *et al.*, (2005) investigated the effect of 900bMHz EMR on TSH and thyroid hormones in rats. The result showed a reduction in the serum levels of TSH, T4 and T3. The aim of the present study was to evaluate the significance of living near cell phone towers by determining the RF EMR effects in vivo on studying the alteration of TSH and thyroid hormones (T4, T3) and histopathological changes of thyroid gland in young and adult male Wistar rats.

Materials and Methods

Forty-eight young and adult males Wistar albino rats were used in this study, these rats were obtained from the animal house at Faculty of Pharmacy Khartoum University. They were kept in plastic cages to adapt for 7 days before starting the experiments. The cages dimensions were 30×20×22cm. The rats were fed by special diet containing carbohydrates, proteins and vitamins. Two experiments were used as long-term exposure.

Experiment No. 1

In this experiment 24 male rats with the age of 4 weeks (young) old were used. Their weights ranged between 35.0 to 54.2 g. They were exposed 8 weeks to radio frequency (RF) electromagnetic radiation emitted by cell tower base station on the roof of one house in south Gebra/Khartoum State. The rats were divided into 4 groups six rats each in separate cage as follows: G0: Control, animals were kept without being exposed to any RF EMR, G1: animals were exposed to RF EMR (57nw/m2) at distance 3 meters from the tower,G2: animals were exposed to RF EMR(37nw/m2) at distance 10 meters from the tower. G3: animals were exposed to RF EMR (10nw/m2) at distance 20 meters from the tower. The radiation emission continued for 8 weeks with daily period of 10 hours at night (8 PM to 6 AM), at temperature 30-33ºC.

Experiment No 2

In this experiment, 24 male rats with the age of 12 weeks (adult) old were used. Their weights ranged between 01.8 to127.2g. They were exposed for 4 weeks to radio frequency (RF) electromagnetic radiation emitted by cell tower base station. Similarly the rats were divided into 4 groups and placed at the same distances and the same power of radiation of rats in experiment No.1. Radiation emission continued for 4 weeks. At the end of experiments (lasting after two months in experiment No.1 and one month in experiments No.2), rats were weighted, anatomized, blood samples were collected from a pericardial puncture by using syringe size 5mm and put into EDTA tubes. Serum was prepared by centrifugation of the blood for 15 min at13000r/min and kept at -20ºc till analyzed. Thyroid functions test (TSH, T4, T3) were estimated using Enzyme Linked Immuno- Sorbent Assay (ELISA) kits (Biotek made in USA).

For histological studies, thyroid glands were removed then placed in10% formal saline to 24 hours; then taken and processed for paraffin embedding. After that the tissues were sectioned by microtome at 5μm then the sections of tissue were prepared and mounted on glass slides. Haematoxylin-eosin stain was used. The general structure and pathological changes were studied and photographs were taken using light microscope provided with digital camera. The data considered changes in the body weight, alteration of TSH, T4 and T3 and histological changes of thyroid gland. These data were presented as mean values ± standard deviation (SD). Differences among the four groups in the two experiments were analyzed by the one-way analysis of variance (ANOVA). The accepted level of significance was set at $P < 0.05$.

Results

The results of the effects on the body weight are shown in Table

(1), which indicate decrease in relative weight increments ranging between 107.26% -131.43% in the 3 groups of young males in comparison to control 187.5%. Similarly decrease was observed in relative body weight increments in the 3 groups of adult male ranging between-7.6%-47.6% compared with control 74% (Table 2).

Table 1: Relative weight increments of young rats exposed to RF EMR

Group	Initial mean body weight (g)	Final mean body weight (g)	Relative weight increments (%)
Control (G 0)	35.00	100.63	187.5
G 1	49.95	103.53	107.26
G 2	34.23	78.51	129.36
G 3	54.21	141.65	131.43

Table 2: Relative weight increments of adult male rats exposed RF EMR

Group	Initial mean body weight (g)	Final mean body weight (g)	Relative weight increments (%)
Control (G 0)	127.21	222.48	74.00
G 1	112.25	103.75	-7.6
G 2	100.4	113.17	12.7
G 3	101.75	150.27	47

Mean serum levels of TSH in G1, G2 and G3 of young male rats exposed to RF EMR showed increase levels compared to the control ranged between 0.256±0.116-0 in G1 to .093±0.035 µu/ml in G3 (Fig.1). The statistical analysis showed significant result in mean levels of TSH between control and G1, G2 and G3 of young male rats (P <0.05=0.004).

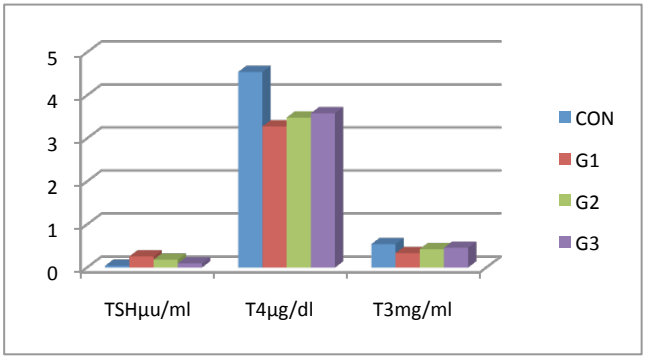

Fig 1: Mean serum levels of TSH, T4 and T3 hormones of young male rats exposed to RF EMR Compared with control

Similarly, an increase of mean serum levels of TSH in G1, G2 and G3 of adult male rats exposed to RF EMR was observed. The mean high level was observed in G1 (0.229± 0.175) and the low level in G3 (0.110± 0.152). No significant different between control and G1, G2 and G3 of adult rats (P > 0.05= 0.099) (Fig. 2).

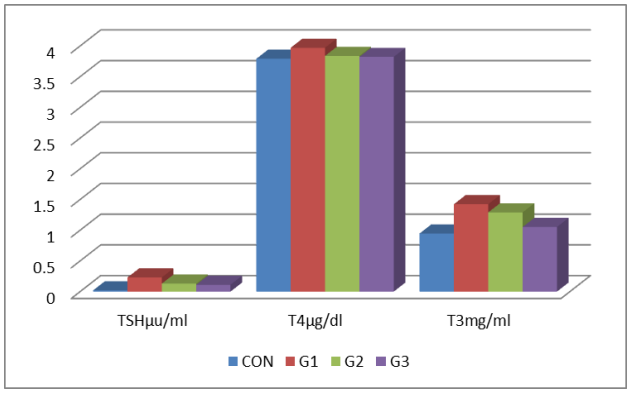

Fig 2: Mean serum levels of TSH, T4 and T3 hormones of adult male rats exposed to RF EMR Compared with control

The mean levels of T4 in young male rats exposed to RF EMR Fig. (1) showed significant decrease compared to control ranged between 3.277 ± 0.375 µg/dl in G1 to 3.584 ± 0.478µg/dl in G3, (P=0.000). In contrast, the mean levels of T4 in adult male groups (G1, G2 and G3) showed slightly increase level in comparison to control ranged between 3.96 ± 0.447 µg/dl in G1 to 3.82 ± 0.499 µg/dl in G3. The statistical analysis showed no significant different in the level of T4 between the three groups and control P =0.834 Fig (2). The mean levels of T3 in young male rats exposed to RF EMR showed non-significant decrease levels in all groups when compared to control ranged between 0.335± 0.106 mg/ml in G1to 0.457 ± 0.270 mg/ml in G3 (P= 0.429) refer to Table (3) and Fig. (1). However, the mean levels of T3 in adult male rats exposed to RF EMR showed increase levels in all groups when compared with control ranged between 1.422± 0.436 mg/ml in G1 to1.05 ± 0.270 mg/ml in G3 Fig. (2). Significant difference was shown among the levels of T3in G1, G2 and G3 of the adult male and the control groups (P < 0.05).

Histopathological changes in thyroid gland

Thyroid gland from the control animals showed normal histological appearance, with cuboidal epithelium lining medium sized follicles. The connective tissues in-between these follicles contained blood capillaries (Plate 1). At histopathological examination, thyroid glands among groups of young and adult males rats exposed to RF EMR showed various changes. Most of thyroid follicles were enlarged with an increase in the colloidal area and lined by thin flattened follicular cells in G3of young males exposed to10nw/m2 RF EMR (Plate 2). Hypertrophy, hyperplasia and vaculation of some follicular cells in G2 of young male exposed to 37nw/m2 RF EMR were observed (Plate 3). Histological changes were detected in G1of young male exposed to 57nw/m2 RF EMR represented by appearance of compact tumor (Plate 4) with total absent of glandular pattern. Minimal increase of follicular cells (started hyperplasia) and less dilated

blood vessels was observed in G3 of adult male exposed to 10 nw/m^{2}RF EMR (plate 5). Hyperplasia, vaculation, some follicles of disruption walls with fusion of neighboring follicles and blood vessels congestion were observed in G2 of adult male exposed to 37 nw/m^2 RF EMR (Plate 6). Some follicles were small and irregular with little colloid storage and more dilated blood vessels were observed in G1 of adult males exposed to 37 nw/m^2 RF EMR (Plate7).

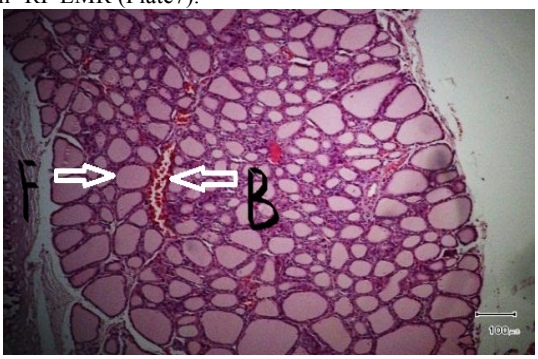

Plate 1. Transverse section of thyroid gland of control Group of male rats exposed to RF EMR (4×20) shows normal follicles (F) with medium sized and cuboidal cells, blood vessels (B) (H&E).

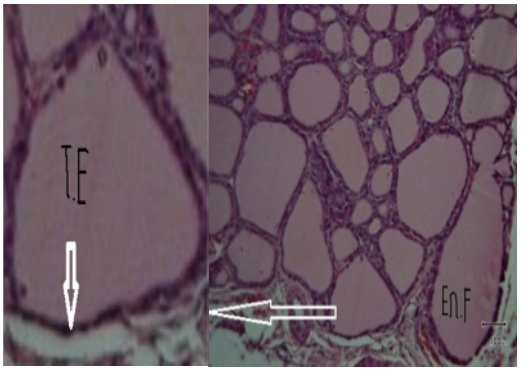

Plate 2. Transverse section of thyroid gland of G3 of young males exposed to 10nw/m2 RF EMR (10×20) shows enlarged follicle (En. F) and thin epithelial cells (T.E) (H&E).

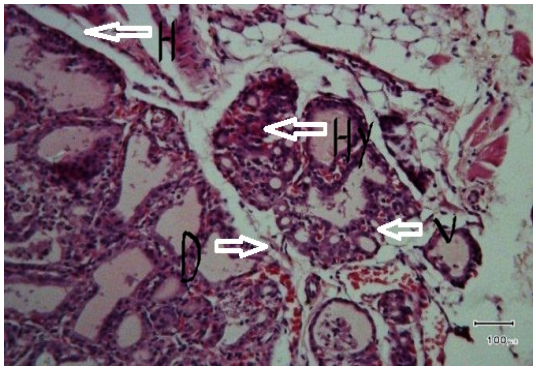

Plate 3. Transverse section of thyroid gland of G2 young males exposed to 37nw/m2 RF EMR X (10×20) shows Hypertrophy (H), Hyperplasia (HY), Vaculation (V) of follicular cells and dilated blood vessels (D) (H&E).

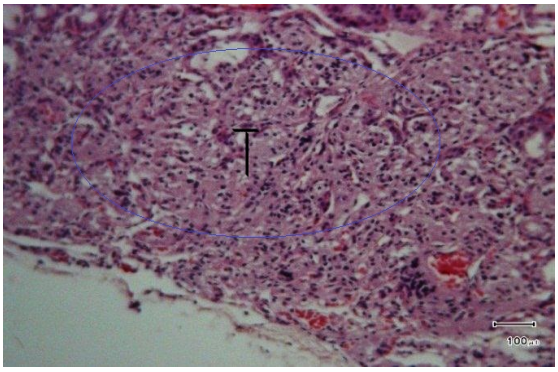

Plate 4 Transverse section of thyroid gland of G1 young males exposed to 57nw/m2 RF EMR X (10×20) shows appearance of compact follicular tumor (T) with total absent of glandular pattern (H&E)

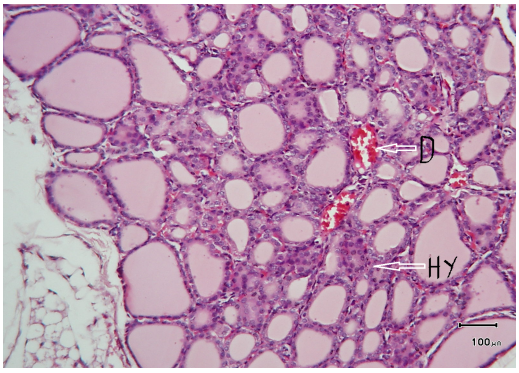

Plate 5 Transverse section of thyroid gland of G3 of adult male exposed to 10nw/m2 RF EMR Shows hyperplasia (HY) in some follicles and dilated blood vessels (D) X (10×20) (H&E) .

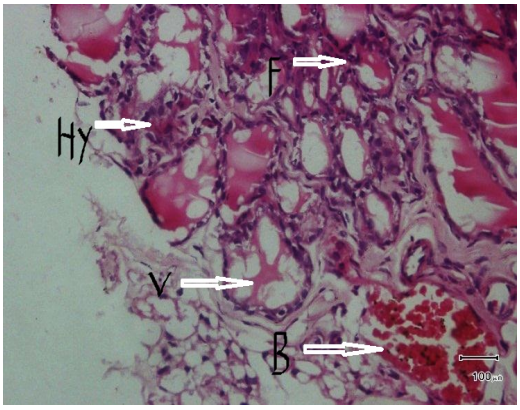

Plate 6 Transverse section of thyroid gland of G2 adult males

exposed to 37nw/m2 RF EMR X (10×20) shows hyperplasia (HY), vaculation (V), some follicles of disruption walls with fusion (F) of neighboring follicles and blood vessels congestion (B) (H&E)

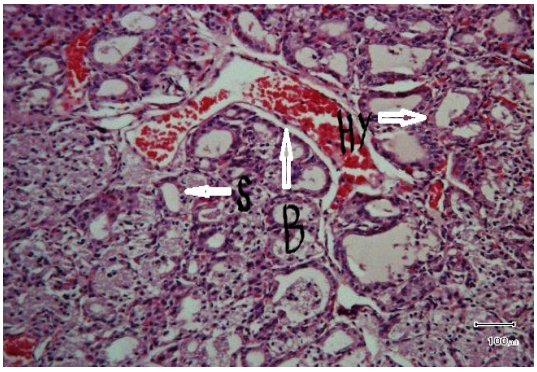

Plate 7 Transverse section of thyroid gland of G1 adult males exposed to 57nw/m2 RF EMR X (10×20) shows some small and irregular follicles with little colloid storage (S), hyperplasia (HY) and more dilated blood vessels (B) (H&E).

Discussion

In this study, the significance of living near cell phone towers was evaluated by determining the RF EMR affection in vivo, by studying the alteration of TSH and thyroid hormones (T4, T3) and histo-pathological changes of thyroid gland of young and adult male Wistar rats. The relative body weight increment was decreased in both young and adult males groups. These findings are in agreement with IIhan *et al.*, (2004) who confirmed that exposure of rats to microwave frequencies at 900 MHz can cause oxidative stress on these animals and decrease their antioxidant activities, leading to weight loss. Also this result agreed with Pourlis, (2009) who reported that rats were exposed at 970 MHz for 22h/day caused reduced (-12%) fetal body weight. Also our findings agreed with Hajioun *et al.*, (2014) who confirm that EM waves can cause weight loss when rats were exposed to microwave frequencies at 900 MHz used in cell phones. According to Aghdam *et al.*, (2009), these changes resulted because magnetic fields can enhance fat breakdown and glycogenesis. Based on the study, the result showed a higher mean TSH level and lower mean T4 and T3 levels than control inG1, G2 and G3 of the young male rats exposed to RF EMR. However, the mean levels of TSH, T4 andT3 in G1, G2 and G3 of adult male rats exposed to RF EMR showed increase level when compared with control. These findings are in agreement with the result of Saddiki *et al.*, (1986) who confirm that single exposure to microwaves increased plasma TSH levels and resulted in a higher activity of the rats' thyroid glands. Also agreed with the result of Rajkovic *et al.*, (2006) who found that thyroid activation increased in 2-month old rats exposed to EMF for 1 month, this suggests an increased level of TSH. In contrast, another study by Quinlan *et al.*, (1985) suggested that levels of TSH were not changed in rats after exposure to 60 Hz and 100Kvm electric field for 1 to 3 hours. The present results agreed with Udintsv *et al.*, (1978) and Zagorskaya, (1989) who confirmed increased thyroid gland activity after 15 min exposure of rats to20mT, 50Hz EMF, and increased T3 and T4, but the same experiment for 2 months after the exposure showed decreased levels of thyroid hormones Zagorskaya and Rodina, (1990). In contrast with our results, histophysiological study of the thyroid in 2 months old rats exposed to 50 Hz electric field for 8 hours a day for 4 weeks showed no effect on plasma TSH, T4 and thyroid structure, but the level of T3 decreased (Portet and Cabanes, 1988). Our results of young male groups of T4 and T3 levels are in line of Koyu *et al.*, (2005) who investigated the effects of 900MHz EMR on TSH and hyroid hormones in rats. Their result showed a reduction in the serum levels of TSH, T4 and T3. Mortavazi *et al.*, (2009) studied the associations between mobile phone use and alterations in the levels of TSH and thyroid hormones in humans. The result showed higher than normal TSH level, low mean T4 and normal T3.These differences may be due to the differences in the dose and duration of exposure to radiation. The results of the young male groups in this study indicated hypothyroid status. Its finding are in agreement with results of Haiying *et al.*, (2006), who found that hypothyroid subjects were diagnosed with biochemical parameters of T3 and T4 below the normal ranges, and TSH above the normal range. The alterations in the levels of TSH, T4 and T3 was followed by histopathological changes in the thyroid gland of young and adult male rats exposed to RF EMR. Our results showed enlarged follicles with increase in the colloidal area and lined by thin flattened follicular cells in G3 of the young males exposed to 10 nw/m^2 RF EMR. This indicates that thyroid glands of the exposed rats were in an inactive state. The increase in the follicular area and diameter in G3 of the young male exposed to10 nw/m^2 RF EMR may be associated with the increase in colloidal area and diameter. So the size of a follicle depends on the amount of colloid (Hartofit *et al*., 2005). Resting follicles are usually associated with increased thyroid colloidal area (Esmekaya et al., 2010). Hypertrophy, hyperplasia and vaculation of some follicular cells in G2 of young male exposed to37nw/m2 and hyperplasia in G1, G2 and G3 of adult male exposed to RF EMR were observed. These agreed with McNabb, (1992) who suggested that TSH is responsible for the morphological appearance of thyroid follicles and hormones secretion leading to hypertrophy and hyperplasia of the follicular cells. Hypertrophy and hyperplasia indicating a condition of higher activation in the thyroid of exposed rats to EMF Galloni *et al.*, (2009). Some follicles of disruption walls with fusion of neighboring follicles were observed in G2 of adult male exposed to 37 nw/m^2 (Plate 6). This is because of hyperplasia in follicular cells. More dilated blood vessels were observed in G1, G2 and less in G3 of adult male rats exposed to 57, 37 and 10 nw/m^2. Rajkovic, (2006) demonstrated and increased volume density of blood capillaries in rats exposed to 50Hz EMF. This vascular effect of RF EMR might be caused by

the action of mast cells situated around blood vessel and or nerve fibers terminating nearby. Both mediators are known to increase thyroid blood flow, and histamine increases the capillary dilated (Michalkiewicz *et al.*,1993).

Conclusions

1. This study has shown that cell tower radiation (RF) caused body weight loss in both young and adult groups.

2. Our results confirm that RFR caused a remarkable decrease in the levels of TSH, T4 and T3 in young male groups and increased them in adult male groups. These changes in the levels of hormones were evident following to the exposure of RFR.

3. Histopathological examination showed various degrees of follicular cells hypertrophy, hyperplasia and tumor were evidenced pointing out a variability in the functional state of the thyroid gland. Further studies should be done to involve the nature of such effects in the thyroid gland under the influence of EMF.

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