Original Article

Histological study on the Effect of Electromagnetic Radiation Emitted from 4G Cell Phones on the Thyroid Gland of the Adult Male Albino Rat

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ABSTRACT

Introduction: The massive increase in cell phone use in the world increases worries about its possible side effects on the human health. The cell phones release nonionizing radiofrequency waves that can affect the thyroid gland due its anatomical position. Thyroid gland regulates the metabolic rate of the body and plays an important role in the human health.

Aim of the Work: The present study aimed to investigate the effects of electromagnetic waves emitted from 4G mobile phone on the thyroid gland using the adult male albino rat as an experimental model.

Material and Methods: Thirty-six adult male albino rats were divided into 2 groups: Group I which was divided into 2 subgroups; IA (Control) and IB (Sham exposed). Group II (4G group) in which the rats were exposed to Lenovo B phones as sources of 4G-LTE radiation emitted by ringing for one hour per day for eight weeks. At the end of the experiment, thyroid gland specimens were processed for histological examination. Also, morphometric study and statistical analysis were done.

Results: Histological sections from the 4G group showed signs of hypoactivity in the form of statistically significant increase in the follicular diameter and statistically significant decrease in the follicular epithelium height. In addition, some follicles showed signs of degeneration in the form of denuded follicles, exfoliated epithelium in the follicular lumen. Some follicular cells showed vacuolated cytoplasm and pyknotic nuclei. Also, accumulation of macrophages was detected at the site of degeneration. In the interstitial tissue between the follicles, dilated and congested blood vessels were noticed and there was apparent increase in the parafollicular and mast cells. These results were confirmed by transmission electron microscopic examination.

Conclusion: Electromagnetic radiation emitted from 4G cell phone resulted in pathological changes in the thyroid gland of adult male albino rat.

Received: 12 March 2022, **Accepted:** 24 March 2022

Key Words: Mobile, rat, thyroid.

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ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Nowadays, human beings are exposed to a wide range of electromagnetic radiation (EMR) from a variety of sources. as power lines, electrical appliances, cabling in buildings, microwaves and cellular phones that are typically held near the ears. As a result, electromagnetic radiation exposure is dangerously increasing, creating a serious health risk[1].

In today's world, cell phones are both popular and necessary[2]. They can not only be used for voice chats, but they may also be used to communicate news, images, and internet information[3]. Cell phone technology is one of the world's wildest-growing technologies[2,4]. There are around 2 billion mobile phone users in the globe today, and 83 percent of worldwide internet users prefer to use their cell phone devices to access the internet[1]. Nearly 5.7 billion people (roughly three-quarters of the population in the world) will have signed up for mobile phone services by 2020[5]. In Egypt, the mobile penetration rate is more than 112% and this means that nearly each Egyptian resident has a mobile device[6].

Internationally, mobile phones work in radiofrequencies from 800 to 3500 MHz[2]. Increased signals are linked to the advancement of mobile phone telecommunication systems. The first generation (the analogue mobile phone system) was introduced in 1980 and works at 450-900 MHz. After a decade, the second generation (the global system of mobile communications GSM) was announced and runs at 902.4 MHz. The third generation uses a radiofrequency of 1,800MHz[7,8]. The fourth-generation long-term evolution communication system (4G-LTE) was just presented, and it can bring internet speeds that are exceedingly fast[9].

The fourth-generation mobile services use the LTE (Long-Term Evolution) technology. All the bands of frequencies used via historical communication systems are compatible with LTE such as (Uplink; 2500 to 2570 MHz and also downlink; 2620 to 2690 MHz), have been added to existing bands, such as (Uplink; 1710 to1785 MHz;

 DOI: 10.21608/ejh.2022.126546.1656

downlink; 1805 to 1880 MHz). LTE is a protocol-based internet technology that is entirely numerical. In 2016, the 4G and 3G technologies reported to be fifty five percent of the entire mobile wideband networks, with 4G accounting for around 41% of all connections[10].

The prolonged use of a cell phone can distress the body via RF-EMR (radiofrequency induced electromagnetic radiation) as non-ionizing radiation. The production of RF-EMR depends mainly on the frequency of the held cell phone. The greater the frequency of the mobile phone, the higher beaming energy absorbed in the human body[8]. Mobile phones, even being carried on belts, purses, or pockets, can produce EMR whether turned on or in standby mode, exposing different portions of the human body to injurious radiations[1]. Electromagnetic radiation is a non-ionizing radiation that can affect the body function through the thermal effects via producing hyperthermia or the non-thermal effects like generating oxidative stress in the cell by increasing the reactive oxygen species or by decreasing the activity of the antioxidant enzymes with subsequent DNA damage and cellular membrane disruption[8].

Previous studies reported that cell phone can have an adverse effect on the human health causing cancer, sleeping disorder, hearing disability, blurring vision[1], defective testicular function[11], neck pain, painful fingers, morning tiredness, restlessness[12]. In addition, experimental studies reported structural changes in the brain tissue & the adrenal gland[13] and the parotid gland[4].

The thyroid gland is an endocrine gland. It is butterfly in shape and situated in the front of the lower part of the neck[14]. Its function is production, storage and the release of the thyroid hormones; triiodothyronine and thyroxine[15] that have a significant impact on body metabolism regulation, growth, development and the activity of the nervous system. Thus, any disturbance in the thyroid gland can have adverse effects in the metabolism of the whole body[16]. Unfortunately, it can be a target for any electromagnetic radiation[17]. Previous study performed on the thyroid gland using 900 MHz radiation (similar to that emitted by 2G-GSM mobile phones) showed pathological changes in the gland and hypothyroidism[16].

It was observed that most of the previous studies focused on the biochemical changes of the thyroid gland resulting from exposure to 2G-GSM mobile phones[17]. So, the aim of the present work was to observe the histological alterations in the thyroid gland resulting from exposure to 4G mobile phones using the adult male albino rat as an experimental model.

MATERIAL AND METHODS

Material

During this study, the presence of other signals from other mobile phones, electromagnetic field releasing devices, Wi-Fi networks were evaded except the used cell phone in the electromagnetic field exposure room.

In the present study, Lenovo B was used as a 2100-2400 MHz (4G-LTE)[18] continuous wave electromagnetic energy generator which has SAR values: 1.557 W/Kg (head)/ 1.714 W/Kg (body-worn).

Each mobile phone was silent to avoid the sound of the bell and thus expose the animals only to the radiofrequency radiation of the mobile phone. The mobile phone was positioned directly under the cage in which the rats stayed during the exposure[19].

Experimental rats

Following permission from CARE; the Animal Research Ethics Committee at the Faculty of Medicine in the University of Ain Shams. Thirty-six adult male albino rats (weighing 180-250 gm) were kept at Ain Shams Faculty of Medicine's animal household of medical research center. Each three rats were kept in one stainless steel cage which measured 30x35x40 cm. The rats were given a cycle of 12 hours of light and 12 hours of darkness and got water and food on a regular basis. Animals were housed for ten days before to the experiment to allow them to acclimate to the conditions.

Two groups of rats were created

Group I: Consisted of twenty-four rats that were equally subdivided into two subgroups.

- Subgroup IA (control group): Consisted of twelve rats that were kept away from any source of EMR including mobile phones.
- Subgroup IB (Sham exposed group): Consisted of twelve rats that were exposed to switched off cell phones 24 hrs. per day for eight weeks. This group was exposed to switched off Lenovo B phones.

Group II (4G group): Consisted of twelve rats that were exposed to Lenovo B phones as sources of 4G-LTE radiation emitted by ringing for one hour per day for eight weeks[6].

Tissue preparation for light microscopy

All the experimental animals were sacrificed by administering sodium thiopental intraperitoneally (25 mg/kg B.W.). Thyroid glands were removed, as well as the trachea and adhering tissue linked to the thyroid gland. Thyroid tissues were put in 10% neutral formalin, then dehydrated in increasing degrees of the alcohol, cleaned in xylol, and paraffin blocks were created. Five-micron-thick thyroid sections were cut and dyed with H&E and Masson's trichrome stains, examined and photographed with light microscope.

Tissue preparation for electron microscopy

The thyroid glands were chopped into minute pieces, fixed in 2.5 percent glutaraldehyde, rinsed in the phosphate buffer, then fixed in one percent osmium tetroxide. Following the fixation, dehydration and embedding in epoxy resin was done. Toluidine blue was used to

stain semithin (1um) sections[20] and examined by light microscope. After that, ultrathin sections were done, then dyed with the lead citrate and the uranyl acetate, inspected and then photographed with a transmission electron microscope (Jewel 1200 EX).

Morphometric and statistical study

Only the thyroid follicles in the middle of the thyroid lobules were measured since they were the active ones. It included the measurement of:

- The diameter of thyroid follicles in hematoxylin and eosin-stained sections at a magnification of x100
- The height of follicular cells in hematoxylin and eosin-stained sections at a magnification of x400.

The measurements were achieved by the image analyzer (Leica Q500 MC) program in the Histology Department of Ain Shams University's Faculty of Medicine. The computer was linked to an Olympus XB microscope Japan. The measurements were carried out per high power field via (X10 or X40) in six different non overlapping fields chosen at random in six different sections from six different animals in each group.

IPM SPSS Statistics Data Editor was used to conduct the statistical examination. Then, the one-way analysis of variance test was applied to do data evaluation. The data was provided as a mean with a standard deviation (SD). The *P value* determined the data relevance.

- A *P value* of more than 0.05 was deemed non-significant.
- A *P value* of less or equal to 0.05 was deemed significant.
- A *P value* of less or equal to 0.001 was deemed highly significant.

RESULTS

Group I(Control group)

The subgroups (IA and IB) of group I showed similar normal histological picture of the thyroid gland, so both groups were named the control group.

A- Light microscopic results

H&E-stained thyroid sections from group I (control group) showed the normal architecture of the rat thyroid gland. The gland was formed of multiple lobules separated by thin connective tissue septa. The thyroid lobule consisted of variable sized rounded or oval follicles with connective tissue and capillary beds in between. The large follicles were found at the periphery of the gland while the smaller ones were found at the center of each lobule (Figure 1). Each follicle was lined with a one layer of flat to cuboidal follicular thyrocytes that had vesicular rounded nuclei. The lumen of each thyroid follicle had acidophilic homogenous colloid. The parafollicular (C) cells were found in groups between the follicles. They had pale cytoplasm and large pale nuclei (Figure 2).

Regarding Masson's trichrome-stained thyroid sections, thin collagen fibers were observed in the connective tissue capsule, in between the thyroid follicles and in the septa between the thyroid lobules (Figure 3).

Examination of semithin sections stained with toluidine blue showed normal thyroid tissue. Each follicle was lined with a one layer of cuboidal follicular thyrocytes that revealed oval or rounded vesicular nuclei with central prominent nucleoli. The parafollicular (C) cells appeared large polyhedral and pale with large pale nuclei. They were embedded between the thyroid follicular cells and the basement membrane within the follicular epithelium. Fibroblasts with elongated dark nuclei were observed around the follicles and in the interstitium between them. In addition, blood vessels were seen in the interstitium (Figure 4).

B- Electron microscopic results

Electron microscopic investigation of ultrathin thyroid gland sections from rats in group I (control group) showed follicular cells with euchromatic rounded to oval nuclei and their apical border showed numerous microvilli that project into the colloid. The lateral surfaces of the thyroid follicular cells showed tight junctions. Their cytoplasm showed cisternae of rough endoplasmic reticulum (rER) that were parallel and regular, mitochondria and dense lysosomal granules (Figure 5). The parafollicular cells were also noticed between the thyroid follicular cells and the basement membrane within the follicular epithelium. Each cell had large oval euchromatic nucleus, mitochondria, Golgi apparatuses and numerous electron dense secretory granules (Figure 6).

4G group

A- Light microscopic results

H&E-stained thyroid sections from the 4G group showed disintegration and disorganization of the thyroid follicles. Many follicles were irregular in shape and large in size (Figure 7). Multiple follicles had interrupted follicular walls with desquamated epithelial cells in their lumina. Fusion of some follicles was also seen in some sections. Many follicles had degenerated colloid in their lumina and some follicles were empty looking and devoid of colloid. Follicular cells varied from flattened to low cuboidal and most of them had flat, dark stained nuclei and vacuolated cytoplasm. The parafollicular cells were noticed frequently in the exposed rats. Congested and dilated blood vessels were also noticed between the follicles (Figure 8).

Regarding Masson's trichrome-stained thyroid sections, excessive amount of collagen fibers was found in the septa between the lobules and also in between the follicles (Figure 9).

Examination of semithin sections stained with toluidine blue from the 4G group showed large thyroid follicles filled

with homogenous colloid and lined with one layer of flat follicular thyrocytes with flat nuclei. Some thyroid follicles were small and degenerated with desquamated epithelial cells in their lumina (Figure 10). Other follicles had cells with heterogenous cytoplasmic granules and pseudopodia extending into the colloid. These cells were most probably macrophages (Figure 11). Vacuolated cytoplasm and pyknotic nuclei were found in some follicular cells (Figure 12). In addition, congested and dilated blood vessels were noticed in the interstitium and there was apparent increase in the parafollicular cells (Figure 10). Also, degranulated mast cells were frequently noticed in many semithin sections of this group (Figures 10,11).

B- Electron microscopic results

Electron microscopic examination of the ultrathin thyroid gland sections from the rats in group II (4G group) showed follicular cell with hyperchromatic nucleus. Its cytoplasm was rarified and filled with multiple electron dense lysosomal granules. Part of the parafollicular cell was also seen (Figure 13). Other follicular cells showed irregular nuclei, markedly dilated cisternae of the rER that lost the lamellar arrangement and swollen mitochondria (Figure 14). In addition, large cell with hyperchromatic nucleus and multiple variable sized cytoplasmic heterogenous granules (electron dense and electron lucent) was also seen. This cell was most probably the macrophage. In addition, large amount of collagen fibers was seen (Figure 15).

Morphometric results and statistical analysis

The mean follicular diameter in µm of the thyroid gland in all groups was illustrated in (Table 1 and Chart 1).

The mean follicular cell height in µm of the thyroid gland in all groups was illustrated in (Table 2 and Chart 2).

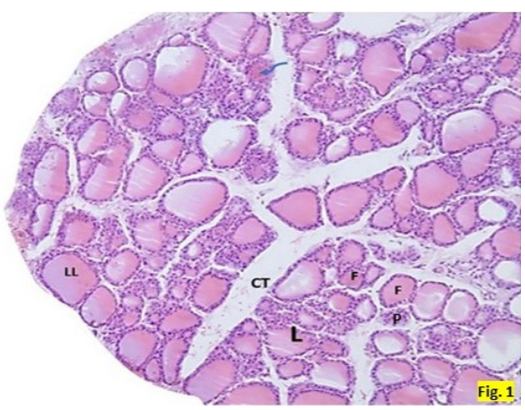

Fig. 1: A photomicrograph of a section of the rat's thyroid gland from the control group showing multiple thyroid lobules (L) separated by connective tissue septa (CT). Each lobule contains variable sized follicles. The larger follicles (LL) are present at the periphery of the lobules and the smaller ones (F) are present at the center of each lobule. Notice the tangential section of the thyroid follicle (blue arrow) and the groups of parafollicular cells (P) in association with the small sized follicles. H&E (X100)

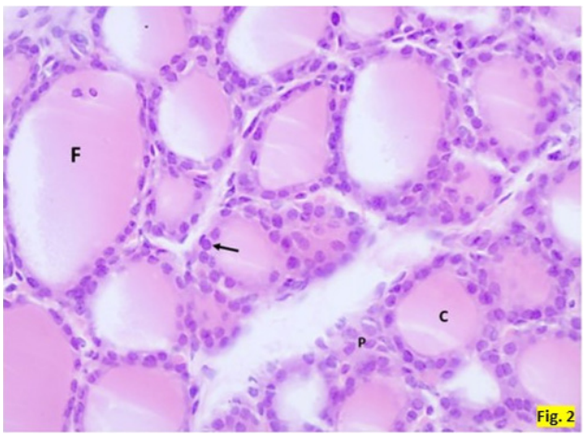

Fig. 2: A photomicrograph of a section of the rat's thyroid gland from the control group showing variable sized follicles (F) lined with a single layer of cuboidal follicular cells that contain central rounded vesicular nuclei (black arrow). The follicles are filled with eosinophilic colloid (C). Notice the groups of parafollicular cells (P) between the follicles. H&E (X400)

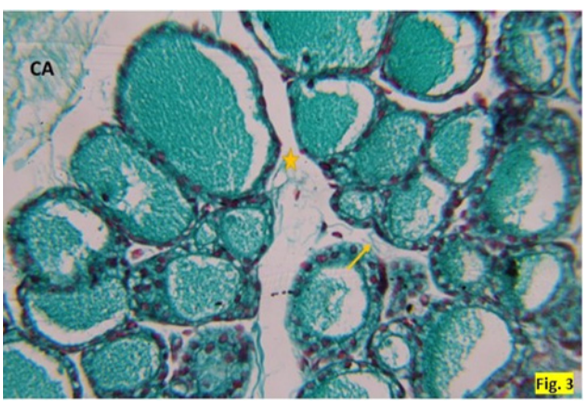

Fig. 3: A photomicrograph of a section of the rat's thyroid gland from the control group showing the collagen fibers in the connective tissue capsule (CA), in between the thyroid follicles (yellow arrow) and in the septa (yellow star) between the thyroid lobules. Masson's trichrome (X400)

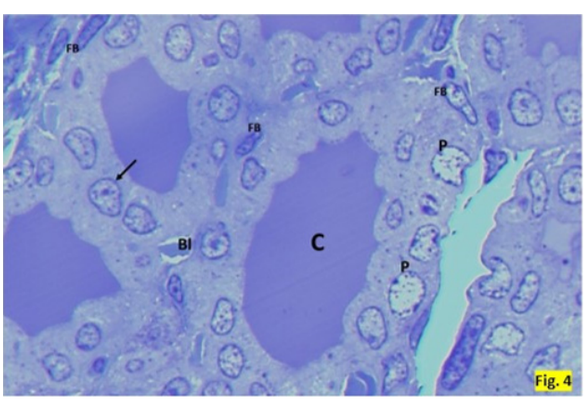

Fig. 4: A photomicrograph of a semithin section of the rat's thyroid gland from the control group showing normal thyroid follicles filled with homogenous colloid (C). Each follicle is lined with a single layer of cuboidal follicular cells with rounded vesicular nuclei (black arrow). Notice the fibroblasts (FB), the blood capillaries (Bl) and the parafollicular cells (P) which are large cells with large vesicular nuclei and pale cytoplasm. Toluidine blue (X1000)

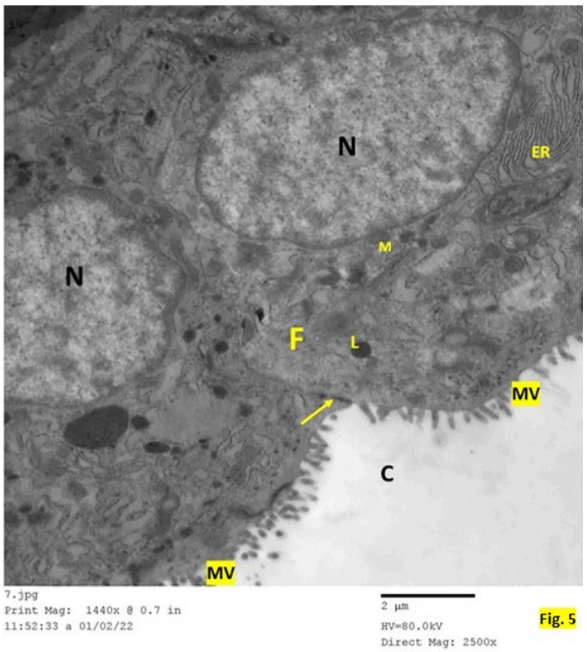

Fig. 5: Electron micrograph of the thyroid gland from the control group showing cuboidal follicular cells (F) with rounded to oval euchromatic nucleus (N) and apical numerous short microvilli (MV) projecting into the colloid (C). Their cytoplasm shows paralleled cisternae of rough endoplasmic reticulum (ER), mitochondria (M) and electron dense lysosomal granules (L). Notice the tight junction (yellow arrow) between the follicular cells. Uranyl acetate &lead citrate (X2500)

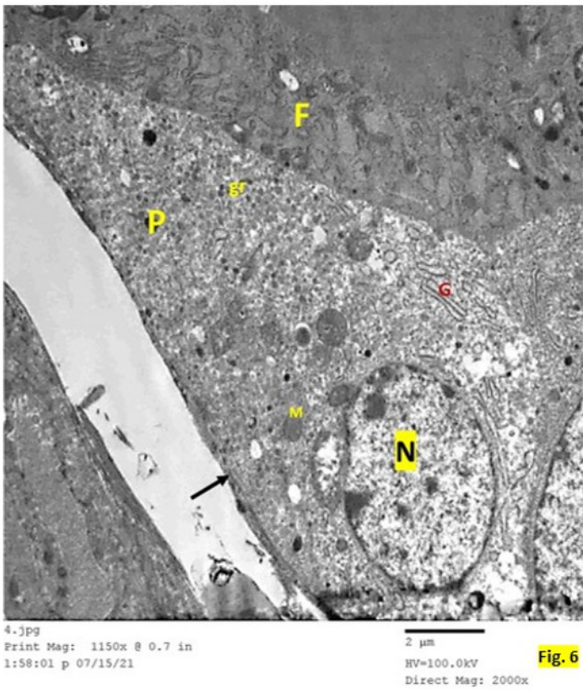

Fig. 6: Electron micrograph of the thyroid gland from the control group showing the parafollicular cell (P) with a large oval euchromatic nucleus (N) lying between the follicular cells (F) and the basal lamina (black arrow). Its cytoplasm contains many small electron dense secretory granules (gr), mitochondria (M) and Golgi complex (G). Uranyl acetate &lead citrate (X2000)

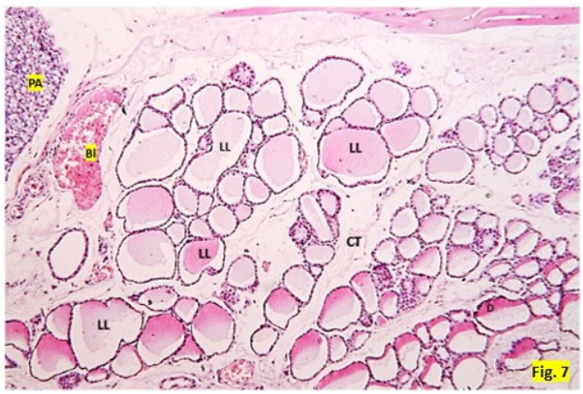

Fig. 7: A photomicrograph of a section of the rat's thyroid gland from group II showing irregular thyroid follicles with apparent increase in the large follicles (LL) and the follicles with degenerated colloid (D). Notice the wide connective tissue septa between the thyroid lobules, the parathyroid gland (PA) and the congested dilated blood vessel (Bl). H&E (X100)

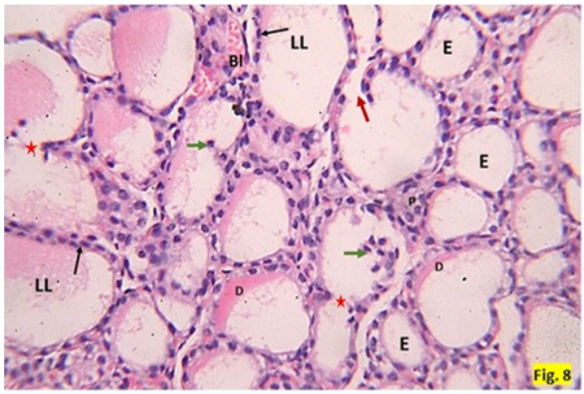

Fig. 8: A photomicrograph of a section of the rat's thyroid gland from group II showing large follicles (LL) lined with follicular cells with flat nuclei (black arrow) and follicles with fused lumina (red star). Some follicles are denuded having disrupted follicular wall (red arrow) and other follicles have exfoliated epithelium within their lumina (green arrow). Some follicles are empty looking (E) and other follicles contain degenerated colloid (D). Notice the dilated congested blood capillaries (Bl) and the parafollicular cells (P) between the follicles. H&E (X400)

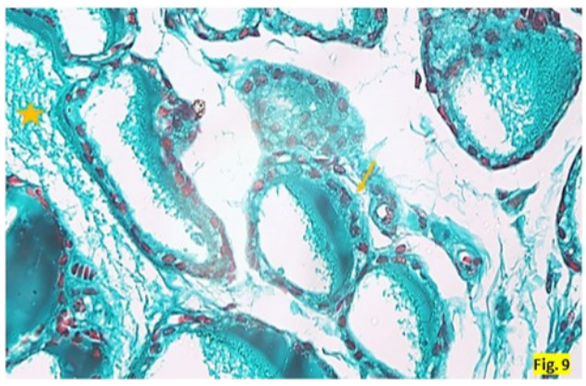

Fig. 9: A photomicrograph of a section of the rat's thyroid gland from group II showing many collagen fibers in the connective tissue septa (yellow star) and between the thyroid follicles (yellow arrow). Masson's trichrome (X400)

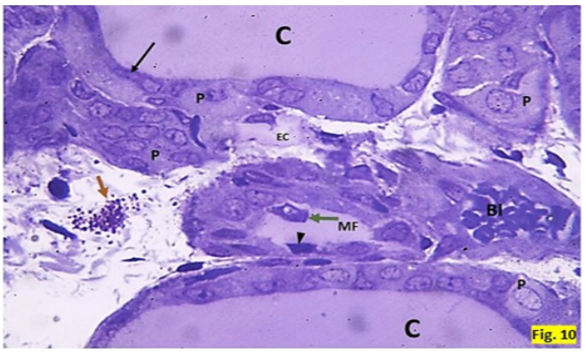

Fig. 10: A photomicrograph of a semithin section of the rat's thyroid gland from group II showing degenerated micro-follicle (MF) lined with follicular cells with hyperchromatic nuclei (black arrowhead) and contains exfoliated epithelium within its lumen (green arrow). The other thyroid follicles are dilated, filled with homogenous colloid (C) and lined with flat follicular cells with flat nuclei (black arrow). The interstitium contains extruded colloid (EC), aggregated parafollicular cells (P), dilated congested blood vessel (Bl) and mast cell with metachromatically stained granules (orange arrow). Toluidine blue (X1000)

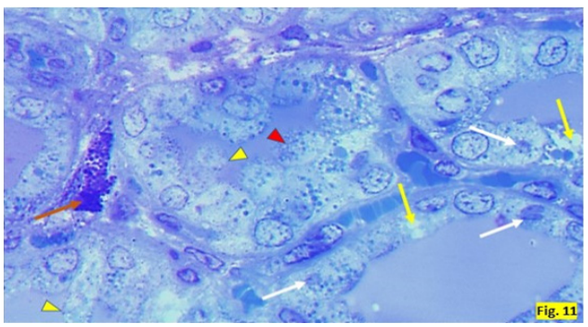

Fig. 11: A photomicrograph of a semithin section of the rat's thyroid gland from group II showing some cells with heterogenous cytoplasmic granules (red arrowhead) and pseudopodia (yellow arrowhead) projecting into the lumen of the degenerated follicle. Notice the follicular cells with pyknotic nuclei (white arrow) and vacuolated cytoplasm (yellow arrow) and the mast cell with its metachromatically stained granules (orange arrow). Toluidine blue (X1000)

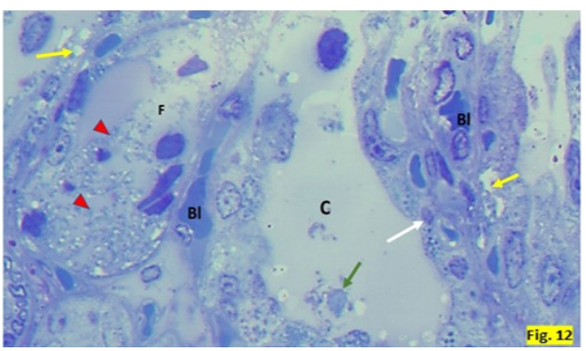

Fig. 12: A photomicrograph of a semithin section of the rat's thyroid gland from group II showing disrupted follicle lined with follicular cells with pyknotic nuclei (white arrow) and contain desquamated epithelial cells (green arrow) and few colloid (C) in its lumen. Some cells with heterogenous cytoplasmic granules (red arrowhead) appeared inside the lumen of the degenerated follicle (F). Notice the congested blood vessels (Bl) between the follicles and the vacuolated cytoplasm in some follicular cells (yellow arrow). Toluidine blue (X1000)

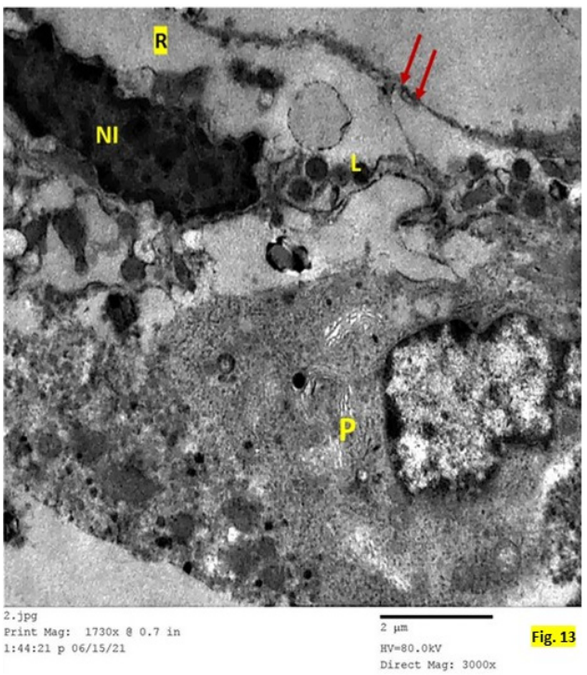

Fig. 13: Electron micrograph of the thyroid gland from group II showing one degenerating follicular cell with irregular hyperchromatic nucleus (NI), rarified cytoplasm (R), multiple electron dense lysosomal granules (L). Notice the complete loss of the apical microvilli (red arrow) and the part of the parafollicular cell (P). Uranyl acetate &lead citrate (X3000)

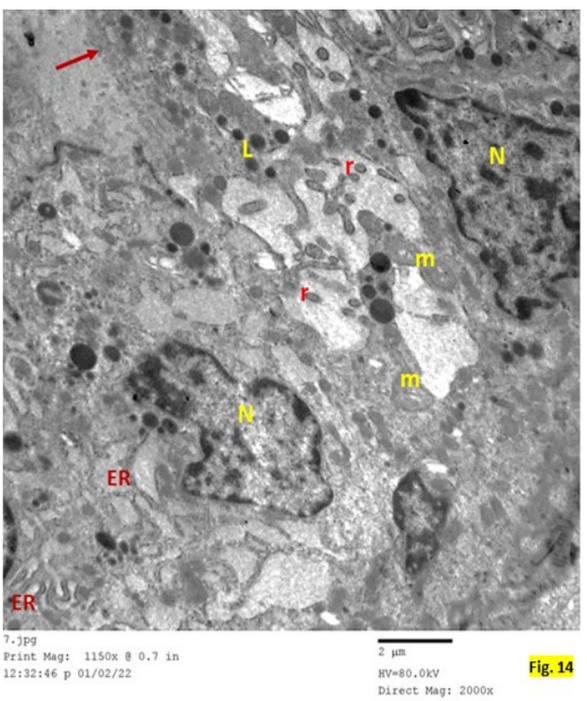

Fig. 14: Electron micrograph of the thyroid gland from group II showing follicular cells with irregular nuclei (N), dilated rough endoplasmic reticulum (ER), fragmented rER (r), swollen mitochondria (m) and multiple lysosomes (L). Notice the complete loss of the apical microvilli (red arrow). Uranyl acetate &lead citrate (X2000)

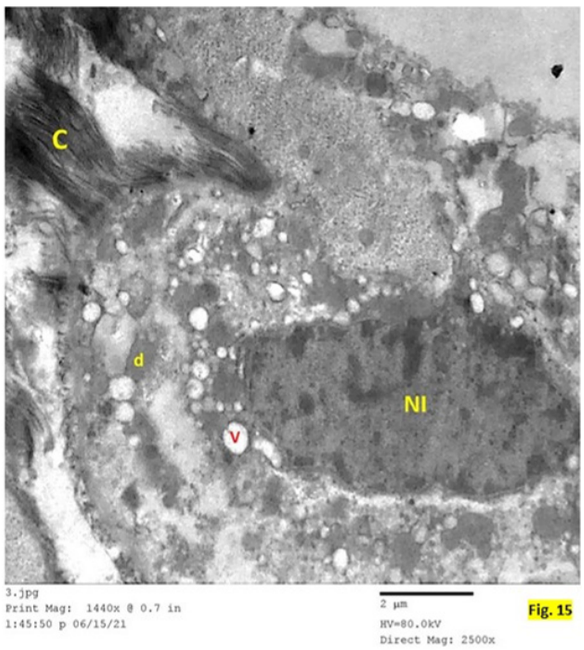

Fig. 15: Electron micrograph of the thyroid gland from group II showing thyroid macrophage. Its cytoplasm shows hyperchromatic nucleus (NI), multiple variable sized cytoplasmic heterogenous granules; electron dense (d) and electron-lucent (V). Notice the multiple collagen fibers (C) in the interstitium. Uranyl acetate &lead citrate (X2500)

Table 1: Mean (µm) ± SD of the mean follicular diameter of the thyroid gland in all groups

	Control group	Sham exposed group	4G group
Mean ± Standard deviation	43.57 ± 2.86	43.2 ± 2.77	61.66 ± 4.15
P-value		(*P*= 0.863) [1]	(*P*<0.001) [2] (*P*<0.001) [3]

(*P*) [1] = non-significant decrease compared to the control group.
(*P*) [2] = highly significant increase compared to the control group.
(*P*) [3] = highly significant increase compared to the sham exposed group.

Table 2: Mean (µm) ± SD of the mean follicular cell height of the thyroid gland in all groups

	Control group	Sham exposed group	4G group
Mean ± Standard deviation	6.15 ± 0.08	6.12 ± 0.07	3.9 ± 0.56
P-value		(*P*= 0.896) [1]	(*P*<0.001) [2] (*P*<0.001) [3]

(*P*) [1] = non-significant decrease compared to the control group.
(*P*) [2] = highly significant increase compared to the control group.
(*P*) [3] = highly significant increase compared to the sham exposed group.

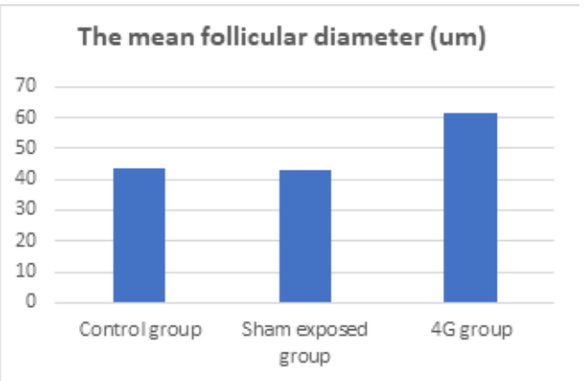

Chart 1: The mean follicular diameter in µm of the thyroid gland in all groups

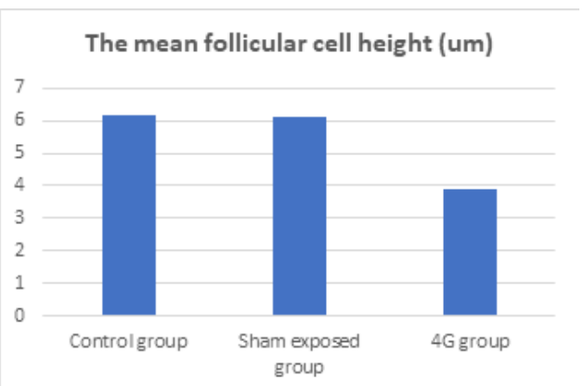

Chart 2: The mean follicular cell height in µm of the thyroid gland in all groups

DISCUSSION

Mobile phones are considered the chief source of electromagnetic radiation as they are used and carried close to the body. The whole-body acts as antenna for electromagnetic radiation absorption. That's why the signals transmitted by a mobile phone can reach all the body parts, penetrate deep into the living tissues, and affect the body at the cellular level[21].

The purpose of this study was to investigate the impact of electromagnetic waves emitted from 4G mobile phones using the adult male albino rat as an experimental model.

In mammals, the glandular activity of the thyroid gland is commonly determined by the thyroid hormone secretion rate. In spite of that, the histological examination of the gland was found to be the most sensitive parameter for the detection of factors which adversely affect the thyroid function[22].

Rodents and humans share similarities in the histopathology of the thyroid lesions. That's why the rat

model is relevant for the assessment of possible thyroid lesions in humans. Male rats were chosen in this study as they have higher circulating TSH levels than female rats, so they are more sensitive to the thyroid toxicants. In addition, estrogen (the female sex hormone) may increase the risk of thyroid diseases[22].

Histopathological examination of the rat's thyroid glands in the 4G group revealed significant light microscopic and ultrastructural changes in the exposed rats' thyroid glands.

In this study, light microscopic examination revealed significant increase in the diameter of the thyroid follicles, decrease in the height of the follicular epithelium and increased colloid in some follicles. These findings were confirmed by morphometric analysis. Similar findings were observed by Esmekaya *et al*., (2010)[16] who reported that these changes could suggest resting and unstimulated follicular cells in the radiofrequency exposed group as the height of the follicular epithelium depends mainly on the functional status of the thyroid gland. The intake of the colloid reduces as the activity of the thyroid follicles declines, which inhibits thyroid hormone secretion and synthesis. As a result, the colloid builds up in the follicular lumen, lowering the height of the nearby thyroid follicular cells. That is to say, the lower the follicular cells' height, the lower their activity. In the same context, Koyu *et al*., (2005)[23] found decreased serum TSH, T3 and T4 in rats that were exposed to 900-megahertz radiofrequency radiation emitted by mobile phones. Therefore, we could hypothesize that decreased serum TSH might be the cause for the hypoactivity of the rats' thyroid glands and the resting follicular thyrocytes.

In this study, H&E-stained thyroid sections showed some follicles displaying evidence of thyroid degeneration such as interrupted walls, exfoliated epithelium, dark stained nuclei and vacuolated cytoplasm in some follicular cells and degenerated colloid that became eccentric in position. These findings were coincided with Mohamed and Elnegris, (2015)[24] and Tuncal *et al*., (2021)[25].

In addition, the semithin sections of the exposed rats showed also denuded follicles with exfoliated epithelium in their lumina and follicular cells with vacuolated cytoplasm and pyknotic nuclei in their walls. Some collapsed micro follicles were also seen. Extruded colloid was also found in the interstitium. Moreover, some degenerated follicles had cells with heterogenous cytoplasmic granules and pseudopodia extending into the colloid. These cells were most probably macrophages. The accumulation of macrophages in the thyroid follicle is called granuloma. These granulomatous lesions are caused by the rupture of thyroid follicles with subsequent invasion by the macrophages as a reaction to extruded colloid[26].

These light microscopic findings were confirmed by electron microscopic examination and many ultrathin sections showed follicular cells with pyknotic nuclei, dilated rough endoplasmic reticulum, degenerated microvilli, swollen mitochondria and increased lysosomes. Similar findings were also observed by Mohamed and Elnegris, (2015)[24] and Rajkovic *et al*., (2006)[27] as a response of the rat's thyroid gland to low frequency electromagnetic field.

It was reported that the cellular responses to electromagnetic field exposure is manifested as reversible and irreversible functional and structural changes to the cells and their organelles, respectively[24]. Previous studies reported that cell phones can affect the thyroid gland tissue through nonthermal and thermal impacts via stimulating the receptors of the cells and disrupting the junctional complexes between the cells. The nonthermal action of the non-ionizing radiation can cause reactive oxygen species to develop and heavy metals to accumulate in the cell[28]. In this aspect, previous studied reported that radiofrequency radiation could induce caspase-9 and caspase-3 dependent apoptotic pathways in follicular cells of rat's thyroid gland with subsequent apoptosis. Furthermore, radiofrequency radiation can activate the NADPH oxidase enzyme found on the cell membrane with subsequent production of reactive oxygen species and triggering of apoptosis. The oxidative stress can disturb the cellular calcium pumps, binding proteins and transporters. Moreover, the radiofrequency radiation itself can act on the Ca pumps leading to Ca efflux from the cells with subsequent cellular apoptosis[16].

In addition, electromagnetic field induces lipid peroxidation which leads to alteration in the lipid/protein ratio within the cell membrane or the cell itself. This lipid peroxidation leads to distraction of the thiol groups in cell membrane and initiation of reactions that leads to cell death[24]. Also, lipid peroxidation could enhance the transport of water and electrolytes into the follicular cell and that leads to cytoplasmic vacuolation[29]. On the other hand, Mohamed and Rateb, (2019)[30] attributed the vacuolated cytoplasm to the presence of dilated rER. The disruption in the protein production within the dilated rough endoplasmic reticulum may hinder the production of apoptosis inhibitors or loss of the critical proteins that are implicated in cellular homeostasis, which results in cell death and the degenerated follicular cells are liable to slough off (Mohamed and Rateb, 2019)[30]

Apparent increase in the mast cells within the interfollicular spaces was characteristic finding in this work. Similar finding was also observed by Rajkovic *et al*., (2005)[31] as a result of low-frequency electromagnetic field exposure in the rat thyroid gland. Rajkovic *et al*., (2005)[32] stated that the increase in degranulated mast cells could be a direct response to radiation. Other researchers reported that TSH promote the release of serotonin from mast cells in the thyroid gland. In turn serotonin activates the follicular cells and stimulates them to engulf thyroglobulin from the colloid[29]. On the other hand, mast cells can release proteases. These enzymes are kept in an active state in the mast cell and when mast cells degranulate, big quantities of proteases are ejected from the cytoplasm into the extracellular area[33]. The activation of protease-

activated receptor 2 (PAR2) by proteases produced by the mast cells contributes to inflammation in the tissues and the disruption of the tight junctions between the cells with subsequent increase in the barrier permeability[34]. This could explain the denuded follicles and the extruded colloid in the interstitium.

Congested blood vessels in the interfollicular spaces were also seen in this study. This finding was also observed by Shaukat *et al*., (2011)[35] as a response of rat thyroid tissue to exposure to mobile phone radiation for 8 weeks. This vascular effect of the electromagnetic field was also in agreement with Rajkovic *et al*., (2006)[27] who attributed it to mediators from the intrathyroid nerve endings and the mast cells that are present around the blood vessels. These mediators increase the thyroid blood flow and the capillary permeability and that enable important molecules as TSH to reach the thyroid by the bloodstream.

Moreover, there was apparent increase in the parafollicular cells in the exposed rats in the present work. This finding was also observed by Lopez-Martín *et al*., (2021)[36] who stated that nonionizing radiation might form a toxic environment that can change the function, morphology, and stress response of the parafollicular cells. In this aspect, Mohamed and Elnegris, (2015)[24] also reported the probable link between the thyroid gland's function and the c cells' activity via one of these mechanisms; TSH indirectly regulates the c cells, the thyroid follicular cells regulate the c cells, or the c cells regulate the thyroid follicular cells. In the same context, some researchers reported that C cells have TSH receptors, and they can produce many regulatory peptides like helodermin, gastrin-releasing peptide and serotonin that can alter follicular cell activity and stimulate thyroid hormone synthesis. Also, C cells can synthesize melatonin that can play a role in the thyroid gland antioxidant defense against any oxidative stress[37].

In this work, thyroid sections stained with Masson's Trichrome demonstrated an increase in collagen fiber deposition in the interstitium. Similar finding was also observed by Mohamed and Elnegris, (2015)[24] who stated that this finding is a direct response of the rat's thyroid tissue to low frequency electromagnetic fields. Some authors reported that oxidative stress and reactive oxygen species can activate TGF-β transforming growth factor beta which is considered the cytokine that is most effective in promoting fibrosis[38]. In addition, radiation could activate TGF-β and once it is activated after radiation, it can promote a chain of cellular events which result in increased collagen deposition and radiation-induced fibrosis. Also, TGF- β has the ability to draft neutrophils and activate the macrophages. The macrophages that are activated can release a variety of cytokines, including TGF- β, which can cause fibrosis by stimulating fibroblasts to secrete some matrix proteins[39].

CONCLUSION

The current study found that exposing the adult male rat's thyroid gland to the radiofrequency radiation of a 4G cell phone caused structural alterations at the light and electron microscopic levels, leading to thyroid hypoactivity.

RECOMMENDATIONS

Make a bigger space between your phone and your head or body. This can be accomplished by:

- utilizing a hands-free accessory such as a wired earpiece or a microphone
- talking on the phone while in speaker mode
- texting instead of chatting
- placing your thumb between the phone and your ear.

ACKNOWLEDGMENT

We would like to thank and express our deepest gratitude to Prof. Dr. Amany Mohamed Hosney El Shawarby, professor of Histology and Cell Biology at Ain Shams University's Faculty of Medicine who generously assisted us with this paper.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة نسيجية لتأثير الإشعاع الكهرومغناطيسي المنبعث من الهواتف المحمولة من الجيل الرابع على الغدة الدرقية لذكر الجرذ الأبيض البالغ

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المقدمة: تثير الزيادة الهائلة في استخدام الهواتف المحمولة في العالم مخاوف بشأن آثارها الجانبية المحتملة على صحة الإنسان. تصدر الهواتف المحمولة موجات ترددات لاسلكية غير مؤينة يمكن أن تؤثر على الغدة الدرقية بسبب موقعها التشريحي. تنظم الغدة الدرقية معدل التمثيل الغذائي في الجسم وتلعب دورًا مهمًا في صحة الإنسان.

الهدف من البحث: لقد هدفت الدراسة الحالية إلى معرفة تأثير الموجات الكهرومغناطيسية المنبعثة من الهاتف المحمول الجيل الرابع على الغدة الدرقية باستخدام ذكر الجرذ الأبيض البالغ كنموذج تجريبي.

المواد وطرق البحث: تم تقسيم ستة وثلاثين من ذكور الجرذان البيضاء البالغة إلى مجموعتين: المجموعة الأولى: التي تم تقسيمها إلى مجموعتين فرعيتين؛ الأولى أ والأولى ب. المجموعة الثانية: حيث تعرضت الفئران في تلك المجموعة الى هواتف لينوفوب كمصادر لإشعاع الجيل الرابع المنبعث من الرنين لمدة ساعة واحدة يوميًا لمدة ثمانية أسابيع. في نهاية التجربة تمت معالجة عينات الغدة الدرقية للفحص النسيجي. كما أجريت دراسات قياسية وتحليل إحصائى.

النتائج: أظهرت المقاطع النسيجية من مجموعة الجيل الرابع علامات نقص النشاط في شكل زيادة ذات دلالة إحصائية في قطر الجريب الدرقى وانخفاض ذات دلالة إحصائية في ارتفاع الخلايا الظهارية. بالإضافة إلى ذلك، أظهرت بعض الجريبات الدرقية علامات تحلل في شكل الجريبات المجردة، مع انفصال بعض من الخلايا المبطنة فى التجويف الجريبي. كما أظهرت بعض الخلايا الجريبية السيتوبلازم المفرغ والنواة المنكمشة الداكنة. وأيضا تم الكشف عن تراكم الخلايا البلعمية في موقع التحلل. ولوحظ توسع واحتقان الأوعية الدموية وزيادة واضحة في الخلايا المجاورة للجريب والخلايا البدينة في النسيج الخلالي بين الجريبات. تم تأكيد هذه النتائج بالمجهر الالكترونى النافذ.

الخلاصة: أدى الإشعاع الكهرومغناطيسي المنبعث من الهاتف الخلوي الجيل الرابع إلى تغيرات مرضية في الغدة الدرقية في ذكر الجرذ الأبيض البالغ.