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ORIGINAL ARTICLE

Decreased level of plasma nesfatin-1 in rats exposed to cell phone radiation is correlated with thyroid dysfunction, oxidative stress, and apoptosis

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ABSTRACT

Context: Exposure to Electomagnetic radiation fields of cell phones causes thyroid dysfunction and a previous study revealed that nesfatin-1 may affect functions of the thyroid gland.
Objective: To study the role of nesfatin-1 on functions of rat's thyroid gland exposed to EMRF.
Materials and methods: Thirty adult male rats were divided equally into 3 groups as group I, group II and group III. The experiment extended for 30 days then the plasma nesfatin-1 level, thyroid functions, and thyroid tissue oxidative stress were assessed. Also; histological and immunohistochemical study studies were done to evaluate structural and apoptotic changes of the thyroid gland.
Results: There was a significant decrease in plasma nesfatin-1 level and thyroid functions with an increase in oxidative stress and apoptosis. Interestingly, there was a correlation between nesfatin-1 level and markers of thyroid function, oxidative stress and apoptosis.
Conclusion: Nesfatin-1 plays a role in thyroid dysfunctions of rats exposed to mobile phone radiation.

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KEYWORDS
Nesfatin-1; cell phone radiation; thyroid function; oxidative stress and apoptosis

Introduction

Up till now, there is an extensive debate about the harmful effects of mobile phones usage on human health as there is a great controversy regarding the induction of physiological and pathological effects in humans after exposure to the emitted Electromagnetic radiation field (EMRF) by mobile phones. The range of emitted wave frequencies from cell phones is 900–1 GHz (Hajioun *et al.* 2014).

Research on the influence of EMRF-driven oxidative stress is still in progress, especially in studies suggesting that EMRF may contribute to the aetiology of thyroid dysfunctions (Antonio *et al.* 2016). The thyroid gland is usually exposed to the irradiation of cell phone as the cell phone lies near it during calling leading to negative effects on the hormones and their receptors in users of cell phones (Lauer *et al.* 2013)

The oxidative processes are important for thyroid hormone synthesis. Although, large quantities of reactive oxygen species (ROS), particularly; hydrogen peroxide (H2O2) are formed in the thyroid gland under physiological conditions but; extra oxidative stress caused by exposure to EMRF, leads to potential damage of macromolecules and thyroid dysfunction (Rai *et al.* 2018).

In addition, apoptosis plays an important role in physiology and pathology of thyroid gland, since the biochemical and morphological characters of programmed cell death were monitored in normal thyroid gland and various thyroid diseases. However, the mechanisms and regulation of apoptosis in thyroid tissues remain indistinct (Zuo *et al.* 2014).

The main functions of nesfatin-1 are regulation of blood glucose, inhibition of food intake and may be regulation of thyroid functions as it is localised in the paraventricular nucleus (PVN) and the thyrotropien releasing hormone (TRH) through influencing the membrane potential of TRH neurons (Tohma *et al.* 2015).

Moreover, previous studies revealed that nesfatin-1 had a role in thyroid dysfunctions in patients suffering from type 2 diabetes mellitus (Liu *et al.* 2014), had anti-apoptotic effect and activated the antioxidant enzymes in many tissues (Korani and Sonbol 2018, Erfani *et al.* 2019).

From these findings we could hypothesise that nesfatin-1 may affect thyroid gland dysfunctions induced by exposure to mobile phone radiation. So this study aimed to assess the effects of EMRF emitted from the mobile phone on nesfatin-1 plasma levels and their correlation with the thyroid functions, oxidative stress and apoptosis of thyroid glands.

Materials and methods

Animals

Thirty adult male albino rats, 2 months old, weighing 200–250 g were used in the present study and housed in the animal house of medical research center/Faculty of Medicine/Benha University at room temperature (24 °C) with 12 h light/dark cycles. Animals were acclimatised for 2 weeks before starting the experiment and allowed to access

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balanced chow diet and tap water. All experimental procedures followed the guidelines of local Ethical Committee at Benha Faculty of Medicine/Benha University and the guidelines established by the publication of USA/NIH (No. 85–23, revised 1996).

Experimental design

The rats were randomly divided equally into three groups (ten rats each) and were exposed to Nokia N70 mobile phones (Nokia Corporation, Finland) for 30 days as follow:

Group I (GI) rats were exposed to the switched off mode.
Group II (GII) rats were exposed to the EMRF for one hour/day (El-Gohary and Said 2017).
Group III (GIII) rats were exposed to the EMRF for two hours/day (El-Gohary and Said 2017).

Exposure to electromagnetic radiation

The presence of signals from other cellular devices, metals, WiFi networks or EMRF-emitting devices were avoided except the used mobile phone in the EMRF exposure room during this study. The rats were exposed to EMRF (900 MHz at SAR of 0.9 W/kg emitted by the mobile phones for one hour/day (8–9 AM) in GII and two hours/day (8–10 AM) in GIII for 30 days. The highest SAR value stated by the manufacturer of the used mobile phone Nokia N70 was 0.95 W/kg of body mass (Sieroń-Stołtny *et al.* 2015). The mobile phone was kept in a silent mode under the cage of animals during the time of exposure to avoid the sound of the bell and expose the animals only to EMRF of the mobile phone (Hammodi 2011, El-Gohary and Said 2017).

Blood sample collection and biochemical assay of *nesfatin-1 and thyroid hormones*

By the end of experiment, rats were anaesthetised with intraperitoneal (IP) injection of sodium pentobarbital (30 mg/kg/BW) then, the blood samples were collected from rats of all groups through intra-cardiac puncture (Koyu *et al.* 2005) to assay levels of nesfatin-1 and thyroid hormones [total T3, total T4 and TSH]. The concentrations of nesfatin-1 levels were measured in plasma using the commercial nesfatin-1 ELISA kit (MyBiosource, San Diego, CA) (Feyza *et al.* 2015) while, the levels of serum thyroid hormones [total T3, total T4 and TSH] were measured by the diagnostic radioimmunoassay (El-Wakf *et al.* 2009).

Biochemical assay of *MDA and antioxidant enzymes (GSH-Px and SOD)*

The thyroid gland of each rat was excised and divided into two parts; one part was homogenised, kept at −80 °C and used to measure lipid peroxidation malondialdehyde (MDA) and antioxidants glutathio peroxidase (GSH-Px) and superoxide dismutase (SOD) in the thyroid tissues while the other part was used for histological and IHC studies. The lipid peroxidation was evaluated by determination of thiobarbituric acid reactive substances to assay MDA levels through using a standard kit purchased from Biodiagnostic, Egypt (Stocks *et al.* 1972, Hussien *et al.* 2017). Also, the contents of thyroid tissues (GSH-Px and SOD) were determined using a commercial kit purchased from Biodiagnostic, Egypt (Hussien and Mousa 2016).

Light microscopic study

The excised specimens of thyroid glands were fixed immediately in buffered formalin solution 10% overnight and processed to get a thin tissue sections (4 um) that were stained by H&E stains to reveal the general structure of thyroid gland and Sirus red technique to evaluate the extent of fibrosis through using the light microscope (Bancroft and Layton 2019).

Immunohistochemistry (IHC) study

Avidin Biotin peroxidase Complex technique was used to detect the IHC reaction of caspase-3 protein in the sections of thyroid gland tissue with the anti-caspase-3 monoclonal antibody and biotinylated secondary antibody (Abcam/UK) according to the manufacturer instructions of primary antibody to detect the extent of apoptosis in the thyroid gland (Abcam/UK) (Mousa *et al.* 2018).

Morphometric study

Digital photomicrographs were taken from the stained sections of thyroid glands and the morphometrical parameters in all groups were measured in 5 random fields from each rat at a magnification of 640×. Then the photomicrographs were analysed by Leica imaging system to determine the thyroid follicular sizes, area % of collagen fibres and area % of caspase-3 immuno-expression in the follicular cells (Cirillo *et al.* 2019).

Statistical analysis

The statistical evaluation of results was performed by using the SPSS programme version 19 (Chicago/USA). Data were expressed as mean ± standard deviation (SD). Intergroup comparisons among all studied parameters, were analysed by One way ANOVA and *post hoc* test. The correlation was analysed by using Pearson's correlation coefficient (r) 2 tailed tests where p values $<.05$ was statistically significant.

Results

Effects of EMF on thyroid hormonal functions and plasma nesfatin-1 levels in all experimental groups

Table 1 showed that exposure of rats to EMF in GII and GIII led to a significant ($p < .05$) decrease in serum T3 and T4 with a significant increase in TSH levels when compared to

Table 1. Effect of EMF on thyroid function and plasma nesfatin-1 level in all experimental groups.

Parameter	GI	GII	GIII
T4 (μg/dL)	5.1 ± 0.44	3.4 ± 0.17*	1.8 ± 0.12**
T3 (μg/dL)	0.44 ± 0.02	0.25 ± 0.02*	0.18 ± 0.01**
TSH (μg/dL)	10.3 ± 0.08	20.3 ± 0.18*	28.4 ± 4**
Nesfatin-1 (ng/mL)	0.163 ± 0.006	0.098 ± 0.019*	0.053 ± 0.009**

Mean ± SD of serum T4, T3, TSH and Nesfatin-1 in all groups: * indicates a significant difference ($p < 0.05$) compared with GI while ** indicates a significant difference ($p < .05$) compared with GII.

GI. By comparing GII and GIII there was a significant decrease in T3 and T4 with a significant increase in TSH levels in GIII. On the other hand, exposure of rats to EMF in GII and GIII led to a significant decrease in plasma nesfatin-1 level when compared to GI. By comparing GII and GIII there was a significant decrease in nesfatin-1 levels in GIII.

Effects of EMF on oxidative stress levels in tissue homogenate of all groups

Table 2 showed that exposure of rats to EMF in GII and GIII led to a significant increase in MDA with a significant decrease in GSH-Px and SOD levels in thyroid tissue when compared to GI. By comparing GII and GIII there was a significant increase in MDA with a significant decrease in GSH-Px levels and SOD in GIII.

The histological and IHC results of all experimental groups

Histological examination of sections from the thyroid glands from GI rat's (control group) stained with H&E showed numerous thyroid follicles of variable sizes lined by cubical follicular cells with rounded nuclei and few para-follicular C cells. The thyroid follicles had a central homogenous acidophilic luminal colloid and separated by inter-follicular cells and thin interstitium of loose connective tissue (LCT) in Figure 1(A) that indicates normal structure of the thyroid glands. The stained sections with picosirus red revealed separation of follicles by a thin BM and interstitium of LACT in Figure 1(D) while the immunostained sections of follicular cells in Figure 1(G) showed a weak cytoplasmic immunoexpression of caspase-3 protein.

Histological examination of thyroid gland sections from GII revealed apparent decrease in the colloid content and size of thyroid follicles. Some follicles had vacuolated colloid while others had no colloid in Figure 1(B) while Figure 1(E) revealed inter-follicular tissue with thick LCT. Additionally; Figure 1(H) showed moderate cytoplasmic immunoexpression of caspase-3 in the follicular cells. All of these changes revealed mild degeneration of the thyroid gland.

Histological examination of thyroid gland sections from GIII revealed dilated BV and shrinkage of multiple follicular cells with a prominent decrease in colloid content as many follicles had no colloid and others had vacuolated colloid in Figure 1(C). Additionally; Figure 1(F) revealed inter-follicular tissue with thick LCT and dilated BV between the follicles while Figure 1(I) showed a strong cytoplasmic immunoexpression of caspase-3 in the follicular cells. All of these changes revealed marked degeneration and apoptosis of the thyroid gland.

Table 2. Effect of EMF on oxidative stress in all experimental groups.

Parameter	GI	GII	GIII
MDA (nmol/g tissue)	2.3 ± 0.12	3.7 ± 0.19*	4.5 ± 0.13**
GSH-Px (U/mg)	4.2 ± 0.19	3.3 ± 0.26*	2.7 ± 0.22**
SOD (U/mg)	7.3 ± 0.32	5.6 ± 0.22*	4.4 ± 0.16**

Mean ± SD of thyroid tissue homogenate MDA, GSH-Px and SOD in all groups: * indicates a significant difference ($p < .05$) compared with GI while ** indicates a significant difference ($p < .05$) compared with GII.

The statistical analysis of thyroid gland morphometric study

Table 3 confirmed the light microscopic results and revealed a highly significant decrease in sizes of thyroid follicles, increased area % of collagen fibres and increased area % of caspase-3 immuno-expression in the follicular cells in GIII. These changes indicated marked degeneration and apoptosis of the thyroid gland in GIII more than GI and GII.

The correlation between plasma nesfatin-1 levels and thyroid functions in all groups

Figure 2(A–C) showed Pearson's correlation analysis which revealed a positive correlation of plasma nesfatin-1 level with levels of T4 ($r = -0.965$; $p = .01$) and T3, ($r = -0.971$; $p = .01$) while, there was a negative correlation with TSH ($r = -0.931$; $p = .01$).

The correlation between plasma nesfatin-1 levels and oxidative stress or area % of caspase-3 immuno-expression in all groups

Figure 3(A–D) showed Pearson's correlation analysis which revealed a positive correlation of plasma nesfatin-1 level with levels of GSH-Px ($r = 0.934$; $p = .01$) and SOD ($r = 0.979$; $p = .01$) while, there was a negative correlation between plasma nesfatin-1 and MDA levels ($r = -0.978$; $p = .01$) and area % of caspase-3 immuno-expression in the follicular cells ($r = -0.927$; $p = .01$).

Discussion

Recently, the abundance of wireless telecommunication technology causes an increase in the number of cell phone users while the studies on health outcomes associated with exposure to EMRF of cell phones are rising. However these effects still uncertain till now and the underlying mechanisms of thyroid dysfunction in rats exposed to EMRF remains poorly understood.

In the current study, we examined the effects of EMRF generated from N70 Nokia mobile phone on plasma levels of nesfatin-1 biomarker and its correlation with thyroid functions, oxidative stress and apoptosis in the thyroid gland of rats.

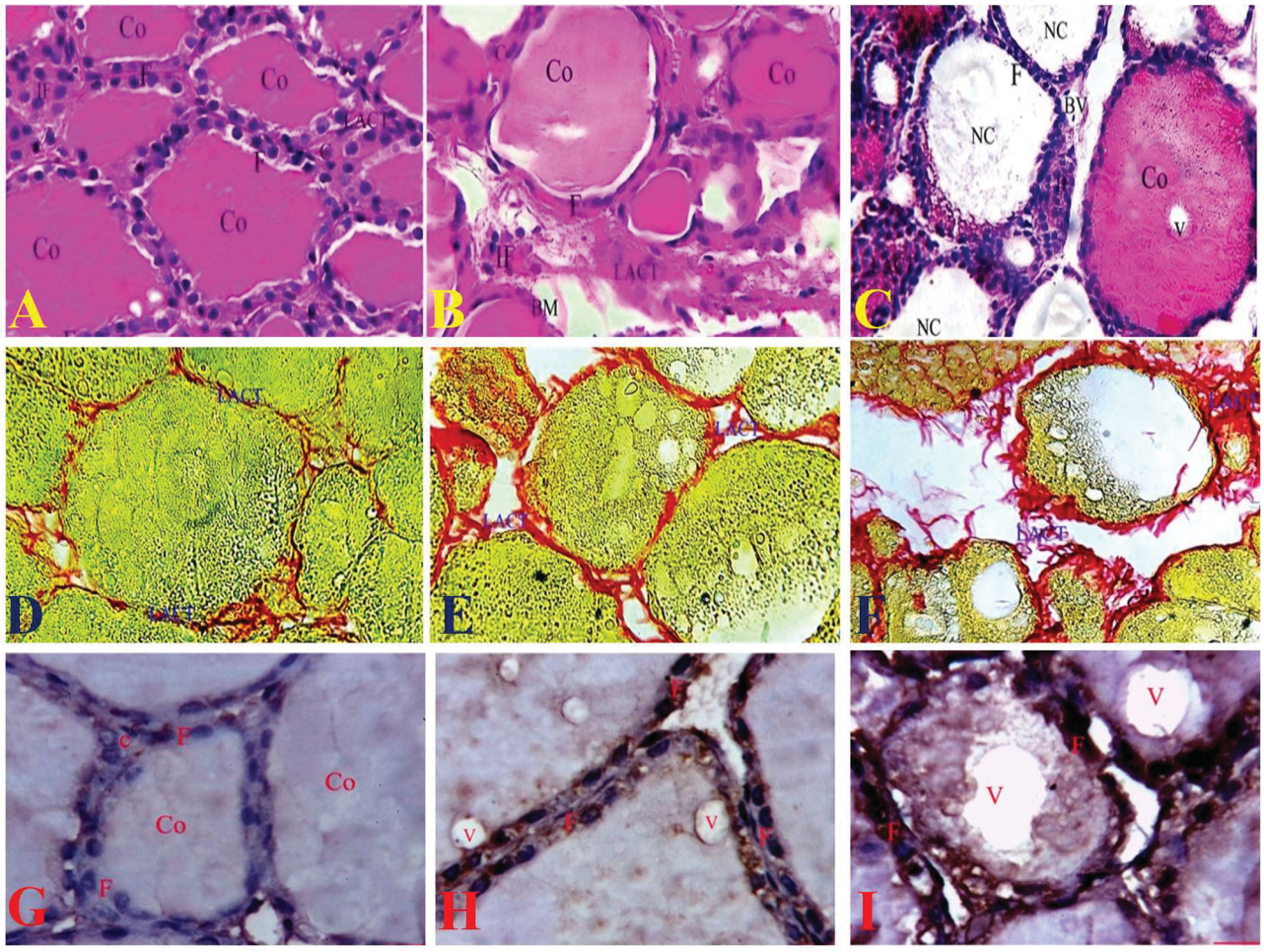


Figure 1. (A–I) show photomicrographs from sections of thyroid glands in all groups. (A–C) are stained with H&E stain X 640 and the bar is 500 μm. (A) Shows numerous thyroid follicles of multiple sizes which are lined with multiple cubical follicular cells (F) and have central homogenous acidophilic colloid (Co) in their lumens. The follicles are separated by inter-follicular cells (IF) and thin interstitium of LACT. (B) The thyroid follicles of GII reveal apparent decrease in follicular sizes and colloid content. Some follicles have vacuolated colloid (V) and others have no colloid (NC). (C) The thyroid follicles of GIII are separated by IF and thick interstitium of LACT while; the thyroid follicles reveal marked reduction in the colloid content with blood vessels (BV). Shrinkage of multiple follicular cells is noticed while; many follicles have no colloid (NC) and few have vacuolated colloid (V). While; (D–F) are stained with Picosirus red stain X 640 and the bar is 500 μm. The thyroid follicles are separated by a thin LACT in GI (D), a thick LACT in GII (E) and a thick LACT with thick collagen fibres in GIII (F). Additionally, (G–I) were stained with PAP immunostain 1000 X and the bar is 200 μm. The cytoplasmic immunoexpression of caspase-3 protein follicular (F) and parafollicular (C) cells is weak with prominent colloid (Co) in GI (G), moderate with some vacuolated colloid (V) in GII (H) and strong with marked vacuolated colloid (V) in GIII (I).

Table 3. Shows morphometrical study and statistical analysis of thyroid glands in all groups.

Parameter	GI	p Value	GII	p Value	GIII	p Value
Size of thyroid follicles/μm	2558 ± 41	–	2075 ± 36	0.03*	1585 ± 79	.02**
Area % of collagen fibres between thyroid follicles	1.3 ± 0.26	–	4.1 ± 1.4	0.04*	7.1 ± 1.4	.01**
Area % of caspase-3 immuno-expression in the follicular cells	0.7 ± 0.37	–	3.2 ± 1.2	0.03*	6.3 ± 1.6	.02**

Mean ± SD of morphometric study in thyroid gland of all groups where * indicates a significant difference ($p < .05$) compared with GI while ** indicates a significant difference ($p < .05$) compared with GII.

Rats of GII showed significant decreases in T3 and T4 while rats of GIII revealed highly significant decreases in T3 and T4 when compared to GI, which are in agreement with Mohamed and Elnegris (2015) and Hajioun *et al.* (2014) who explained that exposure to EMRF may impair exocytosis of thyroid hormones and influence the uptake of iodine in the thyroid gland causing low T3 and T4 levels. Moreover, electromagnetic waves induce stress that alters thyroid functions through elevating the levels of cortisol production, as the increase in glucocorticoid secretion impairs transformation of T4 into T3 (Aghdam Shahryar *et al.* 2009). Additionally, serum TSH level of GII and GIII showed a significant increase when compared with GI due to the active feedback mechanism as the decrease in T3 and T4 serum levels due to induced tissue heating by EMRF. Furthermore, prolonged exposure to the radiations of mobile phone on the thyroid gland induces the anterior pituitary gland to release more TSH which in turn stimulates more production of T3 and T4 (Hashish *et al.* 2008).

Moreover; the current study showed a significant decrease in plasma nesfatin-1 levels of GII rat's with more significant decrease in rats of GIII when compared to GI. While the plasma nesfatin-1 level was negatively correlated with the levels of TSH and positively correlated with T3 and T4 levels

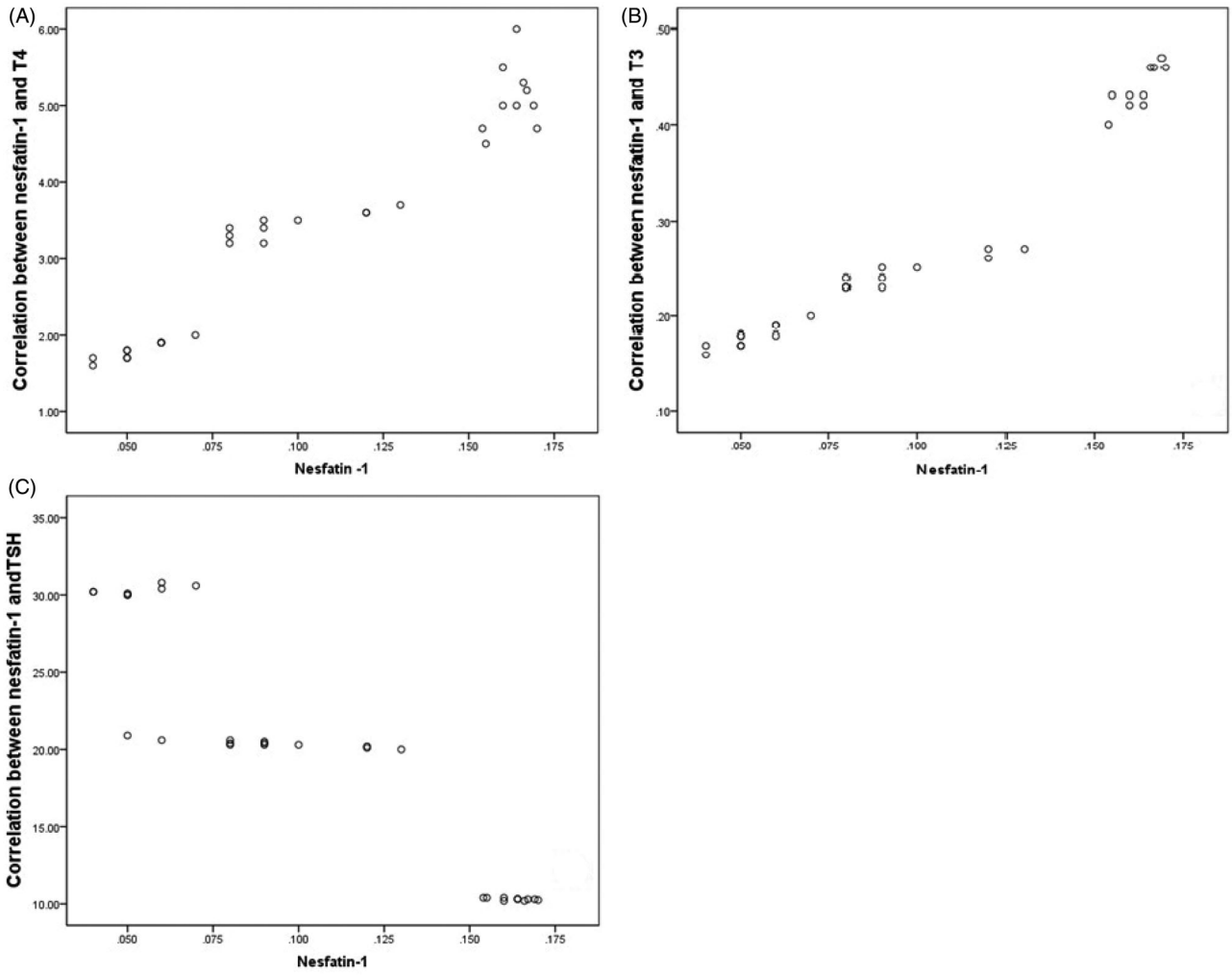


Figure 2. (A–C) Correlation between plasma nesfatin-1 level and T3, T4 and TSH in all experimental groups.(A): correlation of plasma nesfatin-1 level with T4 level (B): correlation of plasma nesfatin-1 level with T3 level (C): correlation of plasma nesfatin-1 level with TSH.

in rats of GII and GIII. These results proposed that nesfatin-1 may act as a regulatory factor of thyroid functions in rats exposed to EMRF.

Furthermore, Li *et al.* mentioned that nesfatin-1 may regulate thyroid gland functions in both aging and type 2 diabetes mellitus (Li *et al.* 2014) through improving insulin sensitivity. This result was explained by Brailoiu *et al.* who mentioned that central nesfatin-1 can stimulate insulin receptor/insulin receptor substrate-1/AMPK/Akt/target the hypothalamus. Also, nesfatin-1 immunopositive neurons combined with TRH neurons in the PVN to induce membrane potential of TRH neurons through central nesfatin-1 (Brailoiu *et al.* 2007). Moreover, the thyroid hormones are controlled by the hypothalamus-pituitary-thyroid axis, through the hypothalamic PVN, that produce TRH to regulate TSH secretion from the anterior pituitary (Artur *et al.* 2012).

As regard oxidative stress, there was a significant increase in MDA with a significant decrease in GSH-Px and SOD in rats of GII and more significant effect in rats of GIII.

These results were in agreement with Sinha (2008) who showed that EMRF caused oxidative stress in the thyroid tissues and damaged the follicular cells due to the imbalance between free radical production and the anti-oxidant defense mechanisms. In case of mitochondrial exposure to electromagnetic radiation; oxidative stress is expressed as a significant increase in ROS, decrease in antioxidant enzymes (GSH-Px and SOD) and increase in MDA (a biomarker of lipid peroxidation). EMRF inhibits the mitochondrial respiratory chain by lengthening the lifespan of free radicals and weakening the anti-oxidant defense system (Ilhan *et al.* 2004, Hao *et al.* 2015).

Furthermore, our study revealed a significant increase in the area % of caspase-3 immuno-expression which indicates apoptosis in the follicular cells of GII rats and more significant effect in rats of GIII. These results coincide with Sinha (2008) who mentioned that electromagnetic radiation induced oxidative stress and lipid peroxidation in the thyroid tissue, which activates the pathway of apoptosis through causing an imbalance between the anti-apoptotic and pro-apoptotic proteins that led to a reduction in the mitochondrial membrane potential and the release of cytochrome C which triggers caspase-signalling pathways and led to cell death (Zuo *et al.* 2014). Additionally, Zuo *et al.* (2014) showed that apoptosis increased after exposure to EMRF that elicited a sequence of interconnected events such as impaired signalling, structural damage, gene mutations, oxidative stress and finally apoptosis.

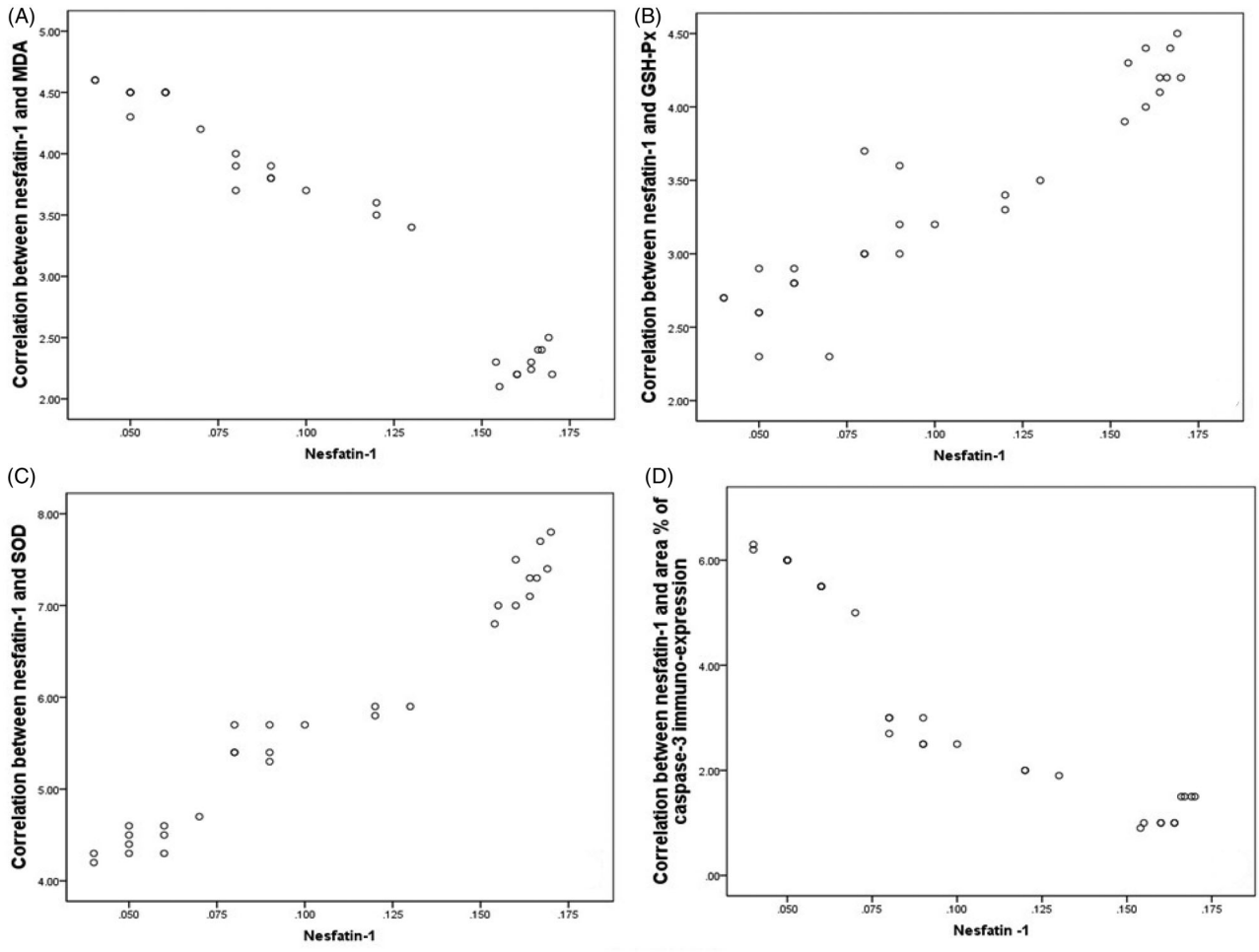


Figure 3. (A–D) Correlation between plasma nesfatin-1 level and MDA, GSH-Px, SOD and area % of caspase-3 immunoexpression in all experimental groups. (A) correlation of plasma nesfatin-1 level with MDA, (B) correlation of plasma nesfatin-1 level with GSH-Px, (C) correlation of plasma nesfatin-1 level with SOD and (D) correlation of plasma nesfatin-1 level with area % of caspase-3 immunoexpression.

Interestingly our study analysed the correlation between nesfatin-1 and MDA, GSH-Px, SOD and area % of caspase-3 immuno-expression in the follicular cells. The plasma nesfatin-1 level was negatively correlated with the level of MDA and the area % of caspase-3 immuno-expression in the follicular cells while, there was a positive correlation between nesfatin-1 and GSH-Px and SOD in rats of GII and GIII.

Tang *et al.* (2012) reported that nesfatin-1 has anti-oxidative and anti-apoptotic effects through its neuro-protective effects against subarachnoid haemorrhage that induced brain injury and inhibited apoptosis in rats. Also Ozsavci *et al.* (2011) and Kolgazi *et al.* (2015) reported that nesfatin-1 can improve gastric injury through its antioxidant effects. Furthermore, Guanjun *et al.* (2015) showed that nesfatin-1 could protect the kidney from ischaemia reperfusion injury by decreasing the oxidative stress and inhibiting apoptosis.

Conclusion

In light of our results we concluded that the plasma nesfatin-1 level is decreased in rats exposed to EMRF of cell phone radiation. And the level of plasma nesfatin-1 is negatively correlated with thyroid dysfunction, oxidative stress, and apoptosis. Moreover, prolonged duration of exposure to EMRF causes more thyroid dysfunction associated with marked reduction of nesfatin-1 plasma levels and a prominent increase in oxidative stress and apoptosis. Further studies on the mechanisms responsible for the decrease in plasma nesfatin-1 concentration in rats exposed to EMFR are recommended. Also, we recommend further studies on the effect of exogenous nesfatin-1 on the thyroid function of rats exposed to EMRF.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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