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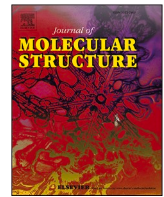

Investigation of gene expression levels in thyroid tissues of rats treated with Wi-Fi electromagnetic wave (2.4–3 GHz Wi-Fi RF-EMF)

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A B S T R A C T

In this study, it was aimed to examine the effects of wireless waves applied to rats in the thyroid tissue by evaluating gene expression levels on 12 determined gene regions. 20 healthy 16-week-old Wistar albino female rats weighting 200–220 g were used. In the experiments, two groups, control and experimental groups, were formed, and ten female rats were used in each group. While WI-FI electromagnetic field was applied to the experimental group of these rats, nothing was applied to the control group. Many different gene regions, including *beta catenin, beta-actin, GAPDH, L3B, HIF 1-Alpha, GSk-3B, TCF, WNT7a, WNT10a, WNT2, Beclin 1 and Beclin 2, ATG5* and *ATG12*, were investigated. *ACTB* and *GAPDH* gene primers were used as a house keeping gene. The fold change values were calculated statistically from the data obtained from the gene expression results. Compared to the control group, it was observed that the experimental group had significant increases in gene expression levels of many gene regions investigated in the study ($P<0.05$). Among the study groups, 12 different genes such as *beta catenin, beta-actin, GAPDH, L3B, HIF 1-Alpha, GSk-3B, TCF, WNT7a, WNT10a, WNT2, Beclin 1* and *Beclin 2, ATG5* and *ATG12,* which we have determined according to the literature, are known to be related to autophagy and oxidative stress. When the expression levels were investigated, it was determined that the expression coefficients of all the genes studied in the wnt/ß catenin pathway in the experimental group of rats exposed to the Wi-Fi EM field, except for *ATG5* and *ATG12,* were quite high, and there was significant differences between the groups.

1. Introduction

With the development of communication technologies and the spread of wireless (Wi-Fi) technology, the effect of technology surrounding us on human health has been investigated. Wi-fi devices produce an electromagnetic field, a type of non-ionizing radiation. While the damage done by ionizing radiation on DNA is known, the harmful effects of non-ionizing radiation on human health are not yet known [1, 2]. Exposure to a low amount of electromagnetic fields has been shown to be effective in DNA, RNA, protein synthesis, cell division, and carcinogenesis [3–5]. Likewise, it has been determined in different studies that non-ionizing radiation affects cellular respiration and hormonal and immune responses [6].

In the current era, technological breakthroughs are in many areas, such as the internet, mobile computers and smartphones, and new energy technologies. All these technological breakthroughs are mostly designed to facilitate human life and enable us to do great things in a short time. However, rapidly changing technologies have changed the global economy and human life in a positive or negative way. While technology is developing rapidly, the effects of all this technology on life are ignored. In the development of communication infrastructures, the use of electromagnetic waves is one of the most important developments in recent years. Wireless internet infrastructures have become more preferred due to their ease of use (Cell phones, television, microwave, etc.). Although the effects of technological tools on living things have been examined in studies, very few studies have been found in the literature on the impact of electromagnetic waves originating from wireless on living things. In some of these studies, it is stated that the effects of electromagnetic waves on living things are essential [1,2,7-9]. In contrast, in some publications, it is noted that no significant effects can be detected [9,10]. Studies with tissue and cell systems have shown that exposure to low-intensity EM fields affects the synthesis of biomolecules (DNA, RNA, and protein), cell division, cancer formation, and calcium entry and exit from the membrane [3–5]. Some epidemiological

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studies show that long-time exposure to RF-EMF causes an increase in certain types of cancer and other diseases [8,11,12]. Some other studies do not support such claims [13,14]. In some studies, the heat shock protein (HSP) family, ornithine decarboxylase (ODC), p38 mitogen-activated protein kinase (MAPK), c-jun, c-myc, p21, bax, GADD45, and Nurr1 have been reported with possible epigenetic effects of RF-EMF exposure showed that genes and the proteins they express can be sensitive to radiofrequency waves [15–18].

In recent years, it has been shown in various studies exposed to EMFs can effect organ development, hematological parameters, hormones and the immune system [19–21]. In a research aticle by Eser et al. (2013) ıt was demonstrated that the EMFs caused the significant oxidative effects in brain tissue and ıt caused stress and inflammatory damage [22]. EMFs have two main effects like oxidative stress and thermal effects on tissues. Especially in tissues with high liquid content, electromagnetic waves frequency causes heat-related damage while the low frequency ones cause damage due to oxidative stress. In addition, this damaging effect is more severe in tissues with high fluid content [23]. Thyroid tissue is close to the surface and its fluid content is high. So ıt is more exposed to the effects of EMF [24]. For this reason, the changes in the thyroid tissue in the researches that study the effects of electromagnetic fields arouse quite a curiosity. Because of this reason, in this study it was preferred to investigate gene expression values of the thyroid tissue of rats.

In some studies, 2450 MHz EMF has important biochemical and chemical effects in rat thyroid tissues and histopathological changes were detected [25]. On the other hand in some research about EMF exposure' s effects on human tissues, RFE exposure seem to have no potential carcinogenic effect on human thyroid cells. Moreover, common biomarkers like HSP 70 also remained unchanged by applying cell phone EMF. These researchers failed to find an association between cell phone-RFE and thyroid cancer [18].

The genes investigated in this study were selected from the genes on the *WNT/beta-catenin* pathway, which have an essential role in the primary events of life such as embryogenesis, tissue regeneration, cell proliferation, and autophagy. In addition, genes thought to have a role in maintaining vitality and minimizing damage in case of oxidative damage were preferred. The *WNT/beta-catenin* pathway has important roles in embryonic development, tissue self-renewal, and various diseases [26,27]. In the literature, it has been shown that WNT signaling regulates programmed cell death through many different pathways. In this study, we investigated the effects of wi-fi on living tissues at the genetic level. Thyroid tissue was chosen as the target tissue because of thyroid cancers, the incidence of which has increased in clinical practice in recent years.

2. Material and method

2.1. Animal models

In our study, 20 healthy Wistar albino female rats, 16 weeks old, weighing 200–220 g, were used following the 3R rules (reduction, replacement, refinement care) adopted as an animal ethics rule in the Experimental Animal Production and Research Laboratory of Cumhuriyet University Faculty of Medicine. Our study was approved by Sivas Cumhuriyet University Experimental Animals local ethics committee (21.12.2021-65202830-050.04.04-613). In the study, rats were randomly selected, and divided into two groups ($n = 10$) as Group 1 for control and, Group 2 ($n = 10$) for wireless electromagnetic waves exposure experimental group. Rats were housed in standart cages at temperature 24 ± 2 °C in Animal Experimentation Unit of Sivas Cumhuriyet University Faculty of Medicine in 2021 year. Animal experimentations were conducted in accordance with the article writing by Durna Daştan et al. (2018) [6]. A common wireless internet router (Zyxel NBG-418N-V2 wireless Access Point/Router) was used for Wi-Fi RF-EMW exposure. The router was able to generate RF-EMW field at 3 GHz for 12 h/day for 30 days and was working in 300 Mbps transmission. External electromagnetic field pollution was eliminated by placing the router and rat cages inside a Faraday Cage [28]. The control group, was kept alive in a Faraday Cage environment for 30 days without being exposed to any electromagnetic environment. Also the experimental group and control group were kept in a similar cage, and in every day, the strength of the Wi-fi wave in both control and experimental cages were examined. Care was taken to ensure that there was no wifi exposure in the control group, and that the wi-fi intensity applied in the experimental group was always the same. At the end of 30 days, the rats were sacrificed, and their thyroid tissues were taken. The obtained tissues were taken into sterile eppendorf tubes, and 1 mL of Ribo Saver (Gene All, Seoul, Korea) solution was added to them. Later they were stored at −80 °C until RNA isolation.

2.2. RNA isolation and reverse transcription (RT) reaction

Total RNA was isolated using GeneAll® Hybrid-R™(GeneAll® Hybrid-R™ - Seoul, Korea) kit following the manufacturer's recommendations. Spectrophotometric measurement was performed to calculate how many nanograms of RNA were in μL of the samples obtained and to check for purity. Approximately 20–40 ng of total RNA was found for each sample. OD260/OD280 ratio was used to verify RNA purity. Care was taken to ensure that the ratio of the pure RNAs obtained was between 1.8 and 2.2 and was also used for other steps.

Obtained RNAs were transformed to the cDNA using a first strand cDNA Synthesis Kit (South Korea) and applied the manufacturer's recommendations in this step. cDNA samples were stored under −20 °C until qRT-PCR, for each reaction was used 100 ng/ml of total RNA. The real-time PCR (Applied Biosystem Step One Plus, USA) device was used for gene expression analysis and the SYBR master mix (GeneAll Real Amp TM, Korea) was used in the Real-Time qPCR stage. In this study, many different gene regions such as *Beta catenin, GAPDH, beta-actin, HIF 1-Alpha, L3B, GSk-3B, TCF, WNT7a, WNT10a, WNT2, Beclin 1 and Beclin 2, ATG5* and *ATG 12* genes were investigated. For each gene region, the temperature conditions in the Real-time PCR step were adjusted according to the amount and type of organic base with N carried by the primers. For the initial denaturation, incubation was performed at 95 °C for 10 min, and temperature and time conditions were applied for 15 s at 95 °C in the denaturation step of the real-time PCR cycle and 1 min at

Table 1
Information on the primers used in this study.

Primer names	Primer sequence
LC3BII-F	5′- TTATAGAGCGATACAAGGGGGAG-3′
LC3BII-R	5′- CGCCGTCTGATTATCTTGATGAG-3′
Beclin1-F	5′- ATGGAGGGGTCTAAGGCGTC-3′
Beclin1-R	5′- TCCTCTCCTGAGTTAGCCTCT-3′
ATG5-F	5′- AGCCAGGTGATGATTCACGG-3′
ATG5-R	5′- GGCTGGGGGACAATGCTAA-3′
ATG12-F	5′- TCCCCGGAACGAGGAACTC-3′
ATG12-R	5′- TTCGCTCCACAGCCCATTTC-3′
Beclin2-F	5′- TCAGCCGGAGACTCAAGGT-3′
Beclin2-R	5′- CACAGCGGGTGATCCACATC-3′
HIF1a-F	5′- ACCTTCATCGGAAACTCCAAAG-3′
HIF1a-R	5′- ACTGTTAGGCTCAGGTGAACT-3′
CateninB-F	5′- ATGGAGCCGGACAGAAAAGC-3′
CateninB-R	5′- CTTGCCACTCAGGGAAGGA-3′
GSK3b-F	5′- TGGCAGCAAGGTAACCACAG-3′
GSK3b-R	5′- CGGTTCTTAAAATCGCTTGCCTG-3′
TCF-F	5′- CGCACCAGCAGTACAGATGAG-3′
TCF-R	5′- CAGCTTGGTCTGCGCCTTA-3′
WNT7a-F	5′- TCAGTTTCAGTTCCGAAATGGC-3′
WNT7a-R	5′- CCCGACTCCCCACTTTGAG-3′
WNT10a-F	5′- GCTCAACGCCAACACAGTG-3′
WNT10a-R	5′- CGAAAACCTCGGCTGAAGATG-3′
WNT2-F	5′- CTCGGTGGAATCTGGCTCTG-3′
WNT2-R	5′- CACATTGTCACACATCACCCT-3′
ACTB-F	5′- GGCTGTATTCCCCTCCATCG-3′
ACTB-R	5′- CCAGTTGGTAACAATGCCATGT-3′

60 °C in the Annealing/Extention step. Information on the primers used in this study is given below (Table 1). ACTB gene primers were used as a housekeeping gene in the Real-Time qPCR step. After the reaction was complete, the data were recorded and analyzed. For quantifications of messenger RNA expression, the *GAPDH* and *beta- actin* gene transcripts were used as a reference house keeping gene and normalized relative to the control group. The "Delta delta Ct Method (ΔΔCt)" was used for the calculation of the realistic quantification.

2.3. Statistical analysis

Statistical calculations were made according to the fold change values obtained from the gene expression results. Whether there were statistically significant differences between each gene expression coefficient between the control and experimental groups were evaluated and plotted with the GraphPad-Prism program. When the normality characteristics of the groups were evaluated with the Shapiro-Wilk test and Kolmogorov-Smirnov tests, it was determined that the non-parametric test assumptions were fulfilled, so the comparisons between the groups were made with the Mann-Whitney statistical analysis test. *P* <0.05 was considered significant. Groups with significant differences in gene expression coefficients were marked with *.

3. Results and discussion

It was determined that there were significant increases in gene expression levels in the experimental group of individuals exposed to the wi-fi electromagnetic field ($p<0.05$). When the *autophagy-related gene 5 (ATG5)* expression coefficients of the study groups were compared, a statistically significant difference was found ($p<0.05$). It was observed that the gene expression coefficients of the experimental group consisting of rats treated with wi-fi EMF were 2.648±0.315, higher than the control group's value of 0.393±0.112 ($p<0.05$). In terms of the expression coefficient of the *ATG5* gene, large differences were observed in individuals between groups. Looking at the group averages, it is observed that the experimental group is expressed more than six times more than the control group. It is seen that the difference in expression of the *ATG5* gene between the two groups was 2.256 (Fig. 1). Also Fig. 1 shows the *autophagy-related gene 12 (ATG12)* expression coefficients of the experimental and control groups. There was no statistically

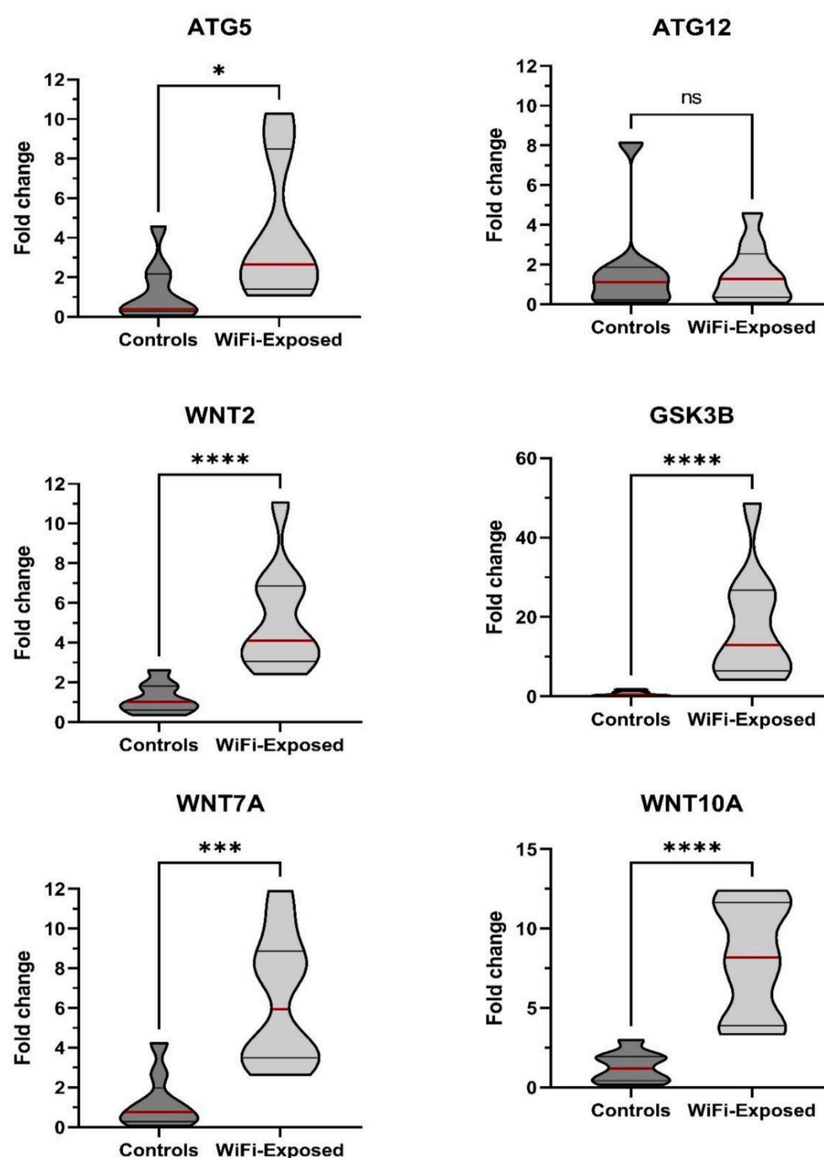

Fig. 1. Expression results of *ATG5, ATG12, WNT2, WNT7, WNT10* and *GSK3B* genes between the experimental and control groups. (Differencies between groups are classified as ns: Not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

significant difference between the groups in terms of the expression level of this gene region ($p>0.05$). The average of the *ATG12* gene expression coefficient of the experimental group was found to be 1.279±0.441, and it is seen that it is close to the gene expression coefficient of the control group, which is 1.124±0.347. In addition, the expression level for the *autaphagia-related gene 12 (ATG 12)* was found to be quite low compared to the expression coefficients of other genes ($p>0.05$, Fig. 1).

The *glycogen synthase kinase 3 beta (GSK3B)* gene expression coefficient of the experimental group was 12.98±1.639, and the expression coefficient of the control group was 0.362±0.059. There was a statistically significant difference between the groups in terms of the *GSK3B* gene in thyroid tissue ($p<0.05$). After wi-fi exposure to the thyroid tissue, the expression level of the *GSK3B* gene increased more than 35 times compared to the control group without wi-fi application, and the difference between the groups was 12.62 (Fig. 1). When the expression coefficients of the *wingless-type MMTV integration site family member 2 (WNT2)* gene were compared between the experimental and control groups, a statistically significant difference was found between them ($p<0.05$). While the *WNT2* gene expression value of the experimental group was found to be 4.100±1.124, the *WNT2* gene expression level of the control group was determined as 1.026±0.737 ($p<0.05$). It is seen that there is an approximately 4-fold higher expression between both groups (Fig. 1). On the other hand, the gene expression coefficients of *wingless-type MMTV integration site family member 10 alpha (WNT10a)* gene expression coefficients in the thyroid tissue were examined, a statistically significant difference was found between the groups in terms of *WNT10a* gene expression coefficients ($p<0.05$). It was determined that the *WNT10a* gene expression coefficient in the thyroid tissue in the experimental group samples was 8.195±1.995, and the *WNT10a* gene expression coefficient obtained from the thyroid tissue of the control group was 1.199±0.538 (Fig. 1). While the difference between the *WNT10a* gene expression coefficients of the experimental and control groups was 6.995, it was determined that the experimental group showed a significant expression difference of more than six times

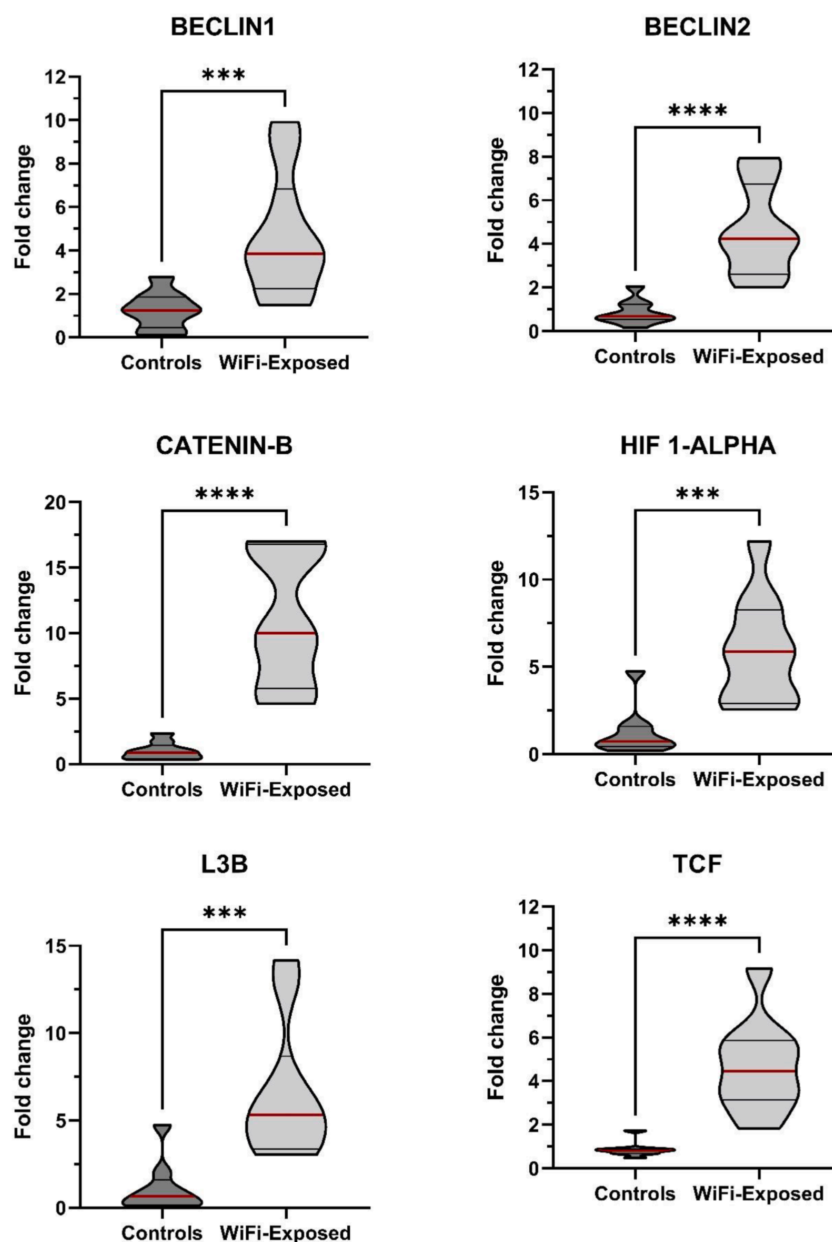

Fig. 2. Expression results of *Beclin 1, Beclin 2, Beta Catenin, TCF, HIF1A* and *LC3B* genes between experimental and control groups (Differencies between groups are classified as ns: Not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

compared to the control group (Fig. 1). When the gene expression coefficients of *wingless-type MMTV integration site family member 7 alpha (WNT7a)* gene expression in the thyroid tissues of our sample groups was examined, the differences between the groups were found to be statistically significant ($p<0.05$). *WNT7a* gene expression coefficient was 5.945±1.389 for the experimental group, while it was 0.765±0.138 for the control group (Fig. 1). It was determined that the *WNT7a* gene expression coefficient of the experimental group showed a significant expression difference, which was more than 7 times higher than that of the control group ($p<0.05$; Fig. 1). In addition, it was determined that the expression level of the *Beclin 1* gene in the experimental group showed statistically significant differences compared to the control group ($p<0.05$). While the expression coefficient of the *Beclin 1* gene was found to be 3.855±0.7344 in the experimental group, the expression coefficient determined for the *Beclin 1* gene was found to be 1.245 ±0.256 in the control group ($p<0.05$). It is seen that there is an expression difference of more than three times between the experimental and control groups (Fig. 2). The differences in the expression level of the *Beclin 2* gene in thyroid tissues between the groups examined within the scope of this study were found to be statistically significant ($p<0.05$) (Fig. 2). *Beclin 2* gene expression coefficient was found to be 4.244±0.956 in the experimental group and 0.686±0.122 in the control group ($p<0.05$). It is seen that the expression coefficient of the *Beclin 2* gene is more than 6-fold, and the difference in expression of the *Beclin 2* gene between the two groups is 3.559 (Fig. 2).

When *beta-catenin (β-catenin, catenin B)* gene expression coefficients in thyroid tissues were compared with the control and experimental groups, the difference between the groups was found to be statistically significant ($p<0.05$, Fig. 2). While it was observed that the *catenin B* gene expression coefficient was 9.995±1.359 in the experimental group, it was 0.856±0.213 in the control group. It is seen that the *beta-catenin* gene expression difference between the two groups was 9.137 (Fig. 2). When the thyroid tissues of the rats belonging to the control and experimental groups were compared in terms of *light chain 3 beta (LC3BII)* gene expression coefficients, it was determined that there was a significant statistical difference between the groups ($p<0.05$; Fig. 2). It was observed that the expression of the *LC3BII* gene was increased in the thyroid tissues of the rats belonging to the experimental group, and the expression coefficient was 5.321±1.058. The expression amount of the *LC3BII* gene in the control group was 0.662±0.039. It was determined that the *LC3BII* gene increased more than eight times in the experimental group compared to the control group (Fig. 2). In this study, when the *transcription factor (TCF)* gene expression coefficients were compared between the experimental and control groups, it was found that the expression amounts between the control and experimental groups were statistically significant ($p<0.05$; Fig. 2). It was observed that *TCF* gene expression increased in the experimental group compared to the control group, and the expression level of the group was 4.467 ±1.012, while it was 0.827±0.167 in the control group. When the expression values of the *TCF* gene were examined, it was determined that the experimental group increased more than five times compared to the control group ($p<0.05$; Fig. 2). In addition, the *hypoxia inducible factor I alpha (HIF1a)* gene expression coefficients between the groups were compared, a statistically significant difference was found between the groups ($p<0.05$; Fig. 2). It was found that *HIF1a* gene expression in the experimental group was increased compared to the control group. *HIF1a* expression level was found to be 5.868±1.218 in the experimental group, while it was 0.735±0.215 in the control group. It was determined that the *HIF1a* gene increased approximately eight times in the experimental group compared to the control group ($p<0.05$; Fig. 2).

In this study, as a result of applying wi-fi electromagnetic field wavelengths to rats for one month, the expression profiles of 12 different genes in thyroid tissue were tried to be revealed. It was observed that the expression coefficients of these 12 genes investigated according to the study data increased compared to the results of the experimental group gene expression results (excluding *ATG12*) compared to the results of the control group. The genes we searched were selected from among the most common genes, which can be an indicator of oxidative stress in living systems and are known to show differences in expression to ensure cell homeostasis, according to the literature. It is also known that these genes investigated in the study are related to the autophagy mechanism in cells.

In previous studies, it has been determined that the *WNT/beta catenin* pathway is essential in many cancer types such as breast, prostate, renal, and cervical. There are also studies showing that RF-EMF has carcinogenic effects on thyroid tissues from different pathways [27]. Conversely in some studies, ıt was seem that RFE exposure had no potential carcinogenic effect on human thyroid cells. The importance of the *WNT/beta catenin* signaling pathway is the regulation of self-renewal and differentiation properties of cancer stem cells. Upregulation of this pathway has been associated with various cancers, and thus, genes of this pathway have been used as promising targets for anti-cancer therapeutics. In the last five years, agents targeting different steps of the *WNT* signaling pathway (Wnt secretion, signal transduction, or β-catenin transcriptional activity) have been developed, and these agents have begun to appear in clinical trials. To find effective treatment methods for diseases associated with the *WNT*/beta catenin pathway, studies are still ongoing on the network covering this pathway and the microenvironment associated with other pathways. In addition, common biomarkers like heat shockh protein genes associated to environmental stress also remained unchanged by applying EMF in previous studies. The researchers did not define an association between cell phone-RFE and thyroid cancer [18]. Although in most cases, MFs increased ROS levels in human, mouse, rat cells, and tissues, there are also studies showing that ROS levels were decreased or not affected by MFs [29]. In another review study, ıt was showed that most animal and many cell studies presented increased oxidative stress caused by RF-EMF and ELF-MF [30]. On the other hand, researchers focused on possible genotoxic analysis of the hematopoietic system after mobile phone type radiation exposure in rats, and there was no significant changes observed in the hematopoietic system of rats after the exposure of RF radiations [31]. In a different study scientist defined that the exposure to radiofrequency radiation (900 MHz, GSM signal) did not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes [32]. On the other hand in a different study in 2021 it was aimed to investigate the effects of radiofrequency radiation emitted by mobile phones and extremely low frequency radiation on thyroid hormones. And in this study it was obtained that the pathological results were generally related to the amount and duration of exposure to EMF radiation [33]. In a study conducted by Eşmekaya et al. in 2010, 900 MHz frequency caused a decrease in thyroid hormone levels by causing damage to the thyroid tissues [34], and in a study writing by Rajkovic et al., it was showed that the 50 Hz EMF caused the damage on thyroid tissues of rats and decreased the T3 and T4 hormone levels [35]. In addition, De Seze et al. found a 21% reduction in TSH concentrations of people exposed to EMF emitted by mobile phones at 900 MHz wave frequency for a month [36]. So it was needed that further human studies exploring thyroid pathology exposured EMF in molecular and cellular level [33].

4. Conclusion

Finally, the experiment of applying wireless electromagnetic wave exposure to rats showed that electromagnetic field application could change the expression coefficients of some genes that we examined related to the *WNT/beta-catenin* pathway. However, studies with other molecules such as proteins and enzymes are needed to determine the effects of these experiments with Wi-Fi-EMF on the gene at the cellular level. Nevertheless, the results of our study are important because they provide data that will raise awareness in people. It also serves as a guide for future work in this field.

CRediT authorship contribution statement

Musa Serin: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization. **Sinan Soylu:** Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Sevgi Durna Daştan:** Formal analysis, Resources, Writing – original draft, Writing – review & editing. **Süleyman Koç:** Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Atilla Kurt:** Software, Validation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Musa Serin reports equipment, drugs, or supplies was provided by Cumhuriyet University. Musa Serin reports a relationship with Cumhuriyet University that includes: speaking and lecture fees. Co-aothor-Sivas Cumhuriyet University.

Data availability

Data will be made available on request.

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