Original Article
Mol Imaging Radionucl Ther 2024;33:19-27 DOI:10.4274/mirt.galenos.2023.04880

Evaluation of the Relationship Between Mobile Phone Usage and miRNA-574-5p and miRNA-30C-5p Levels in Thyroid Cancer Patients

Tiroid Kanserli Hastalarda Cep Telefonu Kullanımı ile miRNA-574-5p ve miRNA-30 Düzeyleri Arasındaki İlişkinin Değerlendirilmesi

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Abstract

Objectives: This study aimed to evaluate the relationship between mobile phone usage and miRNA-574-5p and miRNA-30C-5p levels in patients diagnosed with differentiated thyroid cancer (DTC).

Methods: Fifty patients diagnosed with DTC and 50 healthy volunteers were included in the study. miRNA-574-5p and miRNA-30C-5p gene expression levels in the blood of all subjects were analyzed by real time-polymerase chain reaction, and a questionnaire including various questions was administered to both groups.

Results: Although there was a 7.60-fold increase in miRNA-30C-5p gene expression levels in the patient group compared with the control group, it was not found to be statistically significant. Considering the miRNA-574-5p gene expression levels, although there was a 2.96-fold increase in the patient group compared with the control group, no significant relationship was found. In our study, 85% of our patients were using mobile phones with internet access, whereas 98% of our healthy volunteers were using mobile phones ($p<0.05$). While 53.5% of the patients had their mobile phones with them while they were sleeping, this rate was 83.7% in healthy volunteers ($p<0.05$). However, 93.9% of the healthy volunteers did not have a Wi-Fi device in their bedrooms, and this rate was 75% in the patient group ($p<0.05$).

Conclusion: Although miRNA-30C-5p and miRNA-574-5p gene expression levels were higher in patients than in healthy volunteers, the differences were not statistically significant. Although there was no significant difference in miRNA levels, we believe that due to the higher rate of Wi-Fi device presence in bedrooms in patients compared with healthy volunteers, the effects of electromagnetic radiation on the thyroid can be reduced by paying attention to this simple change.

Keywords: Thyroid cancer, radioiodine, miRNA-574-5p, miRNA-30C-5p

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Received: 14.08.2023 **Accepted:** 26.11.2023

Öz

Amaç: Bizim bu çalışmada amacımız, diferansiye tiroid kanseri (DTK) tanısı konulan hastalarda cep telefonu kullanımının, miRNA-574-5p ve miRNA-30C-5p ile ilişkisini değerlendirmekti.

Yöntem: Çalışmaya DTK tanılı 50 hasta ve 50 sağlıklı gönüllü dahil edildi. Tüm deneklerin kanlarındaki miRNA-574-5p ve miRNA-30C-5p gen ekspresyon seviyeleri gerçek zamanlı polimeraz zincir reaksiyonu yöntemi ile analiz edildi ve her iki gruba çeşitli sorular içeren bir anket uygulandı.

Bulgular: Kontrol grubuna göre hasta grubunda miRNA-30C-5p gen ekspresyon düzeylerinde 7,60 kat artış olmasına rağmen istatistiksel olarak anlamlı bulunmadı. miRNA-574-5p gen ekspresyon seviyelerine bakıldığında hasta grubunda kontrol grubuna göre 2,96 kat artış olmasına rağmen anlamlı bir ilişki bulunmadı. Çalışmamızda hastalarımızın %85'i internet erişimi olan cep telefonu kullanırken, sağlıklı gönüllülerimizin %98'i cep telefonu kullanmaktaydı ($p<0,05$). Hastaların %53,5'i uyurken cep telefonunu yanında bulundururken, sağlıklı gönüllülerde bu oran %83,7 idi ($p<0,05$). Ancak sağlıklı gönüllülerin %93,9'unun yatak odalarında Wi-Fi cihazı bulunmadığı, hasta grubunda ise bu oranın %75 olduğu saptandı ($p<0,05$).

Sonuç: miRNA-30C-5p ve miRNA-574-5p gen ekspresyon seviyeleri hastalarda sağlıklı gönüllülere göre daha yüksek olmasına rağmen istatistiksel olarak anlamlı değildi. miRNA düzeylerinde anlamlı bir fark olmasa da sağlıklı gönüllülere göre hastaların yatak odalarında Wi-Fi cihazı bulunma oranının daha yüksek olması nedeniyle elektromanyetik radyasyonun tiroid üzerindeki etkilerinin bu basit değişikliğe dikkat edilerek azaltılabileceğini düşünüyoruz.

Anahtar kelimeler: Tiroid kanser, radyoiyot, miRNA-574-5p, miRNA-30C-5p

Introduction

Thyroid cancer is the most common endocrine system malignancy, and its incidence is increasing, which can be noticed by all departments working on thyroid gland diseases. Some authors believe that this increase is associated with more frequent health check-ups and increased diagnostic possibilities (1). The etiology of thyroid cancer is currently unknown. However, obesity, smoking, hormonal exposure, and some environmental factors may play a role, especially in childhood exposure to ionizing radiation (2). In addition to diagnostic technological developments against diseases, technological developments that cause electromagnetic radiation exposure, especially mobile phones, have accelerated and become indispensable in our daily lives. The increase in the incidence of thyroid cancer and the parallel increase in mobile phone use raise the question of whether electromagnetic radiation (EMR) plays a role in the etiology of thyroid cancer.

Radiation is divided into two groups, ionizing and non-ionizing radiation, according to its effects on the tissue. Ionizing radiation is also divided into two groups: photon radiation (X and γ-rays) and particulate radiation (such as protons, neutrons, ß and α rays). Non-ionizing radiation consists of ultraviolet, visible light, infrared, microwaves, and radio waves. Biological effects of radiation such as cancer occur mainly with ionizing radiation types. Mobile phones are also a source of non-ionizing radiation. There is an increased risk of developing thyroid cancer in those exposed to high-dose ionizing radiation (3). However, the relationship between the effects of non-ionizing EMR and cancer development has not been definitively proven. According to the results of the INTERPHONE study, no increase was found in the incidence of glioma, meningioma, and acoustic neuroma in long-term mobile phone users (4). The thyroid gland is close to the location where mobile phones are used and is located in the electromagnetic field. Studies have shown that EMR can cause thyroid dysfunction (5). Although Milham and Morgan (6) stated that the incidence of thyroid cancer increases in people with excessive cell phone use, according to most studies in the literature, EMR does not show a carcinogenic effect (7).

Exosomes are extracellular vesicles that are produced and released by different cells, have a size of approximately 30-100 nm, consist of proteins, DNA, mRNAs, microRNAs (miRNAs), and lipids, and provide intercellular communication. Because of the presence of miRNAs located in exosome vesicles in biological fluids, many studies have been conducted on their use as non-invasive biomarkers in cancers (8,9). It plays crucial roles in biological processes, including apoptosis, proliferation, differentiation, and cell growth, with a role in the regulation of gene expression. Each miRNA molecule can bind to several mRNAs and different miRNAs in the mRNA. This may be an important reason why we see different clinical conditions in each patient, even though they have the same tumor type. Numerous studies are ongoing regarding the potential of miRNAs to be used as biomarkers in oncology because of their important role in directing mRNA and subsequent gene expression in both normal and tumor tissues. Changes in the regulation of miRNAs can cause carcinogenesis and cancer progression, and miRNA molecules function as oncogenes in some cancers and as tumor suppressor genes in others. For this purpose, several studies have been conducted on different miRNA panels. For example, it has been reported that miRNA-30 is downregulated after EMR and activates autophagy (10). Recently, the

issue of miRNAs associated with thyroid cancers has also been investigated. Recently, Zhang et al. (11); reported that miR-574-5p/FOXN3 is important in the progression of thyroid cancers. In two other studies conducted with the same miRNA, it was reported that the upregulation of miRNA-574-5p was oncogenic for thyroid cancers (12,13). In studies related to ionizing radiation and miRNA, Li et al. (14) showed that miR-30 plays an important role in radiation-induced apoptosis by directly targeting hematopoietic cells. According to Hao et al. (10), the downregulation of miRNA-30 after EMR exposure is provided by the expression modulation of Beclin, which activates autophagy.

Our aim in this study was to evaluate the relationship between mobile phone use and miRNA-574-5p and miRNA-30 in patients with thyroid cancer.

Materials and Methods

Patient and Healthy Volunteer Selection

Patients diagnosed with differentiated thyroid cancer and evaluated for radioactive iodine treatment by the Departments of Nuclear Medicine and Endocrinology and Metabolism were included in the study. Patients who had received radioactive iodine treatment before and did not agree to participate in the survey were excluded from the study. In addition, patients with another cancer history were not included in the study. While blood was drawn for routine tests during hospitalization for RAI treatments, additional blood was taken to determine miRNA-574-5p and miRNA-30C-5p gene expression levels.

Healthy volunteers were included in the study and the number of patients. Healthy volunteers were selected from those who had not been diagnosed with thyroid cancer and who had no thyroid nodules. The healthy volunteers included in the study were selected from people who were similar to the thyroid cancer patient group in terms of age and gender. Before being included in the study, neck ultrasonography was performed by specialist physicians in the Department of Endocrinology on all healthy volunteers. Blood was collected from healthy volunteers who met the study criteria to determine the gene expression levels of miRNA-574-5p and miRNA-30C-5p.

In addition, questions related to the use of mobile phones by patients and healthy volunteers were answered orally.

Ethical approval for this study was obtained from the Cumhuriyet University Clinical Research Ethics Committee (decision no: 2020-05/02, date: 21.05.2020).

Collection of Blood Samples From the Patient and Healthy Volunteer Groups

During the radioactive iodine treatment of the patients, while routine blood tests (serum thyroid stimulating hormone, thyroglobulin and anti-thyroglobulin antibody) were performed before hospitalization, approximately 2 mL of blood was taken into Paxgene tubes, which will provide RNA stabilization, in the same session, and these blood were stored at -80 °C until all patients were completed. Blood was collected from a similar number of healthy volunteers in the same way as in the patient group and stored.

Gene Expression Analysis Using Real Time-polymerase Chain Reaction (RT-PCR)

RNA Isolation From Blood

RNA isolation was performed according to the manufacturer's protocol using the RNeasy Plus mini isolation kit.

ComplementaryDNA (cDNA) Synthesis

To determine the expression levels of miRNA-574-5p and miRNA-30C-5p by RT-PCR, cDNA synthesis from RNA was performed in accordance with the appropriate kit protocol.

RT-PCR Analysis

RT-PCR analysis of *miRNA-574-5p (YP02116206, A.B.T/ABT-PRMR)* and *miRNA-30C-5p (YP00204783, A.B.T/ABT-PRMR)* genes was performed using the ABT 2X Q-PCR SYBR-Green MasterMix (Without ROX) kit. All cDNA samples were run 3 times under the same conditions. The mean of these three measurements was used in the analyses. In the study, the housekeeping gene *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* (PPH00150F, Qiagen) was used as an internal control to determine the differences in expression levels between the control and study groups.

Analysis of the RT-PCR Data

Analysis of all RT-PCR data of gene expression experiments was performed using Rotor-gene 6000 Series Software Version 1.7. Statistical analysis of the data with the ΔΔCT method was performed using the software "RT2 profiler RT-PCR Array Data Analysis version 3.5" (http://pcrdataanalysissabiosciences.com/pcr/arrayanalysis.php).

Statistical Analysis

SPSS version 24 software was used for statistical analysis. Descriptive quantitative data are expressed as median values, and qualitative data are expressed as percentages. Fisher's exact test and chi-square test were used to compare variables. Whether the variables showed normal

distribution was evaluated by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed using median and interquartile range for non-normally distributed variables. Student's t-test was used in the analysis of normally distributed data, whereas the Mann-Whitney U test was used in the evaluation of data that did not show normal distribution. A p-value of 0.05 was considered to indicate a statistically significant result.

Results

Similar to the literature, the frequency of female patients was much higher, and 41 (82%) of the 50 patients included in the study were female and 9 (18%) were male. The median age was 47 years (range: 17-71 years) in the patients, 46 years (range: 17-71 years) in women, and 51 years (range: 17-81 years) in men. Of the patients, 47 (94%) were papillary and 3 (6%) were follicular. The median tumor size was 17.5 mm (range: 8-70). Of the patients, 23 (46%) were under the age of 45, and 27 (54%) were ≥45 years old. Tumor size was ≤2 cm in 30 (60%) patients, and tumor size was greater than 2 cm in 20 (40%) patients. While the tumor was in one focus in 22 (44%) patients, the tumor was in 2 foci in 10 (20%) patients, the tumor was in 3 foci in 7 patients (14%), and the tumor was in more than 3 foci in 11 (22%) patients. All demographic and clinicopathological data of the patients are presented in Table 1.

When the answers given to the questionnaire in the patient group were evaluated, seven of the patients (14%) had a family history of thyroid cancer. A patient (railroad worker) said he had worked in the radiation field for several years. Except for this patient, the other patients had no history of radiation. Seven of our patients (14%) did not have a history of using mobile phones with internet access. Of these 7 patients, 2 had no history of cell phone use. The median mobile phone usage time of the patients was 10 years (range: 1-25 years), and the median daily cell phone usage time was 3 h (range: 0.5-15 hours). While 81.4% of the patients did not use headphones during long conversations, 83.7% did not turn off their phones while sleeping. 53.5% of the patients had a mobile phone with them while they were sleeping. 5 of the patients did not have a Wi-Fi device at home. In 25.6% of the patients, the Wi-Fi device was in the bedroom, and 90.2% of the patients did not turn off the Wi-Fi device while they were sleeping.

Our study included 50 healthy volunteers. Of these, 33 (66%) were female and 17 (34%) were male. The median age of healthy volunteers was 47.5 (range: 18-66 years). When the

Table 1. Demographic and clinicopathological data of patients

Patient characteristics	**n (%)**
Gender	
Female	41 (82)
Male	9 (18)
Histopathological classification	
Papillary carcinoma	47 (94)
Follicular carcinoma	3 (6)
Age	Median: 47 (range: 17-71)
<45	23 (46)
≥45	27 (54)
Tumor size	Median: 17.5 mm (range: 8-70 mm)
Number of tumor foci	
1	22 (44)
2	10 (20)
3	7 (14)
>3	11 (22)
Presence of lymphovascular invasion	
Present	8 (16)
Absent	42 (84)
Presence of perineural invasion	
Present	3 (6)
Absent	47 (94)
Tumor capsule invasion	
Present	8 (16)
Absent	42 (84)
Thyroid capsule invasion	
Present	14 (28)
Absent	36 (72)
Soft tissue invasion	
Present	14 (28)
Absent	36 (72)
Thyroid parenchyma invasion	
Present	4 (8)
Absent	46 (92)
Lymph node metastasis	
Present	5 (10)
Absent	45 (90)
Distant metastasis	
Present	5 (10)
Absent	45 (90)

responses of healthy volunteers to the questionnaire were evaluated, 9 (18%) had a family history of thyroid cancer. None of the healthy volunteers had a history of high-dose radiation exposure to the neck region. However, 4 patients had a history of laser epilation in the neck area. Only 1 (2%) of the healthy volunteers had a history of using a mobile phone with internet access. The median phone usage time of healthy volunteers was 13 years (range: 2-23 years), and the daily median phone usage time was 4 h (range: 0.5-15 hours). Of the healthy volunteers, 86% did not have a history of using headphones for prolonged conversations, and 90% did not turn off their phones while sleeping. In 83.7% of the healthy volunteers, their mobile phone was with them while they were sleeping. 1 of the healthy volunteers did not have a Wi-Fi device at home. Of the healthy volunteers, 6.1% had a Wi-Fi device in the bedroom, and 93.9% did not turn off the Wi-Fi device while sleeping. The results of the questionnaire answers of the patients and healthy volunteers are given in Table 2.

Statistical Analysis of RT-PCR Results

Expression levels of *miRNA-30C-5p* and *miRNA-574-5p* genes were analyzed by RT-PCR in the differentiated thyroid cancer patient group and healthy volunteer group. *GAPDH* was used as a housekeeping gene in our study. Normalization of the expression levels of genes was achieved with GAPDH.

Table 2. Comparison of the survey results of patient and healthy volunteer groups

	Patient n (%)	**Healthy volunteer n (%)**	**p-value**
Family history of thyroid cancer			
Present	7 (43.8)	9 (56.3)	
Absent	43 (51.2)	41 (48.8)	0.585
High-dose radiation exposure to the neck			
Present	-	-	
Absent	50 (100)	50 (100)	0.315
Smartphone usage			
Present	43 (46.7)	49 (53.3)	
Absent	7 (87.5)	1 (12.5)	0.027*
Phone usage time (years) Median (range)/year	10 (1-25)	13 (2-23)	0.115
Daily usage time (hour) Median (range)/hour	3 (0.5-15)	4 (0.5-15)	0.667
Headphone use			
Present	8 (57.1)	6 (42.9)	
Absent	35 (44.9)	43 (55.1)	0.397
Do you turn off the phone while sleeping?			
Yes	7 (63.6)	4 (36.4)	
No	36 (44.4)	45 (55.6)	0.231
Is the phone with you while you sleep?			
Yes	23 (35.9)	41 (64.1)	
No	20 (71.4)	8 (28.6)	0.002*
Is there a Wi-Fi device in the bedroom?			
Yes	11 (78.6)	3 (21.4)	
No	33 (41.8)	46 (58.2)	0.011*
Do you turn off the Wi-Fi device while sleeping?			
Yes	4 (57.1)	3 (42.9)	
No	37 (44.6)	46 (55.4)	0.522

*Sign indicates p<0.05. Seven patients and one healthy volunteer did not use smartphones. In addition, 5 patients and 1 healthy volunteer did not have a Wi-Fi device at home. For this reason, the number of subjects was missing in some analyses

Statistical analysis of RT-PCR data was performed using Rotor-Gene 6000 software, RT² SYBRGreen qPCR Array Data Analysis version 3.5. The data obtained because of the experiment were analyzed using the ΔΔCT method in the RT-PCR device. RT-PCR data were evaluated using Student's t-test. If the fold change value (Fold change =2-ΔΔCt) is greater than one, it means that the gene expression level is increased, but if the fold change value is less than one, it indicates that the gene expression level is decreased. Fold regulation is the adaptation of fold change results to biological systems. If the fold change value (2-ΔΔCt) is greater than one, it is equal to the fold regulation value; however, if the fold change value (2-ΔΔCt) is less than one, the fold regulation value is the negative inverse of the fold change value. Because of the analysis of the data, the average Ct, fold change, and fold regulation values of each gene region are shown in Table 3.

Although there was a 7.60-fold increase in miRNA-30C-5p gene expression levels in the patient group compared with the healthy volunteer group, it was not found to be statistically significant (p=0.142). Considering the miRNA-574-5p gene expression levels, although there was a 2.96-fold increase in the patient group compared with the healthy volunteer group, no significant relationship was found (p=0.464) (Table 3, Figure 1).

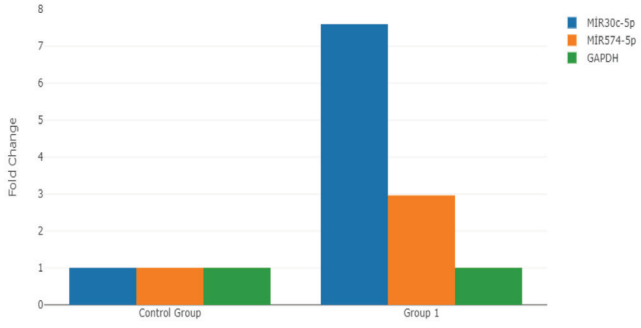

Figure 1. Comparison of expression levels of *miRNA-30C-5p* and *miRNA-574-5p* genes in patient and control groups

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

*Fold change = $2^{(-\Delta\Delta Ct)}$

**Fold regulation = -1/ΔΔCt

ΔCt tumor = Mean Ct target gene tumor - Mean Ct GAPDH tumor

ΔCt control = Mean Ct target gene control - Mean Ct GAPDH control

ΔΔCt = ΔCt (target gene tumor - GAPDH tumor) - ΔCt (target gene control - GAPDH control)

When the histopathological data of the patients were compared with the expression levels of *miRNA-30C-5p* and *miRNA-574-5p* genes, thyroid cancer patients with positive lymph node metastasis, lymphovascular invasion, blood vessel invasion, perineural invasion, tumor capsule invasion, surrounding soft tissue invasion, and thyroid parenchyma invasion were compared with the negative group. Although there were fold increases in miRNA-30C-5p and miRNA-574-5p expression levels in the positive group, these parameters were not statistically significant (Table 4).

"Smartphone usage", "Do you turn off the phone while sleeping?", "Is the phone with you while you sleep?", "Is there a Wi-Fi device in the bedroom?" and "Do you turn off the Wi-Fi device while sleeping?" questions were asked, and the answers given to these questions were evaluated in the patient group. In the patient group who answered yes to the questions, when evaluated in terms of miRNA-30C-5p and miRNA-574-5p expression levels, although there were fold increases, no statistically significant relationship was found. When evaluated in terms of miRNA-30C-5p and miRNA-574-5p expression levels in the control group with the same questions, although there were fold increases, no statistically significant relationship was found. When the answers given to the same questions were evaluated by considering the patient and control groups together, when evaluated in terms of miRNA-30C-5p and miRNA-574-5p expression levels, although there were fold increases, no statistically significant relationship was found (Table 5).

Table 3. CT, fold change, and fold regulation results of RT-PCR data from thyroid cancer and healthy volunteer groups

Genes	Group	Median CT	Standard deviation	Fold-change	p-value
miRNA-30C-5p	Patient Control	21.63 22.69	1.21 1.12	**7.60**	0.142
miRNA-574-5p	Patient Control	18.02 8.11	2.22 3.41	**2.96**	0.464
GAPDH	Patient Control	24.50 22.21	2.51 3.79	1.00	N/A

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, RT-PCR: Real time-polymerase chain reaction, CT: Cycle threshold

Discussion

miRNAs are a group of small, single-stranded, non-coding RNAs approximately 22 nucleotides long. According to various studies, irregularities in miRNAs have been implicated in various pathological processes, including tumorigenesis, making miRNAs a promising diagnostic and therapeutic target in the future (15). Changes in miRNAs cause genetic and epigenetic changes by activating proto-oncogenes or inactivating tumor suppressor genes. Thyroid cancers are the most common endocrine system malignancies, and the aim of this study was to evaluate the relationship between cell phone use and miRNA-574-5p and miRNA-30C-5p expression levels in patients diagnosed with differentiated thyroid cancer.

Human miR-574-5p is encoded by miR-574 (gene ID: 693159) on human chromosome 4p14 and is produced in a multi-step process (16). miR-574-5p is a candidate oncogenic target for different types of cancer (17,18). The first study investigating the relationship between this gene expression and thyroid cancer was conducted in 2013 by Fan et al. (19). According to this in vitro study, an inverse correlation was observed with miRNA-574-5p in thyroid cancer cells. Zhang et al. (13) found that abnormal upregulation of miR-574-5p had an oncogenic effect by regulating the Wnt/β-catenin pathway by targeting quaking proteins (QKI). According to the data of our study, miR-574-5p gene expression levels in the differentiated thyroid cancer patient group were found to be higher than those in healthy volunteers, although the difference was not statistically significant. When the patients were evaluated according to histopathological parameters, although there was an increase in miRNA-30C-5p and miRNA-574-5p expression levels in those with positive lymph node metastasis, lymphovascular invasion, blood vessel invasion, perineural invasion, tumor capsule invasion, peripheral soft tissue invasion, and thyroid parenchyma invasion, there was no statistically significant relationship (Table 4).

The miRNA-30 family is known as a tumor suppressor and plays an important role in the development of many cancer types (20). It has been reported that miRNA-30 is downregulated after EMR and activates autophagy (10). Braun et al. (21) reported that miRNA-30 can reduce the invasive potential of anaplastic thyroid carcinoma

Table 4. Comparison of the expression levels of *miRNA-30C-5p* and *miRNA-574-5p* genes in patients with histopathological parameters

	miRNA-30C-5p		miRNA-574-5p		GAPDH	
	Fold-change	p-value	Fold-change	p-value	Fold-change	p-value
Lymph node metastasis (+/-)	7.21	0.51	9.79	0.56	1.00	N/A
Lymphovascular invasion (+/-)	0.62	0.56	1.06	0.75	1.00	N/A
Blood vessel invasion (+/-)	0.45	0.50	2.10	0.50	1.00	N/A
Perineural invasion (+/-)	3.19	0.95	13.19	0.15	1.00	N/A
Tumor capsule invasion (+/-)	1.76	0.67	1.02	0.30	1.00	N/A
Thyroid capsule invasion (+/-)	2.20	0.98	1.31	0.48	1.00	N/A
Soft tissue invasion (+/-)	1.95	0.84	1.39	0.62	1.00	N/A
Thyroid parenchyma invasion (+/-)	0.82	0.88	0.58	0.54	1.00	N/A

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Table 5. When the patients and healthy volunteers were evaluated together, the relationship between the expression levels of *miRNA-30C-5p* and *miRNA-574-5p* genes was determined according to their responses to smartphone and Wi-Fi usage

	miRNA-30C-5p		miRNA-574-5p		GAPDH	
	Fold-change	p-value	Fold-change	p-value	Fold-change	p-value
Smartphone usage (+/-)	**6.06**	0.45	**2.58**	0.50	1.00	N/A
Do you turn off the phone while sleeping?	**8.07**	0.31	**3.12**	0.75	1.00	N/A
Is the phone with you while you sleep? (+/-)	**7.70**	0.33	**2.82**	0.72	1.00	N/A
Is there a Wi-Fi device in the bedroom? (+/-)	**8.85**	0.46	0.99	0.40	1.00	N/A
Do you turn off the Wi-Fi device while sleeping?	**8.73**	0.33	**3.28**	0.71	1.00	N/A

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

cells. According to the data of our study, miRNA-30C-5p expression levels in patients with differentiated thyroid cancer were found to be higher than those in healthy volunteers, although the difference was not statistically significant.

Epidemiological studies have shown that the incidence of thyroid papillary carcinoma has been increasing since the 1980s. At the same time, a rapid increase is observed in the use of mobile phones (7). Because exposure to ionizing radiation has an important role in the development of cancer, many studies have been conducted and continue to be conducted on whether EMR exposure due to mobile phones will cause cancer. In a study conducted on rats, exposure of the whole body to 900 MHz pulse-modulated RF radiation similar to that emitted by the Global System for Mobile Communications (GSM) caused pathological changes in the thyroid gland (22). According to the results of the INTERPHONE study, no increase was found in the incidence of glioma, meningioma, and acoustic neuroma in long-term mobile phone users (4). According to the results of the prospective study by Benson et al. (23), no association was found between cell phone use and glioma, meningioma, or other cancers not originating from the central nervous system. Silva et al. (7) studied thyroid cells using radiofrequency energy and found that this type of radiation did not show a potential carcinogenic effect. Luo et al. (24) found no relationship between cell phone use and the development of thyroid cancer in their study, which included 462 patients and 498 control groups. However, there was an increased risk of microcarcinoma (tumor size ≤10 mm) in those with a higher frequency of mobile phone use. Wi-Fi is a technology that has an important place in human life. In our study, 85% of our patients were using mobile phones with internet access, whereas 98% of our healthy volunteers were using mobile phones ($p<0.05$). Contrary to our expectations, according to the questionnaire, the habit of keeping a mobile phone in the bedroom while sleeping was higher in healthy volunteers than in patients ($p<0.05$). On the other hand, while there was a Wi-Fi device in their bedrooms in 78.6% of the patients, this rate was much lower (21.4%) in healthy volunteers ($p<0.011$). No difference was detected by turning off the Wi-Fi device while sleeping.

Study Limitations

The biggest limitation of our study was the small number of patients. In addition, some responses to survey studies were not certain (for example, how many hours of phone usage per day? etc).

Conclusion

Although there was a 7.60-fold increase in miRNA-30C-5p gene expression levels in the patient group compared with the control group, it was not found to be statistically significant. When miRNA-574-5p gene expression levels were evaluated, although there was a 2.96-fold increase in the patient group compared with the control group, no significant relationship was detected. Differentiated thyroid cancer patients and their healthy volunteers were asked about "Do you have smart mobile phone use?", "Do you turn off the phone while sleeping?, "Do you have mobile phones near while sleeping?", "Is there Wi-Fi device in bedroom?", "Do you turn off Wi-Fi device while sleeping?" When the answers given to the questions miRNA-30C-5p and miRNA-574-5p expression levels were evaluated, although there were fold increases, no statistically significant relationship was detected. However, because of the limited number of patients, which is the biggest limitation of our study, we recommend that these studies be conducted with larger numbers of patients and healthy volunteers.

Although there was no significant difference between miRNA levels, we believe that the effects of EMR on the thyroid gland can be reduced by paying attention to this simple precaution, since there is a higher rate of Wi-Fi devices in the bedrooms than in healthy volunteers.

Ethics

Ethics Committee Approval: Ethics Committee approval was obtained from Cumhuriyet University Clinical Research Ethics Committee (decision no: 2020-05/02, date: 21.05.2020).

Informed Consent: Written informed consent forms were obtained from patients who agreed to participate in the study.

Authorship Contributions

Surgical and Medical Practices: Z.H., S.A.E., B.S., Ö.U.B., G.D., Concept: Z.H, A.T., S.A.E., B.S., Ö.U.B., Y.S., Design: Z.H., A.T., Ö.U.B., Y.S., Data Collection or Processing: Z.H., A.T., S.A.E., B.S., Ö.U.B., G.D., Y.S., Analysis or Interpretation: Z.H., A.T., Literature Search: Z.H., A.T., Writing: Z.H., A.T., S.A.E.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This project was supported by grants from Sivas Cumhuriyet University Scientific Research Project Unit (T-897).

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