RESEARCH ARTICLE

Genetic Markers for miRNA-16 and miRNA-221 in Women with Thyroid Disorders

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ABSTRACT

This study was conducted in some private laboratories in Baghdad and Salah al-Din for the period from 12/2/2020 to 25/3/2021 to investigate the role of microRNA in women with thyroid disorders (hypothyroidism, hyperthyroidism, and thyroid cancer) by measuring Gene expression levels of two genes (MiR-221 and MiR-16), where a blood sample of 21 hypothyroid women and 15 hyperthyroid women and five representatives of women suffering from thyroid tumors were collected and compared with a healthy control group with 15 samples. The gene expression levels were measured by extracting microRNA from the blood. Scientists use real-time qPCR. The statistical analyses included removing the expression difference Fold of Expression by a value of $2^{(-\Delta\Delta ct)}$. MiR-221.

There is a decrease in gene expression for both hypo and hyperthyroid women and an increase in gene expression in women with thyroid tumors. As for miR-expression levels 16, the results were similar to the effects of MiR-221. There is a decrease in gene expression in cases of hypo and hyperthyroidism and an increase in expression in cases of tumors.

This study clarified the role of miR-221 and miR16 in the possibility of thyroid diseases and tumors. Biologists determine both miR-221 and miR16 functions through the disturbance in the amount of their gene expression. It thus can be used as a biomarker that can be inferred, especially in the case of cancers.

Keywords: Female, miRNA-16, miRNA-221, Qubit-4.0, Thyroid disorders.

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INTRODUCTION

Epigenetic genetics is one of the most important fields of biological sciences in recent years, as it represents a large-scale phenomenon with multiple effects. It also enters the fields of development and influence, especially in medicine and treatment. Epigenetics is a stable genetic phenotype that results from a change in a chromosome without a difference in the DNA sequence. The actual epigenetic definitions express that the entire DNA content is the same in the somatic cells of one species. At the same time, the gene expression patterns have a clear difference in the different types of cells that can be inherited. The mechanisms of this inheritance can affect the gene's activity at the levels of transcription and post-transcription or the level of translation and post-translational modifications.[1]

Epigenetic inheritance contributed to the shift in the medical community to interest in the factors that control the gene rather than the genes themselves. For example, turning off a gene in a person can appear as if a person does not have this gene at all.[2] Epigenetic changes can occur as a result of several environmental factors. Such as pollution, diet, and smoking. These genetic deviations have caused many diseases such as metabolic, cancer, cardiovascular, aging, autoimmune, and neurological and psychological diseases.[3]

The discovery of gene expression is witnessing an expansion of research in various fields, focusing on the mechanisms that affect the development of cancer diseases or other disorders or the effect of exposure to environmental toxins and its impact on the mechanism of our bodies work. miRNAs are small, non-coding pieces of RNAs about 18–25 nucleotides in length involved in post-transcriptional gene repression in all eukaryotes.[4] Scientists discovered miRNAs in 1993 in the nematode worm. It plays a critical role in gene regulatory networks related to thyroid diseases and disorders. Furthermore, the importance of miRNA has emerged as a crucial post-transcriptional regulator in the process of gene expression by regulating cellular activities, such as differentiation, growth, immune regulation, and programmed cell death.[5]

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Approximately 17,000 miRNAs have been identified, with more than 1,900 present in humans. The discovery of this miRNA revolutionized the regulation and understanding of genes and led to the identification of multiple classes of small RNAs involved in gene regulation.[6]

miRNA can be detected in plasma and serum because it circulates stably in the blood and various body fluids, and miRNA molecules may participate in the pathogenesis of the body by working as cellular signals from one cell to another. Broad miRNA expression enables the distinction between benign tissues from their malignant counterparts. The mechanisms of miRNA effect in cancer development are related to downregulation of tumor suppressor genes or upregulation of oncogenes.[7]

It includes different types of miRNAs related to the thyroid gland (miR16, miR22, miR221, miR222, miR375and miR451), which affect thyroid disorders and thyroid cancer.[8]

The miR_16 gene and the miR_221 gene have a role in thyroid disorders and thyroid cancer, especially in regulating the work of many thyroid-specific cellular signals. It causes a decrease in gene expression in cases of hypo and hyperthyroidism and an increase in gene expression in cases of thyroid cancer (Table 1).

MATERIALS AND WORKING METHODS

Collection of Blood Samples

Researchers use sterile medical syringes with a capacity of 5 mL to draw the blood, then 0.5 mL of that blood is taken and added to 0.75 mL of TriZOL solution. The blood is mixed with the solution several times, up and down, until it becomes homogeneous. Then it is preserved and frozen for later use miRNA Quantitation by Qubit 4.0 The assay is highly selective for miRNA over other types of RNA and is accurate for initial sample concentrations from 10 to 100 ng/uL. Scientists perform an assay at room temperature, and the signal is stable for 3 hours. Experiments elucidated that contaminants including salts, free nucleotides, solvents, detergents, or protein are most efficient in the assay.

Biologists prepare the Qubit® working solution by diluting the Qubit® miRNA HS

Reagent 1:200 in the Qubit® miRNA HS buffer. They then add the volume 190 μL from Qubit® working solution to each tube designed to be as a standard, then 10 uL from each provided standard answer has been added into the same pipes, then vortexed. Biologists then add the Qubit® working solution as 197 uL to each tube prepared for the sample, and then 3 uL of the model has been added individually. All composition has been vortexed and incubated at room temperature for 3 mins. Next, laboratories insert standards tubes in the Qubit instrument for creating a concentration curve. They then add tubes one by one to read the concentration for miRNA in each sample.

Primers

All primers used in this study were sourced from Macrogen (South Korea)

RESULTS

miRNA Gene Expression

This study included detecting the gene expression of miRNA16, miRNA221 in patients with thyroid disorders compared with the molecule (U6snRNA) (small nuclear RNA), which is one of the members of the complex composed of several molecules found Inside the nucleus. It has a vital role in modifying the mRNA SpliceoSome in eukaryotic cells. Its expression is constant in all organism cells, so it was relied upon as one of the genes used in measurement or calibration (House Keeping genes) with RT-qPCR results.[9]

Biologists identified the relationship of the change of these molecules (miRNA 16 and miRNA 221) with the clinical characteristics of the patients. It was preserved through the results obtained from experiments using RT-qPCR technology.

Investigators observed the gene expression of miRNA-221 and miRNA-16 by the RT-qPCR technique, where miRNA molecules were extracted and converted into cDNA molecules, then a reaction was performed. RT-qPCR.

miRNA-221 Gene Expression

The CT values for hyperthyroidism samples ranged between (15.15–24.04), while the ΔCT values ranged between (-3.54–-2.27), and the ΔΔCT values ranged between (0.32–3.11). Thus, data depict a decrease in the rate of the Fold Expression (FE) gene expression level. $2^{-\Delta\Delta CT}$ of miRNA-221 by 11.11_50 times in cases of hyperthyroidism.

As for the hypothyroidism samples, the CT values ranged between (15–27.37), the ΔCT values ranged between (-3.55–3.61), and the ΔΔCT values ranged between (0.92.98). Data later concluded that a molecule's decreased gene expression (FE)

Table 1: Prefixes used in the study

Name of Primer	*Sequence (5'→3')*
MiRN 221 ART	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATT GCACTGGATACGACGAAACCC
MiR-221	F-GGAGCTACATTGTCTGCTGG R-CAGTGCGTGTCGTGGAGT
MiRN A16 RT	GTCTCCTCTGGTGCAGGGTCGAGGTATTCGCACCA GAGGAGACCGCCAA
MiR-16	F-CAGCCTAGCAGCACGTAAAT R-GAGGTATTCGCACCAGAGGA
U6	F-CTCGCTTCGGCAGCACA R-AACGCTTCACGAATTTGCGT

might occur (Table 2–4). miRNA-221 in an amount ranging from (1.1–10) in cases of hypothyroidism. Gene expression primarily decreases due to the presence of nuclear receptors. They consist of proteins found inside cells that sense the presence of hormones or molecules that bind to the receptors to work in concert with vital factors to regulate gene expression. Nuclear receptors are transcription factors. They can bind to DNA. Naturally, with its associated genes and in hyperthyroidism disorders, we see a significant decrease in the ability of hormonal binding. This indicates the exhaustion of the number of nuclear receptors. The reduction in gene expression is a protective mechanism against high levels of thyroid hormones in tissues. There is no similar study of decreased gene expression in diseases. Hypothyroidism and hyperthyroidism concerning the miR-221 gene.

As for thyroid tumor samples, the CT values ranged between (14.93–26.01), and the ΔCT values ranged between (-10.31– -3.83), and the ΔCT values ranged between (2.72–0.21). In addition, Tables represented that the FE gene expression level of miRNA-221 increased by between (0.15–3.01) times, noting that one value was deleted due to reading extremes compared to the average FE level of the typical sample.

Differentiated thyroid cancers (DTCs) include the two most common histological types, papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), which make up about 94% of all thyroid cancer cases. However, both PTCs and FTCs may progress to poorly differentiated thyroid carcinomas (PDTCs) or may lose differentiation completely and transform into anaplastic thyroid cancer (ATC).[10]

Some studies by Zembska *et al*. (2019) suggested a vital role for miRNAs as critical factors in the development of thyroid cancer. Moreover, functional studies indicated that miRNA downregulation might play an essential role in thyroid carcinogenesis. Abnormal miRNA expression was found that characterizes PTCs about normal thyroid tissue, especially the vital regulation of miR-221.MiR-221 and miR-222 are endogenous regulators of p27Kip1 protein expression, an essential cell cycle regulator. This study showed that three types of miRNAs (miR221-222- 146) are relatively upregulated in PTC tumors compared to thyroid tissue. Many genes were directly and indirectly regulated by miR-221 and confirmed downregulation of the HOXB5 gene by endogenous or exogenous miR-221.[11]

Table 2: Fold expression values of MiR-16, MiR-221 genes in the first cycle

Fold Expression -16	*Fold Expression-221*	*Sample*	*Number*
0.03	0.28	11	
0.57	0.78	18	2
0.19	0.26	21	3
2.09	1.15	26	4
0.05	0.07	30	5
0.03	0.02	31	6
0.03	0.77	32	7
1.28	2.11	35	8
0.15	0.27	36	9
4.5	1.9	37	10
0.04	0.02	38	11
0.87	0.9	39	12

Gene Expression of miRNA-16 Molecules

The CT values of the hyperthyroidism samples ranged between (12.23–20.3), while the ΔCT values were between (-3.9– -1.89), and the ΔΔCT values ranged between (0.54–2.87), and a decrease in the level of fold expression (FE) was observed.) 2- ΔΔCT of the miRNA-16 molecule by (13-30) times in cases of hyperthyroidism

As for the samples of hypothyroidism disorders, CT values ranged between (16.4–22.2), CTΔ values were between (-4.1_ -3.52), and ΔΔCT values ranged between (0.6–2.03). Furthermore, the Fold Expression level rate decreased (FE) $2^{-\Delta\Delta CT}$ of miRNA_16 molecule ranged between (10-50) times in cases of hypothyroidism.

Decreased gene expression in hyperthyroidism and hypothyroidism happens when cell proliferation and migration decrease. Thus, they promote increased levels of programmed cell death. In addition, the binding of the TSH-stimulating hormone with the receptors designated for binding reduces the activation of the enzyme Adenyl Cyclase located on this membrane, resulting in a defect in the enzymatic activity of the gland cells and thus a decrease in the secretion of thyroid hormones.

The lack of gene expression affects the transport and regulation of iodine, reduces its binding to tyrosine, affects the metabolism of carbohydrates and fats, and reduces the breakdown of proteins. These results are in agreement with what was presented (Figures 1–3).[12]

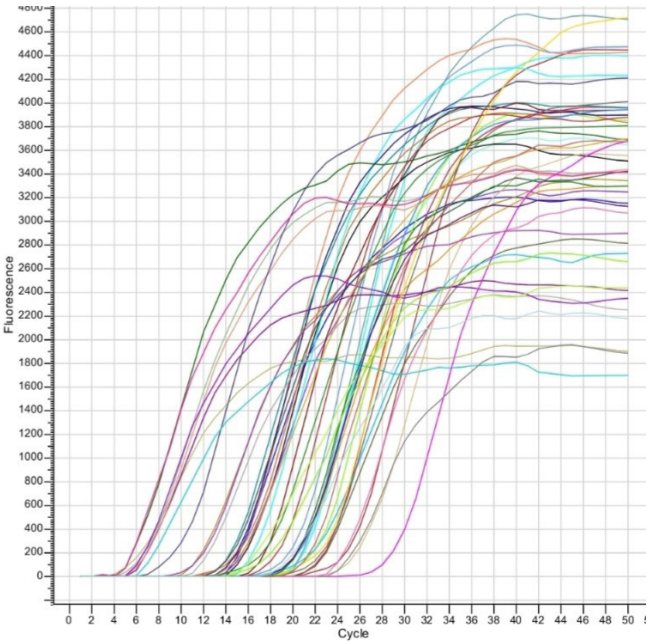

Figure 1: Graph of the amount of gene expression of MiRNA gene for both patients and those infected with the first cycle

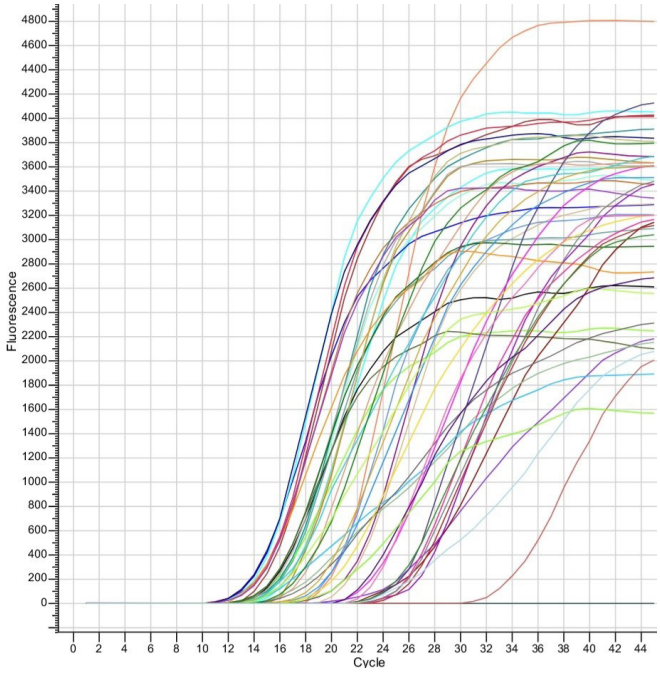

Figure 2: Graph of the amount of gene expression of MiRNA gene for both patients and those infected with the second cycle

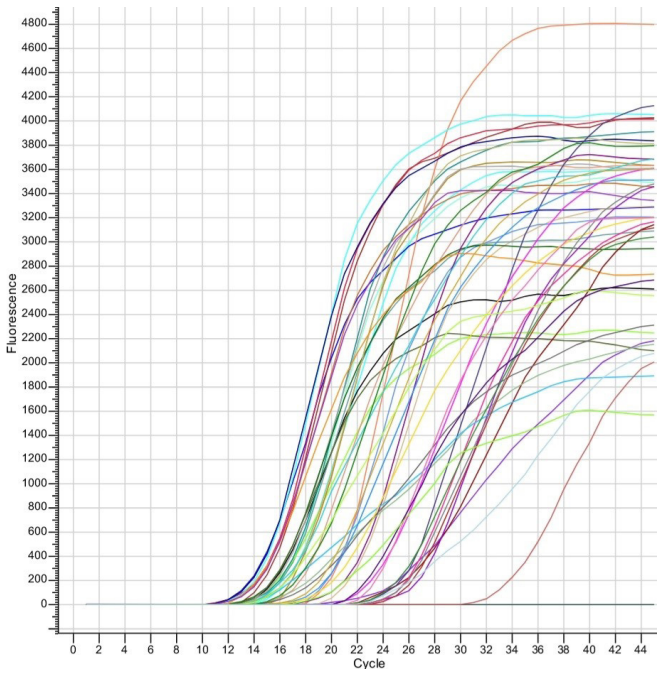

Figure 3: Graph of the amount of gene expression of MiRNA gene for both patients and those infected with the third cycle

Table 3: Fold expression values of MiR-16,MiR-221 genes in the second cycle

Second Run			
Fold expression-16	*Fold expression-221*	*Sample*	*Number*
0.4	0.8	41	1
0.06	0.16	42	2
0.27	0.29	45	3
0.75	0.53	46	4
5.2	4.01	47	5
0.06	0.06	48	6
0.27	0.05	51	7
0.77	0.7	54	8
0.96	0.25	64	9

Table 4: Fold expression values of MiR-16,MiR-221 genes in the third cycle

Third Run			
Fold expression-16	*Fold expression -221*	*Sample*	*Number*
0.07	0.74	67	1
0.9	0.02	82	2
0.33	0.08	85	3
1.7	3.27	88	4
0.09	0.04	89	5
0.14	0.03	90	6
0.35	0.9	65	7
0.44	0.79	63	8
0.05	0.2	69	9
0.26	0.2	77	10
0.03	0.05	81	11
0.06	0.08	49	12
0.25	0.9	55	13
0.09	0.57	58	14
0.38	0.08	59	15
0.08	0.03	60	16
0.5	0.59	0	17
0.04	0.12	25	18
0.67	0.02	27	19
0.19	0.1	28	20

As for thyroid tumor samples, CT values ranged between (13.3–25.5), ΔCT values ranged between (-11.1– -3.87), and ΔΔCT values ranged between (-3.45– -0.18), where results showed an increase in the expression level of FE gene regulation by (10-1) time.

Accelerating cellular proliferation increased cell number, and migration explains gene overexpression. In addition, some recent studies have shown that the expression levels of specific molecular molecules in thyroid tumor tissues are related to pathological characteristics such as tumor size and extra-thyroid expansion. These results are consistent with studies conducted by Chapella & Jazedzewski.[13]

CONCLUSION

Decreased level of miR-16 and miR221 microRNA gene expression, depending on the value of Fold Expression in cases of hypothyroidism and hyperthyroidism.

High level of miR-16 and miR-221 microRNA gene expression in thyroid tumors.

RECOMMENDATIONS

It requires to onduct more comprehensive studies that include diagnosing other types of microRNA in blood samples of people with thyroid disorders, especially thyroid tumors, and comparing them with healthy subjects.

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