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miRNA-155 as a Biomarker for Detection of Graves' Disease

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Abstract

Objective: Main goal of this learn is to know molecular mechanism that links miRNA-155 to the pathophysiology of Graves' disease, as this would help in revealing innovative paths and methods for diagnosing and treating the disease, as this disease is considered an autoimmune problem that affects the thyroid gland and leads to other complications such as eye and skin disorders.

Subjects and Methods: The study included 200 samples, 100 of which were from infected people and the other from healthy people for comparison purposes. Their ages ranged between 20-40 years from visitors to Al-Diwaniyah Teaching Hospital in the period between March to September 2025. 5 ml of blood was collected from all participants, 1 ml of each sample was used in the polymerase chain reaction to detect miRNA155 using PCR technology. The remaining 4 ml of each sample was used to measure interleukin levels by enzyme-linked immunosorbent assay and other biochemical tests in this study.

Results: The yield of the statistical analysis disclose that there were significant statistical variable amidst two groups regarding the level of miRNA-155 (p=0.05). While the difference was clear regarding the level of interleukin-7, as it was high in the serum of patients compared to healthy people (p=0.001), while interleukin-4 was lessen in serum of patients compared to control (p=0.001), As for TRAb test, the statistical result was highly influential between the two groups (p=0.001), and the statistical differences were clear and noticeable for other biochemical tests.

Conclusion: Based on the outputs we obtained, represented by the results, there is a possibility of using miRNA 155 as a diagnostic tool to predict Graves' disease.

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Keywords: Hyperthyroidism, Graves' Disease, Interluken-7, Interlukine-4, Autoimmune Diseases, TRAb, miRNA155

1. Introduction

Graves' disease (GD) is an autoimmune problem caused by targeting of thyroid-stimulating hormone by autoantibodies infect thyroid gland and leads to increased activity and the production of its hormones is disturbed [1]. There is a great possibility that inflammatory and non-inflammatory cytokines and signaling molecules participate in the fluctuation of the immune response, which is a cause of the disease [2], of the signaling molecules are counted MicroRNAs (miRNAs) are important modulators of immune response and gene expression, as they control autoimmune processes through their influence on the development and activation of immune cells [3]. MiRNA-155 is considered one of the main controllers of T helper cell differentiation (Th) as it has the main and leading role in the balance of (Th1) and (Th2) in many autoimmune diseases [4]. It is possible that miRNA-155 stimulates the harmful autoimmunity that leads to Graves' disease, as it does so through its relationship to increased or decreased cytokines, including interleukins, examples of which are interleukin 7 (IL-7) and interleukin 4 (IL-4) used in this study [5]. Interleukin 7 likely stimulates the generation and maintenance of T cells and is therefore important for the survival and repair of these cells in the context of autoimmune processes [6]. As for interleukin-4, the reason it causes autoimmune processes is that it is considered the primary interleukin that is generated through Th2, which stimulates B cells, increases the generation of antibodies, and enhances the IgE response [7]. The relationship of micro155 to IL-7 lies in the fact that the high level of miR-155 stimulates the level of IL-7 [8], and this increases the proliferation of T cells and maintains the autoimmune process, as well as affects the level of IL-4 [9], and ultimately activates immunoglobulin by stimulating B cells.

Complications of Graves' disease and its effects lead to eye and skin disorders associated with the thyroid gland [9]. This study aims to reveal the role of miRNA-155 through its relationship with IL-7 and IL-4 in Graves' disease and the possibility of using it as a diagnostic factor for this disease or the possibility of using it as an innovative treatment by understanding the pathophysiology.

2. Research Methods

Five ml of blood was collected from 200 samples for 100 patients with Graves' disease representing group I after an physician made a clinical diagnosis of Graves' disease by a blood test, and 100 healthy representing group II. Samples were collected after patients' consent and the approval of the ethics committee in College of Dentistry University of al-Qadisiyah (no:880- Date:1-3-2025). In EDTA tubes, peripheral blood samples and were divided into two parts (1 ml) for the purpose of miRNA-55 analysis and preserved using eppendrof tubes and was measured by RT-PCR technique (BioRad-USA). While the remaining (4 ml) was separated into serum by a centrifuge for ten minutes at 3000 rpm in order to measure (IL-7) and (IL-4) using ELISA technique (Mabtech USA). And also, to perform biochemical tests, thyroid receptor antibodies (TRAb) and thyroid function parameters (T3, T4, and TSH). We used a NanoDrop spectrophotometer to measure the concentration and integrity of RNA. All samples were kept at -80°C for testing time, [Figure 1] show study design.

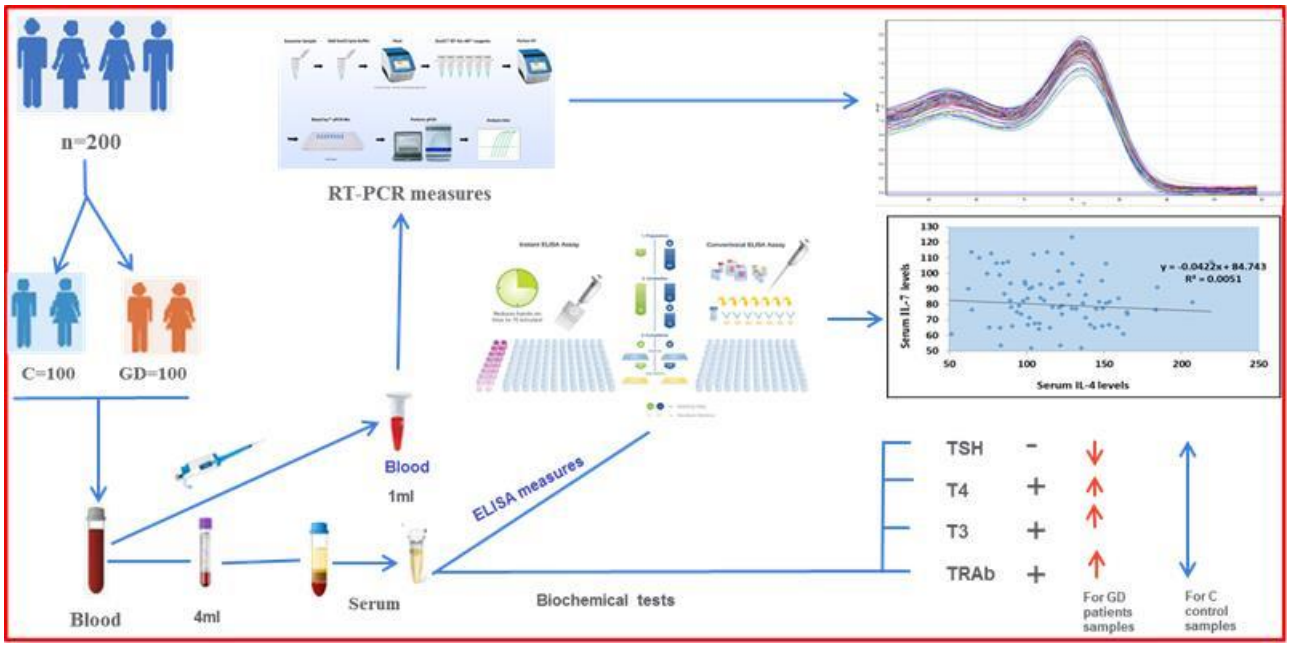

Fig 1: Study Design

Statistical Analysis

SPSS version 26 was employed to describe, analyze, and present the study results. Means and standard deviations (SD) were employed to explain quantitative variables, while frequencies and percentages were used for qualitative description. The T-test was employed to compare independent variables. Pearson's correlation coefficient was used to correlate quantitative variables. A P value of ≤0.05 was set as statistical significance.

3. Results and Findings

The results of age distribution for all the studied groups are shown in [Table 1]. The value (P = 0.699) indicates a statistically insignificant difference between the means of the two groups. [Table 2] includes the results of comparing the gender variable between the two groups, where the value (P = 0.287) indicates no statistically significant difference amidst two groups. Thyroid hormone (T3, T4 and TSH) levels were compared between the two study groups as shown in [Table 3]. Mean triiodothyronine (T3) levels were 4.51 ± 0.91 and 0.98 ± 0.05, in Graves' disease patients and healthy controls, respectively; T3 was elevated in Graves' disease patients and this was demonstrated by a significant variance amidst two groups (P < 0.001). Also mean levels of Thyroxin (T4) were 39.66 ± 5.12 and 9.86 ± 1.45, in patients and control subject respectively; The level was more in the patient compared control group, but variance was significant. The value (P < 0.001) indicates a high statistical variance amidst both groups, as T4 concentration is higher in the serum of patients than in healthy individuals. TSH levels were 2.54 ± 0.177 in patients, compared to 9.70 ± 1.48 in healthy controls, P value indicated a statistically significant variance (P < 0.001). The results in [Table 4] compared the levels of interleukin 4 and 7 amidst two groups. Present result show serum IL-7 concentrations in patients was significantly higher than healthy control subjects 205.5± 28.3 versus 157.3 ± 25.1 respectively, P < 0.001). But the serum IL-4 concentrations in patients was significantly lower than healthy control subjects 79.9 ± 11.5 versus 135.7 ± 20.2 respectively, P < 0.001). Some interleukin markers, including IL-7, are indirectly linked to IL-4, as demonstrated in patients and shown by the logistic regression model in Figure 2. This may indicate that Graves' disease condition enhances production of IL-7 in suppression the expression of IL-4. In this study, a quantitative analysis of RT-PCR analyzed expression of miR155 and comparison of its expression amidst seemingly, control and patients group. Change in gene expression was calculated utilizing relative quantitative measurement (Levac and Schmitten, 2008). Based on normalization, the Ct values used to calculate ΔCt represent the variance between the mean Ct values of the miR155 cDNA amplification transcript for each case and the U6 case.

In calculating the relative expression of the miR155 gene that included the two study groups, the 2-ΔCt results are also usable in [Table 5]. Mean of ΔCt (normalization Ct values) for each group were (-1.28) and (0.028) in the Graves' disease patients and control groups, respectively and mean of 2-ΔCt values of control (0.981) and that for Graves' disease (2.428). Gene expression was significantly larger in Graves' disease than control group. According to an analysis of RT-qPCR data, miR-155 was up regulated in Graves' disease compared to controls and variance was highly significant ($P < 0.05$). The relative fold change average of miR-155 was (2.4) in patients group compared to the control (1.0).

Table 1: Variation amidst two study groups in terms of age variable

Groups	Mean ±	SD	p-value
Graves' disease patients	34.56	7.22	0.699
Control	33.12	7.89	NS

SD: standard deviation; NS: non-significant at P > 0.05

Table 2: Comparability between patients and control groups in sex

Groups	SEX		Total	p-value
	Male	Female		
Graves' disease	28 (28.0%)	72 (72.0%)	100	0.287
Control	35 (35.0%)	65 (65.0%)	100	
Total	63 (31.5%)	137 (68.5%)	200	NS

SD: standard deviation; NS: non-significant at P > 0.05

Table 3: Comparability of thyroid hormone (T3, T4 and TSH) in patients and healthy controls

Groups		T3 (nmol/L)	T4 (µg/dL)	TSH (mIU/L)	TR-Ab (IU/L)
Graves' disease Patients	Mean	4.51	39.66	2.54	3.11
	SD	0.91	5.12	0.177	0.81
Control	Mean	0.98	9.86	9.70	1.01
	SD	0.05	1.45	*I*.48	0.133
p-value		0.001**	0.001**	0.001**	0.001**

SD: standard deviation; **: significant at P > 0.05

Table 4: Comparability of some Interleukin (IL-7 and IL-4) parameters in patients and healthy controls

Groups		IL-7 pg/mL	IL-4 pg/mL
Graves' disease Patients	Mean	205.5	79.9
	SD	28.3	11.5
Control	Mean	157.3	135.1
	SD	25.1	20.2
p-value		0.001**	0.001**

SD: standard deviation; **: significant at P > 0.05

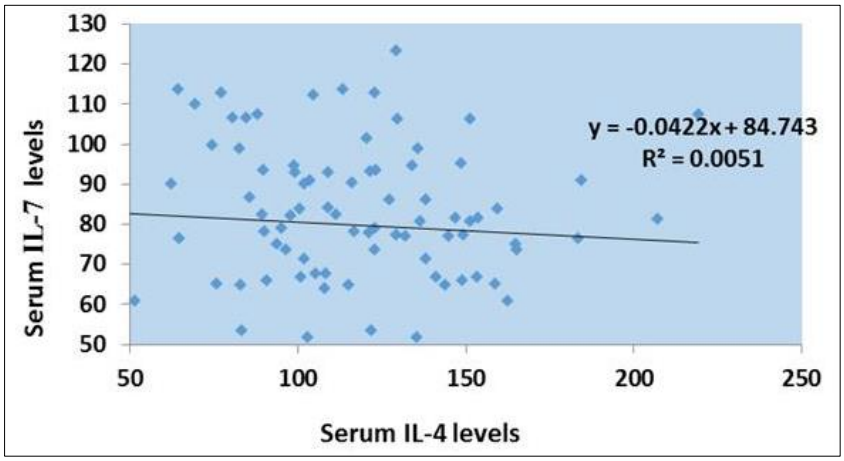

Fig 2: Logistic regression correlations between some Interleukin (IL-7 and IL-4) parameters in Graves' disease patients

Table 4: Comparison of (Ct, $2^{-}\Delta ct$ and Folding) between patients and healthy controls

Groups	Means Ct of miRNA-155	Means Ct of U6	Δ^{Ct} (Means Ct of miR155	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Graves' disease	17.26	18.54	-1.28	2.428	2.428/0.981	2.4
Control	17.59	17.56	0.028	0.981	0.981/0.981	1.00

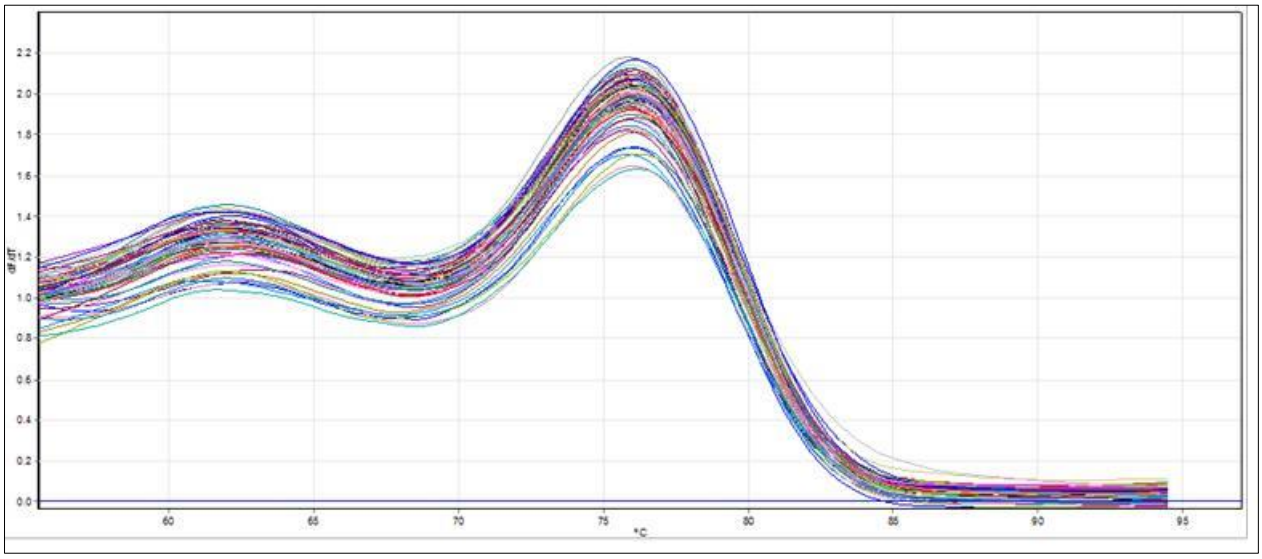

Fig 3: The schematic diagram shows the amplification of the miRNA-155 gene employed quantitative polymerase chain reaction (qPCR). Samples from both study groups were included. The image was taken directly from the Agilent qPCR machine.

4. Discussion

In this study, our results didn't indicate the presence of statistically significant differences in terms of age and sex, as the two groups were identical in terms of age and sex [10]. On the other hand, the result showed a significant statistical difference in the hormones T3 and T4 between the patient and healthy groups, meaning that their concentrations were higher in serum of patients than in healthy individuals [11]. This is attributed to the fact that this condition is an autoimmune disease, in which the thyroid gland becomes overactive, leading to an autoantibody response, particularly immunoglobulin, which activates the thyroid gland and boosts its hormone production. This conversion is exacerbated by hyperthyroidism. Conversely, elevated T3 and T4 levels cause decreased TSH. This is called negative feedback. When the hypothalamus detects elevated T3 and T4 levels, it causes a decrease in TRH, which in turn causes a decrease in TSH [12]. In another context, the results showed a significant enlarge in IL-7 concentrations in serum of Graves' disease compared to serum of control. This increase is due to the disease being an autoimmune condition, in which the immune system is at its peak activity, leading to increased production of IL-7, which is vital for the survival and proliferation of T cells [13]. On the other hand, the increase in this interleukin may be the result of an immune system disorder, where some cytokines increase and others decrease as a result of inflammation [14]. The results also showed that IL-4 levels were inconsistent with IL-7, as they were lessen in serum of patients compared to serum of the control group [15]. This is due to several reasons, including acute inflammation. On the other hand, there may be a decrease in cytokines that prevent and combat inflammation, including IL-4, which directs the direction towards regulating the autoimmune attack on the thyroid gland [14]. On the other hand, our study revealed a close relationship between miRNA155 and Graves' disease, as high levels of the gene increase the severity of the onset and persistence of inflammation [16]. This occurs due to the activation of lymphocytes, particularly T cells, it may be active in the immune response to this disease. This gene also has an additional function, promoting cell proliferation and tissue formation, which leads to augmentation of the thyroid gland attributable to this disease [17].

5. Conclusion

In the end, the serum miR-155 level in GD patients was significantly larger than in the control group. The serum miR-155 level in patients was associated with the extent of thyroid enlargement. In clinical molecular diagnostics, serum miR-155 in patients may represent a potential marker for GD and It is involved in the pathogenesis and has an active role. However, further studies are needed to investigate this gene in GD diagnosis and aetiology.

Abbreviations

GD	Graves' disease
PCR	Polymerase Chain Reaction
IL-7	Interlukein-7
IL-4	Interlukein-4
Th	T helper
Th1	Type 1 T helper
Th2	Type 2 T helper
ELISA	Enzyme-linked Immunosorbent Assay
IgE	Immunoglobulin E
TRAb	Thyrotropin receptor antibodies
T4	Thyroxine and tetraiodothyronine
T3	Triiodothyronine
HbA1c	Glycosylated haemoglobin
TG	Triglycerides
PTH	Parathyroid hormone

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Authors Contribution

Author contributions to the preparation of the manuscript were as follows: ATH; Subject selection and study design, ATH, ASS; Writing and editing the manuscript, ASS; Sample collection and statistical analysis, ATH; Discussion of results All authors read and approved the manuscript.

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Availability of Data Materials

Supplementary data for the study are available from the corresponding author upon request.

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