



Study of serum Micro-RNA 221 Expression in Patients with Thyroid Nodules and Its Relation to Outcome

Thesis

Submitted for the Partial Fulfillment of
M.D. Degree in *Internal Medicine*

By

Sherief Samy Bayomy Mohamed

M.B.B.Ch, M.SC-Faculty of Medicine-Ain Shams University

Supervised by

Prof. Dr./ Raef Malak Botros

*Professor of Internal Medicine and Endocrinology
Faculty of Medicine - Ain-Shams University*

Prof. Dr./ Emad Eldin Farid Ibrahim

*Professor of General Surgery
Faculty of Medicine, Ain Shams University*

Dr./ Alyaa Ahmed ElSherbini

*Associate Professor of Internal Medicine and Endocrinology
Faculty of Medicine, Ain Shams University*

Dr./ Shaimaa El-Metwaly ElDayasty

*Lecturer of Radiology
Faculty of Medicine, Ain Shams University*

Dr. Hanan Mahmoud Ali

*Lecturer of Internal Medicine and Endocrinology
Faculty of Medicine, Ain Shams University*

**Faculty of Medicine
Ain Shams University
2020**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العليم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgment

*First and foremost, I feel always indebted to **AUAH**, the
Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Prof. Dr. Raef Malak Botros**, Professor of Internal Medicine and Endocrinology for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Prof. Dr. Emad el din Farid**, Professor of General surgery, Faculty of Medicine, Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to **Dr. Alyaa Ahmed Elsherbiny**, Assistant Professor of internal medicine and endocrine, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

*Special thanks are due to **Dr. Shaimaa El-Metwaly ElDayasty**, Lecturer of Radiology, Faculty of Medicine Ain Shams University, for her sincere efforts, fruitful encouragement.*

*Special thanks are due to **Dr. Hanan Mahmoud Ali**, Lecturer of Internal Medicine and Endocrinology, Faculty of Medicine Ain Shams University, for her sincere efforts, fruitful encouragement.*

*I wish to introduce my deep respect and thanks to **Prof. Dr. Lamiaa Salem**, Consultant of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her kindness, supervision and cooperation in this work.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Sherief Samy Bayomy

List of Contents

| | Title Page No. |
|--|----------------|
| List of Tables | I |
| List of Figures | III |
| List of Abbreviations..... | V |
| Review of Literature | |
| Introduction | 1 |
| Aim of the Work..... | 4 |
| • CHAPTER 1: Thyroid Nodules and Maliganancy | 5 |
| • CHAPTER 2: Thyroid Ultrasound..... | 50 |
| • Chapter 3: Microrna In Thyroid Nodules | 66 |
| Patients and Methods..... | 88 |
| Results | 113 |
| Discussion | 129 |
| Summary and Conclusion | 137 |
| References | 138 |
| Arabic Summary | ١ |

List of Tables

| Table No. | Title | Page No. |
|-------------------|---|----------|
| Table (1): | Risk factors for development of thyroid nodules | 7 |
| Table (2): | Clinical features that increase the possibility of a malignant versus a benign thyroid nodule..... | 8 |
| Table (3): | The Bethesda System for Reporting Thyroid Cytopathology: diagnostic categories..... | 25 |
| Table (4): | The Bethesda system for reporting thyroid cytopathology: Diagnostic Categories and risk of malignancy | 26 |
| Table (5): | Survival rates for different types of thyroid cancers..... | 49 |
| Table (6): | Comparison of miRNA and siRNA origin and function..... | 68 |
| Table (7): | Studies of miRNA in papillary thyroid carcinomas .. | 75 |
| Table (8): | miRNA expression in thyroid tumors other than papillary carcinoma..... | 78 |
| Table (9): | A master mix was prepared according to manufacturer's instruction as illustrated. | 105 |
| Table (10): | The thermal cycler values..... | 107 |
| Table (11): | Components of the Reaction Mix in each PCR Reaction. | 108 |
| Table (12): | The PCR cycling protocol. | 109 |
| Table (13): | Showing distribution of group 1 and group 2 as regard age, sex, thyroid functions and micro-Rna 221 | 114 |
| Table (14): | Descriptive for Nodule size, TIRADS score, Bethesda classification system, pathology in group 1 | 115 |
| Table (15): | Age in group 1 and group 2: | 116 |
| Table (16): | Showing range of age in both benign and malignant pathologies..... | 116 |
| Table (17 a,b,c): | Showing comparisons regarding thyroid stimulating hormone, free t3 and free t4 in both groups: | 118 |

List of Tables (Cont...)

| Table No. | Title | Page No. |
|-------------------|--|----------|
| Table (18 a,b,c): | Showing comparisons regarding thyroid stimulating hormone, free t3 and free t4 in subgroups 1A and 1b..... | 119 |
| Table (19): | Showing distribution of malignant and benign pathologies with different TIRADS values. | 120 |
| Table (20): | Showing distribution of malignant and benign pathologies with different sizes of dominant nodules. | 122 |
| Table (21): | Showing distribution of malignant and benign pathologies with different Bethesda values. | 123 |
| Table (22): | MicroRna 221 in sera of both groups | 124 |
| Table (23): | Showing range of microRna 221 in both benign and malignant pathologies: | 124 |
| Table (24): | ROC curve between Pathology Benign and Malignant in Patients:..... | 126 |
| Table (25): | Summary for different correlations with benign and malignant outcomes and controls: | 127 |

List of Figures

| Fig. No. | Title | Page No. |
|---------------------|--|----------|
| Figure (1): | An algorithm for evaluation of a thyroid nodule | 17 |
| Figure (2): | Algorithm for evaluation and management of patients with thyroid nodules based on US pattern and FNA cytology..... | 23 |
| Figure (3): | Normal thyroid ultrasound in transverse view | 51 |
| Figure (4): | A predominantly cystic nodule..... | 53 |
| Figure (5): | An entirely cystic nodule with comet-tail artifact (<i>arrow</i>)..... | 53 |
| Figure (6): | Hypoechoic solid nodule with both micro and macrocalcifications..... | 54 |
| Figure (7): | Smooth margin | 58 |
| Figure (8): | Ill defined margin..... | 58 |
| Figure (9): | Irregular margin | 58 |
| Figure (10): | Lobulated margin..... | 58 |
| Figure (11): | Microcalcifications within thyroid nodule | 59 |
| Figure (12): | miRNA regulation of gene expression through the RNAi pathway..... | 67 |
| Figure (13): | Translational repression and inhibition of protein synthesis..... | 70 |
| Figure (14): | Putative role of suppressor miRNAs and oncogene miRNAs in carcinogenesis..... | 72 |
| Figure (15): | LOGIQ™ P7, GE Healthcare, Innovative ultrasound imaging inc.2020 | 91 |
| Figure (16): | Representative images of TIRADS scoring in different thyroid nodules | 94 |
| Figure (17): | Papillary thyroid cancer hematoxylin and eosin | 97 |
| Figure (18): | The miRNeasy mini kit spin column extraction procedure..... | 104 |

List of Figures (Cont...)

| Fig. No. | Title | Page No. |
|---------------------|--|----------|
| Figure (19): | PCR detection system (5 Plex Rotor Gene RealTime PCR Analyzer (Qiagen, Germany)..... | 109 |
| Figure (20): | Showing range of age in both benign and malignant pathologies..... | 117 |
| Figure (21): | Showing distribution of malignant and benign pathologies with different TIRADS values..... | 120 |
| Figure (22): | Showing distribution of malignant and benign pathologies with different TIRADS values..... | 121 |
| Figure (23): | Showing distribution of malignant and benign pathologies with different sizes of dominant nodules..... | 122 |
| Figure (24): | Showing distribution of malignant and benign pathologies with different Bethesda values. | 123 |
| Figure (25): | Showing range of microRna 221 in both benign and malignant pathologies..... | 125 |
| Figure (26): | ROC curve between Pathology Benign and Malignant in Patients | 126 |
| Figure (27): | microRna 221 in different groups benign and malignant and controls | 128 |

List of Abbreviations

| Abb. | Full term |
|------------------------------|--|
| <i>⁹⁹TC</i> | <i>Thyroid radioactive technetium-99m scan</i> |
| <i>ACR</i> | <i>American College of Radiology ACR</i> |
| <i>ATA</i> | <i>American thyroid association</i> |
| <i>ATC</i> | <i>Anaplastic thyroid cancer</i> |
| <i>AUS/FLUS</i> | <i>Atypia of uncertain significance/follicular lesion of undetermined significance</i> |
| <i>CEA</i> | <i>Carcinoembryonic antigen</i> |
| <i>DTC</i> | <i>Differentiated thyroid cancer</i> |
| <i>EDTA</i> | <i>Ethylene diamine tetra-acetate</i> |
| <i>FA</i> | <i>Follicular adenoma</i> |
| <i>FC</i> | <i>Follicular carcinoma</i> |
| <i>FN</i> | <i>Follicular neoplasm/suspicious for follicular neoplasm</i> |
| <i>FNA</i> | <i>Fine needle aspiration</i> |
| <i>FTC</i> | <i>Follicular thyroid carcinoma</i> |
| <i>GEC</i> | <i>Gene expression classifier</i> |
| <i>Gy</i> | <i>Gray unit of absorbed radiation dose</i> |
| <i>IQR</i> | <i>Inter-quartile range</i> |
| <i>MEN</i> | <i>Muliple endocrine neoplasia</i> |
| <i>miR</i> | <i>micro-Rna</i> |
| <i>miRNA</i> | <i>micro rna</i> |
| <i>MTC</i> | <i>Medullary thyroid carcinoma</i> |
| <i>PC</i> | <i>Papillary carcinoma</i> |
| <i>PDC</i> | <i>Poorly differentiated carcinoma</i> |

List of Abbreviations (Cont...)

| Abb. | Full term |
|---------------------|--|
| <i>PDC</i> | <i>Poorly differentiated carcinoma</i> |
| <i>PET</i> | <i>Positron emission tomography</i> |
| <i>PTC</i> | <i>Papillary thyroid carcinoma</i> |
| <i>Rad</i> | <i>Unit of absorbed radiation dose</i> |
| <i>RISC</i> | <i>RNA-induced silencing complex</i> |
| <i>RLN</i> | <i>Recurrent laryngeal nerve</i> |
| <i>RT</i> | <i>Reverse Transcription</i> |
| <i>siRNA</i> | <i>Short interfering RNA</i> |
| <i>SMC</i> | <i>Suspicious for malignant cells</i> |
| <i>TBSRTC</i> | <i>The Bethesda System for Reporting Thyroid Cytopathology</i> |
| <i>Tg</i> | <i>Thyroglobulin</i> |
| <i>TIRADS</i> | <i>Thyroid Imaging, Reporting and Data System</i> |
| <i>Us</i> | <i>Ultrasound</i> |
| <i>UTR</i> | <i>Untranslated region</i> |

Abstract

A major dilemma in the diagnostic management of thyroid nodules is to determine whether it is a benign or malignant lesion and hence to determine decision for surgery. The majority of individuals with thyroid nodules are asymptomatic. The current first line of evaluation of thyroid nodules encompasses thyroid hormone laboratory tests, and ultrasonography of the thyroid gland, fine-needle aspiration (FNA) biopsy is often used to rule out cancer in thyroid nodules, in 20–30 % of cases, however, FNAB yields indeterminate cytological results and suspicious for malignancy. Surgery was classically recommended for such indeterminate nodules for their risk of malignancy, which, overall, is about 25 % when confirmed histopathologically upon thyroidectomy. As a result, about 75 % of patients with cytologically indeterminate thyroid nodules would undergo unnecessary thyroid surgeries for nodules that prove to be benign only after surgery. Recent advances in research on thyroid carcinogenesis have yielded applications of diagnostic molecular biomarkers and profiling panels in the management of thyroid nodules. Among these markers are MicroRNAs (miRs) are small RNA sequences (19–25 nucleotides) that function to regulate the expression of genes. In this paper we aim to detect a possible of Micro-RNA 221 expression in sera of Patients With thyroid nodules and its relation to outcome after surgery.

INTRODUCTION

Thyroid nodules are extremely common and are mostly benign. Only 4%-6.5% of all thyroid nodules are cancerous (*Lin et al., 2005*).

The majority of individuals with thyroid nodules are asymptomatic. The nodules are usually found during routine physical examination with some incidental findings seen on diagnostic imaging (e.g., ultrasound [US], computed tomography, magnetic resonance imaging, or positron emission tomography) performed for other indications (*Mendel et al., 2004*).

Rarely, patients with thyroid nodules may complain of pain in the neck, jaw, or ear. If a nodule is large enough to compress the trachea or esophagus, it may cause difficulty with breathing, swallowing. Even less commonly, hoarseness can be caused if the nodule invades the recurrent laryngeal nerve that controls the vocal folds but this is usually related to thyroid cancer (*Knudsen et al., 2012*).

The current first line of evaluation of thyroid nodules encompasses thyroid hormone and thyroid-stimulating hormone laboratory tests, and ultrasonography of the thyroid gland (*Khadra et al., 2014*).

Ultrasonography is noninvasive and reveals many features relevant to the pathology of nodules. For example, an increased risk of malignancy has been associated with the presence of microcalcifications, irregular or speculated margins with no halo, marked hypoechogenicity, mostly solid composition, and taller than wider shape (*Moon et al., 2010*).

On the other hand, presence of peripheral vascularity, round shape, isoechogenicity, spongiform appearance, smooth margins, and cystic composition are associated with benignancy (*Moon et al., 2008*).

As such, fine-needle aspiration (FNA) biopsy is often used to rule out cancer in thyroid nodules (*Haugen et al., 2016*).

Although FNA is a safe and widely used procedure, complications such as discomfort or local pain and self-limited small hematomas may occur. Approximately 60%- 80% of FNAs result in benign findings (*Cibas et al., 2016*).

In 20–30 % of cases, however, FNAB yields indeterminate cytological results and suspicious for malignancy. Surgery was classically recommended for such indeterminate nodules for their risk of malignancy, which, overall, is about 25 % when confirmed histopathologically upon thyroidectomy. As a result, about 75 % of patients with cytologically indeterminate thyroid nodules would undergo unnecessary thyroid surgeries for nodules that prove to be benign only after surgery (*Alexander et al., 2015*).

This historically represents a major dilemma in the diagnostic management of thyroid nodules, to which other conventional diagnostic modalities, such as ultrasonography, are also unable to provide definitive solution (*Haugen et al., 2015*).

Recent advances in research on thyroid carcinogenesis have yielded applications of diagnostic molecular biomarkers and profiling panels in the management of thyroid nodules. The specific utility of these novel, clinically available molecular tests is becoming widely appreciated, especially in perioperative decision making by the surgeon regarding the need for surgery and the extent of initial resection (*Robert et al., 2014*).

Among these markers are MicroRNAs (miRs) are small RNA sequences (19–25 nucleotides) that function to regulate the expression of genes. MiRNAs have been shown to play a key role in the regulation of gene expression and there is evidence that they are involved in a wide variety of physiological cellular processes including differentiation, proliferation, and apoptosis (*Hatfield et al., 2005*).

AIM OF THE WORK

To detect value of Micro-RNA 221 expression in sera of Patients With thyroid nodules and its relation to outcome after surgery.