

**Assessment of Serum Midkine Level in benign
and Malignant Thyroid Nodules: Can Midkine
be a marker of thyroid malignancy?**

Thesis

Submitted for partial fulfillment of Master Degree
in Endocrinology

By

Maha Mohamed Mostafa EL-Nabarawy

M.B, B.CH

Under supervision of

Prof Dr./ Nermin Ahmed Sheriba

Professor of Internal Medicine, Diabetes and Endocrinology
Faculty of Medicine – Ain Shams University

Dr./ Maram Mohamed Maher Mahdy

Assistant Professor of Internal Medicine,
Diabetes and Endocrinology
Faculty of Medicine – Ain Shams University

Dr./ Rana Hashem Ibrahim EL Attary

Lecturer of Internal Medicine, Diabetes and Endocrinology
Faculty of Medicine – Ain Shams University

**Faculty of Medicine
Ain Shams University
2019**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

لسببناك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدقة الله العظيم

سورة البقرة الآية: ٣٢



Acknowledgments

*First and foremost, I feel always indebted to **Allah**, the **Most Beneficent and Merciful** who gave me the strength to accomplish this work,*

*My deepest gratitude to my supervisor, **Prof. Dr. /Nermin Ahmed Sheriba**, Professor of Internal Medicine, Diabetes and Endocrinology, Faculty of Medicine – Ain Shams University, for her valuable guidance and expert supervision, in addition to her great deal of support and encouragement. I really have the honor to complete this work under her supervision.*

*I would like to express my great and deep appreciation and thanks to **Dr./Maram Mohamed Maher Mahdy**, Assistant Professor of Internal Medicine, Diabetes and Endocrinology Faculty of Medicine – Ain Shams University, for her meticulous supervision, and her patience in reviewing and correcting this work,*

*I must express my deepest thanks to my **Dr./Rana Hashem Ibrahim EL Attary**, Lecturer of Internal Medicine, Diabetes and Endocrinology Faculty of Medicine – Ain Shams University for guiding me throughout this work and for granting me much of her time. I greatly appreciate her efforts.*

*Special thanks to my **Parents** and all my **Family** members for their continuous encouragement, enduring me and standing by me.*

*✍ **Maha Mohamed Mostafa EL-Mabarawy***

List of Contents

<i>Subject</i>	<i>Page No.</i>
List of Abbreviations.....	i
List of Tables.....	ii
List of Figures	vii
Introduction	1
Aim of the work	3
Review of Literature	
Approach to Thyroid Nodules.....	4
Midkine	34
Midkine as a Novel Tumor Marker	61
Subjects and Methods	75
Results.....	84
Discussion	100
Summary	107
Conclusion.....	109
Recommendations	110
References	111
Arabic Summary	—

List of Abbreviations

<i>Abbr.</i>	<i>Full-term</i>
ACR	: American College of Radiology
AFP	: α -fetoprotein
ALK	: Anaplastic lymphoma kinase
ALK	: Anaplastic lymphomakinase
AUS	: Atypia Undetermined Significance
BIRADS	: Breast image reporting and data system
CA	: Carbohydrate antigen
CEA	: Carcinoembryonic antigen
CT	: Computed tomography
DTC	: Differentiated thyroid cancer
EAE	: Experimental autoimmune encephalitis
EIA	: Enzyme-linked immunoassay
ELISA	: enzyme-linked immunosorbent assay
ERK	: Extracellular signal-regulated kinase
ESCC	: Esophageal squamous cell carcinoma
FNA	: Fine needle aspiration
FNAB	: Fine needle aspiration biopsy
H&E	: Hematoxylin and eosin
HCC	: Hepatocellular carcinoma
HIF1α	: Hypoxia-inducible factor 1 α
LDL	: Low-density lipoprotein

LRP	: LDL receptor-related protein
MCP	: Monocyte chemoattractant protein
MFP	: Medial floor plate
MK	: Midkine
mRNA	: Messenger ribonucleic acid
MTNS	: McGill Thyroid Nodule Score
NF	: Nuclear factor
ODNs	: Oligodesoxy-ribonucleotides
OSCC	: Oral squamous cell carcinoma
PA	: Plasminogen activator
PCR	: Polymerase chain reaction
PTC	: Papillary thyroid cancer
PTN	: Pleotropin
RT	: Real-time
SD	: Standard deviation
siRNA	: Small interfering RNA
SPSS	: Statistical package for social science
Tg	: Thyroglobulin
TIRADS	: Thyroid image reporting and data system
TPO	: Thyroid peroxidase
TSH	: Serum thyrotropin
WT	: Wilms' tumor
ζ (PTPζ)	: Protein tyrosine phosphatase

List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (1):	Management of Nodules	30
Table (2):	Actions of MK to cultured cells	42
Table (3):	Acute and chronic effects of midkine in vessel and kidney diseases	58
Table (4):	Midkine gene and protein overexpression in various cancers in tissue, blood and urine.....	62
Table (5):	Comparison between Group A (malignant nodule), Group B (Benign nodule) and Group C (Control) as regards age.....	85
Table (6):	Comparison between Group A (malignant nodule), Group B (Benign nodule) and Group C (Control) as regards sex.....	85
Table (7):	Comparison between Group A (malignant nodule), Group B (Benign nodule) and Group C (Control) as regards thyroid profile (TSH, fT3 and fT4)	86
Table (8):	Comparison between Group A (malignant nodule), Group B (Benign nodule) and Group C (Control) as regards Midkine.	88
Table (9):	Comparison between Group A (malignant nodule) and Group B (Benign nodule) as regards contour of nodules.....	90
Table (10):	Comparison between Group A (malignant nodule) and Group B (Benign nodule) as regards calcifications.....	91

Table (11): Comparison between Group A (malignant nodule) and Group B (Benign nodule) as regards sonographic features of nodules..... 92

Table (12): Comparison between Group A (malignant nodule) and Group B (Benign nodule) as regarding size of nodules 94

Table (13): Comparison between all parameters as regards Midkine level..... 95

Table (14): Comparison between FNA results as regards Midkine level..... 96

Table (15): Correlation between Midkine and all variables (Age,TSH,ft3,ft4 and size of nodules) in all patients groups. 97

Table (16): The ROC curve between patients and controls as regard Midkine..... 99

List of Figures

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
Figure (1):	Anatomy of thyroid gland	4
Figure (2):	Follicles of the thyroid gland, consisting of a layer of simple epithelium enclosing a colloid-filled cavity.....	5
Figure (3):	Thyroid nodule	6
Figure (4):	ACR TI-RADS criteria, levels and recommendations.....	19
Figure (5):	Technetium-99m (99mTc) thyroid scan of a large, nontoxic multinodular goiter.	23
Figure (6):	Histologic pattern of a mildly differentiated follicular thyroid carcinoma (250 X).....	26
Figure (7):	Diagnostic sequence and therapeutic decisions in managing a patient with an apparent single nodule of the thyroid.....	28
Figure (8):	The domain structure of MK and the three-dimensional structure of its C-terminal domain.....	36
Figure (9):	Midkine and its roles in atherogenesis.	60
Figure (10):	Comparison between Group A (malignant nodule), Group B (Benign nodule) and Group C (Control) as regarding Midkine.....	89
Figure (11):	Comparison between Group A (malignant nodule) and Group B (benign nodule) as regarding contour of nodules.....	90

Figure (12): Comparison between Group A (malignant nodule) and Group B (benign nodule) as regarding calcifications 91

Figure (13): Comparison between FNA results as regards Midkine level 96

Figure (14): Correlation between Midkine and age in all patients groups..... 98

Figure (15): Correlation between Midkine and size of nodules in all patients groups..... 98

Figure (16): The Cutpoint and Sensitivity and Specificity 99

Abstract

Background: Thyroid nodules are a common clinical problem. The prevalence of malignancy in thyroid nodules is currently about 5–15%. Optimal prediction of malignancy in nodular thyroid disease is needed to achieve the best medical and surgical intervention. Midkine (MK), a novel heparin-binding growth factor, plays critical roles in a variety of biological phenomena such as carcinogenesis, inflammation/immunity, blood pressure, cellular proliferation, survival, migration of cellular functions, angiogenesis, fibrinolysis, and host defense and tissue protection. **Aim of the Work:** The aim of this study is to evaluate the value of serum Midkine as a marker of Malignancy in Patients with Nodular Thyroid Disease. **Patients and Methods:** This comparative study was conducted on 75 subjects with age ranging from 25-80 years selected from outpatient clinic of Internal Medicine and Endocrinology of Ain Shams University Hospital. **Results:** Serum Midkine level showed a significant increase in cases with malignant thyroid nodules having irregular borders and microcalcifications than in cases with benign thyroid nodules. **Conclusion:** Serum Midkine might be the indicator of malignant thyroid cytopathology, suggesting that midkine might serve as a novel biomarker in assessment of thyroid nodules. The present study explored the usefulness of midkine as a biomarker in the differentiation between benign and malignant thyroid nodules in samples from serum.

Key words: Serum Midkine, benign/malignant thyroid nodules, thyroid malignancy

Introduction

Thyroid nodule is typically asymptomatic, and 33% to 68% of adults have thyroid nodules when evaluated by ultrasound. Most thyroid nodules are benign, but about 7% to 15% of individuals with thyroid nodules harbor thyroid cancer. Thyroid nodules may also cause morbidity due to hyperthyroidism or local compression. Population-based studies suggest a doubling in thyroid cancer incidence in recent decades, but nearly all of this increase is attributable to clinically occult cancers detected incidentally on imaging or pathology (**Ronald and Andrew, 2017**).

Optimal prediction of malignancy in nodular thyroid disease is needed to achieve the best medical and surgical intervention. Fine needle aspiration biopsy (FNAB) is widely used and has improved preoperative prediction of malignancy but still has disadvantages including operator variability and nondiagnostic reports. Therefore researchers have focused on identifying novel biologic markers that might be associated with malignancy in thyroid nodules (**Jin and McHenry, 2012**).

Midkine is a multifunctional cytokine predominantly expressed during embryogenesis, while in adult organisms, its expression is resumed during inflammation, tissue repair, and carcinogenesis. Midkine displays a number of activities that might be relevant for cancer development, e.g., it has

been demonstrated to act as a mitogen, an antiapoptotic, and angiogenic factor, a chemoattractant and haptotactic factor, an immunomodulator, and an inducer of synthesis of several cytokines and growth factors, such as IL-8, TGF- β , MIP-2, and MCP-1 (**Muramatsu, 2010**).

There is release of Midkine from cancer tissue into the blood. Serum midkine is found in 87% percent of various cancers. High serum and tissue Midkine levels have been proposed as indicative of malignancy in numerous tumors and to be a prognostic marker of their behavior (**Kadomatsu et al., 2013**).

In thyroid cancer, tissue Midkine overexpression has been reported to be in correlation with clinicopathological features of the tumor, hypothesizing that Midkine might play a role as a biomarker for diagnosis and more aggressive behavior of thyroid cancer such as lymph node metastasis and extrathyroidal invasion. Also they found that benign adenomatoid nodules showed less Midkine overexpression than the malignant nodules (**Kato et al., 2000**).

The studies for Serum Midkine in differentiated thyroid cancer were very rare but it was found higher in differentiated thyroid cancer than benign thyroid lesions (**Meng et al., 2015**).

Aim of the work

The aim of this study is to evaluate the value of serum Midkine as a marker of Malignancy in Patients with Nodular Thyroid Disease.

Approach to Thyroid Nodules

Introduction

The thyroid gland is a highly vascular, brownish-red gland located anteriorly in the lower neck, extending from the level of the fifth cervical vertebra down to the first thoracic. The gland varies from an H to a U shape and is formed by 2 elongated lateral lobes with superior and inferior poles connected by a median isthmus, with an average height of 12-15 mm, overlying the second to fourth tracheal rings (Sakr, 2016).

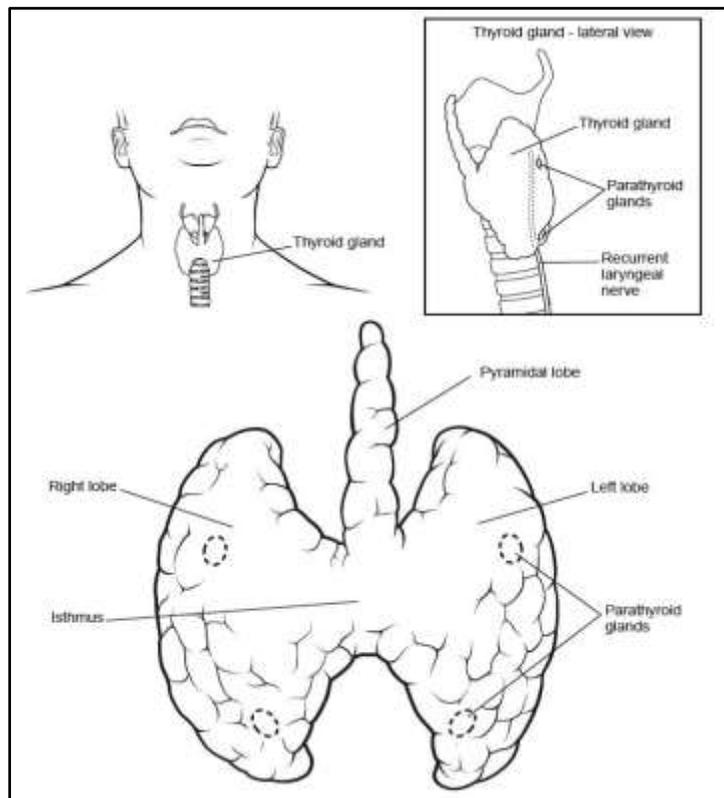


Figure (1): Anatomy of thyroid gland (Dorion, 2015).