



**SERUM MICRORNA-222 EXPRESSION FOR  
MALIGNANCY PREDICTION IN EUTHYROID PATIENTS  
WITH INDETERMINATE THYROID NODULE**

*Thesis*

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**In Internal Medicine**

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## List of Abbreviations

<b>18F-FDG-PET</b>	F18-fluorodeoxyglucose-PET
<b>ACE</b>	American College of Endocrinology
<b>ACR</b>	American College of Radiology's
<b>ATA</b>	American Thyroid Association
<b>ATCs</b>	Anaplastic thyroid carcinomas
<b>ATP</b>	Adenosine triphosphate
<b>AUS/FLUS</b>	Atypia of Undetermined Significance /Follicular Lesion of Undetermined Significance
<b>BIRADS</b>	Breast Imaging, Reporting, and Data System
<b>Ca</b>	Calcium
<b>cDNA</b>	Complementary Deoxyribonucleic acid
<b>CE-US</b>	Contrast-enhanced ultrasound
<b>CISH</b>	Chromogenic in situ hybridization
<b>CLL</b>	Chronic lymphocytic leukemia
<b>CT</b>	Computed tomography
<b>D1</b>	Type I iodothyronine deiodinase
<b>D2</b>	Type II iodothyronine deiodinase
<b>D3</b>	Type III iodothyronine deiodinase
<b>DIT</b>	Diiodotyrosine
<b>DNA</b>	Deoxyribonucleic acid
<b>dsRNA</b>	Double-stranded RNA
<b>DTC</b>	Well-differentiated thyroid cancer
<b>DWI</b>	Diffusion-weighted imaging
<b>ELISA</b>	Enzyme-linked immunosorbent assay
<b>FAP</b>	Familial adenomatous polyposis
<b>FCD</b>	Follicular cell derived

## List of Abbreviations

<b>FISH</b>	Fluorescence in situ hybridization
<b>FLUS</b>	Follicular lesion of undetermined significance
<b>FN</b>	Follicular Neoplasm
<b>FNA</b>	Fine needle aspiration
<b>FNAB</b>	Fine-needle aspiration biopsy
<b>FNMTc</b>	Familial nonmedullary thyroid carcinoma
<b>FSH</b>	Follicle stimulating hormone
<b>FTAs</b>	Follicular thyroid adenomas
<b>FTC</b>	Follicular thyroid carcinoma
<b>GH</b>	Growth hormone
<b>H&amp;E</b>	Haematoxylin and Eosin
<b>H2O2</b>	Hydrogen peroxide
<b>IFVPC</b>	Invasive follicular variant of papillary carcinoma
<b>K-TIRADS</b>	Korean Society for Thyroid Radiology
<b>MEN</b>	Multiple endocrine neoplasia
<b>MEN2</b>	Multiple endocrine neoplasia type 2
<b>MiRNA</b>	Micro Ribonucleic acid
<b>MIT</b>	Monoiodotyrosine
<b>MRI</b>	Magnetic resonance imaging
<b>MRNAs</b>	Messenger Ribonucleic acid
<b>MTC</b>	Medullary thyroid carcinoma
<b>MTCs</b>	Medullary thyroid carcinomas
<b>NADPH</b>	Nicotinamide adenine dinucleotide phosphate

## List of Abbreviations

<b>NFVPTC</b>	Noninvasive follicular variant of papillary thyroid carcinoma
<b>NIFTP</b>	Non-invasive follicular thyroid neoplasm with papillary-like nuclear features
<b>nt</b>	Nucleotides
<b>PCR</b>	Polymerase chain reaction
<b>PET</b>	Positron emission tomography
<b>PGE2</b>	Prostaglandin E2
<b>Pol II</b>	RNA polymerase II
<b>Pol III</b>	RNA polymerase III
<b>pre-miRNA</b>	Precursor miRNA
<b>PRL</b>	Prolactin
<b>PTC</b>	Papillary thyroid carcinoma
<b>PTH</b>	Parathormone
<b>qRT-PCR</b>	Quantitative real-time polymerase chain reaction
<b>RAI</b>	Radioactive iodine
<b>RAIU</b>	Radioactive iodine uptake
<b>RISC</b>	RNA-induced silencing comple
<b>RISC</b>	RNA-induced silencing complex
<b>RNA</b>	Ribonucleic acid
<b>RNAi</b>	RNA interference
<b>RT</b>	Reverse Transcription
<b>rT3</b>	Reverse Triiodothyronine
<b>RXRs</b>	Retinoic acid X receptors
<b>SFN</b>	Suspicious for Follicular Neoplasm
<b>siRNA</b>	Small interfering RNA

## List of Abbreviations

<b>SPECT</b>	Single photon emission computed tomography
<b>T2</b>	Diiodothyronine
<b>T3</b>	Triiodothyronine
<b>T4</b>	Thyroxine
<b>TBG</b>	Thyroxin-binding globulin
<b>Tg</b>	Thyroglobulin
<b>TGF-<math>\beta</math></b>	Transforming growth factor beta
<b>TIRADS</b>	Thyroid Imaging, Reporting, and Data System
<b>TR</b>	Thyroid receptor
<b>TREs</b>	Thyroid response elements
<b>TRH</b>	Thyrotrophin-releasing hormone
<b>TSH</b>	Thyroid stimulating hormone
<b>US</b>	Ultrasound

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## **Abstract**

**Background:** The Prevalence of thyroid nodules is rising nowadays, luckily most of them are benign. The risk of malignancy 5-15%, which necessitates the ultimate need to accurately distinguish benign from malignant nodule to avoid unnecessary thyroidectomy with risk of recurrent laryngeal nerve injury, postoperative hypothyroidism and lifetime thyroid replacement therapy, and other complications related to surgery and anaesthesia. Recent evidence suggests that circulating miRNA might have probable advantage as diagnostic or prognostic markers for numerous cancers. Given their reproducible and constant presence in sera, miRNA profiles have emerged as a non-invasive method to categorise a wide variety of human cancers.

**Aim of the study:** To evaluate a possible relationship between the expression level of circulating miRNA-222 and the histological outcome of euthyroid patients undergoing thyroidectomy for thyroid nodules with indeterminate FNAB cytology.

**Subjects and Methods:** 45 euthyroid patients with indeterminate thyroid nodules diagnosed with ultrasound and FNAC which planned for thyroidectomy. Quantitative assay of serum micro RNA-222 expression by quantitative Real-Time polymerase chain reaction (qRT-PCR) performed preoperatively, and results compared with postoperative histopathology.

**Results:** The incidence of thyroid nodules was predominant in female gender in benign group and malignant group. Risk of malignancy increases as TI-RADS and Bethesda scores increases. Also, larger nodule in size has a more risk of malignancy ( $p=0.027$ ). Expression level of circulating miRNA-222 in serum can't differentiate between benign and malignant patients where there was no significant difference between them statistically ( $p=0.905$ ). Circulating miRNA-222 was a poor predictor for malignant nodules with sensitivity of 50%, specificity of 32.43%, with high negative predictive value (NPV=75%).

**Conclusion:** Although circulating miRNA-222 has been identified as novel minimally invasive biomarker for preoperative prediction of malignant nodules, but in our study, it did not show a value as a tool for discrimination of malignant nodules. Ultrasound remained important procedure in preoperative prediction and management of thyroid nodules especially when correlating to nodule size which had positive correlation with malignancy in our study.

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**Key words:** Thyroid nodules, microRNA-222, FNAC, Bethesda, TI-RADS

## INTRODUCTION

Thyroid cancer is the most common endocrine neoplasms, which accounts for approximately 1.7% of all cancer diagnoses (*Ferlay et al., 2010*). However, most thyroid nodules are benign and only 5% harbor malignancy (*Gharib, 2004*). Therefore, it is essential to develop safe and accurate test to differentiate between benign nodules and malignant ones (*Dean and Gharib, 2008*).

Currently, the most important diagnostic method in the detection of thyroid cancer is fine-needle aspiration biopsy (FNAB). Nevertheless, up to 16.6–22.5% of the detections cannot be diagnosed definitely, owing to wrong diagnosis or sampling errors (*Papini et al., 2002*).

**Cibas and Ali published in 2009** the Bethesda Uniform System for Reporting Thyroid Cytopathology. Six different categories were described: nondiagnostic, benign, malignant, atypia/follicular lesion of undetermined significance (FLUS), follicular neoplasm, and suspicious for malignancy. Whereas the first three categories are straight forward to manage, it is the last three categories that pose a management challenge. Biopsy results that include FLUS, follicular neoplasm, and suspicious for malignancy carry a malignancy rate of 5%-15%, 15%-30%, and 60%-75% respectively. The recommended approach for indeterminate FNAB results varies between repeat FNAB,