Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases:
*From circulating chemistry to molecular cytogenetics.***"**

Thesis submitted to

Faculty of Medicine

BLDE (Deemed to be University)

Vijayapura, Karnataka, India.

For the award of the degree of

Doctor of Philosophy

in

Medical Pathology

By

Anita P Javalgi

PhD Research Scholar

Registration No: 14PHD001

Department of Pathology,

BLDE (Deemed to be University)'s Shri B. M. Patil Medical College,

Hospital and Research Centre, Vijayapura, Karnataka, India.

December 2019

BLDE (DEEMED TO BE UNIVERSITY)

Shri B.M.Patil Medical College, Hospital and Research Centre

Vijayapur, karnataka, India

Certificate

I hereby declare that this thesis entitled "*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.*"is a bonafide and genuine research work carried out by me under the guidance of Dr B R Yelikar, Professor and Head,Dept. of Pathology and Dr Kusal K. Das, Professor Dept. of Physiology, BLDE (Deemed to be University), Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapur, Karnataka, India.

No part of this thesis has been formed the bases for the award of any degree or fellowship previously.

Signature of the Candidate

Dr. Anita P Javalgi, M.B.B.S, M.D.

Ph.D. scholar

Reg No.14PHD001

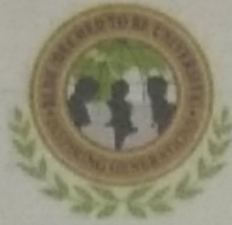Department of Pathology,

BLDE (Deemed to be University),

Shri B.M.Patil Medical College,

Hospital and Research Centre,

Vijayapur, Karnataka, INDIA

Date: 30.12.19

Place: Vijayapur

BLDE (DEEMED TO BE UNIVERSITY)

ShriB.M.Patil Medical College, Hospital and Research Centre

Vijayapur, karnataka, India

Certificate by the Guide

This is to certify that this thesis entitled "*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from Circulating Chemistry to Molecular Cytogenetics.*" is a bonafide research work carried out by Dr. Anita P Javalgi under my supervision and guidance in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Centre, Vijayapura,Karnataka, India in the fulfilment of the requirements for the degree of Doctor of Philosophy in Pathology.
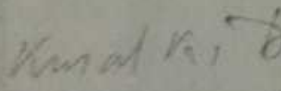
Signature of the guide

Dr B R Yelikar MBBS,MD(PATHOLOGY)
Professor
Department of Pathology,
Medical College for Women
Surathul rendn uthbullapur

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Certificate by the Co Guide

This is to certify that this thesis entitled *"Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics."* is a bonafide research work carried out by Dr. Anita P Javalgi under my supervision and guidance in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

Professor
Physiology
Signature of the Co-guide Shri B.M Patil Medical
Prof. Kusal K. Das, PhD, FRSB Hospital & R.C.
Co- Guide   Vijayapur-586103.
Professor, Laboratory of Vascular Physiology and Medicine,
Department of Physiology,
Shri B.M.Patil Medical College, Hospital & Research Centre,
BLDE (Deemed to be University), Vijayapur, Karnataka,India.

BLDE (DEEMED TO BE UNIVERSITY)

Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Copyright

Declaration by the candidate

I hereby declare that the BLDE (Deemed to be University), Shri B. M.Patil Medical College, Hospital and Research Center, Vijayapur, Karnataka, shall have the rights to preserve, use and disseminate this declaration/thesis in print or electronic format for academic/research purpose.

© BLDE (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Center, Vijayapura Karnataka, India.

Signature of the Candidate
Dr. Anita P Javalgi, M.B.B.S, M.D.
Ph.D. scholar
Reg No.14PHD001
Department of Pathology,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA.

BLDE (DEEMED TO BE UNIVERSITY)

Shri B.M.Patil Medical College, Hospital and Research Centre

Vijayapur, karnataka, India

Endorsement by the Head of Department

This is to certify that this thesis entitled "*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.*" is a bonafide research work carried out by Dr. Anita P Javalgi in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology..

Dr S U Arakeri

Prof and Head
Dept. of Pathology,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA

Prof. & HOD
Dept. of Pathology.
B.L.D.E.U's Shri B. M. Patil
Medical College, BIJAPUR-586103

BLDE (DEEMED TO BE UNIVERSITY)

Shri B.M.Patil Medical College, Hospital and Research Centre

Vijayapur, karnataka, India

Endorsement by the Principal/Head of Institution

This is to certify that this thesis entitled "*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.*" is abonafide research work carried out by Dr. Anita P Javalgi in the Department of Pathology, Shri. B. M Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

Dr Aravind V Patil

PRINCIPAL,
BLDE (Deemed to be University),
Shri B.M Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA
PRINCIPAL
LDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR-586103

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Copyright

Declaration by the candidate

Signature of the Candidate
Dr. Anita P Javalgi, M.B.B.S, M.D.
Ph.D. scholar
Reg No.14PHD001
Department of Pathology,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA.

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Certificate

I hereby declare that this thesis entitled **"*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.*"**is a bonafide and genuine research work carried out by me under the guidance of Dr B R Yelikar, Professor and Head,Dept. of Pathology and Dr Kusal K. Das, Professor Dept. of Physiology, BLDE (Deemed to be University), Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapur, Karnataka, India.

No part of this thesis has been formed the bases for the award of any degree or fellowship previously.

Signature of the Candidate
Dr. Anita P Javalgi, M.B.B.S, M.D.
Ph.D. scholar
Reg No.14PHD001
Department of Pathology,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA.

Date:

Place:

BLDE (DEEMED TO BE UNIVERSITY)
ShriB.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Certificate by the Guide

This is to certify that this thesis entitled **"***Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from Circulating Chemistry to Molecular Cytogenetics.***"** is a bonafide research work carried out by Dr. Anita P Javalgi under my supervision and guidance in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Centre, Vijayapura,Karnataka, India in the fulfilment of the requirements for the degree of Doctor of Philosophy in Pathology.

Signature of the guide

Dr B R Yelikar MBBS,MD(PATHOLOGY)
Professor
Department of Pathology
ShriB.M.Patil Medical College, Hospital & Research Centre,
BLDE (Deemed to be University)
Vijayapur, Karnataka,India.

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Certificate by the Co Guide

This is to certify that this thesis entitled **"***Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.***"** is a bonafide research work carried out by Dr. Anita P Javalgi under my supervision and guidance in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

Signature of the Co-guide
Prof. Kusal K. Das, PhD; FRSB
Co- Guide
Professor, Laboratory of Vascular Physiology and Medicine,
Department of Physiology,
Shri B.M.Patil Medical College, Hospital & Research Centre,
BLDE (Deemed to be University), Vijayapur, Karnataka,India.

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Endorsement by the Head of Department

This is to certify that this thesis entitled **"***Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.***"** is a bonafide research work carried out by Dr. Anita P Javalgi in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology..

Dr S U Arakeri

Prof and Head
Dept. of Pathology,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA

BLDE (DEEMED TO BE UNIVERSITY)
Shri B.M.Patil Medical College, Hospital and Research Centre
Vijayapur, karnataka, India

Endorsement by the Principal/Head of Institution

This is to certify that this thesis entitled **"*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from circulating chemistry to molecular cytogenetics.*"** is abonafide research work carried out by Dr. Anita P Javalgi in the Department of Pathology, Shri. B. M.Patil Medical College, Hospital and Research Center, Vijayapura,Karnataka, India in the fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

Dr Aravind V Patil

PRINCIPAL,
BLDE (Deemed to be University),
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapur, Karnataka, INDIA

Acknowledgement

Success is an acknowledgement honored by a group who values your actions in the specificity of the venture they cherish - Nothing More! They may not represent the values you personally cherish."

Rejean Nantel

I express my gratitude to all the study participants included in this study

It gives me immense pleasure to express my deep sense of gratitude and heartfelt thanks to my guide **Dr. B R Yelikar** Professor of Pathology, BLDE (Deemed to be University)'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka for his valuable guidance, advice and constant support for completing my research work successfully.

I express my sincere gratitude to **Prof. Kusal K. Das**, Professor, Laboratory of Vascular Physiology and Medicine, Department of Physiology, BLDE (Deemed to be University)'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, my mentor and co- guide, for his endless support, suggestions, full faith and trust in me. It is under his privileged guidance that I have been able to complete my research work. It has been a very rewarding experience to work under him. His enthusiasm, eye for perfection and his keen interest in research has always been very inspiring.

I am grateful to **Dr. M. S. Birder**, Vice-Chancellor BLDE (Deemed to be University), Vijayapura, **Dr. J. G. Ambekar**, Registrar, BLDE (Deemed to be University), Vijayapura, **Dr. Aravind V. Patil,** Principal, BLDE (Deemed to be University)'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, **Dr. S. P.**

Dedication

I dedicate my work to my parents

Dr Pandit G Javalgi MA,Phd

Dr Smt Indumati P Javalgi MPhil Phd

&

My Husband

Dr Vishwanath G Shettar MBBS,MS(Orthopaedics)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Sl no	Abbreviations	
1.	ABCA1	ATP-Binding Cassette Transporter A1
2.	ACC1	Acetyl-Coacarboxylase 1
3.	ACC	Acetyl Coa Carboxylase
4.	AD	Autoimmune Disease
5.	AFLUS	Atypia Follicular Lesion Of Undetermined Significance
6.	AITDs	Autoimmune Thyroid Diseases
7.	AR	Adrenergic Receptor
8.	BAT	Brown Adipose Tissue
9.	CB	Core Biopsy
10.	CCH	C Cell Hyperplasia
11.	CEA	Carcino Embryonic Antigen
12.	ChREBP	Carbohydrate Response Element Binding Protein;
13.	CK	Cytokeratin
14.	COX	Cyclo-Oxygenase
15.	CPT-I	Carnitinepalmitoyltransferasei
16.	CRD	Crohns Disease
17.	CYP7a1	Cytochrome P-450 7A1
18.	CYP7A1	Cholesterol 7-Hydroxylase
19.	D2	5=-Deiodinase Type
20.	DC	Diagnostic Categories
21.	EA	Eosin Stain
22.	ELFA	Enzyme Linked Fluroscent Assay
23.	ERK	Extracellular Signal Regulated Kinase
24.	FAS	Fatty Acid Synthase
25.	FN	Fibronectin
26.	FNAC	Fine Needle Aspiration Cytology
27.	FoxO3	Forkhead Box O3
28.	FTC	Follicular Thyroid Carcinoma
29.	FVPTC	Follicular Variant Of PTC
30.	Gal 3	Galectin- 3

31.	GD	Graves Disease
32.	GLUT4	Glucose Transporter 4
33.	HBME 1	Hector Battifora Mesothelial Cell 1
34.	HCG	Human Chorionic Gonadotropin
35.	HOMGCR	3-Hydroxy-3-Methylglutaryl-Coa Reductase
36.	HT	Hashimoto's Thyroiditis
37.	HTA	Hyalinising Trabecular Adenoma
38.	I2	Iodine
39.	LDL-R	Low-Density Lipoprotein Receptor.
40.	LDL-R	Low-Density Lipoprotein Receptor;
41.	LXR	Liver X Receptor
42.	MAPK	Mitogen Activated Protein Kinase
43.	MCT8	The Monocarboxylate Transporter 8
44.	MHC	Myosinheavy Chain
45.	MMTC	Micro Medullary Thyroid Carcinoma
46.	MS	Multiple Sclerosis
47.	MTC	Medullary Thyroid Carcinoma
48.	mTOR	Mammalian Target Of Rapamycin
49.	NCI	National Cancer Institute
50.	NCoR	Nuclear Corepressor
51.	NE	Norepinephrine
52.	NIFTP	Non Invasive Follicular Thyroid Carcinoma With Papillary Like Nuclear Features
53.	NIMA	Non Inherited Maternal Antigens
54.	OG	O Green Stain
55.	PBN	Parvalbuminergic Neurons
56.	PCR	Polymerase Chain Reaction
57.	PI3K	Phosphatidylinositol Triphosphate Kinase
58.	PKC	Protein Kinase C
59.	POMC	Proopiomelanocortin
60.	PPAR	Peroxisome Proliferator-Activated Receptor
61.	PPAR1	Peroxisome Proliferator Activated Receptor
62.	PTC	Papillary Thyroid Carcinoma

63.	PTOS6K	Ribosomal Protein S6 Kinase Beta-1
64.	RA	Rheumatoid Arthtritis
65.	RET	Receptor Signaling
66.	RTH	Resistance to Thyroid Hormone
67.	RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
68.	RXR	Retinoid X Receptor
69.	SD	Sjogrens Disease
70.	SERCA	Sarcoplasmic Reticulum Calcium Atpase
71.	SFN	Suspicious Of Follicular Neoplasm
72.	SM	Suspicious For Malignancy
73.	SMRT	Silencing Mediator For Retinoic And Thyroid Hormone Receptor
74.	SNS	The Sympathetic Nervoussystem
75.	SPR	Solid Phase Receptacle
76.	STAT	Stimulates Signal Transducer And Activator Oftranscription
77.	STAT3-P	Stimulates Signal Transducer And Activator Oftranscription 3 Phosphorylation
78.	T	Thyroxine
79.	T1DM	Type 1 Diabetes Mellitus
80.	T3	Triiodothyronine
81.	TBG	Thyroxine Binding Globulin
82.	TBPA	Prealbumin
83.	TBSRTC	The Bethesda System Of Reporting Thyroid Cytology
84.	Tg	Thyroglobulin
85.	Tg Ab	Thyroglobulin Antibody
86.	TH	Thyroid Hormone
87.	TPO Ag	Thyroid Peroxidase Antibody
88.	TRAb	TSH Receptor Antibody
89.	TRE	Thyroid Hormone Receptor Elements
90.	TRH	Thyrotropin Releasing Hormone
91.	TRH	Thyrotropin-Releasing Hormone
92.	TSH	Thyroid Stimulating Hormone
93.	TSH	Thyroid-Stimulating Hormone

94.	TTF1	Thyroid Transcription Factor 1
95.	TTR	Transthyretin
96.	UCP	Uncoupling Protein
97.	US	Ultrasonograph
98.	VMH	Ventromedial Nucleus
99.	VPN	Paraventricular Nucleus
100.	α-MSH	A-Melanocytestimulating Hormone

ABSTRACT

ABSTRACT

OBJECTIVES: This study aims to understand the role of auto antibodies in the structural derangement of thyroid tissue and to identify the specific immune marker for diagnosing the thyroid lesions. Hence the objectives of this research work were to know the significance of FT3, FT4, TSH, anti TPO antibody, anti thyroglobulin antibody in various thyroid lesions, to study immune marker expression of HBME-1 and Galectin 3 in the resected thyroid specimen, to correlate between serum markers and tissue immune markers in the thyroid lesions and to know commonest mutation of RET proto-oncogene in thyroid specimens in familial thyroid malignancies

METHODOLOGY: This was a two year prospective study carried out from 2015 to 2016 at Dept. of pathology, BLDE (Deemed to be University), Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura. The study group included out patients and in patients with thyroid abnormality. All cases referred to cytology section were included and individuals on hormone therapy or antithyroid drugs as treatment were excluded from the study. So total of 165cases were studied during this period. Thyroid function test (serum TSH, Free T4 and Free T3), anti TPO and anti TG antibodies were measured from the preferable early morning 5ml venous drawn blood sample. Fine needle aspiration was performed in all cases. Histopathology correlation of cases was done of resected thyroid tissue and immune markers HBME1 and Galectin 3 was done where ever required. R*et* oncogene mutational study was carried out on few prophylactic thyroidectomy specimens which accounted to 14cases.

RESULTS: Total of 165 cases of thyroid swellings were studied and observed females preponderance with 81% and male 19% distribution respectively. Commonest age group affected was 3rd and 4th decade. Youngest patient was 12yrs old and oldest 72years.

Thyroid hormones assay were within normal range of all individuals. Anti thyroperoxidase (Anti TPO) and anti-thyroglobulin (Anti-TG) levels were significantly raised in autoimmune thyroiditis and in few cases of papillary carcinoma. All 165 cases were subjected for Fine Needle aspiration cytology and diagnoses were categorized according to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). 24cases were non diagnostic(excluded from the study), 83cases categorised as II, 32cases as categorised III, 16cases as cat IV, 9 cases belonged to cat v and 15 cases were categorised in cat VI. Seventy one cases (71 cases) had histopathological correlation. Commonest histopathological diagnosis was colloid goitre (21cases), 16 cases showed chronic thyroiditis (Lymphocytic thyroiditis 11 cases, Granulomatous thyroiditis 3cases and Graves' disease 2 cases), 10 cases of follicular adenoma and 24 cases were thyroid malignancy. Papillary carcinoma was commonest malignancy with 15 cases, 4 cases of follicular carcinoma, 2 cases of medullary carcinoma and 2 cases of metastatic carcinoma (both were squamous cell carcinoma metastasis). One rare case of mucoepidermoid carcinoma was diagnosed.

CONCLUSION: Anti TPO is significantly raised in chronic thyroiditis and papillary carcinoma. The Bethesda system of Reporting Thyroid Cytology (TBSRTC) is strongly recommended for thyroid cytopathology.HBME 1 and Galectin 3 have high specificity and sensitivity in diagnosing thyroid malignancies. C634R was commonest exon seen in Ret oncogene mutations.

CHAPTER I

INTRODUCTION

INTRODUCTION

Endocrine disorders are common disorders noticed worldwide. The prevalence of endocrine disorders especially thyroid diseases are also increasing in Indian population and it is estimated that nearly forty million people suffer with thyroid abnormalities[1].

The thyroid abnormality could be functional or structural derangement with thyroid swelling as commonest clinical presentation. Thyroid nodules being the frequent finding in clinic, its appearance is noticed frequently with increasing age. Nodular thyroid disease refers to the presence of a solitary nodule or multiple nodules, solid to cystic lesions. The incidence of thyroid lesions has increased due to early detection of lesions due to advanced radiological techniques which pick up the nodules of any size from few millimeters to centimeters. Majority of the nodules turn benign after investigations, but also the incidence of malignancies has increased to 2.4 fold lately.

Recent studies have highlighted that 12% of adult population suffers from palpable goiter in iodine deficient areas. This is followed by increased incidence of autoimmune thyroid diseases, especially in iodine sufficient area[2,3].

Antibodies against Thyroperoxidase and Thyroglobulin are two autoantibodies which are commonly found in patients with thyroid diseases[4]. Few studies in literature show the association of thyroperoxidase antibody levels and the severity of lymphocytic infiltration in autoimmune thyroiditis and thus playing a role in pathogenesis of antibody-dependent cell-mediated cytotoxicity[5,6]. Research done by sir Boelaert K. et al. noticed strong association between thyroperoxidase antibodies levels in serum and thyroid lesions and he commented that less attention has been paid for measuring thyroid autoantibodies, in addition to thyroid stimulating hormone, which will help in predicting the progress thyroid nodules. Thus their research included all thyroid nodules

with ultrasound findings and autoantibodies level measurements, and confirmed that antibodies against thyroperoxidase and thyroglobulin are two important thyroid autoantibodies to assess the risk of thyroid nodules[7].

Gold standard investigation to evaluate thyroid structural abnormalities is Fine needle aspiration cytology and histopathological study of thyroid tissue. However sometimesthe pathologists are confronted with difficulties in reaching an accurate diagnosis between the indeterminate thyroid lesions. To overcome these constrains various immunohistochemical markers as ancillary tests are available now. Galectin-3, CK19, HBME-1 and TPO are immune markers which are studied extensively and their role in diagnosing the thyroid lesions have been established in the previous studies[8].

Galectin-3 belongs to galectin family and chemical structure has an N-terminal tail composed of nine collagen-like repeats. It is found that it has some role in pathogenesis of thyroid malignancies[9]. Hector Battifora mesothelial -1 is a surface antigen localized in the microvilli of the mesothelial cell and its role in thyroid malignancies have been studied and thus studies reveal that its immune expression is wider in papillary carcinoma compared to follicular neoplasms [10, 11,12].

Thyroid cancer is the most common endocrine malignancy compared to other endocrine organs and it is sixth most common cancer in women and increased incidence in younger females[13]. The common thyroid carcinoma are papillary carcinoma, follicular carcinoma and medullary carcinoma followed by other morphological variants, these carcinoma's occur both in sporadic form and hereditary form, commonest malignancy with hereditary occurrence is medullary carcinoma which is associated with Multiple endocrine Neoplasm Syndromes.

Multiple Endocrine Neoplasm syndrome is rare entity which is an autosomal dominant inheritance caused by missense germ line ret proto-oncogene mutations. These

mutations are at different locus and thus produce different variants of disease categorized as type I and II[14,15,16] . Recent studies have identified preneoplastic lesion in thyroid tissue of high risk individuals who have familial history of medullary carcinoma. These lesions are identified as C-cell hyperplasia and micro medullary carcinoma[17,15].

With increased prevalence of thyroid autoimmune disorders and increased incidence of thyroid malignancies in past decade affecting the mortality and morbidity of human population, literature has few studies to understand the pathogenesis of thyroid cancer. Also there is a paucity of prospective clinical studies that are capable of evaluating various tests which may significantly reduce patient morbidity and unnecessary surgery in benign thyroid disease. The present study was undertaken to study the significance of FT3, FT4, TSH, anti TPO antibody, antiTG antibody in various thyroid lesions. And also to understand the importance of The Bethesda Reporting System of Thyroid cytopathology of thyroid nodules, Histopathological correlation in available cases and to study immune marker expression of HBME-1 and Galectin 3 in the resected thyroid specimen in applicable cases.

References

1. J.Larry Jameson and Anthony P. Weetman. Disorders of the Thyroid Gland (Endocrinology and Metabolsim) Harrison's principles of Internal Medicine. 18ᵗʰ edition McGraw Hill publications. 2012: 2; 16: 2911-14.

2. Unnikrishnan AG and   MN Usha. Thyroid disorders in India: An epidemiological perspective, Indian J EndocrinolMetab. 2011; 15(2): 78–81.

3. Grogan RH , Mitmaker EJ, and Clark OH. The Evolution of Biomarkers in Thyroid Cancer- From Mass Screening toa Personalized Biosignature.Cancers2010; *2*: 885-912.

4. Roberts, C.G.; Ladenson, P.W. Hypothyroidism. Lancet 2004, 363, 793–803.

5. Mitchell, J.D.; Kirkham, N.; Machin, D. Focal lymphocytic thyroiditis in Southampton. J. Pathol. 1984;144: 269–73.

6. Kasagi, K, Kousaka T, Higuchi K, Iida Y, Misaki T, Alam M.S, Miyamoto S, Yamabe H, Konishi J. Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: Comparison with histological findings. Thyroid 1996; 6: 445–50.

7. Boelaert K, Horacek J, Holder R.L,Watkinson J.C, Sheppard M.C, Franklyn J.A. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J. Clin. Endocrinol. Metab. 2006; 91: 4295–301.

8. Arcolia V, Journe F, Renaud F, Leteurtre E, Gabius HJ, RemmelinkM.Combination of Galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer 2017;14: 4183-89

9. Kopitz J, Vértesy S, André S, Fiedler S, Schnölzer M and Gabius HJ: Human chimera-type galectin-3: Defining the critical tail length for high-affinity

glycoprotein/cell surface binding and functional competition with galectin-1 in neuroblastoma cell growth regulation. Biochimie 2014;104: 90-99.

10. Ippel H, Miller MC, Vértesy S, Zheng Y, Cañada FJ, Suylen D, Umemoto K, Romanò C, Hackeng T, Tai G, *et al*: Intra-and intermolecular interactions of human galectin-3: Assessment by full-assignment-based NMR. Glycobiology 2016;26: 888-03.

11. Prasad ML, Pellegata NS, Huang Y, Nagaraja HN, de la Chapelle A and Kloos RT: Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunohistochemistry is useful for the differential diagnosis of thyroid tumors. Mod Pathol 2005;18: 48-57.

12. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N and Smit JW: Combined immunostaining with galectin-3, fibronectin-1, CITED-1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-{gamma}, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. Eur J Endocrinol 2008 ;158: 375-84.

13. Knudsen N, Bulow I, Jorgensen T, Perrild H, Ovesen L, Laurberg P. Serum Tg-A sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. J ClinEndocrinolMetab. 2001; 86: 3599–603.

14. Krampitz, G.W. and J.A. Norton, RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Cancer, 2014. 120(13): 1920-31.

15. Donis-Keller, H., et al., Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Human molecular genetics, 1993. 2(7): 851-56.

16. Mulligan, L.M., et al., Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 1993; 363(6428): 458-60.

17. Wells Jr, S.A., et al., Revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma The American ThyroidAssociation Guidelines Task Force on Medullary Thyroid Carcinoma. Thyroid, 2015.

CHAPTER II

AIMS AND

OBJECTIVES

RESEARCH HYPOTHESIS

Autoantibodies against thyro-peroxidase (Anti TPO) enzyme and thyroglobulin (Anti TG) plays a role in pathogenesis of chronic thyroiditis as well as papillary carcinoma.

AIM

- To understand the role of auto antibodies in the structural derangement of thyroid tissue.
- To identify the specific immune marker for diagnosing the thyroid lesions.

OBJECTIVES OF THE STUDY:

1. To know the significance of FT3, FT4, TSH, anti TPO antibody, anti thyroglobulin antibody in various thyroid lesions.

2. To study immune marker expression of HBME-1 and Galectin 3 in the resected thyroid specimen.

3. To correlate between serum markers and tissue immune markers in the thyroid lesions.

4. To know commonest mutation of RET proto-oncogene in thyroid specimens in familial thyroid malignancies

CHAPTER III

REVIEW

OF LITERATURE

INDEX

I.   ANATOMY PHYSIOLOGY AND BIOCHEMISTRY

Thyroid gland is a part of the human body's endocrine system. It is the largest organ specialized for endocrine function in human body.

It is a butterfly - shaped gland Fig (1) with high vascular supply anchored around the front of the throat near the voice box [1].
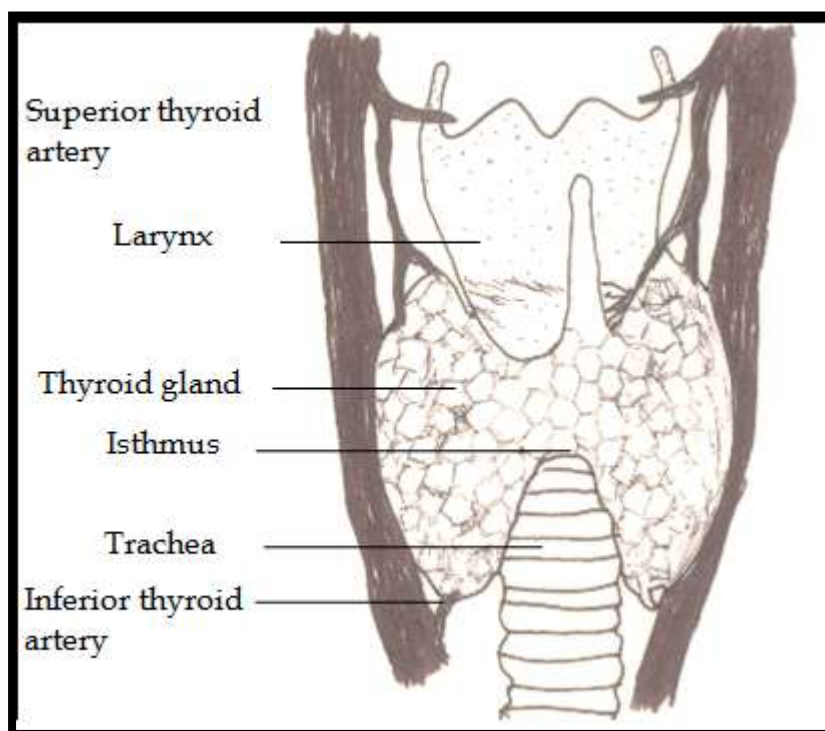
Figure1: Gross appearance of thyroid gland

The gland is essential to normal body growth in infancy and childhood. It absorbs iodine from the diet and releases thyroid hormones -iodine- containing compounds that help govern body's basal metabolic rate, controlling body temperature, regulating protein, fat and carbohydrate catabolism in all cells[2]

The specific actions of thyroid hormone(TH) is seen in metabolic regulation. These include the molecular mechanisms of TH action[3], lipidregulation[4], cross-talk with

nuclear receptors[5], the role of repressors in metabolic regulation[6], thyroid hormone adrenergic interactions[7], facultative thermogenesis[8], and the metabolic influences on central regulation of TH[9,10] as shown in figure 2 .
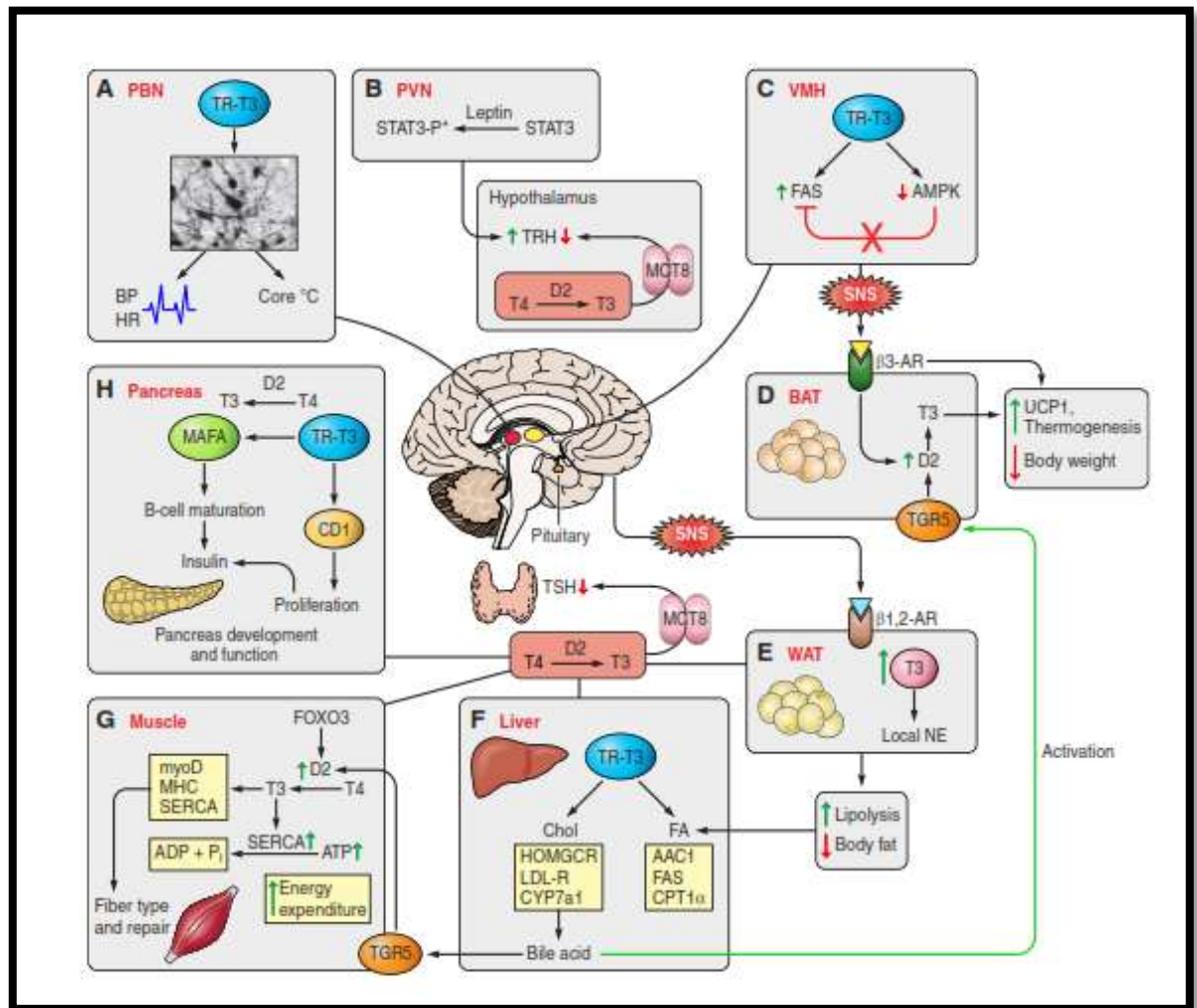
Figure 2:TH in metabolic regulation[10]

Overview of sites of thyroid hormone regulation of metabolism. *Hypothalamic-Pituitary-Thyroid axis:* thyrotropin releasing hormone(TRH) and thyroid stimulating hormone (TSH) respond primarily to circulating serum T4, converted in the hypothalamus and pituitary to T3 by the5=-deiodinase type 2 (D2). The monocarboxylate transporter 8 (MCT8) is required for T3 transport into the pituitary and hypothalamus. *A,parvalbuminergic neurons (PBN):* PBN are a population of newly

discovered neurons in the anterior hypothalamus that are directly linked to theregulation of cardiovascular function, including heart rate, blood pressure, and body temperature. Thyroid hormone receptor signaling is required for the normal development of PBN neurons linking thyroid hormone to cardiac and temperature regulation. *B, paraventricularnucleus of the hypothalamus (VPN):* leptin, produced in peripheral fat tissue, provides feedback at the VPN, stimulates signal transducer and activator of transcription (STAT)3 phosphorylation (STAT3-P*), which directly stimulates TRH expression. Leptin also stimulates TRH indirectly in the arcuatenucleus by inhibiting neuropeptide Y and agouti-related protein, stimulating proopiomelanocortin (POMC), and the POMC product α-melanocyte stimulating hormone (α-MSH) stimulates CREB in the TRH neuron .*C, ventromedial nucleus of the hypothalamus (VMH):* hyperthyroidism or T3 treatment stimulates de novo fatty acid synthesis in the VMH, which inhibits AMPK phosphorylation and increases fatty acid synthase (FAS) activity. Increased hypothalamic lipid synthesis is associated with activation of the sympathetic nervous system (SNS) which stimulates brown adipose tissue (BAT). *D, BAT:* adrenergic signaling through the β 3-adrenergic receptor (AR) stimulates*UCP1* gene expression, stimulates D2 activity by deubiquitination, and promotes thermogenesis and weight loss. The metabolic signal from bile acid via the G protein-coupled membrane bile acid receptor (TGR5) has been shown in one model to stimulate D2 activity and local T3 production, which further stimulates BAT lipolysis, UCP1 expression, and thermogenesis. *E, white adipose tissue (WAT):* SNS signals via _1- and _2-ARstimulate WAT lipolysis. T3 stimulates local production of norepinephrine (NE), increasing lipolysis and reducing body fat. *F, liver*: HOMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; ACC1, acetyl-CoAcarboxylase 1; CYP7a1, cytochrome *P*-450 7A1; CPT-1_, carnitinepalmitoyltransferase 1_; LDL-R, low-density lipoprotein receptor. *G,*

*muscle:*Forkhead box O3 (FoxO3) induces D2 expression, increases local T3 in skeletal muscle, and promotes T3-target gene expression; myoD, myosin

heavy chain (MHC) and sarcoplasmic reticulum Ca2_-ATPase *(SERCA)*. Local T3 also determines the relative expression level of MHC and SERCAisoforms. Expression level of these isoforms determines muscle fiber types and initiation of repair. *SERCA2a* is primarily expressed in slow-twitch fibers and *SERCA1* in fast-twitch fibers. T3 stimulates SERCA, which hydrolyzes ATP and increases energy expenditure. *H, pancreas:* T3 and TRare required for normal pancreatic development and function. In rat pancreatic β cells, expression of TR and D2 are activated during normal development. T3 treatment enhances *Mafa*(v-mafmusculoaponeuroticfibrosarcoma oncogene homolog A) transcription factor gene expression

and increases MAFA protein content, the key factor for maturation of β cells to secrete insulin in response to glucose. T3 stimulates *cyclin D1*(CD1) gene expression and protein level and promotes proliferation. Increasing cyclin D1 activates the cyclin D1/cyclin-dependent kinase/retinoblastoma protein/E2F pathway.

TableI: Specific actions of thyroid hormone in metabolic regulation.

Process	Elements that regulates metabolism	Basic mechanism	Examples of physiological actions.
Thyroid hormone action	TR isoforms Corepressor action (NCoR and SMRT) Nutrient feedback	TR isoform specificity Histone modification Sumoylation	Increased Basal metabolic rate(BMR) Stimulate lipolysis/lipogensis

	Nongenomic action Tissue selective thyroid hormone transport	Corepressor interactions Modulation of signal transduction pathways Stimulation of Na^+-K^+-ATPase and SERCA1	Increase in adaptive thermogenesis Stimulate β oxidation of fatty acids
Central regulation of TRH/TSH	T4/T3 feedback Leptin AMPK activation Oxregenic/anorectic peptides/appetite regulations Thyronamines (T1AM) Circadian rhythms	Integration of TRH/TSH regulation with metabolic signals Thyroid hormone transport into hypothalamus and pituitary. Integration of adrenergic signalling	TSH measurement for the diagnosis of thyroid diseases and to monitor treatment
Local ligand activation by D2	D2 expression and activity D2 polymorphism	Regulation of D2 ubiquitination/ deubiquitination Increase in D2	TSH/T4 set point T4/T3 replacement therapy of hypothyroidism

		activity with reduction in serum T4 concerntration Development and tissue selective deiodinase expression.	Stimulates adaptive thermogenesis
Thermogenesis and body weight	Basal metabolic rate Adaptive thermogenesis Body weight and body composition Appetite	Integration of adrenergic signalling Central and local adrenergic actions Stimulation of CPT1α expression. Stimulation of UCP1 expression.	Reduces body fat Increases βoxidation of fatty acids Stimulates adaptive thermogenesis
Cholesterol and triglycerides	Cholesterol synthesis Reverse cholesterol transport Lipolysis/lipogenesis Hepatic steatosis	Stimulates LDL-R Stimulates ABCA 1	Reduces serum cholesterol, triglycerides and hepatic steatosis
Carbohydrate metabolism	Pancreatic islet development and proliferation Insulin production	TR expression in developing islets and its function D2 required for	Stimulates gluconeogenesis Reduces insulin sensitivity

	Gluconeogenesis Insulin sensitivity and its metabolism	development Insulin signalling Stimulation of mitochondrial respiration Increase expression of GLUT4,ACC1, ChREBP	Increase insulin metabolism

ABCA1, ATP-binding cassette transporter A1; ACC1, acetyl CoA carboxylase; ChREBP, carbohydrate response element binding protein; CPT-I_, carnitinepalmitoyltransferaseIa; CYP7A1, cholesterol 7-hydroxylase; D2, 5=-deiodinase type 2; GLUT4, glucose transporter 4; LDL-R, low-density lipoprotein receptor; LXR, liver X receptor; NCoR, nuclear corepressor; PPAR_, peroxisome proliferator-activated receptor; RXR, retinoid X

receptor; SERCA, sarcoplasmic reticulum calcium ATPase; SMRT, silencing mediator for retinoic and thyroid hormone receptor; T3 , triiodothyronine; T , thyroxine; TGR5, G protein-coupled receptor bile acid receptor; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; UCP, uncoupling protein.

Thyroid hormone is critical for normal growth, differentiation, development and maintenance of metabolic homeostasis (Yen, 2001). The thyroid hormone influences diverse metabolic pathways for lipid and glucose metabolism. There are two principal thyroid hormones: thyroxine (T4, L-3,5,3',5'-tetraiodothyronine) and triiodothyronine (T3, L-3,5,3'-triiodothyronine). The biologically active form of thyroidhormone is T3,

whereas T4 is the most abundant thyroidhormone in the blood. In target tissues, type I or type II 5'-deiodinase (DI or DII) catalyzes the deiodination of T4 and controls the local T3 concentration in cells (Braverman et al.,1970; Köhrle, 2000).The physiological function of thyroid hormone is mainly carried through thyroid hormone nuclear receptors (TRs) in nucleus. TRs belong to the nuclear receptor superfamily, act as ligand-inducible transcription factors, and occupy a central position in mediating the action of T3. TRs bind to specific cis elements called thyroidhormone response elements (TREs) which are located in the promoter of target genes as homodimers or heterodimers with retinoid X receptor (RXR) (Cheng, 2000). The transcription mediated by TRs requires extensive cooperation and dynamic interplay with many nuclear receptor coregulators(McKenna et al., 1999; Yen, 2001)[11].

Effect of thyroid hormone on specific bodily mechanisms in human body[12].

- Stimulation of carbohydrate metabolism.

- Stimulation of fat metabolism.

- Stimulation of protein metabolism.

- Effect on plasma and liver fats.

- Increase requirements for vitamins.

- Increase basal metabolic rate (BMR).

- Effect on cardiovascular system.

- Excitatory effect on the central nervous system.

- Effect on sleep.

- Effect on other endocrine glands.

- Effect on sexual function.

REGULATION OF THYROID HORMONE PRODUCTION

TH is secreted from the thyroid gland under the regulation of the hypothalamic-pituitary axis (figure 3). Thyroid Releasing Hormone (TRH), secreted from the hypothalamus, acts upon the pituitary gland, binding to G protein-coupled TRH receptors on the thyrotrope, resulting in an increase in intracellular cAMP, and subsequent thyrotropin (TSH) release[13].
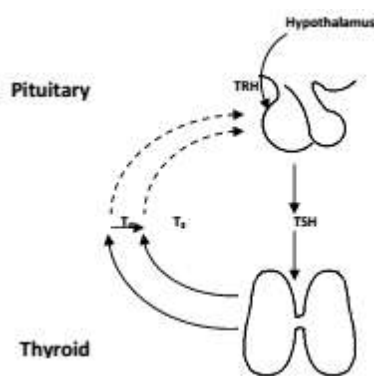
Figure 3: Hypothalamus-pituitary axis

The secretion of TSH from the anterior pituitary gland is controlled by

- Circulating concentration of thyroid hormones.

- Thyrotrophin-releasing hormones (TRH).

The effect of thyroid hormones is to reduce TSH secretion. This negative feedback, is achieved by binding of T3 to anterior pituitary nuclear receptors. In the anterior pituitary gland most of the intracellular T3 is derived from circulating free T4. Therefore this gland is more sensitive to changes in plasma T4 than T3concentrations[13]..

Specific effects of TSH on the thyroid gland[12,13]:

TSH is increases the secretion of thyroxine and triiodothyronine by the thyroid gland. Its specific effects on the thyroid gland are as follow:

- Increased proteolysis of the thyroglobulin that has already been stored in the follicles, with resultant release of the thyroid hormones into the circulating blood and diminishment of the follicular substance itself.

- Increased activity of the iodide pump, which increases the rate of "iodide trapping" in the glandular cells, sometimes increasing the ratio of intracellular to extracellular iodide concentration in the glandular substance to as much as eight times normal.

- Increased iodination of tyrosine to form the thyroid hormones.

II.   HORMONE ASSAY AND FUNCTIONAL DISORDERS

Thyroid hormone (TH) regulates metabolic processes essential for normal growth and development as well as regulating metabolism in the adult[14]. It is well established that thyroid hormone status correlates with body weight and energy expenditure[15].

Hypothyroidism results from any condition that leads to thyroid hormone deficiency, while *Hyperthyroidism* refers to the conditions with over secretion of thyroid hormone. *Resistance to thyroid hormone (RTH)* is a syndrome in which patients have hyposensitivity to thyroidhormone, elevated circulating serum T3 and T4, and elevated or no suppressed thyroid stimulating hormone (TSH) levels. The clinical manifestations of RTH patients are variable among families. It is noteworthy that RTH patients can have clinical symptoms that have features of both hypothyroidism and hyperthyroidism, suggesting variable resistance in

differenttissues[11].

Hyperthyroidism (Thyrotoxicosis)[2,16,17]:

Hyperthyroidism, excess thyroid hormone, promotes a hypermetabolic state characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis[16]. Conversely, hypothyroidism, reduced thyroid hormone levels, is associated with hypometabolism characterized by reduced resting energy expenditure, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis[17].

The major causes and clinical features of hyperthyroidism are[18]:

- Grave's disease

- Toxic multinodulargoitre (Solitary toxic adenoma)

- Exogenous iodine and iodine-containing drugs,e.g. amiodarone

- Excessive T4 or T3 ingestion

- Ectopic thyroid tissue, e.g. strumaovarii

- functioning metastatic thyroid cancer HCG dependent e.g. choriocarcinoma, pituitary tumour (very rare)

- Congenital (neonatal) hyperthyroidism

- Non autoimmune hereditary hyperthyroidism

- Subacute thyroiditis

- Silent thyroiditis

- Postpartum thyroiditis

- Hyperemesis gravidarum

- TSH receptor mutation

- Thyrotoxicosis factitia

- Pituitary resistance to thyroid hormone

Hypothyroidism[2,19,20].

The commonest cause

Adult

- Hashimoto's disease

- 131I therapy for hyperthyroidism

- Subtotal thyroidectomy for hyperthyroidism or tumour

- Previous antithyroid drug therapy

- Postpartum (60%−70% transient)

- Drugs (lithium, amiodarone, iodides interferon alfa)

- Pituitary hypothyroidism (secondary)

- Hypothalamic hypothyroidism (tertiary)

- Subacute thyroiditis, silent thyroiditis

- Iodine deficiency

- Generalised resistance to thyroid hormone

Child/neonate

- Neonatal hypothyroidism

- Thyroid agenesis

- Thyroid ectopia

- Thyroid dyshormonogenesis

- Others

- Resistance to thyroid

Clinical features of Hyperthyroidism are Weight loss (but normal appetite), Sweating, heart intolerance, Fatigue, Palpitation: sinus tachycardia or atrial fibrillation angina, heart failure (high output), Agitation, tremor, Generalized muscle weakness, Proximal myopathy, diarrhoea, Oligomenorrhoea, infertility, Goitre, Eyelid retraction, Lid lag[2].

Clinical features of hypothyroidism are lethargy, tiredness, cold intolerance, dryness, coarseness of skin and hair, weight gain, hoarseness, anemia, dementia, psychosis constipation, bradycardia, angina, pericardial effusion, carpel tunnel syndrome, menorrhagia, galactorrheoa[2].

Subclinical hypothyroidism, defined as the presence of a low normal serum thyroxin accompanied by a moderately raised TSH (grade 1, 5–10; grade 2, 10.1–20; grade 3, >20) should nearly always be treated[2].

LABORATORY EVALUATION OF THYROID FUNCTION

A multitude of tests are currently available for testing thyroid function[21]:

• Serum-based methods are available for measuring both total (TT4 and TT3) and free (FT4 and FT3) thyroid hormone concentrations.

• In addition, measurements can be made of the thyroid hormone binding proteins, Thyroxine Binding globulin (TBG), Transthyretin (TTR)/Prealbumin (TBPA) and Albumin, as well as for the pituitary thyroid stimulator, thyrotropin (thyroid stimulating hormone, TSH) and the

thyroid hormone precursor protein, Thyroglobulin (Tg).

• The recognition of autoimmunity as the leading cause of thyroid dysfunction, has led to the development and incorporation of tests to determine thyroid autoantibodies – thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb), and TSH receptor antibodies (TRAb).

Reference values of thyroid function test

Test Range

TSH: 0.5 -4.7mU/L

T3: 0.92-2.78nmol/L

FT3: 0.22-6.78 pmol/L

T4: 58-140 nmol/L

FT4: 10.3-35pmol/L

TSH

Serum TSH level remains the single best test of thyroid function. Thyroid-stimulating hormone testing is the preferred approach because[22]:

1. TSH is central to the negative-feedback system

2. Small changes in serum thyroid function cause logarithmic amplification in TSH secretion

3. The most advanced (third-generation) chemiluminescent TSH assays can now detect both elevation and significant lowering of TSH levels, and are capable of reliably measuring values <0.1mU/L, thus aiding detection of subclinical thyrotoxicosis.

TSH testing should be commonly carried out in the following settings:

• In patients presenting with suspected goitres: Serum TSH levels must be measured.

• As screening for congenital hypothyroidism: A heel-prick blood specimen is used for determining serum TSH levels. This is an established screening test for congenital hypothyroidism .

• In patients with atrial fibrillation, dyslipidaemia, osteoporosis, and infertility: Serum TSH levels should be measured at presentation.

Total T4 and T3

Several laboratories measure the total T4 and total T3 which is not a true reflection of the thyroid status of an individual. This is because thyroid hormones circulate in the body largely

in the inactive form, bound to carrier proteins (thyroid binding globulin (TBG), transthyretin and albumin) while only the small unbound fraction is metabolically active[23,24].

Considerations which Alter Thyroid Levels

• Binding protein abnormalities can increase totalT3 in the absence of hyperthyroidism, notably during estrogen treatment contraceptive pills, and pregnancy. If necessary, a

T3-uptake test or thyroxine-binding globulin measurement can be used to calculate a free-T3 index, or a free-T3 test can be obtained to clarify an ambiguous increased total-T3 result.

• When hypothyroidism is suspected, a free-T4 estimate is appropriate because total-T3 and free-T3 tests have inadequate sensitivity and specificity in this setting.

• When hyperthyroidism is suspected, the combination of a free-T4 estimate and a total- or free-T3 estimate provides the most complete assessment of the severity of hyperthyroidism and identifies cases of "T3-toxicosis", i.e. a selective increase of the serum T3 concentration.

• In some centres, free-T4 and -T3 tests are routinely used when the TSH is increased, but in others, serum T3 measurements are obtained only when the TSH is low and the free T4 is within the reference interval. It is preferable to monitor both serum free T4 and T3 in patients with low serum TSH (other than hypothyroid patients taking T4),even after the thyroid diagnosis is known, to establish patterns of increasing or decreasing values over time[25,26].
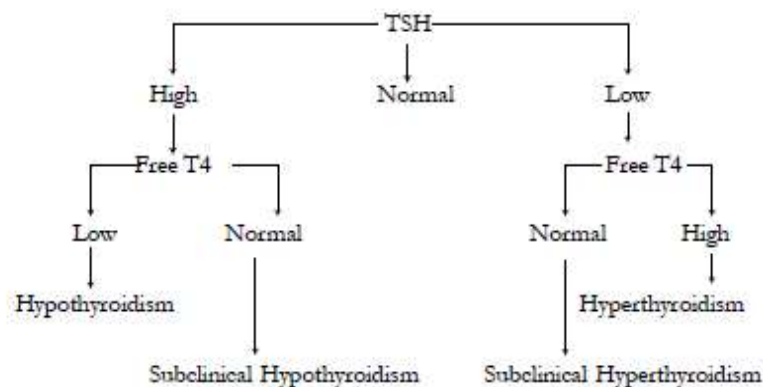
Figure 4: Algorithm for the diagnosis of Thyroid dysfunction

THYROID AUTOIMMUNITY THYROIDSPECIFICAUTOANTIBODIES

(TPOAb, TgAb and TRAb)

Anti TPO (anti thyroperoxidase)

Approximately 70-80 % of patients with Graves' disease and virtually all patients with Hashimoto's, atrophic thyroiditis or post-partum thyroiditis have TPOAb detected. In the future, TPOAb measurement may be used as a prognostic indicator for thyroid dysfunction. The paradoxical absence of TPOAb in some patients with unequivocal TSH abnormalities likely reflects the suboptimal sensitivity and/ or specificity of current TPOAb tests or non-autoimmune thyroid failure (atrophic thyroiditis). Although changes in autoantibody concentrations often reflect a change in disease activity, serial thyroid autoantibody measurements are not recommended for monitoring treatment for AITD. The enhanced sensitivity and specificity of the TPO immunoassay methods make them a more cost-effective option over the older semi-quantitative AMA agglutination tests, since they obviate the need for additional TgAb measurements in the routine diagnosis of autoimmune thyroid disorders[27].

Thyroglobulin antibodies

Auto antibodies against Tg are encountered in autoimmune thyroid conditions, usually in association with TPOAb. According to the current guidelines, all sera should be prescreened for TgAb by a sensitive immunoassay method prior to Tg testing. Therefore, TgAb is primarily used as an adjunct test for serum Tg estimation[28].

TSH receptor autoantibodies (TRAb)

TRAb tests are used in the differential diagnosis of hyperthyroidism, the prediction of fetal and neonatal thyroid dysfunction due to transplacental passage of maternal TRAband prediction of the course of Graves' disease treated withantithyroid drugs

Thyroid disorders have diverse clinical manifestations therefore, on part of vigilant clinician every suspected case of thyroid disease needs to be evaluated with laboratory investigations.

Thereby appropriate treatment for thyroid disorders can be instituted or conservative monitoring carried out to anticipate potential future consequences. The enhanced sensitivity and specificity of TSH assays have greatly improved the assessment of thyroid function tests.

Since TSH levels change dynamically in response to the alterations of T3 and T4, the approach to evaluate whether the patient has thyroid disorder is to test the TSH levels first.

When hypothyroidism is suspected, a free-T4 estimate is appropriate because total-T3 and free-T3 tests have inadequate sensitivity and specificity in this setting. When hyperthyroidism is suspected, the combination of a free-T4 estimate and a total- or free-T3estimate provides the most complete assessment of the severity of hyperthyroidism and identifies cases of "T3-toxicosis", i.e. a selective increase of the serum T3 concentration[29].

Table 2: Characteristics of thyroid disorders according to results of thyroid function tests.

Disorder	TSH	T_4	T_3	FT_4	Tg	TBG	rT_3	ATPO	ATG	TBII	TSI	TBA
Primary hypothyroidism	↑	↓	N or ↓	↓	N or ↓	N	↓	N or ↑	N or ↑	N or ↑	n	n or ↑
Transient neonatal hypothyroidism	↑	↓	↓	↓	N or ↓	N	↓	N	N	↑	n	↑
Hashimoto thyroiditis hypothyroidism	↑	N or ↓	N or ↓	N or ↓	N or ↓	N	↓	↑	↑	n or ↑	n	n or ↑
Graves' disease	↓	↑	↑	↑	↑	N	↑	↑	↑	↑	↑	n or ↑
Neonatal Graves' disease	↓	↑	↑	↑	↑	N	↑	n or ↑	n or ↑	↑	↑	n or ↑
TSH deficiency	N or ↓	↓	↓	↓	↓	N	↓	n	N	n	n	n
Thyroid dishormonogenesis	↑	↓	↓	↓	N, ↓ or ↑	N	↑	n	N	n	n	n
Thyroid hormone resistance	N or ↑	↑	↑	↑	↑	N	↑	n	N	n	n	n
TSH-dependent hyperthyroidism	↑	↑	↑	↑	↑	N	↑	n	N	n	n	n
T_4 protein-binding abnormalities[*]	N	V	V	N	N	V*	V	n	N	n	n	n
Nonthyroidal illness	V	N or ↓	↓	V	N	N	N or ↑	n	N	n	n	n
Subacute thyroiditis[†]	↓ or ↑	↑ or ↓	↑ or ↓	↑ or ↓	↑ or ↓	N	↑ or ↓	n	n	n	n	n

TSH = thyroid-stimulating hormone; T_4 = thyroxine; T_3 = triiodothyronine; FT_4 = free thyroxine; Tg = thyroglobulin; TBG = thyroxine-binding globulin; rT_3 = reverse T_3; ATPO = antithyroidperoxidase; ATG = antithyroglobulin; TBII = TSH-binding inhibiting immunoglobulin; TSI = thyroid-stimulating immunoglobulin; TBA = TSH receptor-blocking antibody; N = normal; n = negative; V = variable.

[*]The spectrum of binding protein abnormalities includes increased or decreased TBG binding, increased or decreased transthyretin binding, and ↑ albumin binding.

[†]Subacute thyroiditis involves a transient period of hyperthyroidism followed by a transient hypothyroid state.

(Reprinted from Fisha DA (ed) : Disorders of Thyroid Function, Quest Diagnostic Manual. 3rd Editor, p 268.)

III.   STRUCTURAL ABNORMALITIES OF THYROID GLAND

Thyroid nodules may be single, multiple, solid, or cystic and may or may not be functional. The thyroid epithelial cells, induced by random mutations or rearrangements, will grow from a normal state to an abnormal state. This induction of growth exacerbates cellular mutagenesis that generates the nodules[30,31,32].

A thyroid nodule is a discrete lesion within the thyroid gland that is radiologically distinct from the surrounding thyroid parenchyma. Some palpable lesions may not correspond to distinct radiologic abnormalities[33]. Such abnormalities do not meet the strict definition for thyroid nodules. Nonpalpable nodules detected on US or other anatomic imaging studies are termed incidentally discovered nodules or ''incidentalomas.'' Nonpalpable nodules have the same risk of malignancy as do sonographically confirmed palpable nodules of the same size[34]. Generally, only nodules >1 cm should be evaluated, since they have a greater potential to be clinically significant cancers. Occasionally, there may be nodules <1 cm that require further evaluation because of clinical symptoms or associated lymphadenopathy[35].

 The broad understanding of the etiological factors for thyroid lesions are[36]

Environmental chemicals and drugs that are goitrogenic in humans.

1. Complex anions and inorganic atoms (iodine, lithium, $CLO4^-$, $TcO4^-$, $BF4^-$), thiocyanate (SCN^-) (cigarette smoking not convincingly demonstrated the cause of goitre).

2. Thionamides, a family of compounds that share the NH-CS grouping, are used in the treatment of hyperthyroidism. One such compound is goitrin, isolated in plants of the genus *brassica*.

31

3. Aniline derivatives (sulfonamides, tolbutamide, sulfaguanidine, sulfamethoxazole, etc.) are drugs that are usually used as antidiabetics or antibacterials in too low a dosage to have important anti-thyroid effects.

4. Phenol derivatives and polyhydroxyphenols. These compounds pollute the shale and may arise from degradation of humid substances and waste water from coal conversion. Such substances have been indicated as potential causes of goitre. Resorcinol has been found in vitro to be more potent than methimazole.

5. Flavonoids* act on thyroid metabolism by interacting with the nuclear receptor for thyroid hormones. Flavonoids such as vitaxin and others are inhibitors of TPO and are considered goitrogenic in populations fed by millet as staple food.

*Exclusive of the vegetable kingdom, more than 4,000 compounds have been identified in plants. The basic structure is represented by flavone, formed by two benzenic rings linked to a third pyranic ring.

6. Inducers of hepatic drug metabolizing enzymes: these compounds induce the synthesis of hepatic enzymes for their detoxication, producing also an excess of thyroid hormone conjugation and thereby wasting of the hormone. If thyroid hormone wasting is severe, TSH

increases and goitre results. Such a mechanism has been proven in a number of substances: pesticides, environmental toxins such as isomers of DDD and DDT, polychlorinated biphenyls.

Chemical carcinogens known to produce thyroid tumors in animals without clinical or epidemiologic evidence of their role as cause of thyroid carcinomas in men[36].

1. Thionamid compounds: thiourea, methimazole, ethylenethiourea (ETU), thiouracil, propylthiouracil. Use: medicine, ETU degradation product of a fungicide.

2. Aminotriazole. Use: herbicide. 3. Acetylaminofluorene (AAF). Use: insecticide.

4. Methylene benzenamine. Use: Dye intermediate.

5. Oxydianiline (ODA). Use: Azo-Dye.

6. Nitrosamines (DPN, DHPN, BOP). Use: lubricant additive, antioxidant.

7. Nitrosoureas (NMU), (NBU), (ENU). Use: derivatives (BCNU, CCNU, MeCCNU) are drugs against tumors. Streptozocin (naturally occurring nitrosourea) is used in the treatment of islet-cell carcinoma of the pancreas).

'Thyroid nodule' is a term with a complex pathological pattern. In order to understand the pathogenesis the thyroid nodule is categorized into five types, which have a distinct anatomo-clinical picture:hyperplastic nodule; neoplastic nodule; colloid nodule; cystic nodule; and thyroiditic nodule (nodularHashimoto's thyroiditis). Although nontoxic goiter and thyroid nodules are the most common thyroid disorders in adult population, the etiology of both conditions is not completely understood. Some risk factors for goiter and thyroid nodules in middle-aged euthyroid subjects. Female gender, thyroid nodules, smoking, BMI, and lower TSH levels were found to be independently associated with higher risk of goiter. The risk of increased prevalence for thyroid nodules was significantly associated with thyroid volume, female gender, and TSH levels [36,37].

COLLOID NODULE:

Colloid accumulation occurs in thyroid pathology either as a nodule or as areas of colloid in a hyperplasticgoitre. Histology shows a marked dilatation of follicles– sometimes enormous–and flattening of the epithelium. Nodules contain a dense viscous material made up of a concentrated solution of thyroglobulin. It is conceivable that these nodules are produced as a defect of intraluminal thyroglobulinreabsorption[38].

Cystic nodule:Euthyroid nodular goitre a large number of nodules is entirely or partly cystic.

It is estimated that between 15–40% of thyroid nodules are partly or entirely cystic[39] :

– 'true cyst': abnormal dilatation of a pre-existing cavity, lined with epithelium, or 'thyroglossal duct cysts', lined by either squamous epithelium or respiratory-type columnar epithelium. The true cyst is rare: 3/71 (i.e., 4%) cases could be defined as 'true cysts' and generally this kind of cyst is benign[40]; however, a coexistent papillary thyroid carcinoma of the thyroid was present in 25% of cases of thyroglossal duct cysts[41]. more than 95% of thyroid cysts are 'pseudocysts', frequently found in a nodular goitre. In 6 to 15% of cases there might be degenerating thyroid carcinomas[42,43] . Degenerative pseudocysts may have two different clinical presentations: 'acute' and 'long-standing'. Longstanding

pseudocysts' are asymptomatic, occasionally found during ultrasonography. With acute pseudocysts patients can accurately report the onset of the nodule, which is preceded or accompanied by mild neck discomfort and sometimes by inflammatory symptoms[43].

HYPERPLASTIC NODULE

The thyroid gland very easily produces diffuse and nodular hyperplasia–particularly in areas of endemic goitre–the proliferative activity of the thyroid is very low. The thyroid however shows the ability to proliferate rapidly in response to appropriate stimuli. Among those, iodine deficiency directly or indirectly is the principal potent stimulator of the replicative potential of the gland[38] .

THYROIDITIS AND AUTOIMMUNITY

Autoimmune diseases (AD) represent a spectrum of disorders caused by inflammation of organs due to production of antibodies against self-structures and cytotoxic action of T cells. Data from Europe, North America, Australia, New Zealand (defined as area 1) and Asia, Middle East, Caribbean, South America (defined as area 2) differ in the reported prevalence (cases/100,000 individuals) of the most frequent AD (1) as follows: Graves' disease (GD, area 1: 50–626, area 2: 20), Hashimoto's thyroiditis (HT, chronic autoimmune thyroiditis, autoimmune hypothyroidism, area 1: 300–2,980, area 2: 350), rheumatoid arthritis (RA, area 1: 310–810, area 2: 120–550), type 1 diabetes mellitus (T1DM, area 1: 310–570, area 2: no data), Crohn's disease (CRD, area 1: 28–201, area 2: 6–113), multiple sclerosis (MS, area 1: 177–358, area 2: 4–101), and Sjögren disease (SD, area 1: 93–3,500, area 2: 330–1,560). The disparity by sex is high in most of these diseases with a female preponderance of ≥85%; only in some childhood-onset AD, such as T1DM, is the risk equal in both sexes. There are three age peaks for AD onset, namely, 8–10 years (juvenile RA, T1DM), 33–50 years (myasthe-nia gravis, MS, systemic lupus erythematosus, scleroderma, GD), and 52–63 years (HT, SD, adult RA, etc.) (2). Autoimmune thyroid diseases (AITDs) include several inflammatory thyroid[44].Multiple mechanisms for autoimmune disorders is shown in figure 5[45].
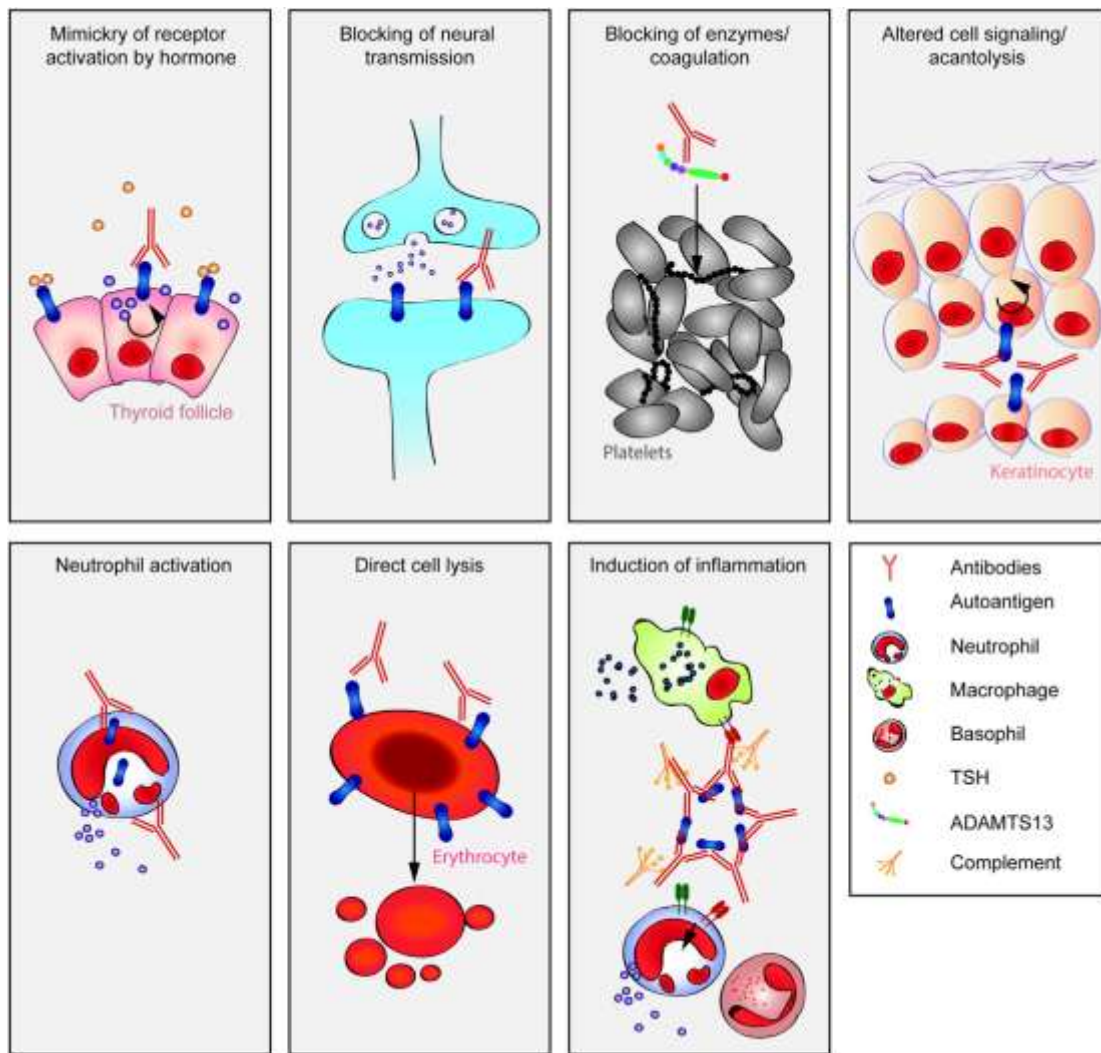
Figure 5: Mechanisms of autoimmunity

Multiple pathways lead to autoantibody-induced pathology. Depending on the targeted autoantigen, and sometimes even depending on the targeted epitope within a single autoantigen, autoantibodies induce pathology through specific and distinct mechanisms. Some are highlighted in this cartoon (from upper left to lower right): antibodies against the thyrotropin receptor (TSHR) mimic hormone stimulation of the TSHR receptor leading to hyperthyroidism, blockade of neural transmission by autoantibody binding to the corresponding receptors may lead to severe neurological diseases such as anti-*N*-methyl-d-aspartate encephalitis, autoantibody-mediated blockade of enzymes of the primary hemostasis may trigger uncontrolled microthrombosis, in pemphigus, autoantibodies induce an altered signaling in

keratinocytes, which either reflects or leads to, a loss of cell–cell adhesion, resulting in severe skin blistering, autoantibodies to antigens expressed by neutrophils can lead to their uncontrolled activation, resulting in severe tissue injury, in autoimmune idiopathic thrombocytopenia autoantibodies trigger thrombocytopenia and severe bleeding, Fcγ-mediated functions may trigger tissue inflammation in many autoimmune diseases, e.g., rheumatoid arthritis and pemphigoid disease[44].

Autoimmune disease and gender influence

Generally, women have a stronger humoral and cellular immuneresponse compared to men. They show a higher CD4:CD8 ratio because of a higher absolute CD4 cell count and a higher level of circulating antibodies[46,47]. Compared to men, they have more rapid rejection of allograft and reduced incidence and regression of tumors[48].Estrogens, androgens and prolactin are hormones that have been studied for increasing susceptibility to ADs and can affect both innate and adaptive immune systems[49,50]. Estrogens seem to direct the immune system to T-helper 2 (Th2) lymphocyte dominance with the consequence of more B cell activation and antibodyproduction[51]. In contrast, androgen favors the development of

a T-helper 1 (Th1) response and CD8þ cell activation[52,53].Prolactin appears to stimulate both cell and humoral-basedimmunity[54] . For this modulation of the immune system to be

possible, these hormones have to be able to bind to receptors expressed by immune cells. B cells have been shown to express both androgen and estrogen receptors. In murine models, CD8 cells have been shown to express estrogen receptors along with monocytes, neutrophils and natural killer cells[55,56].Otherstudies suggest that sex

hormones like estrogen can regulate antigen presentation by macrophages and dendritic cells through production of transforming growth factor beta[57,58].
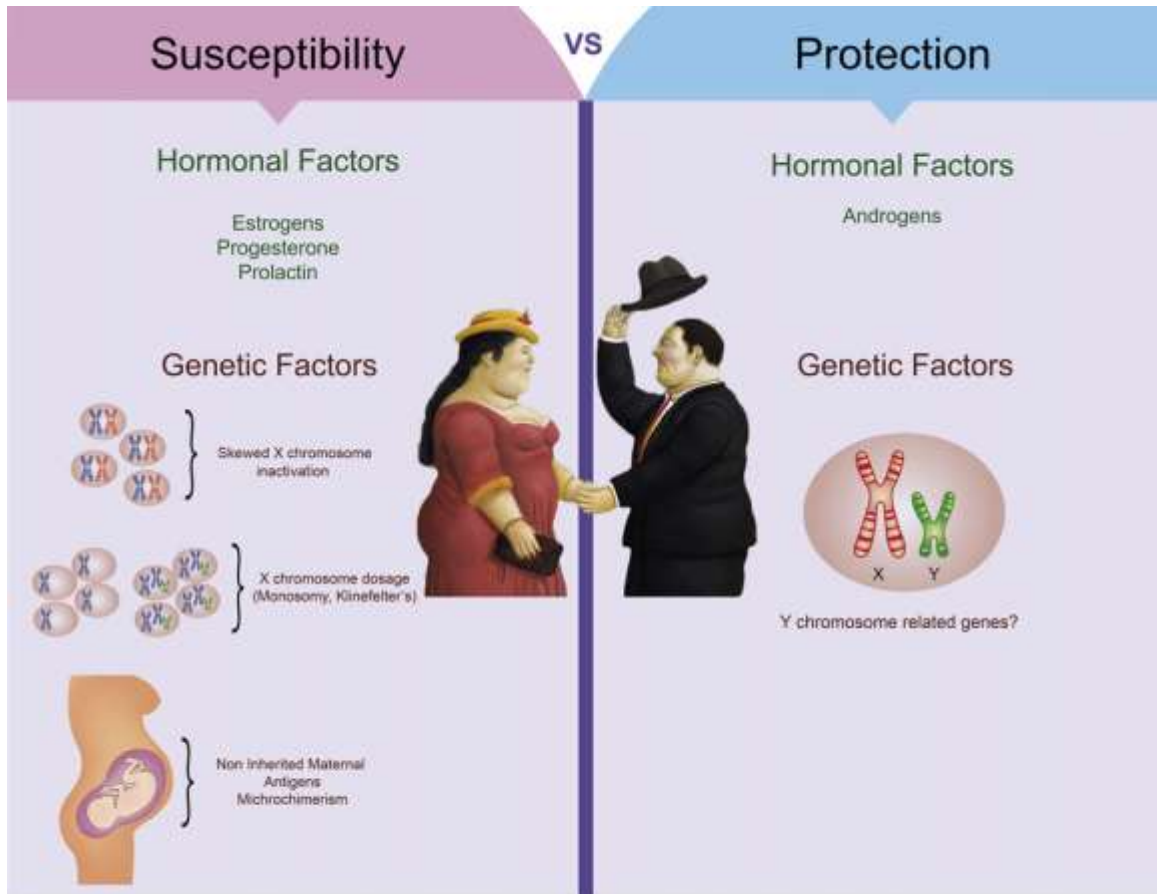
Figure 6: Gender Susceptibility

Gender related factors influencing the development of autoimmune diseases (ADs). Main factors explaining the higher prevalence of several ADs in women include hormonalfactors,since estrogens and prolactin are immune stimulants while androgens are immune suppressors.

Genetic factors include[46]:

1.) Skewed X chromosome inactivation, a potential mechanism whereby X-linked self-antigens may escape presentation in the thymus or peripheral sites that are involved in tolerance induction

2.) X chromosome dosage:

a) Xmonosomy: through the generation of autoreactive T cells that are not exposed to

self-antigens encoded by one of the two X chromosomes

b) Klinefelter's syndrome: incidence of SLE is higher in XXY males;

3.) Non-inherited maternal antigens (NIMAs) acting as modulators of the immune repertoire; 4.) Microchimerism: microchimeric cells might be targeted as foreign cells they could be implicated in the pathogenesis of ADs

5.) Although genetic protective associations of genes in the Y chromosome have not been found in humans, rodent models suggest that unexplored regions or genes in the Y chromosome may play a role in the protection of men to the most relevant ADs.

Circulating immune complexes seen in various thyroid diseases which include Graves' disease, Hashimoto's thyroiditis, Spontaneous myxedema, Asymptomatic thyroiditis, Goitre with thyroiditis, Diffuse goitre, Nodular goitre, Cold nodule and Hot nodule[59].

Autoimmune thyroiditis is defined as an inflammatory state of the thyroid gland characterized

byintrathyroid lymphocytic infiltration, ultrasonographic signs of inflammation, and antibodies to thyroglobulin, thyroid peroxidase (TPO) or both. The generic term autoimmune thyroiditis encompasses Hashimoto thyroiditis (the most frequently encountered form), Graves' disease and some rare variants, such as painless sporadic thyroiditis, postpartum thyroiditis, drug induced thyroiditis and thyroiditis that accompanied polyglandular autoimmune syndromes. Hashimoto thyroiditis is subdivided into the classic form, characterized by goiter and an autoimmune response, and the atrophic form[60].

Environmental factors that contribute to the development of autoimmune thyroiditis[60,61].

- Dietary factors

Iodine

Selenium

- Pollutants

    Radioiodine

    Tobacco smoking

    Polychlorinated biphenyls

    Effects of global warming (possible)

- Hormones

    Pregnancy and parity

- Stress

- Therapy

    Interferon α

    Interferon β

    Interleukin 2

    Amiodarone

    Antiretroviral agents

- Infections

    Yersinia enterocolitica

    Hepatitis C virus

- Socioeconomic environment (hygiene hypothesis)

Pathogenesis of Hashimoto's disease (A) and Graves' Disease (B).

(**A**) Autoreactive CD4+ T cells in HT induce antibody production by B cells. The antibodies bind to the basal membrane of the thyroid follicle, activate complement, and induce necrosis of thyrocytes. The activation of cytotoxic CD8+ T cells leads to the

induction of apoptosis by action of perforin. Finally, the expression of Fas (CD95) and FasL (CD95L) by thyrocytes perpetuates HT[61,62].

(B) Autoreactive CD4+ T cells in GD induce only anti-thyroid-stimulating hormone receptor antibody-producing B cells. These antibodies act stimulatory by increasing I2 metabolism (cAMP/PKA) and promoting proliferation and survival (PI3K/PKC/ERK) of thyrocytes. Blocking antibodies are characterized by lack of effect (not shown), and neutral antibodies activate various pathways, such as PI3K/Akt, mTOR/p70S6K, and MAPK/ERK1/2 and induce thyrocyte apoptosis[44,62,63].
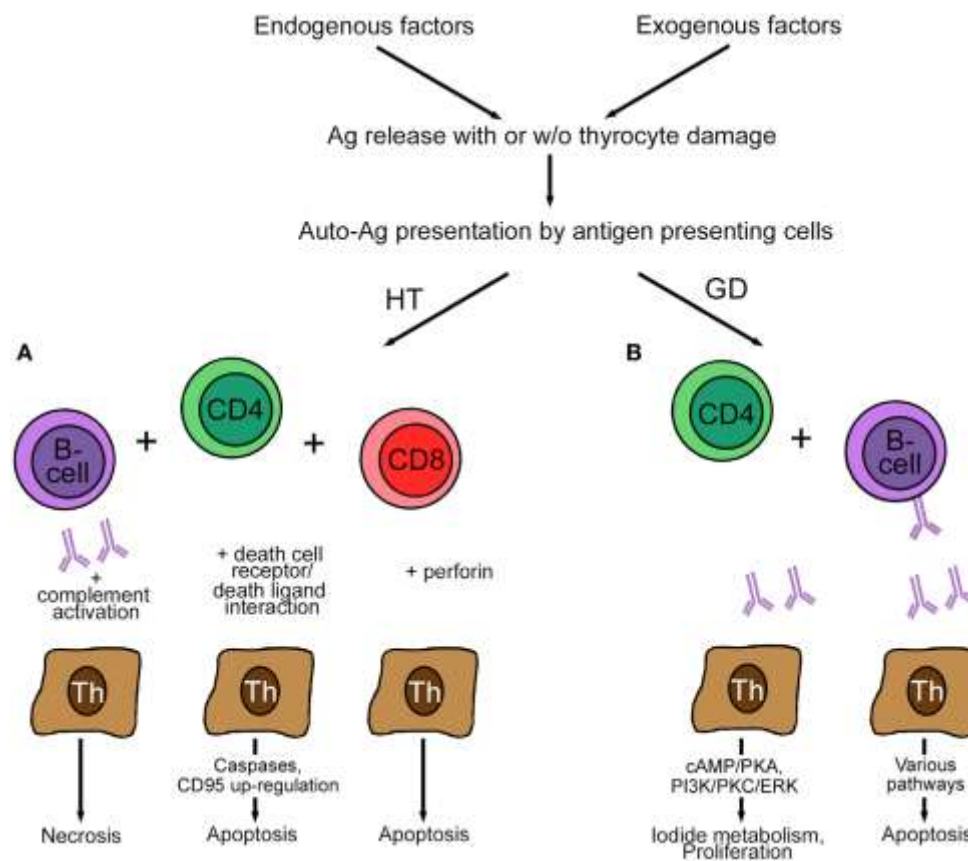
Figure 7: Pathogenesis of Autoimmune Thyroiditis

HISTOLOGIC VARIANTS OF HASHIMOTOS THYROIDITIS(HT)

The classic form: HT typically presents during the 5th decade of life and is overwhelmingly more common in women. The thyroid gland is enlarged and firm. Most patients (approximately 75%) are euthyroid at diagnosis, whereas the remaining

minority show a range of dysfunctions from subclinical hypothyroidism (defined by elevatedTSH levels but with thyroid hormones still within the normal range)to overt hypothyroidism[64,65].

The fibrous variant: The fibrous variant of HT is also more common in older women. It also presents goiter, which is however often symptomatic. The thyroid is lobulated, imparting the appearance of bona fide thyroid nodules. Most patients are hypothyroid and require prompt thyroid hormonereplacement[66]. In older ages this fibrous form evolves into a severe form of thyroid atrophy that manifests clinically as idiopathic myxedema. The thyroid gland is not palpable, and patients have the symptoms of hypothyroidism, which are however more difficult to identify given the overlap with the manifestations of aging[70].

The IgG4-related variant: The IgG4-related variant of HT, like the classic form, occurs most commonly during the 5th decade of life but at a younger age . Like for other IgG4-related diseases, men are frequently affected, decreasing the extremely high female to male ratio of the classic form to 3:1. TheIgG4-related variant tends to have a more rapid and aggressive course, so that many patients are still found to have subclinical hypothyroidism despite being replaced with synthetic thyroid hormones. Thyroid autoantibodies reach the highest values [72].

The juvenile variant: The juvenile variant is the form of HT presenting before 18 years of age,with a mean age at presentation of 11 years. It is more common in females, although with a smaller female to male ratio .Most children have goiter but are usually asymptomatic. At the time of diagnosis, 43% of the children are euthyroid, 24% have subclinical hypothyroidism, 21% overt hypothyroidism, 9% overt hyperthyroidism, and 3subclinical hyperthyroidism. The natural history is variable,with remission, recurrence, and evolution into permanent hypothyroidism all being described[73].

The Hashitoxicosis variant: The Hashitoxicosis variant, initially described by Fatourechi in 1971, has the clinical features of Graves's hyperthyroidism and the pathological appearance of HT. The initial hyperthyroid phase is virtually indistinguishable from Graves's disease, including the elevated thyroid uptake of radioactive iodine and the presence of thyroid-stimulating immunoglobulins. The hyperthyroidism however, is transient, and after a period of 3 to 24 months it evolves into permanenthypothyroidism[74].

Painless (or silent) thyroiditis: It is a lymphocytic inflammation of the thyroid gland that can occur sporadically or, more commonly, within12 months after delivery. The two forms are indistinguishable except for the relation of the latter form to pregnancy, so that is called postpartum thyroiditis. Painless thyroiditis tends to be more prevalent in areas of higher dietary iodine intake, and is scholarly described as having a triphasic pattern consisting of an initial phase of thyrotoxicosis, followed by hypothyroidism, and then recovery[73,74].

THYROID NEOPLASMS

Thyroid cancer has a wide spectrum of morphologies and behaviors that include the most common and indolent tumors as well as the most aggressive and rapidly lethal malignancies. The importance of pathology in the identification, diagnosis, and prognostication of thyroid cancer cannot be underestimated[75].

Similar to other neoplasias also in thyroid carcinomas, activated oncogenes (Table 3) are considered the underlying event leading to uncontrolled cell growth. Activated oncogenes have been identified in thyroid malignancies and also in adenoma and hyperplasia[38,76]. These oncogenes with genetic lesion results in biochemical abnormality and the effect of such abnormality on signal transduction leads to unregulated growth, and finally the histological type is associated with a distinct activated oncogene[77].

Most of the products of activated oncogenes are proteins that operate through the mitogen activated protein kinase cascade (MAPK) and cAMP-dependent protein kinase (PKA). Normally, signals proceed from plasma membrane into cytosolic and nuclear targets, activating a series of regulatory molecules that govern fundamental cellular processes such as proliferation, differentiation, stress response, apoptosis and cell cycle progression. A defect in this finely regulated network might lead to neoplastic transformation[78].

Histological variants of thyroid malignancies

There is general appreciation that thyroid cancer can be divided clinically into a large group of well- differentiated neoplasms of slow growth and high curability, and a smaller group of highly anaplastic tumors with a uniformly fatal outlook. A small group of slowly growing tumors derived from the parafollicular or C cell has been established as a distinct entity called as medullary carcinoma[79].

Table 3: Principal oncogenes and growth factors involved in thyroid carcinogenesis[79].

Oncogene/GF	Lesion	Mechanism	Effect	Neoplasia type	%
ras-Family N&H ras	point mutation ki-ras radiation-induced	ras-constitutively bound to GAP (GTPase-activating protein)	Activation of adenylate cyclase and calcium channels	Adenoma Ca. papillary Follicular Anaplastic	35 18 32 58
gsp	Point mutation	Ribosylated GS-α at arginine 201	Impairing of GTPase activity	Hot adenomas	27
RET (Receptor for glial-derived neurotrophic GF)	RET/PTC Paracentric inversion at chromosome 10 PTC ¾ radiation-induced	Fusion proteins with constitutive TK activities	Mitogenic through constitutive activation of TKR	Ca. papillary: Italy Saudi Arabia	33.3 2.5
RET (Receptor for glial-derived neurotrophic GF)	RET/MTC point mutation germline (fam.) point mutation somatic (sporadic)	Dimerization of RET TKR	Increased auto-phosphorylation and alteration of substrate specificity	Exon 10-11 MEN 2A FMTC Exon 16 MEN 2B (codon 918) Sporadic	97 87 94 23-85
c-MET (α and β subunit)	Amplification of gene for HGF-R or splicing of α and β	Increased receptors for HGF/SF	Enhancement of receptor kinase activity	Ca. papillary (aggressive) Follicular	74 22
TRK	Rearrangement NRTK/Tropo-myosin or cyto-skeletal proteins	Receptor for NGF	Mitogen activated TK cascade	Ca. papillary	12.6
EGF / EGF-R	Overexpression c-Erb B1/c-Erb B2/neu	Competence factor in cell cycle	Transition through G_0-G_1 phase	Ca. anaplastic	
p53	Point mutation at multiple sites, late action, negative dominant effect	Lack of activation of p21/Waf 1 gene expression	Loss of regulation at the critical G_1 to S phase	Ca. anaplastic Papillary Follicular	40 6.2 2.7

Sir Lewis B. Woolner produced data based on a large series of personally observed patients whose condition was diagnosed and who were treated at the clinic at some time between 1926 and 1960. During a period of 35 yrs., 1181 thyroid carcinomas were diagnosed, and the patients were treated at the Mayo Clinic (table 4). The classification used and the relative incidence of various subtypes was presented[80].

Table4: Classification of Thyroid Carcinoma (Mayo Clinic, 1926-1960, 1181 Cases)

Types of Thyroid Carcinoma	Cases
Well-differentiated	
• Papillary	736
• Follicular	208
	77

Undifferentiated	
• Medullary (solid)	160
• with amyloid	
Anaplastic	1,181

The classification of thyroid cancers has undergone multiple alterations over the last century. At the time of publication of the first Armed Forces Institute of Pathology Fascicle on thyroid tumors, published in 1953, the classification of differentiated thyroid cancer was very simple (Table 5): there were benign and malignant tumors and they each came in 2 variants, papillary and follicular, that were distinguished based on architecture. The classification of a lesion as malignant was dependent on identifying invasive behavior that could be infiltration of the tumor capsule, surrounding tissue and/or vessels[81].

Table 5: Classification of primary thyroid neoplasms of follicular cell derivation circa 1953

Benign	Follicular adenoma
	Papillary cystadenoma
Malignant – Differentiated	Follicular carcinoma
	Papillary carcinoma
Malignant - Undifferentiated	Anaplastic carcinoma

The classification based on architecture was complicated by the reality that many "papillary carcinomas" had prominent areas of follicular architecture. Thus came the era of "mixed papillary and follicular" carcinomas, despite the fact that these tumors

behaved in the same fashion as classical papillary carcinomas. The following decades also saw new variants of papillary carcinomas that behaved in a more aggressive way than conventional papillary thyroid carcinoma (PTCs); these included tall cell carcinomas, the very rare columnar cell type and most recently, the hobnail cell variant. Many of these arose within differentiated carcinomas, and some were associated with anaplastic dedifferentiation, paving the way for the concept of progressive dedifferentiation in thyroid carcinoma[82,83].

Table 6: Classification of primary thyroid neoplasms of follicular cell derivation circa 1992[84]

Benign	Follicular adenoma • Conventional • Variants Papillary cystadenoma
Malignant – Differentiated	Follicular carcinoma Papillary carcinoma • Conventional • Variants
Malignant -Poorly differentiated	Insular carcinoma Others
Malignant - Undifferentiated	Anaplastic carcinoma

By 2004, the World Health Organization (WHO) book on endocrine tumors classified all tumors that displayed "distinctive nuclear features" as papillary carcinoma variants[85].

Table 7: Classification of primary thyroid neoplasms of follicular cell derivation circa 2004

Benign	Follicular adenoma- Multiple variants Hyalinizing trabecular tumor
Malignant – Differentiated	Papillary carcinoma • Multiple variants Follicular carcinoma • Minimally invasive • Widely invasive
Malignant -Poorly differentiated	Poorly differentiated carcinoma
Malignant - Undifferentiated	Anaplastic carcinoma

However, the threshold for the diagnosis of follicular variant papillary thyroid carcinoma

(FVPTC) became one of the most controversial areas in pathology with significant

intraobserver and interobserver variation. In 2016, an entire study was dedicated to developing consensus for the recognition and grading of nuclear atypia. Despite the many controversies and changes that have evolved over the past 70 years, it remains

irrefutable that thyroid cancer manifests a spectrum of morphologies that correlates with clinical behavior[86,87,88].

Table 8: World Health Organization classification of primary thyroid neoplasms of follicular cell derivation 2017

Benign	Follicular adenoma	
Borderline/uncertain	Hyalinizing trabecular tumor, Other encapsulated follicular patterned tumors	• Follicular tumor of UM P (Uncertain Malignant Potential) • Well-differentiated tumor of UMP • Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features (NIFT-P)
Malignant	PTC	• Papillary carcinoma • Follicular variant of PTC • Encapsulated variant of PTC • Papillary microcarcinoma • Columnar cell variant of PTC

	FTC	• Oncocytic variant of PTC
		• FTC, minimally invasive
		• FTC, encapsulated Angioinvasive
		• FTC, widely invasive
	Hurthle (oncocytic) cell tumors	• Hurthle cell carcinoma
	Others • Poorly differentiated thyroid • Carcinoma • Anaplastic thyroid carcinoma • Squamous cell carcinoma • Medullary thyroid carcinoma • Mixed medullary and thyroid carcinoma • Mucoepidermoid carcinoma • Sclerosingmucoepidermoid carcinoma with eosinophils. • Mucinous carcinoma • Ectopic thymoma	

	• Spindle epithelial tumor with thymus like differentiation. • Intrathyroidthymic carcinoma Paraganaglioma and mesenchymal /stromal tumors Hematolymphoidtumours Germ cell tumors Secondary tumors	

PROGNOSTIC FACTORS

A major clinical goal for those who manage patients with thyroid cancer is to identify morphologic and molecular features that provide the ability to identify tumors of any given type that will behave more aggressively than others. In differentiated thyroid cancers, several biomarkers, molecular alterations, and epigenetic features, including microRNAs, have been proposed. Morphologic features that predict progression are also important. Clinical parameters, including age, gender, and risk factors, are also of critical relevance. It is generally accepted that size and rate of growth are important clinical parameters that distinguish a classical papillary microcarcinoma that can be subjected to active surveillance from a large, infiltrative classical PTC that requires surgery. Minimally invasive FTCs and encapsulated FVPTCs or NIFT-P lesions, and even low-risk classical PTCs in the clinically detectable range, can be considered adequately treated by surgical resection without total thyroidectomy or lymph node dissection. In contrast, PTCs with morphologic features, such as hobnail, tall, or

columnar cell features, warrant more careful assessment to determine whether they have developed extra thyroidal extension or regional lymph node metastases. Carcinomas of either papillary or follicular type that show unequivocal angioinvasion are more likely to give rise to distant metastases. Any tumor that exhibits areas of dedifferentiation should be considered for more aggressive therapy. It remains important to balance the risks of total thyroidectomy and radioactive iodine therapy with the benefit that is likely to be gained, and this requires insight into the many and various factors that alter the predicted outcome[38].

IV. IMMUNOHISTOCHEMISTRY IN THYROID NEOPLASMS

Most thyroid tumors can be readily diagnosed using histopathologic criteria, which allow the pathologist to differentiate benign from malignant lesions and guarantee an accurate classification for the majority of the variants of carcinomas derived from follicular epithelial cells. However, in most cases, the pathologist is confronted with thyroid lesions in which the distinction between benign and malignant can be quite subtle. The decision favoring one or another has clinical consequences and implies different modalities of treatment. On one hand, there is the need to avoid excessive treatment and psychological discomfort to the patient. On the other hand, patients with potentially aggressive disease need to be guaranteed effective management at the initial stages of disease when it is still curable. For this reason, the approach to these challenging tumors should include ancillary techniques, immunohistochemistry and molecular profiling, that can improve the standard morphologic assessment both in surgical specimens and in cytology samples obtained by fine-needle aspiration[89,90].

Several immunohistochemical markers using different antibodies, alone or combined in panels, have been postulated to improve diagnostic accuracy of follicular-pattern thyroid lesions. They belong to different categories and are involved in cell adhesion (galectin-3, E-cadherin, fibronectin [FN]), receptor signaling (RET), gene transcription control (thyroid transcription factor 1 [TTF-1]), secretion (thyroglobulin [TG], calcitonin, carcinoembryonic antigen [CEA]), cell cycle regulation (p27, cyclin D1), and cellular structure (cytokeratin [CK] 19). They are detected in different cellular compartments such as membrane and/or cytoplasm (Hector Battiforamesothelial (cell) 1 [HBME-1], β-catenin) and nucleus (p53)[91].

i.   Ret oncogene

The identification of *RET/PTC* rearrangements has increased the ability to diagnose PTC. The utility of aberrant*RET*expression by rearrangement as a diagnostic marker in borderline thyroid lesions and preoperative evaluation of thyroid aspirates is supported by its high specificity for PTC. RET immunostaining has been shown to be useful in the assessment of thyroid lesions with incomplete and/or focal features of PTC in which positive staining has been demonstrated in more than 50% of cases in close parallel with the morphologic features suggestive of PTC. RET protein detection by immunohistochemistry has also been reported in the so called HTAs, and this finding was confirmed by detectionof*RET/PTC-1* rearrangements by RT-PCR, suggesting that HTA may represent a variant of PTC. Similarly, it has been shown that oncocytic tumors that exhibit nuclear features of PTC also harbor *RET/PTC* rearrangements[92].

RET protein–positive immunostaining can also be demonstrated in the oncocytic cells of this subset of tumors, supporting the revised classification system for oncocytic tumors that recognizes an oncocytic follicular variant of PTC. RET/PTC oncogene product immunostaining has been reported as a useful adjunct when used in combination with other antibodies including CK19, galectin-3, and HBME-1 in the assessment of thyroid specimens and aspirates[93].

ii.   Cytokeratin 19

Different subtypes of keratin filaments are grouped according to molecular weight. High-molecular-weight CKs (CK1, CK4, CK10, and CK13) are detected in stratified squamous epithelium. Simple or glandular epithelium expresses CK7, CK8, CK18, and CK19.

Normal thyroid follicular epithelium is often negative, although focal staining for

CK19 is usually identified in the compressed thyroid parenchyma surrounding nodules and in follicular cells within lymphocytic thyroiditis. This pattern of staining is consistent with the intense pattern of staining seen in reactive follicular epithelium within thyroid nodules around the site of degeneration, especially at the site of a previous needle biopsy. However, the finely dispersed positivity seen in the cells of PTC is distinctive. Although this feature is usually diffuse throughout the lesion, focal staining for CK19 does not rule out a diagnosis of PTC, particularly in nodules with nuclear features of PTC that are seen focally. CK19 has also been considered by many investigators to be a useful ancillary tool for the diagnosis of papillary carcinoma in FNAC, especially in cytologically suggestive but indeterminate cases. The reported sensitivity and specificity using CK19 as a single marker is as high as 92% and 97%, respectively[94,95,96].

iii. Galectin3

Galectin-3 (31-kd molecular weight) is one of the members of a family of non-integrin _-galactoside–binding lectins that have related amino acid sequences in the carbohydrate binding site. Galectin-3 has affinity for CEA, immunoglobulin(Ig) E,lamnin, and other mucins. Kovacs *etal*found that IHC expression of galectin-3 may help in the differential diagnosis of solitary encapsulated follicular lesions, especially the minimally invasive follicular carcinoma. Several other investigators showed thatgalectin-3 is very useful in distinguishing benign from malignant tumors, especially PTC, with high sensitivity and specificity. Galectin-3 can aid in identifying FVPC, and distinguishing minimally invasive FC from FA. Galectin-3 shows 85.2% sensitivity for immunoexpression distinction between carcinomas and benign nodules [97,98].

iv.HBME 1

HBME-1 is a monoclonal antibody that recognizes an unknown antigen in the microvilli of mesothelioma cells, normal tracheal epithelium, and adenocarcinoma of the lung, pancreas, and breast. HBME-1 has also been reported by several investigators to be a useful marker of malignancy in thyroid nodules. Overall in the thyroid, HBME-1 stains mostly follicular-derived malignant tumors, including well-differentiated and poorly differentiated carcinomas, with a variable sensitivity and specificity in different series[99].

It has been found to be reactive mostly in papillary thyroid carcinoma and some follicular carcinomas, but usually negative in follicular adenomas. Papotti*et al* in a study of well-differentiated thyroid tumors of uncertain malignant potential found that a diffuse and strong expression of HBME-1, and to a lesser extent galectin-3,is preferentially observed in the tumors with nuclear changes suggestive of papillary carcinoma. However, they concluded that the diagnosis of these tumors should also depend on previously defined morphologiccriteria[99].

v. Cyclo-Oxygenase 2

Cyclooxygenase (COX) or prostaglandin H synthase is involved in the formation of prostaglandins from arachidonic acid. Recently, COX-2 has been detected in the thyroid, although the expression varies widely in tumors. Papillary and follicular carcinomas of the thyroid show a significantly higher perinuclear cytoplasmic immunoreactivity, compared with normal follicular cells and follicular adenomas[100].

vi. Peroxisome Proliferator-Activated Receptor.

PAX8-PPAR_1 rearrangements were initially reported in a subset of angioinvasive follicular carcinomas as a result of the translocation t(2;3)(q13;p25), which leads to fusion of the DNA-binding domains of the thyroid transcription factor PAX8 to

domains A to F of the PPAR_1.90 the presence of PAX8-PPAR_1 expression by RT-PCR and shows strong nuclear

stainingby immunohistochemistry in 53% to 69% of follicular carcinomas, as well as in a small number of papillary carcinomas, and its absence in nodular hyperplasia[101].

vii. E-Cadherin

Normal thyroid follicular cells express uniformly high levels of E-cadherin mRNA and have a strong cell surface pattern of staining. E-cadherin staining is variably reduced in well-differentiated thyroid carcinomas and frequently absent in poorly differentiated and anaplastic carcinomas. Loss of Ecadherin expression is an adverse prognostic factor in differentiated thyroid carcinomas[102].

viii. β-catenin

Thyroid tumors express different patterns of immunoreactivity, including strong to weak membranous positivity reported in follicular adenomas and well-differentiated thyroid carcinomas and loss of membranous staining with nuclear and cytoplasmic staining in poorly differentiated and anaplastic carcinomas. Along with E-cadherin, loss of membrane _-catenin immunostaining is an indicator of loss of differentiation and adverse prognosis. Aberrant nuclear immunoreactivity of β-catenin is associated with stabilizing *CTNNB1* exon 3 mutations that are found almost exclusively in poorly differentiated and anaplastic carcinomas. The cribriform-morular variant of PTC, which is pathognomonic of familial adenomatous polyposis–associated thyroid carcinoma, has been reported to demonstrate

cytoplasmic and nuclear accumulation of β-catenin and *CTNNB1* exon 3 mutations[103].

ix.   Fibronectin-1

Fibronectins are multifunctional adhesive glycoproteins found in the extracellular matrix and body fluids. Fibronectin emerged as a potential marker of thyroid carcinoma in microarray studies in which it was reported to be up-regulated compared with normal tissue. An

immunohistochemical panel consisting of FN, galectin-3, and HBME-1 has been reported to be effective in the diagnosis of follicular cell–derived thyroid tumors. However, FN expression is reduced in more aggressive cancers and in the invasive components of differentiated carcinomas. In fact, modulation to up-regulate FN may represents therapeutic target to prevent invasion and metastasis in thyroid carcinoma[104].

x.   Thyroid Peroxidase

Thyroid peroxidase (TPO) is a thyroid-specific enzyme involved in the synthesis of thyroid hormone. Gene suppression and mutations of the *TPO* gene were reported in some differentiated thyroid carcinomas. Reduced TPO immunoreactivity has been proposed by some investigators as a marker to distinguish benign from malignant thyroid tumors at preoperative assessment of thyroid nodules by FNAC. The use of TPO in combination with other immunohistochemical markers has been reported as useful for the diagnosis of papillary carcinoma in surgical thyroid specimens[105].

xi.   P53

Mutations of *TP53* represent late genetic events in thyroid carcinogenesis. As a result, accumulation of p53 can be detected by immunohistochemistry most often in anaplastic and poorly differentiated thyroid carcinomas. Rarely, it can be seen in well-differentiated papillary and follicular carcinomas as well as in medullary carcinomas. Positive immunoreactivity for p53 is an independent prognostic factor for overall survival of patients with thyroid cancer[106].

xii. Thyroid Transcription Factor 1

TTF-1 was identified in 1989 as a nuclear tissue-specific protein with DNA-binding activity that interacted with the *TG* gene in the rat.144 TTF-1 regulates gene expression in the thyroid, lungs, and diencephalon during embryogenesis. In the thyroid, nuclear reactivity for TTF-1 is present in follicular cell–derived benign and malignant lesions and medullary carcinomas. Poorly differentiated carcinomas often show decreased and focal staining for TTF-1, and most anaplastic carcinomas lack TTF-1 reactivity. When used in combination with TG, TTF-1 is an effective marker for thyroid origin. Lack of TTF-1 immunoreactivity in a thyroid tumor should prompt investigation of other differential diagnoses, including parathyroid tumors, paragangliomas, and metastatic lesions[107].

xiii. Thyroglobulin

Thyroglobulin is the primary product synthesized in the thyroid, and the macromolecular precursor of the iodinated thyroid hormones thyroxine (T4) and triiodothyronine (T3) Compared with TTF-1, TG is also a highly sensitive histogenetic marker for follicular cell origin, although patchy staining pattern, particularly in the less well-differentiated tumors, may produce less reliable results in small biopsies[108].

xiv. Calcitonin

Medullary thyroid carcinoma (MTC) and C cells stain positively for calcitonin, a secreted protein produced by parafollicular C cells, which causes a rapid but short-lived drop in the level of calcium and phosphate in blood by promoting the incorporation of those ions in the bones. Calcitonin, together with CEA, chromogranin, synaptophysin, and calcitonin gene-related peptide are the most useful immunohistochemical markers for the diagnosis of MTC, especially when facing histologic subtypes such as the follicular, papillary, or encapsulated variants that can pose diagnostic difficulties with

follicular cell–derived carcinomas and paraganglioma. Calcitonin is also a diagnostic marker to confirm C-cell hyperplasia, which is usually associated with familial medullary carcinoma[109,110].

V. THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY (TBSRTC)

A standardized nomenclature for the interpretation of thyroid fine needle aspirates (FNAs), known as the Bethesda system for reporting thyroid cytopathology was introduced in 2007, which describes six diagnostic categories of lesions as described in table 9.

Table 9: CATEGORIES OF TBSRTC

I	Non Diagnostic or Non Satisfactory	Cyst fluid only Acellular material Hemorrhage/clot/ artifact
II	Benign	Consistent with benign nodules/ lymphocytic thyroiditis/ granulomatous thyroiditis
III	AUS/FLUS (Atypia of undetermined significance or follicular lesion of undetermined significance)	
IV	Follicular neoplasm or suspicious for follicular neoplasm.(Includes Hurthlecell change)	
V	Suspicious for malignancy	Suspicious for • Papillary carcinoma • Medullary carcinoma • Metastatic carcinoma • Lymphoma

		• Others
VI	Malignant	Malignant lesions.

The description of cytology smears to categorize under Bethesda system includes[111]

Non‑diagnostic or unsatisfactory

An adequate smear has at least six well‑preserved follicular groups which contains at least ten follicular cells. But abundant thick colloid may not have a requirement for a minimum number of follicular cells. Smears not fulfillin the criteria or showing onlyhistiocytes are considered inadequate.

Benign

Cytological features of colloid goiter/adenomatoid goiter, Hashimoto's thyroiditis, thyrotoxicosis, de Quervain's thyroiditis,or granulomatous thyroiditis due to Koch's are categorized as benign thyroid lesions.

AUS/FLUS

Smears with cytological features showing few cells with atypia, which cannot be categorized definitely in benign or malignancy or suspicious for malignancies are categorized in this group.

FN/SFN

Smears with high cellularity showing predominant micro follicular or trabecular pattern of arrangement of follicular cells (hurthle cell change also) are categorized under this group.

Suspicious for malignancy

Cellular smears with marked atypia and which cannot be definitely categorized as malignancy is included in this group.

Malignant

Aspirates that appeared unequivocally malignant are placed in this category

Based on the reporting system risk of malignancies is calculated and the management is as shown in table 10.[112]

Diagnostic Category	Risk of Malignancy	Usual Management
Nondiagnostic	——	Repeat FNA with u/s
Benign	0-3%	Clinical follow-up
Atypical Follicular Lesion of Undetermined Significance	5-15%	Repeat FNA
Suspicious for Follicular Neoplasm	15-30%	Surgical lobectomy
Suspicious for Malignancy	60-75%	Near-total thyroidectomy or surgical lobectomy
Malignant	97-99%	Near-total thyroidectomy

VI. References

1. Aboud R S. Iraqi Journal of Science 2011; 52(4): 440-444.

2. Alaa H, Yousif E. Thyroid Gland and Its Rule in Human Body Research Journal of Pharmaceutical , Biological and Chemical Sciences Thyroid Gland and Its Rule in Human Body . 2017;1-7.

3. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. EndocrRev., 2010;31: 139–70.

4. Webb P. Thyroid hormone receptor and lipid regulation. CurrOpin Invest Drugs 2010;11:1135–42.

5. Liu YY, Brent GA. Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. Trends EndocrinolMetab2010;21: 166–73.

6. Motomura K, Brent GA. Mechanisms of thyroid hormone action. Implications for theclinical manifestation of thyrotoxicosis. EndocrinolMetabClin N Am 1998;27: 1–23.

7. Silva JE, Bianco SD. Thyroid-adrenergic interactions: physiological and clinical implications.Thyroid2008;18: 157–65.

8. Silva JE. Physiological importance and control of non-shivering facultative thermogenesis.Front Biosci.2011;3: 352–71.

9. Hollenberg AN. The role of the thyrotropin-releasing hormone (TRH) neuron as a

   metabolic sensor. Thyroid.2008;18: 131–39.

10. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev. 2014;94(2):355–82.

11. Song Y, Yao X, Ying H. Thyroid hormone action in metabolic regulation. Protein Cell. 2011;2(5):358–68.

12.   Guyton A C,Hall J E. "Text Book of medical physiology" 10th ed. 2000; 7: 862-865.

13.   Hershman JM. Clinical application of thyrotropin-releasing hormone. N Engl J Med.1974; 290: 886–90.

14.   Oetting A, Yen PM. New insights into thyroid hormone action. BestPract Res ClinEndocrinol Metab.2007;21: 193–208.

15.   Knudsen N, Laurberg P, Rasmussen LB, Bulow I, Perrild H, Ovesen L, Jorgensen T.Small differences in thyroid function may be important for body mass index and theoccurrence of obesity in the population. J ClinEndocrinol Metab.2005;90: 4019–24.

16.   Motomura K, Brent GA. Mechanisms of thyroid hormone action. Implications for theclinical manifestation of thyrotoxicosis. EndocrinolMetabClin N Am.1998;27: 1–23.

17.   Brent GA. Hypothyroidism and thyroiditis. In: Williams Textbook of Endocrinology,edited by Melmed SP, Larsen PR, and Kronenberg HM. Philadelphia, PA: Elsevier,2012.

18.   Roderick N, Macsween J, Whaley K: "Nuirs Text book of pathology" 13th ed. 1994.

19.   William M J., "Clinical chemistry" 4 th ed., Harcourt publisher limited 2009; 9154-157.

20.   Lazarus JH, Obuobie K. Thyroid disorders - An update. Postgrad Med J. 2000;76(899):529–36.

21.   Joshi SR. Laboratory evaluation of thyroid function. J Assoc Physicians India. 2011;59(SUPPL JAN2011):14–20.

22.   Spencer C. Thyroid Function Tests: Assay of Thyroid Hormonesand Related

Substances, www.thyroidmanager.com. 2010.

23. Supit EJ, Peiris AN. Interpretation of Laboratory Thyroid Function
Tests: Selection and Interpretation. Southern Medical Journal.2002;95:481-85.

24. Werner SC, Ingbar SH. Werner &Ingbar's The Thyroid: afundamental and
clinical text, 9th edition. Lippincott Williams &Wilkins.

25. Mascarenhas JMA. RxPG AIPG 2004 Book, 2006.

26. Daniels GH, Amiodarone-Induced Thyrotoxicosis, The J Clinical
Endo &Metab2000;86:3-8.

27. BoelaertK,Horacek J, Holder R.L,Watkinson J.C, Sheppard M.C, Franklyn J.A.
Serum thyrotropin concentration as a novel predictor of malignancy in thyroid
nodules investigated by fine-needle aspiration. J. Clin. Endocrinol. Metab. 2006;
91: 4295–301.

28. Walfish PG. Triiodothyronine and thyroxine interrelationships inhealth and
disease. Can Med Assoc J 1976;115:338–42.

29. Düsünsel R, Poyrazoglu HM, Gündüz Z et al. Evidence of centralhypothyroidism
in children on continuous ambulatory peritonealdialysis. AdvPerit Dial.
1999;15:262-8.

30. Krohn, K.; Fuhrer, D.; Bayer, Y.; Eszlinger, M.; Brauer, V.; Neumann, S.;
Paschke, R. Molecular pathogenesisofeuthyroid and toxic multinodular goiter.
Endocr. Rev. **2005**, 26, 504–24.

31. Erdogan, M.F.; Gursoy, A.; Erdogan, G. Natural course of benign thyroid
nodules in a moderatelyiodine-deficient area. Clin. Endocrinol. Oxf. **2006**, 65,
767–71.

32. Xu W, Huo L, Chen Z, Huang Y, Jin X, Deng J, et al. The relationship of TPOab
and TGAb with risk of thyroid nodules: A large epidemiological study. Int J

Environ Res Public Health. 2017;14(7):1–11.

33. Marqusee E, Benson CB, Frates MC, DoubiletPM,Larsen PR, Cibas ES, Mandel SJ 2000 Usefulness ofultrasonography in the management of nodular thyroid disease. Ann Intern Med 133:696–700.

34. Hagag P, Strauss S, Weiss M 1998 Role of ultrasoundguidedfine-needle aspiration biopsy in evaluation ofnonpalpablethyroid nodules. Thyroid 8:989–95.

35. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26(1):1–133.

36. Dauksiene D, Petkeviciene J, Klumbiene J, Verkauskiene R, Vainikonyte-Kristapone J, Seibokaite A, et al. Factors Associated with the Prevalence of Thyroid Nodules and Goiter in Middle-Aged Euthyroid Subjects. Int J Endocrinol. 2017;2017.

37. Anderson CE, McLaren KM. Best practice no 171: Best practice in thyroid pathology. J Clin Pathol. 2003;56(6):401–5.

38. Salabè GB. Pathogenesis of thyroid nodules: Histological classification? Biomed Pharmacother. 2001;55(1):39–53.

39. Salabè GB. Physiopathology of thyroid cysts in euthyroidnodulargoitre. ThyroidolClinExp 1991 ; 2 : 125-8.

40. De los Santos E, Keyhani-Rofagha S, Cunningham JJ, MazzaferriEL. Cystic thyroid nodules. Arch Intern Med 1990 ;150 : 1422-7.

41. Heshmati HM, Fatourechi V, van Heerden JA, Hay ID, GoellnerJR. Thyroglossalduct carcinoma: report of 12 cases. MayoClinProc 1997 ; 72 : 315-

9.

42.   Grant CS, Goellner JR. Cystic thyroid nodules: the dilemma ofmalignant lesions [editorial]. Arch Intern Med 1990 ; 150 :1376-7.

43.   Sarda AK, Bal S, Dutta Gupta S, Kapur MM. Diagnosis andtreatment of cystic disease of the thyroid by aspiration. Surgery1988 ; 103 : 593-6.

44.   Fröhlich E, Wahl R. Thyroid autoimmunity: Role of anti-thyroid antibodies in thyroid and extra-thyroidal diseases. Front Immunol. 2017;8(MAY).

45.   Ludwig RJ, Vanhoorelbeke K, Leypoldt F, Kaya Z, Bieber K, McLachlan SM, et al. Mechanisms of autoantibody-induced pathology. Front Immunol. 2017;8(MAY).

46.   Quintero OL, Amador-Patarroyo MJ, Montoya-Ortiz G, Rojas-Villarraga A, Anaya JM. Autoimmune disease and gender: Plausible mechanisms for the female predominance of autoimmunity. J Autoimmun [Internet]. 2012;38(2–3):J109–19. Available from: http://dx.doi.org/10.1016/j.jaut.2011.10.003

47.   Amadori A, Zamarchi R, De Silvestro G, Forza G, Cavatton G, Danieli GA, et al. Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med 1995;1: 1279e83.

48.   Shames RS. Gender differences in the development and function of the immune system. J Adolesc Health 2002;30:59e70.

49.   Cutolo M, Capellino S, Sulli A, Serioli B, Secchi ME, Villaggio B, et al. Estrogensand autoimmune diseases. Ann N Y AcadSci 2006;1089:538e47.

50.   Lleo A, Battezzati PM, Selmi C, Gershwin ME, Podda M. Is autoimmunity a matter of sex? Autoimmun Rev 2008;7:626e30.

51.   McCarthy M. The" gender gap" in autoimmune disease. Lancet 2000;356: 1088.

52.   Gleicher N, Barad DH. Gender as risk factor for autoimmune diseases. J Autoimmun 2007;28:1e6.

53.   Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol Med Microbiol 2003;38:13e22.

54.   McMurray RW. Estrogen, prolactin, and autoimmunity: actions and interactions. IntImmunopharmacol 2001;1:995e1008

55.   Raveche ES, Steinberg AD, Berczi I. Sex hormones in autoimmunity. Pituitary FunctImmun; 1986:283e301.

56.   Rubtsov AV, Rubtsova K, Kappler JW, Marrack P. Genetic and hormonal factors in female-biased autoimmunity. Autoimmun Rev 2010;9:494e8.

57.   Jansson L, Holmdahl R. Estrogen-mediated immunosuppression in autoimmune diseases. Inflamm Res 1998;47:290e301.

58.   Prabhala RH, Wira CR. Sex hormone and IL-6 regulation of antigen presentation in the female reproductive tractmucosal tissues. JImmunol1995;155:5566e73

59.   Brohee D, Delespesse G, Debisschop MJ, Bonnyns M. Circulating immune complexes in various thyroid diseases. Clin Exp Immunol. 1979;36(3):379–83.

60.   Duntas LH. Environmental factors and autoimmune thyroiditis. Nat Clin Pract Endocrinol Metab. 2008;4(8):454–60.

61.   Pearce E et al. (2003) Thyroiditis. N Engl J Med **348:**2646–2655

62.   Weetman AP and McGregor AM (1994) Autoimmunethyroid disease: further developments in ourunderstanding. Endocr Rev **15:** 788–830

63.   Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: Clinical and diagnostic criteria. Autoimmun Rev [Internet]. 2014;13(4–5):391–7. Available from: http://dx.doi.org/10.1016/j.autrev.2014.01.007

64.   Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated populationburden of selected autoimmune diseases in the United States. ClinImmunolImmunopathol 1997;84:223–43.

65.   McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine2012;42:252–65.

66.   Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Clinical review: prevalenceand incidence of endocrine and metabolic disorders in the United States: acomprehensive review. J ClinEndocrinolMetab 2009;94:1853–78.

67.   Li Y, Zhou G, Ozaki T, Nishihara E,Matsuzuka F, Bai Y, et al. Distinct histopathologicalfeatures of Hashimoto's thyroiditis with respect to IgG4-related disease. Mod Pathol2012;25:1086–97

68.   Li Y, Nishihara E, Hirokawa M, Taniguchi E, Miyauchi A, Kakudo K. Distinct clinical,serological, and sonographic characteristics of Hashimoto's thyroiditis based withand without IgG4-positive plasma cells. J ClinEndocrinolMetab 2010;95:1309–17.

69.   Demirbilek H, Kandemir N, Gonc EN, Ozon A, Alikasifoglu A, Yordam N. Hashimoto'sthyroiditis in children and adolescents: a retrospective study on clinical, epidemiologicaland laboratory properties of the disease. J PediatrEndocrinol Metab2007;20:1199–205.

70.   Rallison ML, Dobyns BM, Keating FR, Rall JE, Tyler FH. Occurrence and natural historyof chronic lymphocytic thyroiditis in childhood. J Pediatr 1975;86:675–82.

71.   Wasniewska M, Corrias A, Salerno M, Mussa A, Capalbo D, Messina MF, et al. Thyroid function patterns at Hashimoto's thyroiditis presentation in childhood and adolescence are mainly conditioned by patients' age. Horm Res Paediatr

2012;78:232–6.

72.   Fatourechi V, McConaheyWM,Woolner LB. Hyperthyroidism associated with histologicHashimoto's thyroiditis. Mayo ClinProc 1971;46:682–9.

73.   Wasniewska M, Corrias A, Salerno M, Lombardo F, Aversa T, Mussa A, et al. Outcomesof children with hashitoxicosis. Horm Res Paediatr 2012;77:36–40.

74.   Nishimaki M, Isozaki O, Yoshihara A, Okubo Y, Takano K. Clinical characteristics offrequently recurring painless thyroiditis: contributions of higher thyroid hormonelevels, younger onset, male gender, presence of thyroid autoantibody and absenceof goiter to repeated recurrence. Endocr J 2009;56:391–7.

75.   Asa SL. The Current Histologic Classification of Thyroid Cancer. Endocrinol Metab Clin North Am. 2019;48(1):1–22.

76.   Farid NR, Shi Y, Zou M. Molecular basis of thyroid cancer.Endocr Rev 1994 ; 15 : 202-32.

77.   Said S, Schlumberger M, Suarez HG. Oncogenes and antioncogenesin human epithelial thyroid tumors. J EndocrinolInvest 1994 ; 17 : 371-9.

78.   Wynford-Thomas D. Molecular genetics of thyroid cancer. CurrOpinEndocrinol Diabetes 1995 ; 2 : 429-36.

79.   Woolner LB. Thyroid carcinoma: Pathologic classification with data on prognosis. Semin Nucl Med [Internet]. 1971;1(4):481–502. Available from: http://dx.doi.org/10.1016/S0001-2998(71)81042-5

80.   Berndorfer U, Wilms H, Herzog V. Multimerization of thyroglobulin (TG) during extra-cellular storage: isolation of highlycross-linkedTG from human thyroids. J ClinEndocrinol Metab1996 ; 81 : 1918-26

81.   Hicks MJ, Batsakis JG. Tall cell carcinoma of the thyroid gland. Ann OtolRhinol

Laryngol 1993;102:402–3.

82. Wenig BM, Thompson LD, Adair CF, et al. Thyroid papillary carcinoma of columnar cell type: a clinicopathologic study of 16 cases. Cancer 1998;82(4): 740–53.

83. Motosugi U, Murata S, Nagata K, et al. Thyroid papillary carcinoma with micropapillaryand hobnail growth pattern: a histological variant with intermediate malignancy?Thyroid 2009;19(5):535–7.

84. DeLellis RA, Lloyd RV, Heitz PU, et al. Pathology and genetics of tumours of endocrine organs. Lyons (France): IARC Press; 2004.

85. Ito Y, Miyauchi A, Kudo T, et al. Trends in the implementation of active surveillancefor low-risk papillary thyroid microcarcinomas at kuma hospital: gradualincrease and heterogeneity in the acceptance of this new management option.Thyroid 2018;28(4):488–95.

86. Bai S, Baloch ZW, Samulski TD, et al. Poorly differentiated oncocytic (hurthle cell) follicular carcinoma: an institutional experience. EndocrPathol 2015; 26(2):164–9.

87. Hughes DT, Rosen JE, Evans DB, et al. Prophylactic central compartment neckdissection in papillary thyroid cancer and effect on locoregional recurrence. Ann

SurgOncol 2018;25(9):2526–34.

88. WHO classification of thyroid lesions

89. Ozolins A, Narbuts Z, Strumfa I, Volanska G, Gardovskis J. Diagnostic utility of immunohistochemical panel in various thyroid pathologies. Langenbeck's Arch Surg. 2010;395(7):885–91

90. Fischer S, Asa SL. Application of immunohistochemistry to thyroid neoplasms.

Arch Pathol Lab Med. 2008;39(132):359–72.

91.   Rezk S, Khan A. Role of immunohistochemistry in the diagnosis and progression of follicular epithelium-derived thyroid carcinoma. ApplImmunohistochem MolMorphol. 2005;13:256–64.

92.   Erickson LA, Lloyd RV. Practical markers used in the diagnosis of endocrine tumors. AdvAnatPathol. 2004;11:175–89.

93.   Fusco A, Chiappetta G, Hui P, et al. Assessment of RET/PTC oncogene activation and clonality in thyroid nodules with incomplete morphological evidenceof papillary carcinoma: a search for the early precursors of papillary cancer.Am J Pathol. 2002;160:2157–67.

94.   Cheung CC, Boerner SL, MacMillan CM, et al. Hyalinizing trabecular tumor of the thyroid: a variant of papillary carcinoma proved by molecular genetics. Am J SurgPathol. 2000;24:1622–26.

95.   Belchetz G, Cheung CC, Freeman J, et al. Hurthle cell tumors: using molecular techniques to define a novel classification system. Arch Otolaryngol Head Neck Surg. 2002;128:237–40.

96.   Cerilli LA, Mills SE, Rumpel CA, et al. Interpretation of RET immunostaining in follicular lesions of the thyroid. Am J ClinPathol. 2002;118:186–193.

97.   Nasser SM, Pitman MB, Pilch BZ, et al. Fine-needle aspiration biopsy of papillary thyroid carcinoma: diagnostic utility of cytokeratin 19 immunostaining. Cancer. 2000;90:307–11.

98.   Gómez Sáez JM. Diagnostic and Prognostic Markers in Differentiated Thyroid Cancer. Curr Genomics. 2011;12:597–608.

99.   Saleh HA, Jin B, Barnwell J, Alzohaili O. Utility of immunohistochemical markers in differentiating benign from malignant follicular-derived thyroid

nodules. Diagn Pathol. 2010;5(1):1–11.

100. Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8-PPAR_ rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses.Am J SurgPathol. 2002;26:1016–23.

101. Rocha AS, Soares P, Fonseca E, et al. E-cadherin loss rather than betacatenin alterations is a common feature of poorly differentiated thyroid carcinomas. Histopathology. 2003;42:580–87.

102. Xu B, Yoshimoto K, Miyauchi A, et al. Cribriform-morular variant of papillary thyroid carcinoma: a pathological and molecular genetic study with evidence of frequent somatic mutations in exon 3 of the beta-catenin gene. J Pathol. 2003;199:58–67.

103. LiuW, Asa SL, Ezzat S. 1_,25-Dihydroxyvitamin D3 targets PTEN-dependent fibronectin expression to restore thyroid cancer cell adhesiveness. MolEndocrinol 2005;19:2349–57

104. Weber KB, Shroyer KR, Heinz DE, et al. The use of a combination ofgalectin-3 and thyroid peroxidase for the diagnosis and prognosis of thyroid cancer. Am J ClinPathol. 2004;122:524–31

105. Hosal SA, Apel RL, Freeman JL, et al. Immunohistochemical localization of p53 in human thyroid neoplasms: correlation with biological behavior. Endocr Pathol. 1997;8:21–8.

106. Ratnatnga N, Ramadasa S. Immunohistochemical staining for thyroglobulin in poorly differentiated carcinoma of the thyroid. Ceylon Med J. 1993;38: 113–6.

107. Cho AA, Kong SY, Shin A, Lee J, Lee KK, Lee JJ, et al. Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: A

nested case-control study. BMC Cancer. 2014;14(1):1–10

108. Carlson RW, Larsen JK, McClure J, Fitzgerald CL, Venook AP, Benson AB III, Anderson BO 2014 International adaptations of NCCN Clinical Practice Guidelines

in Oncology. J NatlComprCancNetw 12:643–48.

109. Makki FM, Taylor SM, Shahnavaz A, Leslie A, Gallant J, Douglas S, et al. Serum biomarkers of papillary thyroid cancer. J Otolaryngol - Head Neck Surg. 2013;42(FEB):1

110. Grogan RH, Mitmaker EJ, Clark OH. The evolution of biomarkers in thyroid cancer-from mass screening to a personalized biosignature. Cancers (Basel). 2010;2(2):885–912.

111. Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK. The Bethesda system for reporting thyroid fine needle aspirates: A cytologic study with histologic follow-up. J Cytol. 2013;30(2):94–9.

112. Guidelines of the Papanicolaou Society of Cytopathology for fine‑needleaspiration procedure and reporting. The Papanicolaou society ofcytopathology task force on standards of practice. Diagn Cytopathol1997;17:239‑47

CHAPTER IV

MATERIALS AND

METHOD

METHODOLOGY

INDEX

MATERIALS AND METHODS:

TYPE OF STUDY   : PROSPECTIVE STUDY.

STUDY DESIGN   : Cross sectional study

DURATION OF COLLECTION OF DATA: This was a two year prospective study carried out from 2015 to 2016 at BLDE Deemed to be University, Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura.

STUDY POPULATION: The study group included out patients and in patients with thyroid abnormality of Shri B M Patil Medical College, Hospital& Research Centre, Vijayapura.

SAMPLE SIZE   : Using expected prevalence of thyroid diseases among adults in India as 3.4% per 10000 population[2] , expected sensitivity of HBME -1 marker as 1 and expected specificity of HBME1 as 0.96, as HBME 1 marker as best marker and desired precision as 3% the minimum sample size is 165 with 95% confidence level. This sample size will give the precision of 3% or less for both sensitivity and specificity.

The formula used in this calculation: $n = Z^2 P(1-P) / d^2$

Where   n= sample size

Z = Z Statistic for a level of confidence,

P= expected prevalence or proportion

d= Precision

SELECTION CRITERIA

INCLUSION CRITERIA: All patients with thyroid swelling from ENT & Surgery clinic being referred to Department of Pathology for FNAC/Histopathology study.

EXCLUSION CRITERIA: Patients with thyroid swelling with thyroid hormone therapy or antithyroid drugs.

PLAN OF STATISTIC ANALYSIS :

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft® Excel for Mac 2011 programs. In the present study, descriptive statistics as well as 95% confidence interval for a single proportion and a mean, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was calculated.

ETHICAL CLEARANCE

Ethical clearance was obtained by the Institutional Ethical Committee of BLDE(Deemed to be University) (IEC No-114/2015-16, dated 10/04/2015). Purpose of study was explained and informed consent in local(kannada) language was taken from all the patients. A data collection sheet was designed to gather all the necessary information of the patients. The written official permission was also taken from the hospital administrator.

DATA COLLECTION PROCEDURE:

Parameters estimated:

Serum markers estimation of Free thyroxine (T4), Free tri-iodothyronine (T3), thyroid stimulating hormone (TSH), Anti thyroglobulin antibody (AntiTG) and anti thyroperoxidase (Anti-TPO)

Immune Markers: HBME 1 and Galectin 3

Ret proto-oncogene mutation.

STUDY PLAN FLOW CHART

> Patient with Thyroid swelling visiting our hospital

⬇

> clinical details: onset of swelling associated symptoms and other important history obtained.
>
> informed consent was taken

⬇

> clincal examination of swelling done
>
> 5ml venous blood collected in plain vac-cutainer and processed for analysis and sample run in Vidas hormone analyser

⬇

> Fine needle aspiration was performed and slides processed for further staining and reporting
>
> The Bethesda reporting Sytem for Thyroid cytopatholgy was used for cytology diagnosis

⬇

> Ressected specimens (surgery indicated cases) were obtained and procssed in Histopathology laboratory under standard FFPE(Formalin Fixed paraffin Embedded) procedure and Hematoxylin and Eosin staining done

⬇

> Iummnohistochemistry(IHC) performed in difficult cases
>
> HBME1 and galectin-3 were two immune markers used

SERUM BIOMARKER ASSAY

Principle of a biomerieuxMinividasChemiluminescence Immunoassay

The Solid Phase Receptacle acts a stable state aspiration device for the test. Reactants for the test are prepare-to-use and pre allocate in the covered reagent strips. Most of the experiment steps are done automatically by the mini vidas immunological analyzer. In and out of cycled mechanisms of SPR test processed regularly. Free ingredients are removed through cleaning buffer and last reaction step is 4-Methyl-umbelliferyl phosphate cycled in and out of the SPR. Then, hydrolysis of this substrate into a luminance product by immobilized enzyme, the 4-Methylumbelliferone luminance is detected at 450 nm. The power of the luminance based on the absorption of alkaline phosphatase present on the SPR that alter the substrate. At the end, results are automatically measured by the analyzer. For some tests, two detection steps are performed successively. For antigen detection, the SPR is generally coated on the interior with capture antibody or sometimes with byproducts of the analyte. For antibody detection, the SPR is coated with an encapsulated antigen or antibody administered to the antigen.[1]

Principle: The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After preliminary wash and sample dilution steps, the anti- TPO and anti TG antibodies present in the sample will bind to the recombinant protein coating the interior of the SPR®.

Unbound components are eliminated during a washing cycle. Anti-human IgG antibodies conjugated with alkaline phosphatase, will attach to the immune complex coating the interior of the SPR.A final wash step eliminates the excess conjugate. During the final detection step, the substrate (4-Methyl- umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl- umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of anti-TPO and anti TG antibodies present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.[2]

FINE NEEDLE ASPIRATION CYTOLOGY

A 20 ml plastic disposable syringe is the most satisfactory size coupled with a 21, 23, or 25 gauge needle, after the skin has been cleaned with antiseptic, the thyroid swelling/nodule is held firmly with one hand and the needle is inserted directly into it. The plunger of the syringe is pulled back, thus exerting suction. This is maintained with the thumb, and the needle is moved through the tumour three or four times in different directions. Still with the needle in the tumour, suction is slowly released. The needle is then removed from the tumour and the syringe from the needle. The syringe is then filled with a little air, reconnected to the needle, and the contents of the needle blown on to one or more clean dry slides, which are rapidly air dried[3].

The aspirator should have a writing surface and a microscope available to record the macroscopic findings and check the adequacy of the aspirated material whilst the patient is still present in the clinic[4].

The needle is passed into the lesion and multiple fast jabbing movements in and out of the lesion as well as in different directions are performed. Once the material is seen in the hub of the needle, there is usually sufficient material. Several passes may be performed safely, although the average number of passes may vary according to the experience of the aspirator and the nature of the lesion.[4]

Image-guided FNAC is particularly advantageous in cases of small, non-palpable or multiple lesions. In the case of the thyroid, some centres advocate the use of image-guided FNAC. Karstrup et al. report ultrasound-guided FNAC of the thyroid to be superior to both ultrasound-guided core biopsy (CB) and the combination of ultrasound guided FNAC and CB.[4]

STAINING PROCEDURES

    A. ***Hemotoxyline and eosin staining*** on cytology slides[5]

Begin with step 1 for paraffin sections and step 8 for smears.

1. Xylol	5 minutes
2. Xylol	5 minutes
3. Absolute ethyl alcohol	15 dips
4. Absolute ethyl alcohol	25 dips
5. 95% Ethyl alcohol	15 dips
6. 80% Ethyl alcohol	15 dips
7. 70% Ethyl alcohol	15 dips
8. Wash in distilled water	15 dips

9. Harris's hematoxylin (modified—2 minutes or according to preference)

10. Wash in running tap water until excess stain is removed (approximately 1 minute)

11. Acid alcohol (1.5 ml of concentrated HCl in 650 ml of 70% ethyl alcohol)—two to three dips or until specimen is red in color

12. Wash in running tap water   30 seconds

13. Lithium carbonate solution (1.5 ml saturated lithium carbonate solution, 650 ml of 70% ethyl alcohol   1 minute

14. Wash in tap water   15 dips

15.50% Ethyl alcohol   15 dips

16.Eosin   approximately 20 seconds

17.Wash in running tap water until excess stain is removed (approximately 1 minute)

18.95% Ethyl alcohol   15 dips

19.95% Ethyl alcohol   15 dips

20.Absolute ethyl alcohol   15 dips

21.Absolute ethyl alcohol   1 minute

22.Xylol   15 dips

23.Xylol   15 dips

24.Xylol   5 to 10 minutes

25.Mount

B. Giemsa stain[5]

1.Wash in distilled water   15 dips

2.Giemsa working stain   2 hours

3.1% Acetic acid   1 quick dip

4.Blot slide with bibulous paper

5.100% ethyl alcohol—until there is only a slight bluish tint to the alcohol that runs off

   the slide

6.Xylene	10 dips
7.Xylene	10 dips

8.Mount with permanent mounting medium

C. *Papanicolaou staining*[6]

Fixation: Fix smear according to standard procedure. For example, 95% alcohol or 100% methanol.

Procedure 1 (Standard Method):

1. 95% Ethanol 15 minutes (fixation)Rinse in tap water

2. Harris or Gill Hematoxylin 1-3 minutes (Time vary with selection of hematoxylin solution)

3. Rinse in tap water or Scott's tap water

4. 95% Ethanol 10 dips

5. OG-6 stain for 1.5 minutes.

6. 95% Ethanol 10 dips

7. EA-50, or Modified EA-50, or EA-65 stain for 2.5 minutes.

8. 95% Ethanol 10 dips, 2 changes

9. 100% Ethanol 1 minute

10. Clear in 2 changes of xylene, 2 minutes each

11. Mount with permanent mounting medium

BETHESDA REPORTING SYSTEM[7]

The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories

I. Nondiagnostic or Unsatisfactory

Cyst fluid only

Virtually acellular specimen

Other (obscuring blood, clotting artifact, etc)

II. Benign

Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)

Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context

Consistent with granulomatous (subacute) thyroiditis

Other

III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm

Specify if Hürthle cell (oncocytic) type

V. Suspicious for Malignancy

Suspicious for papillary carcinoma

Suspicious for medullary carcinoma

Suspicious for metastatic carcinoma

Suspicious for lymphoma

Other

VI. Malignant

Papillary thyroid carcinoma

Poorly differentiated carcinoma

Medullary thyroid carcinoma

Undifferentiated (anaplastic) carcinoma

Squamous cell carcinoma

Carcinoma with mixed features (specify)

Metastatic carcinoma

Non-Hodgkin lymphoma

Other

THYROID GROSSING[8]

Specimen Type: THYROIDECTOMY (hemi/total)

Weigh (fresh), orient, and measure

Examine for defects on surface and Comment on presence/absence of skeletalmuscle

Ink: anterior blue, posterior black, orange isthmic margin

Check clinical record for location of suspected lesions(imaging/FNA)

Draw diagram with locations and sizes

Serially section from superior to inferior (keeping order in case you need to return to case and nodule/region)

Identify other structures (lymph nodes, pyramidal lobeetc)

Describe cut surfaces

Size (*staging size cutoffs: 1 cm, 2 cm, 4cm*)

Number, location, characteristics (color, consistency, hemorrhage, necrosis, fibrosis, calks) of nodules

Encapsulation of nodules

Distance to margins

Remaining parenchyma

Indicate in which cassettes the nodules are located, **<u>Cassette Submission:</u>**4-7 cassettes

IMMUNOHISTOCHEMISTRY PROCEDURE.[9]

FFPE tissue specimens is cut in 3-μm-thick sections on charged slides

⬇

Deparaffinization and rehydration carried out by routine treatment{xylene for 4×5 minutes in graded ethanol for 2×3 minutes (99.9%), 4×3 minutes (96%), and 5 min

⬇

(70%) }.

Endogenous peroxidase activity is blocked by 0.5% hydrogen peroxide in methanol x

⬇

10min.

Rinse in the TBS buffer (pH 7.6, Trisbuffered saline, THAM-HCl 50 mmol/L, NaCl

150 mmol/L) for 5 minutes

⬇

The slides were subjected to heat-induced antigen retrieval (HIER) treatment in a

domestic microwave oven for 3×5 minutes at maximum power in basic buffer

⬇

The slides are allowed to cool at room temperature for 20 minutes in the HIER buffer.

⬇

Rinse with TBS buffer for 5 minutes

⬇

Incubate with primary antibodies at room temperature for 60 minutes.

⬇

Unbound primary antibodies later removed by repeated rinses with TBS buffer for 2×5

minutes.

⬇

The slides are incubated in a humid chamber for 30 minutes with EnVision+ with

following rinses in TBS for 2×5 minutes

⬇

The slides are then rinsed in water and counterstained with hematoxylin for 3 minutes.

After color development in tap water for 5 minutes, the slides are cover-slipped using

an aqueous mounting medium paramount.

The slides of positive and negative control were included in each run[9].

The staining intensity was graded as negative (0), weakly positive (1), moderately positive

(2),or positive (3).

Control slides

Galectin 3: Endothelial cells - nuclear and cytoplasmic staining

HBME 1: Mesothelial cell- membranous staining

RET PROTO-ONCOGENE MUTATION STUDY.[10]

DNA extraction and molecular genetic analyses

Paraffin blocks of prophylactic thyroidectomy specimens were retrieved and 7micron thin sections taken and total genomic DNA was extracted. Following DNA extraction, the RET exons 10, 11, 13, 14, and 15 were amplified by polymerase chain reaction (PCR). The PCR products were purified with Thermo Scientific ExoI-FastAPmixture and the final volume of sequencing mixture. After the PCR sequencing, the products were precipitated with sodium acetate/ethanol procedure and sequenced by Sanger's sequencing method.

REFERENCES

1. The European Journal of Biotechnology and Genetic Engineering Vol. 5 No. 1, 2018 ISSN 2397-2076 Progressive Academic Publishing, UK Page 16 www.idpublications.org

2. VIDAS® Anti-TPO (ATPO) 9300916 F - en - 2015/06 CLSI is a trademark belonging to Clinical and Laboratory Standards Institute, Inc. www.biomerieux.com 1-6

3. Lever, Trott, Webb Fine needle aspiration cytology.JClinPathol 1985;38: 1-11

4. Svante R. OrellThe techniques of FNA cytology chap 2Orell and Sterrett's fine needle aspiration cytology 2012, Elsevier Limited. 9-11

5. Carol E. BalesIII - Techniques in Diagnostic Cytology > 44 - Laboratory Techniques Editors: Koss, Leopold G.; Melamed, Myron R.Title: Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition Copyright ©2006 Lippincott Williams & Wilkins page 1596-609

6. https://www.ihcworld.com/_protocols/special_stains/papanicolaou_stain.htm

7. Cibas and Ali / The Bethesda System for Thyroid CytopathologyAm J ClinPathol2009;132:658-65

8. Seethala RR, Asa SL, Carty SE, Hodak SP, McHugh JB, Richardson MS et al, Protocol for the Examination of Specimens FromPatients With Carcinomas of the Thyroid Gland Based on AJCC/UICC TNM, 7th edition Thyroid 3.1.0.0 Protocol web posting date: August 2014 page 5-9

9. Kopitz J, Vértesy S, André S, Fiedler S, Schnölzer M and Gabius HJ: Human chimera-type galectin-3: Defining the critical tail length for high-affinity glycoprotein/cell surface binding and functional competition with galectin-1 in neuroblastoma cell growth regulation. Biochimie 2014;104: 90-99.

10. Zeugin JA, Hartley JL. Ethanol Precipitation of DNA. Focus.1985; 7: 1-2.

Photograph
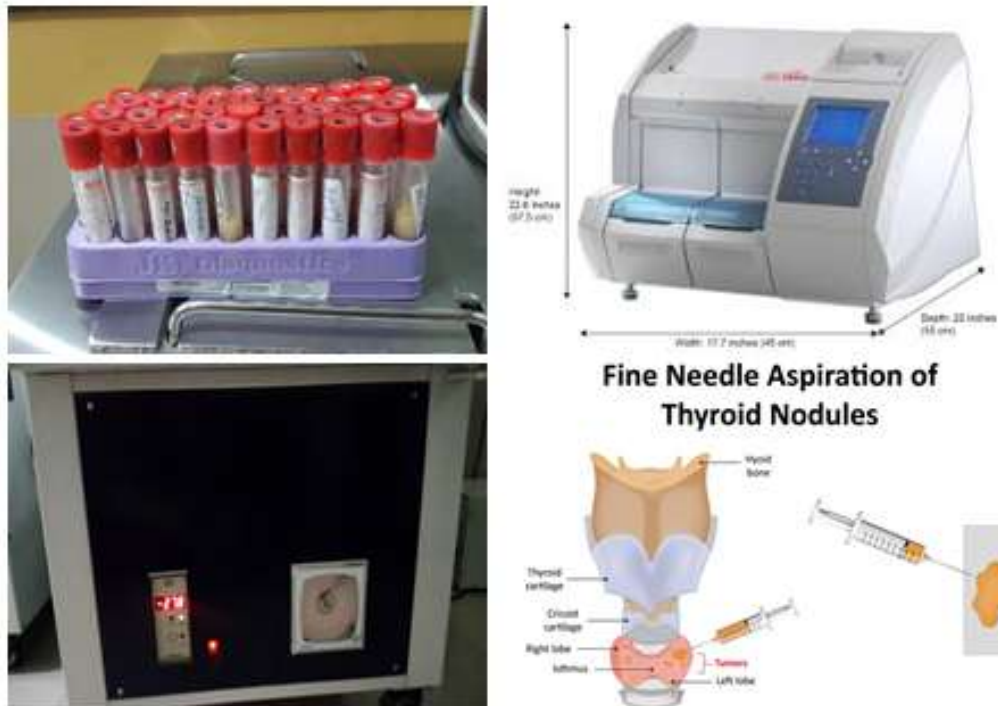
Figure8: Courtesy: Photograph from department of Blood bank, Dept. of Biochemistry

and Internet www.fnacimage .com

Grossing   **Staining**   **IHC procedure**

Figure 9: Dept. of Pathology

CHAPTER V

RESULTS

INDEX

RESULTS

AGE AND SEX DISTRIBUTION

Total of 165 thyroid nodules (cases) were studied and observed females preponderance with 81% and male 19%. Thyroid lesions have wide age distribution from 10year to 75years in our study. Commonest age group affected was 3rd and 4th decade. Youngest patient was 12yrs old and oldest 72years.
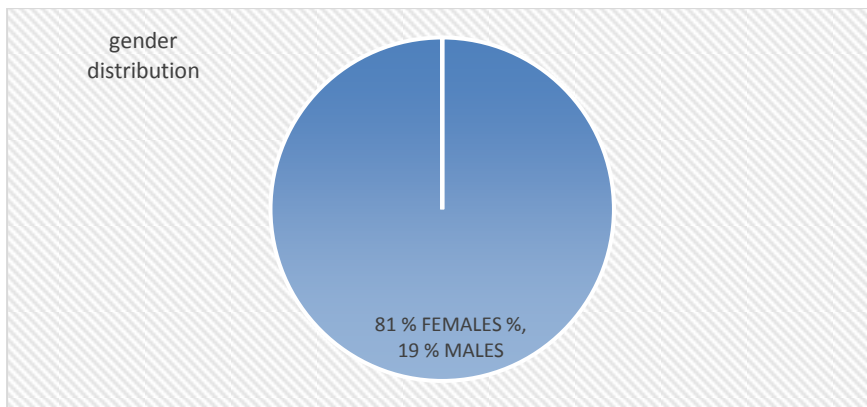
Figure 10: Pie Chart showing Sex distribution.

Table 11: Age Distribution

Sex/age	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Female	15	45	33	15	18	6	2	134
Male	3	6	11	6	2	3	-	31

SERUM BIOMARKERS: THYROID HORMONES AND AUTO-ANTIBODIES

Serum biomarkers of thyroid function, which are Free T3, Free T4 and thyroid stimulating hormone (TSH), were with in normal range of all individuals. Anti thyroperoxidase (Anti TPO) and anti-thyroglobulin (Anti-TG) levels were significantly raised in autoimmune thyroiditis and in few cases of papillary carcinoma.

Normal values of serum biomarkers are TSH = 0.4-4.0 mu IU/ ml, FT3= 3.5-7.8 pmol/L FT4= 9 – 25 pmol/L, Anti TG =<20 IU/ml and   Anti TPO=< 35 IU/ml.

In present study, Mean and SD was calculated for all serum markers and observed that thyroid hormones were within normal range.

Table 12: Serum Biomarkers: thyroid hormones and antibodies level non-neoplastic thyroid lesions

Thyroid lesion	TSH 0.4-4.0 muIU/ml	FT3 3.5-7.8 pmol/L	FT4 9 – 25 pmol/L	Anti TG <20 IU/ml	Anti TPO < 35 IU/ml
Goitre (colloid/nodular/toxic)	1.62±0.76	3.02±042	1.22±0.24	16.63±3.42	35.2±3.23
Lymphocytic thyroiditis	20.66±4.05	1.42±0.37	0.53±0.16	43.25±7.46	63.26±5.96
Granulomatous thyroiditis	2.22±0.43	6.24±1.56	19.45±6.5	26±3.50	35.2±2.50
Graves' disease	0.02±0.01	13.3±3.69	30±4.79	34.85±6.76	42.28±5.92

Table 13: Serum Biomarkers: thyroid hormones and antibodies level neoplastic thyroid lesions

Thyroid lesion	TSH 0.4-4.0 muIU/ml	FT3 3.5-7.8 pmol/L	FT4 9 – 25 pmol/L	Anti TG <20 IU/ml	Anti TPO < 35 IU/ml
Follicular neoplasm; Follicular adenoma Follicular carcinoma	0.83±0.16	4±1.73	11.3±1.67	12.6±3.77	25±0.04
	2±0.03	3.9±-0.1	20±0.02	12±1.41	17.5±2.42
Papillary carcinoma	2.1 ± 0.54	4.65±1.16	13.8±4.16	30.5±14.53	33.3±17.93
Medullary carcinoma	0.9±0.03	4±0.56	13±3.2	20±0.9	39±1.2

Table 14: Significance of Autoantibodies

Thyroid lesion	Anti TG	Anti TPO
Goitre (colloid/nodular/toxic)	0.61	0.12
Lymphocytic thyroiditis	0.05	0.002
Granulomatous thyroiditis	0.06	0.001
Graves' disease	0.015	0.025
Follicular neoplasm	0.215	0.071

Papillary carcinoma	0.02	0.04

Anti TPO and anti TG were statistical significant in chronic thyroiditis which includes

- Lymphocytic thyroiditis (Anti TG= 43.25 ± 7.46 and Anti TPO=63.26 ± 5.96),

- Granulomatous thyroiditis(Anti TG=26±3.50 and Anti TPO =35.2±2.50) and

- Graves' disease (Anti TG=34.85±6.76 and Anti TPO =42.28±5.92) and

- Neoplastic lesions these autoantibodies were significant raised in papillary carcinoma (Anti TG= 30.5±14.53 and Anti TPO=33.3±17.93).

FINE NEEDLE ASPIRATION CYTOLOGY:

All 165 cases were subjected for Fine Needle aspiration cytology and diagnoses were categorized according to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). We observed 83cases (51%) were benign cases (Bethesda category II), 9% cases were frank malignant (Bethesda Category VI), 9.7% cases were follicular neoplasm (Bethesda category IV) and **30.3% cases were falling in grey area which is Bethesda category V, IVand category III**.

Table 15:The Bethesda Reporting system of Thyroid Cytology (TBSRTC)

Bethesda system		No of cases	
I	Acellular/ only fluid/ blood	24	
II	Adenomatoid nodule/ nodular goitre/thyroiditis	83	
III	AUS/FUS	32	Total 165 cases
IV	Follicular neoplasm	16	
V	Suspicious for papillary ca, medullary ca, anaplastic ca, lymphoma & other rare tumors	09	
VI	Confirms malignancy	15	

HISTOPATHOLOGY AND CYTOPATHOLOGY CORRELATION:

Histopathological diagnosis was correlated in 71 cases.

Table 16: Histopathology Diagnosis

Thyroid lesion	Histopathology cases
Goitre (colloid/nodular/toxic)	21
Lymphocytic thyroiditis	11
Granulomatous thyroiditis	3
Graves' disease	2
Follicular neoplasm; Follicular adenoma Follicular carcinoma	10 4
Papillary carcinoma Medullary carcinoma Metastatic carcinoma Mucoepidermoid carcinoma	15 2 2 1
TOTAL	71 CASES

Seventy one cases (71 cases) had histopathological correlation. Commonest histopathological diagnosis was colloid goitre (21cases), 16 cases showed chronic thyroiditis (Lymphocytic thyroiditis 11 cases, Granulomatous thyroiditis 3csaes and Graves' disease 2 cases), 10 cases of follicular adenoma and 24 cases were thyroid malignancy. Papillary carcinoma was commonest malignancy with 15 cases, 4 cases of follicular carcinoma, 2 cases of medullary carcinoma and 2 cases of metastatic

carcinoma and both were squamous cell carcinoma deposits. One rare case of mucoepidermoid case was diagnosed.

Table 17: Histopathology and Cytopathology Correlation of cases.

Bethesda system	Includes	Cytology cases	HPR correlation Cases	Final diagnosis
II	Benign	83	24	Goitre (colloid/nodular/toxic) Lymphocytic thyroiditis Granulomatous disease Graves' disease Adenomatoid nodule
III	Atypia of unknown significance (AUS)	32	13	
IV	Follicular neoplasm/suspicion for follicular neoplasm	16	14	Follicular neoplasm; Follicular adenoma Follicular carcinoma
V	Suspicious for other malignancy	09	20	Papillary carcinoma Medullary carcinoma Metastatic carcinoma Mucoepidermoid carcinoma
VI	Malignancy	15		
Total	-	165	71	-

IMMUNOHISTOCHEMISTRY PROFILE

Resected thyroid specimens with morphological challenges having overlapping features of benign and malignancies which were difficult to render definitive diagnosis on H&E slide were subjected immunohistochemistry. HBME1 and galectin 3 immune expressions was studied. Gal-3 expression in thyroid papillary neoplasms was found to have a sensitivity of 86.67%, specificity of 85%, and positive predictive value of 89.66% and negative predictive value of 80.95%. HBME expression had sensitivity of 84.62%, specificity of 79%, positive predictive value 81.32% and negative predictive value 69.23%. Gal-3 immune marker is more specific for papillary lesions.

Table 18: Immune marker expression in different lesions.

HPR diagnosis	No of cases	HMBE 1	Galectin 3
Papillary carcinoma	15	Positive	Positive (15case)
MNG with papillary like features	12	Negative	Positive (1case)
Lymphocytic thyroiditis	2	Negative	Negative
Graves' disease	1	Negative	Positive
Follicular carcinoma	3	positive	Negative

Table 19: Gal-3 expression in thyroid neoplasms

sensitivity	86.67%
specificity	85%
positive predictive value	89.66%
negative predictive value	80.95%.

Table 22:HBME 1 expression in thyroid lesions.

sensitivity	84.62%
specificity	79%
positive predictive value	81.32%
negative predictive value	69.23%.

Ret oncogene mutation

Fourteen prophylactic thyroidectomy cases done in family with history of medullary carcinoma were studied for morphological changes and ret proto-oncogene mutations. We observed that C-cell hyperplasia (CCH) was noted in 13cases and 9 cases also showed micro medullary carcinoma of thyroid (MMTC). Commonest exon mutated was at 634(85.7%) with C634R (50%), C634W (21.4%), C634Y (14.3%), and 609(14.3%) mutations. 75% of the exon 634 mutations had C Cell Hyperplasia association thyroid. This observation was statistically significant (p = 0.036).
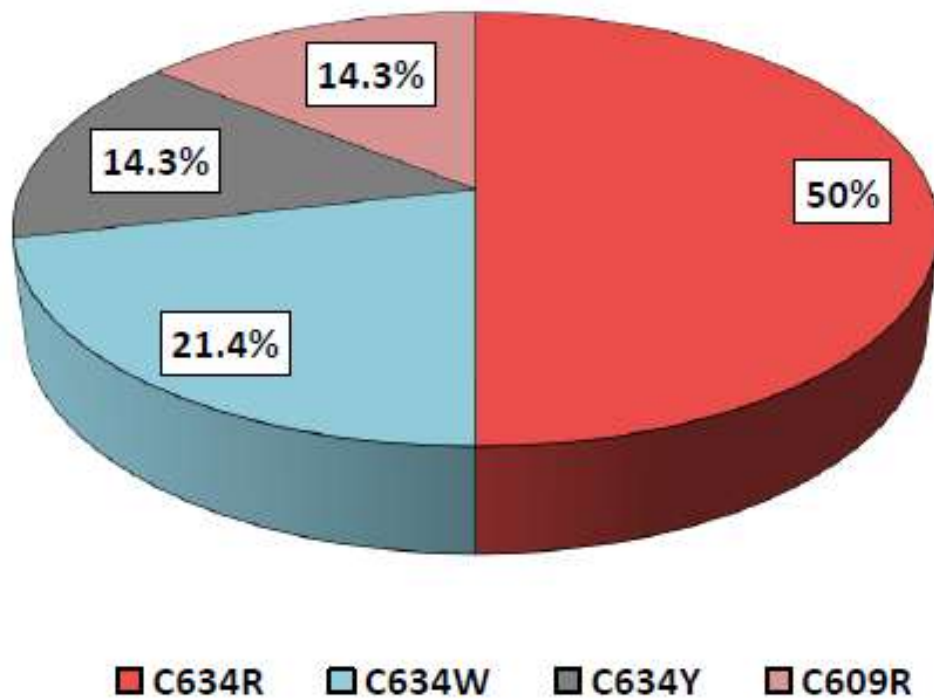
Figure11: showing exon mutation
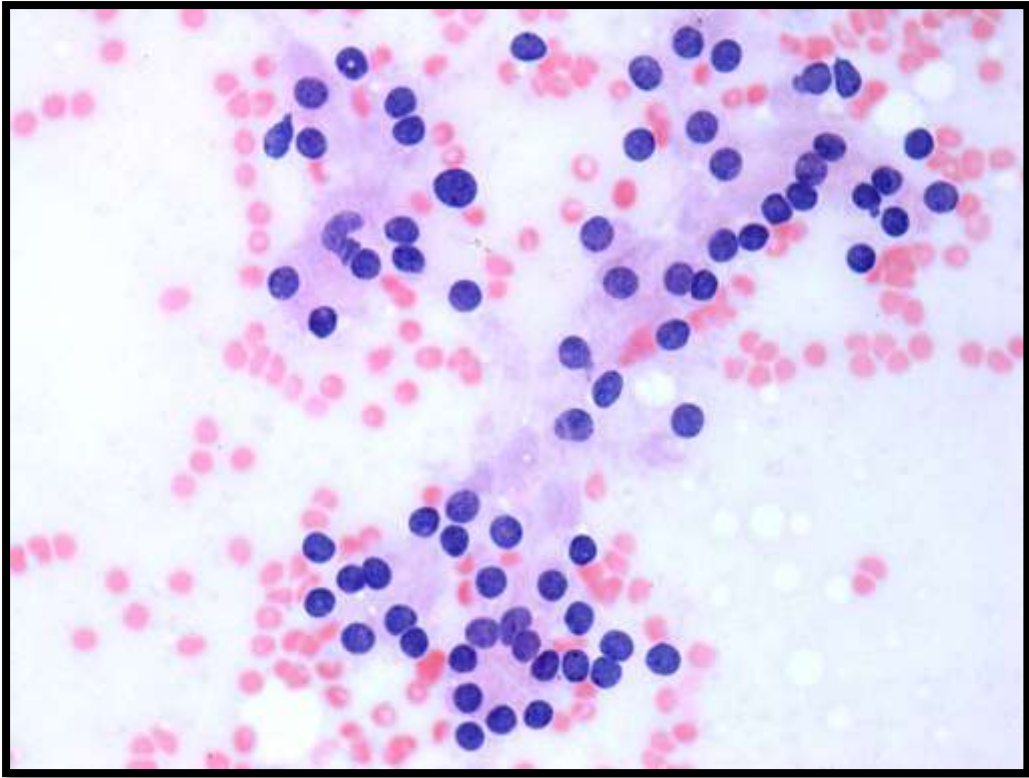
Figure12: Goitre (40x H&E stain)
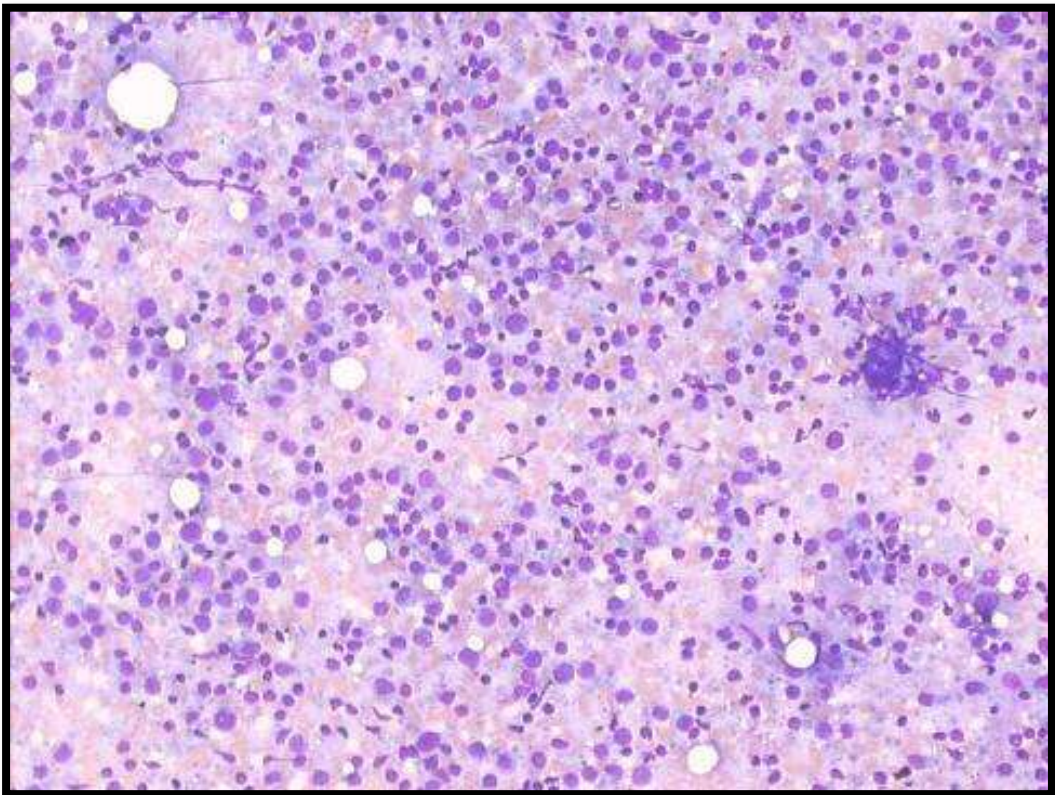
Figure 13 :Hashimotos thyroiditis (10x leishman stain)
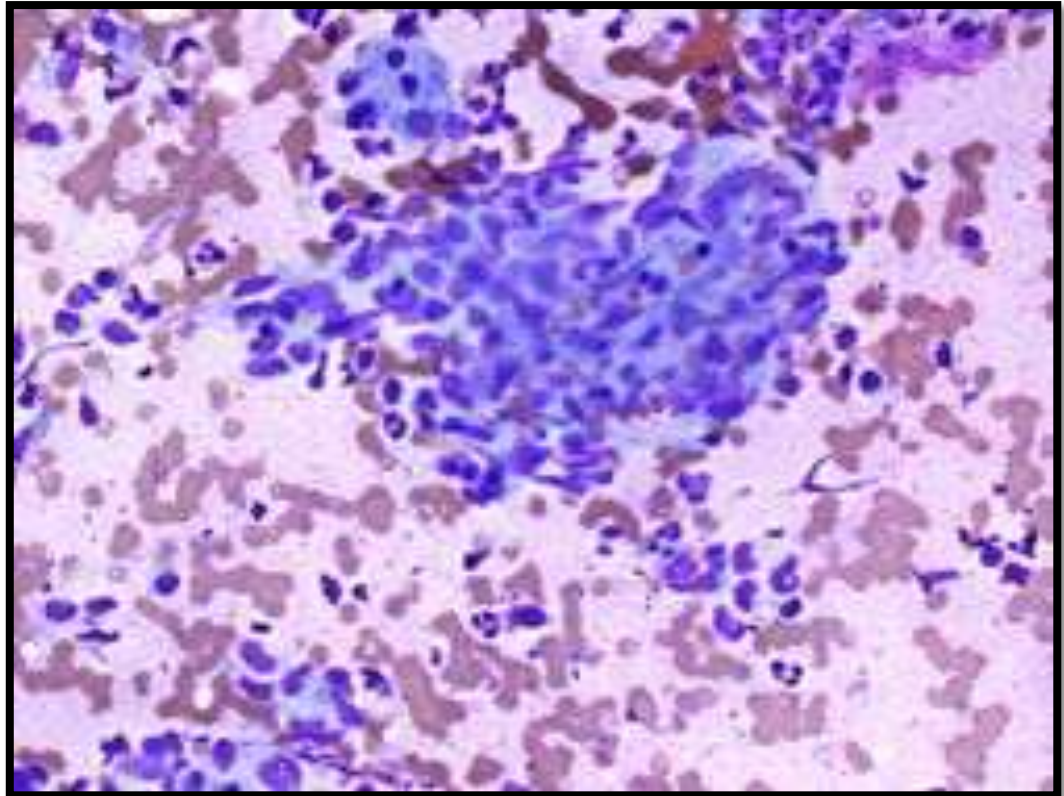
Figure14:Granulomatous thyroiditis10x leishman stain
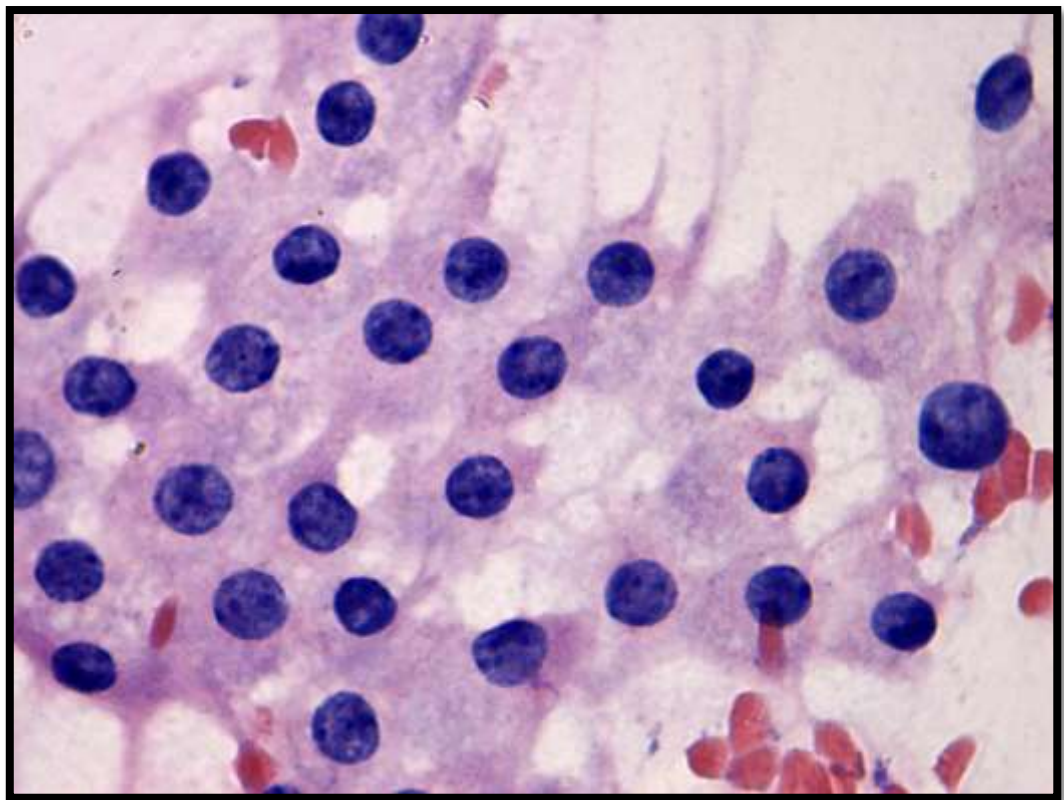
Figure15: Graves'disease 40xleishman stain
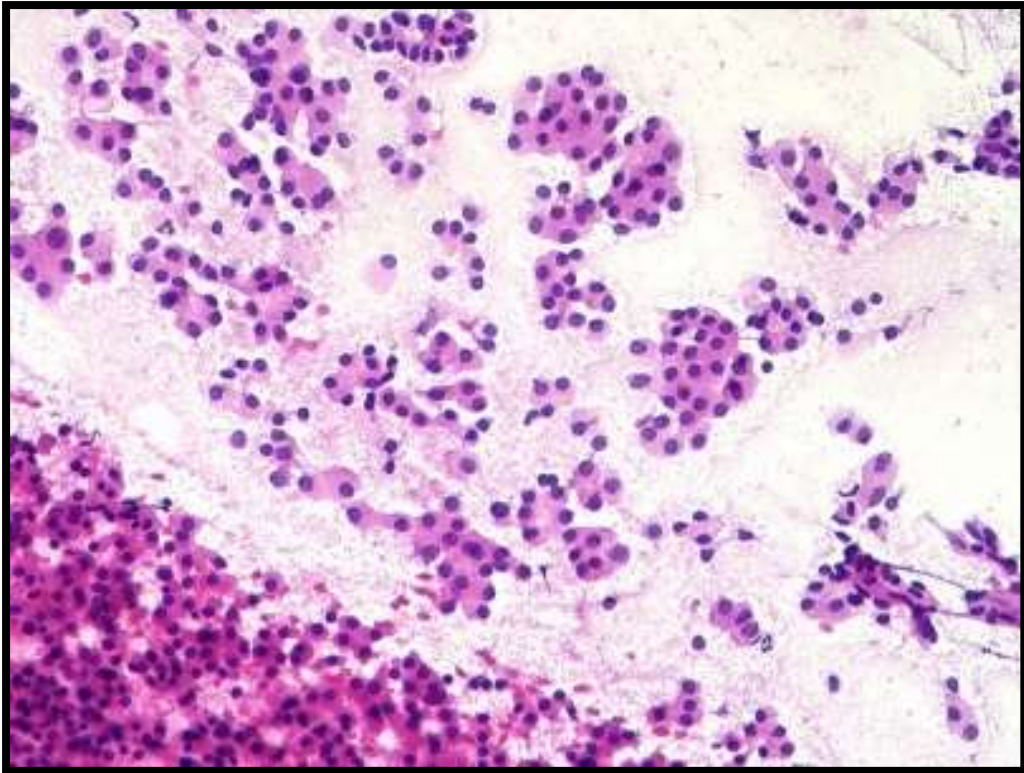
Figure16 :Follicular neoplasm 40x H&E stain
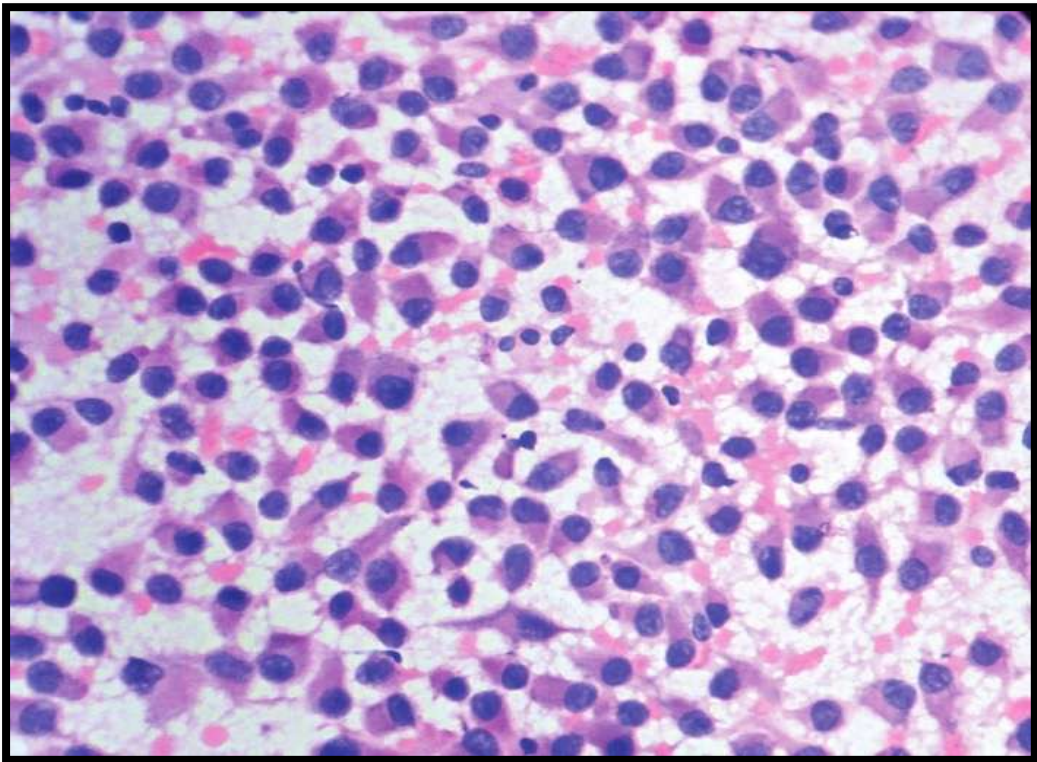
Figure17: Medullary carcinoma 40x H&E stain

Figure18 :Papillary carcinoma10x leish
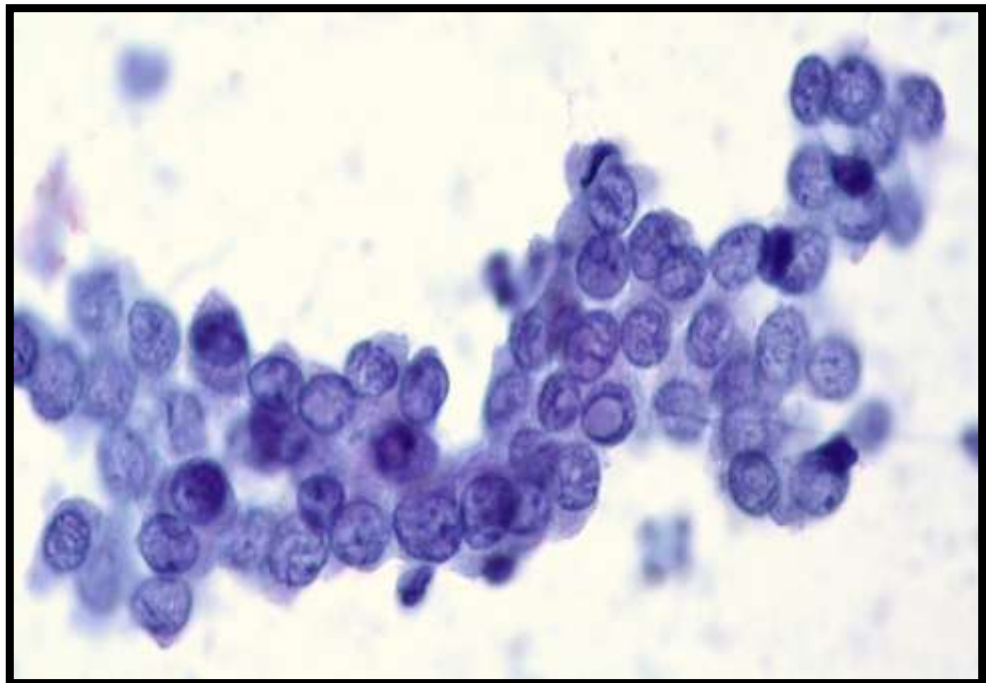
Figure19 :Papillary carcinoma40x leishman stain
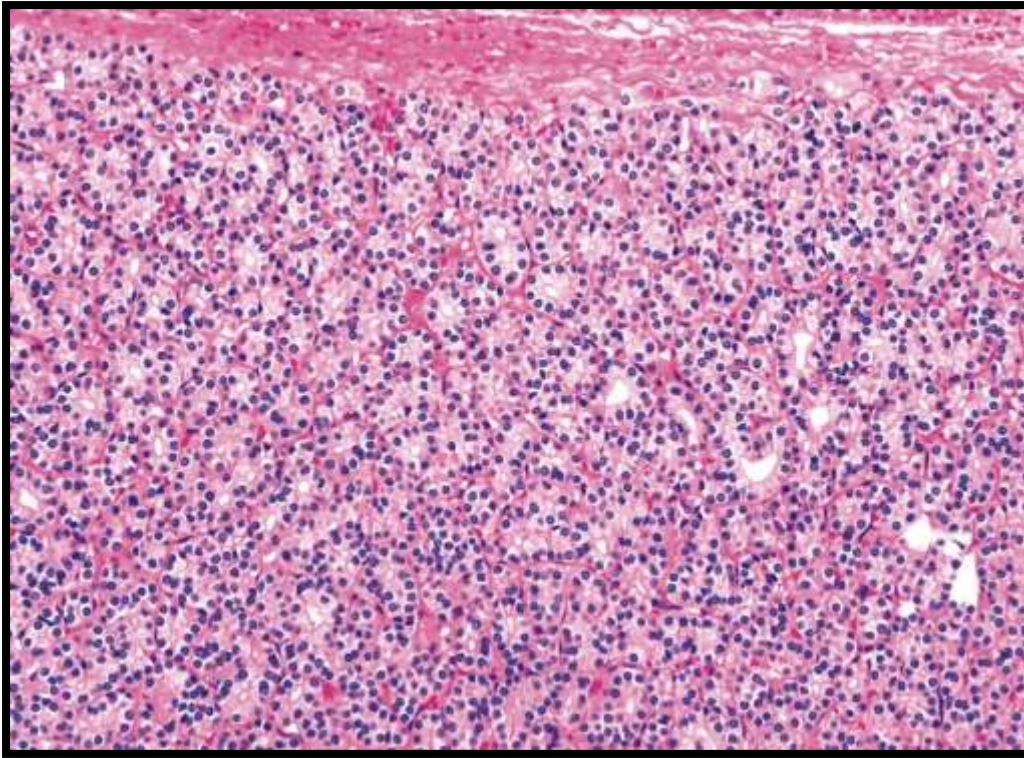
Figure20:10x H&E stain Follicular adenoma
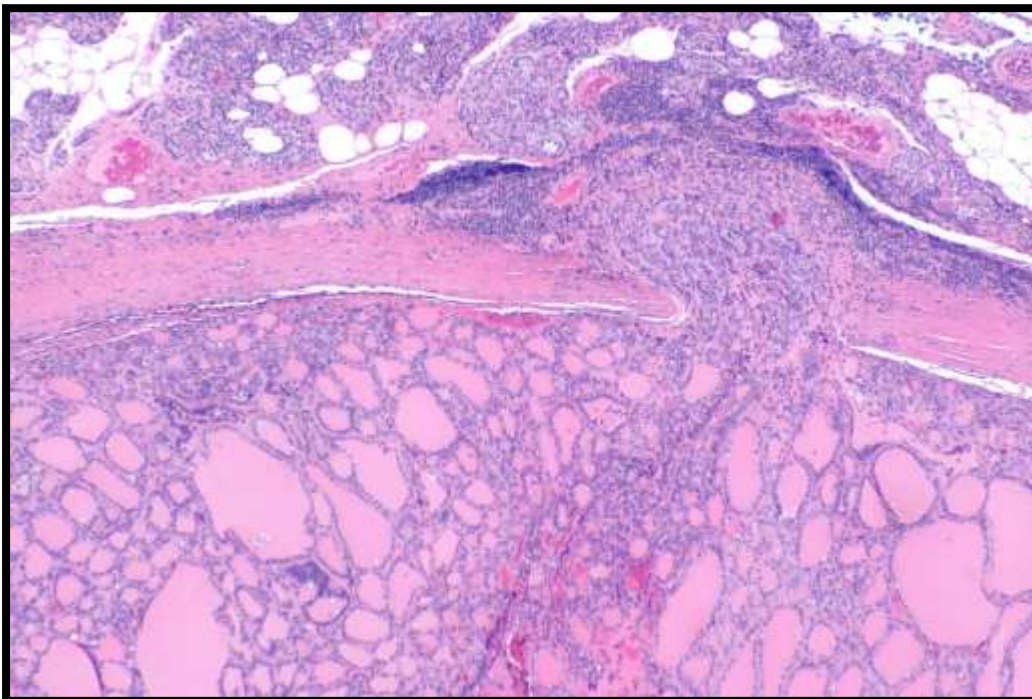
Figure21:10x H&E stain Follicular carcinoma with capsular invasion
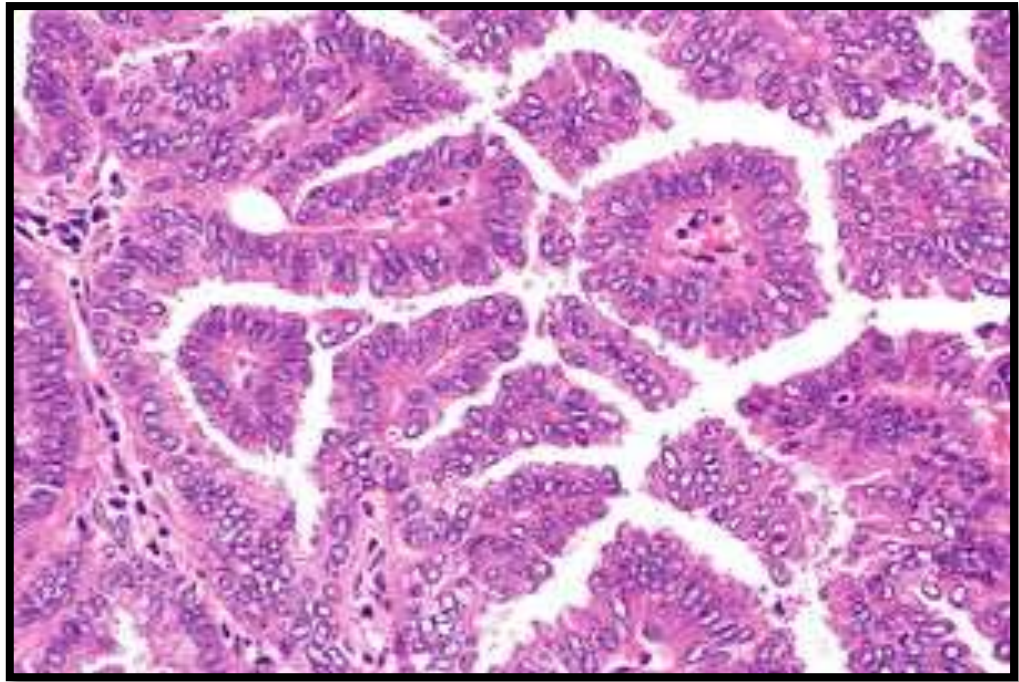
Figure 22: 10x H&E stain Pap Ca
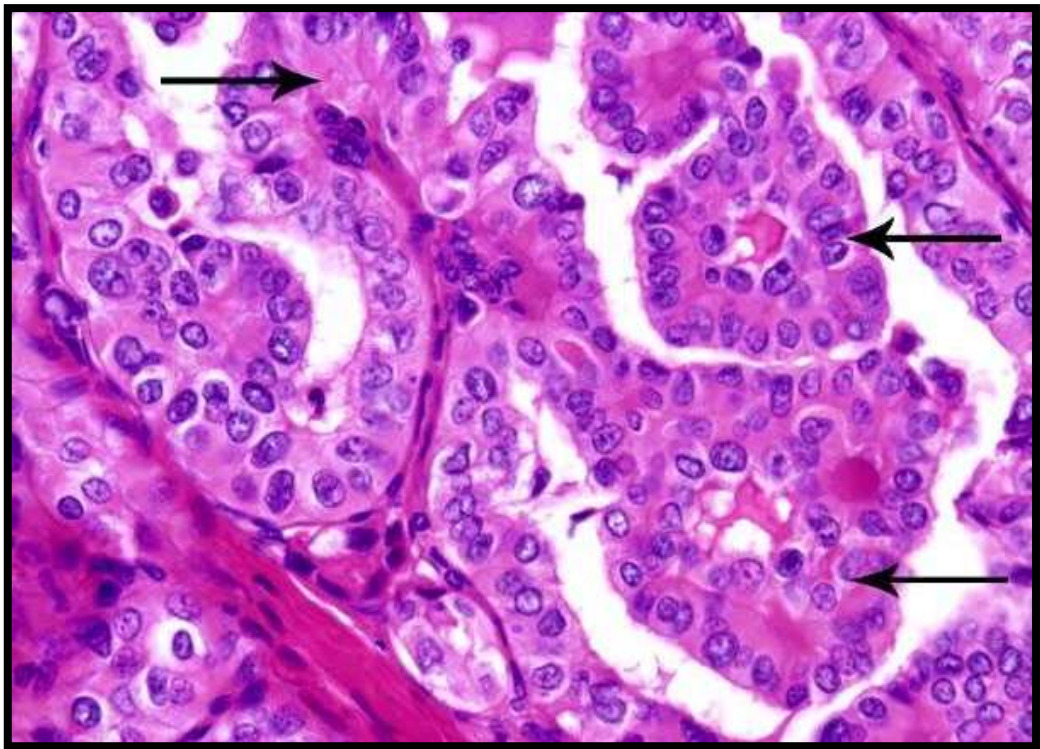
Figure 23: 40x H&E stain Pap Ca
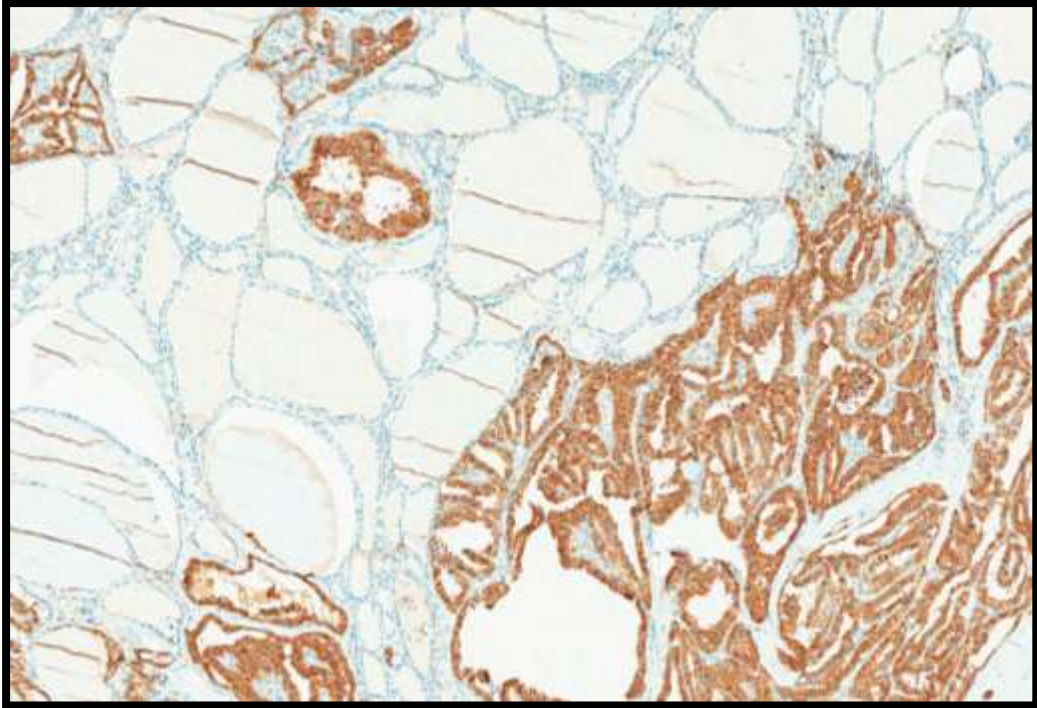
Figure 24: 10x IHC HBME-
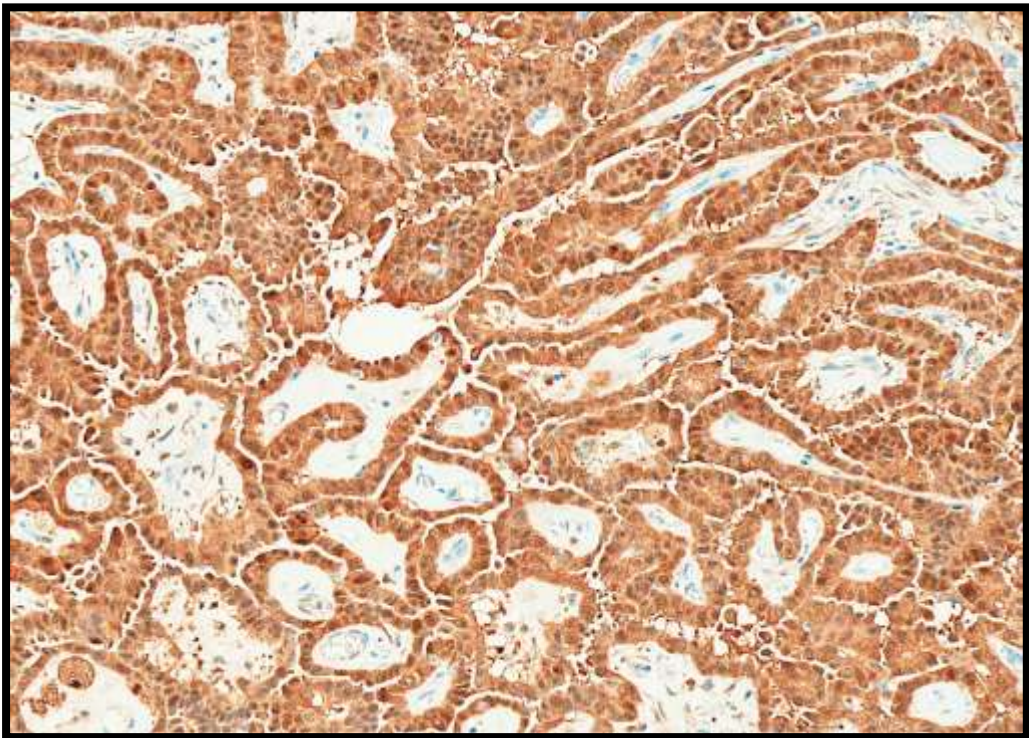
Figure 25: 10x IHC GALECTIN 3 cytoplasmic and nuclear staining

CHAPTER VI

DISCUSSION

INDEX

AGE AND SEX DISTRIBUTION

In present study we had 165 thyroid lesion subjects with thyroid lesions and we observed female preponderance 81% in comparison to the study done by Karimi F *et al* in their cross-sectional study, 655 out of 981 participants (66.8%) were female and 326 out of 981 participants (33.2%) were male. The mean age of the study subjects was 39.13 ± 14.36 years and the mean TSH concentration was 3.02 ± 5.98 mIU/L (95% CI: 2.62 to 3.41) [1]. Studies done by Weimin Xu *et al*and Howrah *et al* also observed female predominance in their studies[1,2].

Knudsen et al. observed 2 to 10 fold increased incidence of goiter in females though he noted that thyroid volume was larger in men than in women [3]. And thus an association between thyroid volume and gender was reported in literature. Studies have shown that gender-related differences became evident only after puberty, suggesting that sex hormones may play a role in thyroid volume[4]. Also there are studies in literature which have attempted to show an association between thyroid volume and parity statusand suggested that pregnancy increases thyroid volume, particularly when combined with tobacco smoking and iodine deficiency [5,6].

Causes for the higher incidence of thyroid nodules in women are unclear. Sex hormones like estrogen and progesterone play major role in female preponderance in thyroid nodular lesions and this was also observed by Kung et al. and he reported that pregnancy was associated with new thyroid nodule formation and an increase in size of preexisting thyroid nodules[7].

Female gender inclination is not only restricted to goiter but also the incidence of autoimmune thyroid diseases in noted more commonly in females. Main factorsinclude

hormonal factors, since estrogens and prolactin are immune stimulants while androgens are immune suppressors[8]. Genetic factors also play a role in autoimmune disorders and they include

a. Skewed X chromosome inactivation, a potentialmechanism whereby X-linked self-antigens may escape presentation in the thymus or peripheral sites that are involved in tolerance induction.

b. X monopsony: through the generation of autoreactive T cells that are not exposed to self-antigens encoded by one of the two X chromosomes, and Klinefelter's syndrome: incidence of autoimmune disorders is higher in XXY males

c. Non-inherited maternal antigens (NIMAs) acting as modulators of the immune repertoire.

d. Microchimerism

Circulating immune complexes are seen in various thyroid diseases which includeGraves' disease, Hashimoto's thyroiditis, Spontaneous myxedema, asymptomatic thyroiditis, Goitre with thyroiditis, diffuse goitre, Nodular goitre, Cold nodule and hot nodule[7,9].

Autoimmune thyroiditis is defined as an inflammatory state of the thyroid gland characterized by intrathyroid lymphocytic infiltration, ultrasonographic signs of inflammation, and antibodies to thyroglobulin, thyroid peroxidase (TPO) or both. The generic term autoimmune thyroiditis encompasses Hashimoto thyroiditis (the most frequently encountered form), Graves' disease and some rare variants, such as painless sporadic thyroiditis, postpartum thyroiditis, drug induced thyroiditis and thyroiditis that accompanied polyglandular autoimmune syndromes[10].

SERUM HORMONES AND AUTOANTIBODIES.

Cahoon KE *et al* had population based cohort study which measured serum Tg, urinary iodine, TSH, anti-thyroglobulin, anti- thyroid peroxidase levels and Ultrasound to assess the presence of nodules and estimate thyroid volume and concluded that serum Tg is significantly related to presence of thyroid abnormalities as well as indicators of thyroid function and iodine deficiency and, therefore, could be used to characterize the iodine status and thyroid function of individuals in the context of epidemiological study[10].

In spite of iodine sufficient belt the incidence of thyroid diseases are increasing, with the literature supporting with the autoimmune cause. In present study the antibodies levels were raised in autoimmune thyroid disease and, levels were also high in papillary carcinoma and medullary carcinoma suggesting they could play role in etiopathogenesis.Similar findings were also observed by Young Ah Cho et al in "Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study"[11] .Eun Sook Kim et al, reported that TGAb was associated with an increased risk of thyroid cancer in thyroid nodules[12]. Variousprevious studies show such an analogous association between raised autoantibodies specific to thyroid organ and thyroid malignancies.

Anti Thyroperoxidase:Anti TPO

Autoimmune thyroid diseases which largely include Grave's disease, Hashimoto's thyroiditis or post-partum thyroiditis have risen anti TPO levels in serum. Thus TPOAb measurement may be used as a prognostic indicator for thyroid dysfunction. The paradoxical absence of TPOAb in some patients with unequivocal TSH abnormalities likely reflects the suboptimal sensitivity and/ or specificity of current TPOAb tests or

non-autoimmune thyroid failure (atrophic thyroiditis). Variability in autoantibody concentrations usually reflectschange in disease activity. The enhanced sensitivity and specificity of the TPO immunoassay methods make them a more cost-effective method in routine diagnosis of autoimmune thyroid disorders[11].

Antibodies against Thyroglobulin: Anti TG

Anti TG is also seen raised along with anti TPO in autoimmune diseases of thyroid. Current guideline suggests that anti TG should be tested prior to thyroglubin levels in highly suspected cases of autoimmune thyroiditis. High sensitive immunoassay should be used to pre-screen the individual's serum for antiTG levels. TgAb is primarily used as an adjunct test for serum TG estimation[12].

TSH receptor autoantibodies (TRAb)

Antibodies against TSH receptor are formed not only in autoimmune thyroiditis but also observed in hyperthyroidism, neonatal thyroid dysfunction where the maternal antibodies have crossed the placenta.

The TRAb levels are also used for monitoring the progress of course of Grave's disease in patients with anti-thyroid drugs.

Clinical manifestations of thyroid disorders are diverse hence the clinician should be extremely vigilant and should evaluate the case with various laboratory investigations which not only includes hormone assay but also various autoantibodies level in serum for appropriate management and monitoring of the disease.

The enhanced sensitivity and specificity of TSH assays have greatly improved the assessment of thyroid function tests. TSH levels dynamically alter based on circulating T3 and T4 levels.

In functional abnormalities of thyroid like hypothyroidism assessing free T4 is better than total T3 and and free T3. In cases of hyperthyroidism combined levels of free T4 and free T3 levels gives appropriate assessment of disease. Also these levels help in identifying selective T3-toxicosis where only free T3 levels are raised. Also along with these hormone assay antibodies level assessment helps in confirmation of disease.[11,12].

Prevalence of the thyroid autoantibody positivity is relatively high worldwide. It is well-known that in iodine-sufficient areas there is a higher rate of AT prevalence than in iodine-deficient ones[13]. Iodine intake is probably one of the most important factors that affects thyroid autoimmunity and the incidence of AT. In a random sample of general, euthyroidpopulation living in Holland (n = 2703) the prevalence of the TPOAbs positive cases was 8.4%.In Denmark among 4649 randomly selected subjects between 18 and 65 years of age 13.1% were TPOAbs positive and 18.1% had increased concentrations of either TPO or TG autoantibodies[14] .In the United States, TPOAbs were positive in 11.3% and TGAbs in 10.4% in a population sample of 13,344 subjects. In Japan among 1818 adults 31.4% of women and 17.7% of men were positive for TgAbs or TPOAbs.In another study from the same country presence of TPOAbs or TGAbs was reported in 12.8% of the studied cases[15].A very similar number (12.4% TPOAb positivity) was noted also by Australian authors[16].In India TPOAbs were detected in more than 13% subjects from a group of 4409 adults and 22% from a sample of 5376 adults[15,16,1718].

Similar research carried out by Sir Xu W et al over 1000 adults residing in coastal area of China observed strong association of raised autoantibodiesi.e. Anti TPO and anti TG of thyroid in various thyroid nodules. And also he noticed females were more affected than males[19, 20]. This kind of similar observation we had in present study. TPO Ab levels vary based on severity of lymphocytic thyroiditis and this indicates the role of

antibody dependent cell mediated cytotoxicity as pathogenetic pathway in structural abnormality and lymphocyte infiltration[21, 22].

Boelaert K. et al. reported that TPOAb and TGAb are most important antibodies to assess the risk of thyroid nodules. In his research he analysed all cases with ultrasound findings, clinical assessment and antibody measurements and the author states that TPOAb is dependently associated with thyroid diseases[19]. Generally, TPOAbs are present in 12–26% of euthyroid subjects, more often in women[23].

The association between TSH levels and thyroid malignancies has been studied since decades and studies show that there is strong association between raised TSH levels and cancer and thus playing a key role in thyroid carcinogenesis[24]. Recently prevalence of thyroid cancer in individuals with autoimmune thyroiditis has increased suggesting the role of autoimmunity in thyroid carcinogenesis and increased risk. Literature shows studies with association between raised antibodies, autoimmune thyroiditis and later developed thyroid cancer, like author Kim et al found an elevated risk of papillary carcinoma in Korean patients with hashimotos thyroiditis who also had raised TPOAb levels[25,26].

Also systemic autoimmune disease carries increased risk of developing thyroid cancer and a study done by Antonelli et al reported the higher prevalence of papillary thyroid cancer in systemic lupus erythematosus patients, particularly in patients with thyroid autoimmunity[27,28]. We also observed that the presence of TPOAb may affect the association between hormone levels and the risk of thyroid cancer, as we noticed raised TPOAb and TGAb in papillary carcinoma.

CYTOLOGICAL DIAGNOSIS

In present study, cytological diagnosis of all cases was categorized according to the Bethesda system of reporting thyroid cytopathology (TBSRTC) thus signifying its importance for surgical management of thyroid nodules. All 165 cases were subjected for Fine Needle aspiration cytology and diagnoses were categorized according to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). We observed 83cases (51%) were benign cases (Bethesda category II), 9% cases were frank malignant (Bethesda Category VI), 9.7% cases were follicular neoplasm (Bethesda category IV) and **30.3% cases were falling in grey area which is Bethesda category V, IVand category III**.

Author Mondal et al studied 1000 above cases of thyroid nodules in three year study period. FNAC was performed and the cytological diagnosis was rendered using the Behesda system and the results were 6.8% categorized as non-diagnostic, 83% as benign lesions of thyroid, 0.9% cases as follicular lesions of undetermined significance (FLUS), 4.1% cases of suspicious for follicular neoplasm, 1.4% cases as suspicious for malignancies and 3.8% cases were diagnosed as malignant lesions. 31.66% of his study cases had histopathological correlation and he noticed 68.7% cases were benign lesions on histopathology and 22.29% cases as malignant[29].

Study done by Zarif, *et al* demonstrates higher risks of malignancy in diagnostic categories(DC) I, DC II,DC III and DC IV than that of the original TBSRTC definition, along with a higher specificity and positive predictive value for cancer diagnosis, and a lower sensitivity and negative predictive value[30].

Similar study done by SafaAlshaikh et al titled 'Classification of thyroid fine-needle aspiration cytology into Bethesda categories: An institutional experience and review of

the literature' where a total of 632 patients underwent 681 FNAs during the study period. The incidence of each Bethesda category is summarized in Briefly, 69 (10.1%) were Category I/Bethesda I (nondiagnostic), 469 (68.8%) were Category II/Bethesda II (benign), 85 (12.4%) were Category III/Bethesda III (AUS), 20 (2.9%) were Category IV/Bethesda IV (SFN, 18 (2.6%) were Category V/Bethesda V (suspicious for malignancy), and 28 (4.1%) were Category VI/Bethesda VI (malignant)[31]. Various studies have been done to study the effect of implementation of the Bethesda system of reporting as shown in table 21.

Table 21:TBSRTC in present study and other studies comparison.

Diagnostic category	Present study	Jo *et al*	Yassa*et al*	Yang *et al*	Nayar and Ivanovic*et al*
Non diagnostic	1.2	18.6	7	10.4	5
Benign	87.5	59.0	66	64.6	64
AFLUS	1	3.4	4	3.2	18
SFN	4.2	9.7	9	11.6	6
SM	1.4	2.3	9	2.6	2
Malignancy	4.7	7.0	5	7.6	5

Numerical in percentage

HISTOPATHOLOGY AND CYTOPATHOLOGY CORRELATION:

In present study Seventy one cases (71 cases) had histopathological correlation. Commonest histopathological diagnosis was colloid goitre (21cases), 16 cases showed chronic thyroiditis (Lymphocytic thyroiditis 11 cases, Granulomatous thyroiditis 3csaes and Graves' disease 2 cases), 10 cases of follicular adenoma and 24 cases were thyroid malignancy. Papillary carcinoma was commonest malignancy with 15 cases, 4 cases of follicular carcinoma, 2 cases of medullary carcinoma and 2 cases of metastatic carcinoma and both were squamous cell carcinoma deposits. One rare case of mucoepidermoid carcinoma was diagnosed.

Study done my Mondal et al, out of 1020 cases, 323 cases was available for follow‑up histopathology. Out of these 323 cases, 3 cases had original FNA diagnoses as non‑diagnostic, 222 cases as benign, 5 cases as AFLUS, 36 cases as SFN, 12 cases as SM, and 45 cases as malignant. We compared the original FNA diagnoses of these 323 cases with the diagnoses obtained on HPE and calculated the malignancy risk for each category[29].

Elaborative study done by SafaAlshaikh and coauthors observed that out of 681 thyroid nodules from 632 patients which underwent FNAC, surgery was done for 126 (18.5%) nodules for 119 patients, all of whom had histopathology available for review. This comprised 16/69 (23.1%) Category I cases, 60/469 (12.7%) Category II cases, 25/85 (29.4%) Category III cases, 9/20 (45%) Category IV cases, 11/18 (61.1%) Category V cases, and 12/28 (42.8%) Category VI cases Of the 126 nodules with histopathology follow-up, carcinoma was identified in 35 cases yielding an overall rate of malignancy of 27.8% (35/126 nodules and 34/119 patients). There were 15 Bethesda I nodules

(nondiagnosis) with follow-up histopathology. Of these, malignancy was found in only one case, which was a papillary thyroid microcarcinoma (6.67%, 1/15). The rest were all benign thyroid lesions which also included one case ofHurthle cell neoplasm and one case of follicular adenoma [29].

Of the 60 nodules diagnosed as Bethesda II (benign) on preoperative FNAC, nine nodules found to be malignant, yielding a malignancy rate of 15% (9/60) for those undergoing surgery, which represented around 2% of the total number of Category II nodules. Of the 85 nodules diagnosed as Bethesda III (AUS/FLUS), 25 were followed up with surgery and malignancy identified in 7 cases (all were papillary thyroid carcinomas) with an estimated risk of malignancy of 28%.There were 20 Bethesda IV nodules (Follicular neoplasm/SFN), with follow-up histopathology available in 9 cases and malignancy identified in 2 cases only (22% of those undergoing surgery, and 10% of the entire cohort), one of which turn out to be a poorly differentiated carcinoma. Of the remaining cases, there were one Hurthle cell neoplasm, two follicular adenomas, and four adenomatoid nodules in multinodulargoiter. There were 18 Bethesda V nodules (suspicious for malignancy), of which 11 underwent surgery and 8 (72.7%) were confirmed to be carcinoma, all are papillary carcinomas including three cases with follicular variant papillary carcinomas. There were 28 Bethesda VI nodules (malignant), of which 12 underwent surgery and all (100%) were confirmed to be carcinoma, all papillary carcinomas including one case of follicular variant papillary carcinoma[29].

As previously mentioned in cytological diagnosis discussion, study done by Mondal et al had correlated 31.66% cases with histopathologicaldiagnosis and observed that 68.7% cases were benign lesions on histopathology and 22.29% cases as malignant[29].

IMMUNE MARKER EXPRESSION IN THYROID NEOPLASM.

Various immune markers represent different component of thyroid follicular cells. They could be membrane antigen, cytoplasmic protein or the nucleus protein. Hence many different antibodies against these proteins or substances have been studied since decades, to mention few important antibodies studied are galectin-3, Hector Battifora mesothelial cell antibody (HBME-1), cytokeratin-19, RET, TTF-1, hTERT, telomerase, p27 and p53.Galectin-3 and HMBE-1 are two markers which are extensively studied to understand their specificity and sensitivity[32].

Saleh HA et al studied immunohistochemical markers like galectin-3, HBME-1, CK19 and Ret oncoprotein to differentiate benign and thyroid nodules and concluded that immunomarkers are significantly more expressed in malignant tumours compared to benign lesions and may be of additional diagnostic value when combined with routine histology[33].

Author Arcolia et al studied gal-3, HBME-1 and CK 19 immune marker expression in thyroid resected specimens and concluded that gal-3 is a useful immunohistochemical marker to discriminate malignant tumours from benign thyroid nodules and also, is a sensitive marker for the diagnosis of thyroid malignancy. Combination of gal-3 with CK19 and HBME-1 gives high specific result,for the diagnosis of well-differentiated thyroid cancer[34,35].

In the present research carried out, 48 resected thyroid specimens had diagnostic difficulty due to overlap of morphological features on H&E stains. These cases were subjected to immunohistochemistry where galectin-3 and HBME-1 were studied and

observed Gal-3 expression in thyroid papillary neoplasms was found to have a sensitivity of 86.67%, specificity of 85%, and positive predictive value of 89.66% and negative predictive value of 80.95%. HBME expression had sensitivity of 84.62%, specificity of 79%, positive predictive value 81.32% and negative predictive value 69.23%. Gal-3 immune marker is more specific for papillary lesions. This observation is with concordance with other studies mentioned.

Zhang et al observed the potential of triple immunohistochemical staining to be used as an ancillary test to clarify cytological diagnoses of indeterminate thyroid nodules. He demonstrated the diagnostic value of dual positive/colocalization of Galectin-3 and HBME-1 for thyroid malignancy[36] .similarly the present study highlights the importance of galectin 3 and HBME-1 immune markers in diagnosing thyroid malignancies. And thus the study concluded that cumulative approach including thyroid serum markers and tissue immune marker gives a complete diagnostic approach of thyroid lesions. Gal-3 is a promising marker in the diagnosis of papillary carcinoma and HBME-1 is useful marker for its expressions in thyroid carcinoma compared with benign neoplasms thus representing a promising target for therapy of thyroid cancers[37].

RET PROTO-ONCOGENE

The *RET* proto-oncogene encodes a receptor-type tyrosine kinase with an extracellular domain, a Trans membrane domain, and an intracellular tyrosine kinase domain. This was reviewed byItoh et al (1989)[38].In the past 5 years, the general interest in studying the *RET* proto-oncogene has been growing exponentially.The *RET* proto-oncogene was identified as the susceptibility gene for multiple endocrine neoplasia type 2 (MEN 2), an inherited cancer syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma (Pheo), and parathyroid hyperplasia (PTH) [39].

Status of RET proto-oncogene germline mutation can be used to distinguish between the sporadic and the hereditary MTC. Moreover, the knowledge about the precise RET mutations (genotype) may suggest a predilection toward a particular phenotype and clinical course. The results of RET proto-oncogene mutation analysis in MTC patients guides the physicians for surveillance including tumor marker measurement and management of pheochromocytoma and hyperparathyroidism. Also, knowledge of the RET mutations can guide the treating physicians for decisions regarding prophylactic thyroidectomy and intra-operative management of the parathyroid glands in the family members of the patients that are at risk for developing MTC.[27]Multifocality and bilaterality of the CCH was commonly associated with prophylactic than index cases in our study. This is in agreement with previous studies on prophylactic thyroidectomies[38,39].

We found codon 634 as the most common mutated codon of RET proto-oncogene in our study. Most common codon mutated in this group was 634(12 out of 14 cases), which is in agreement with the literature[40] .We found that C634R mutation was the most common (7/14 cases), and most aggressive form as it was associated with majority of the cases of MMTC; and single case of MTC with LN metastases in our study. This is

in agreement with other limited previous studies done by Frank-Raue K *et al* (2006) and Alvandi E *et al* (2011)[41] .

Table 22 : comparison   of 634 exon mutation of ret proto-oncogene in other studies.

	Rita Abi-Raad et al	DemetEtit et al	Our study
No.of prophylactic cases studied	22	42	14
CCH	9(41%)	26(62%)	9(64.3%)
634 exon mutation	80%	76.6% cases	85.7% cases

REFERNCES

1. Karimi F, Kalantarhormozi MR, Dabbaghmanesh MH, Omrani GR. Thyroid disorders and the prevalence of antithyroid antibodies in shiraz population. Arch Iran Med. 2014;17(5):347–51.

2. WeiminXu ,LiangliangHuo , Zexin Chen , Yangmei Huang , Xingyi Jin , Jing Deng.The Relationship of TPOAb and TGAb with Risk of Thyroid Nodules: A Large Epidemiological StudyInt. J. Environ. Res. Public Health 2017 14: 723;1-22

3. Dauksiene D, Petkeviciene J, Klumbiene J, Verkauskiene R, Vainikonyte Kristapone J, Seibokaite A, et al. Factors Associated with the Prevalence of Thyroid Nodules and Goiter in Middle-Aged Euthyroid Subjects. Int J Endocrinol. 2017;2017.

4. Y. Fleury, G. Van Melle, V. Woringer, R. C. Gaillard, and L. Portmann, "Sex-dependent variations and timing of thyroid growth during puberty," The Journal of Clinical Endocrinology and Metabolism, vol. 86, no. 2, pp. 750–754, 2001.

5. M. Rotondi, G. Amato, B. Biondi et al., "Parity as a thyroid size-determining factor in areas with moderate iodine deficiency," The Journal of Clinical Endocrinology and Metabolism, vol. 85, no. 12, pp. 4534–4537, 2000.

6. N. Knudsen, I. Bülow, P. Laurberg, L. Ovesen, H. Perrild, and T. Jørgensen, "Parity is associated with increased thyroid volume solely among smokers in an area with moderate to mild iodine deficiency," European Journal of Endocrinology, vol. 146, no. 1, pp. 39–43, 2002.

7. G. Popoveniuc and J. Jonklaas, "Thyroid nodules," Medical Clinics of North America, vol. 96, no. 2, pp. 329–349, 2012.

8. W. C. Kung, M. T. Chau, T. T. Lao, S. C. F. Tam, and L. C. K. Low, "The

effect of pregnancy on thyroid nodule formation," The Journal of Clinical Endocrinology and Metabolism, vol. 87, no. 3, pp. 1010–1014, 2002.

9. Quintero OL, Amador-Patarroyo MJ, Montoya-Ortiz G, Rojas-Villarraga A, Anaya JM. Autoimmune disease and gender: Plausible mechanisms for the female predominance of autoimmunity. J Autoimmun [Internet]. 2012;38(2–3):J109–19.

10. Elizabeth K Cahoon, , Alexander Rozhko, Maureen Hatch, Olga Polyanskaya, , EvgeniaOstroumova, , MinTang, et al   Factors associated with serum thyroglobulin levels in a population living in Belarus.ClinEndocrinol (Oxf). 2013 July; 79(1): 1-16.

11. Cho et al.: Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study. BMC Cancer 2014 14:873

12. Kim, E.S.; Lim, D.J.; Baek, K.H.; Lee, J.M.; Kim, M.K.; Kwon, H.S.; Song, K.H.; Kang, M.I.; Cha, B.Y.; Lee, K.W.; et al. Thyroglobulin antibody is associated with increased cancer risk in thyroid nodules. Thyroid 2010, 20, 885–891

13. Lind P, Langsteger W, Molnar M, Gallowitsch HJ, Mikosch P, Gomez I. Epidemiology of thyroid diseases in iodine sufficiency. *Thyroid*. 1998;8(12):1179–1783.

14. Pedersen IB, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Laurberg P. Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *ClinEndocrinol (Oxf)*. 2003;58(1):36–42.

15. Kasagi K, Takahashi N, Inoue G, Honda T, Kawachi Y, Izumi Y. Thyroid

function in Japanese adults as assessed by a general health checkup system in relation with thyroid-related antibodies and other clinical parameters. *Thyroid*. 2009;19(9):937–944.

16. Takeda K, Mishiba M, Sugiura H, Nakajima A, Kohama M, Hiramatsu S. Evaluated reference intervals for serum free thyroxine and thyrotropin using the conventional outliner rejection test without regard to presence of thyroid antibodies and prevalence of thyroid dysfunction in Japanese subjects. *Endocr J*. 2009;56(9):1059–1066.

17. O'Leary PC, Feddema PH, Michelangeli VP, et al. Investigations of thyroid hormones and antibodies based on a community health survey: The Busselton thyroid study. *ClinEndocrinol (Oxf)*. 2006;64(1):97–104

18. Jóźków P, Lwow F, Słowińska-Lisowska M, Mędraś M. Trends in the prevalence of autoimmune thyroiditis in the leading private health-care provider in Poland. Adv Clin Exp Med. 2017;26(3):497–503.

19. Xu W, Huo L, Chen Z, Huang Y, Jin X, Deng J, et al. The relationship of TPOab and TGAb with risk of thyroid nodules: A large epidemiological study. Int J Environ Res Public Health. 2017;14(7):1–11.

20. Roberts, C.G.; Ladenson, P.W. Hypothyroidism. Lancet 2004, 363, 793–803.

21. Mitchell, J.D.; Kirkham, N.; Machin, D. Focal lymphocytic thyroiditis in Southampton. J. Pathol. 1984, 144,269–273.

22. Kasagi, K.; Kousaka, T.; Higuchi, K.; Iida, Y.; Misaki, T.; Alam, M.S.; Miyamoto, S.; Yamabe, H.; Konishi, J.Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: Comparison with histological findings. Thyroid 1996, 6, 445–450.

23. Prummel MF, Wiersinga WM. Thyroid peroxidase autoantibodies in euthyroid subjects. *Best Pract Res ClinEndocrinolMetab*. 2005;19(1):1–15.

24. Cho AA, Kong SY, Shin A, Lee J, Lee KK, Lee JJ, et al. Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: A nested case-control study. BMC Cancer. 2014;14(1):1–10.

25. Kim KW, Park YJ, Kim EH, Park SY, Park DJ, Ahn SH, Park do J, Jang HC, Cho BY: Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. Head Neck 2011, 33:691–695.

26. Azizi G, Malchoff CD: Autoimmune thyroid disease: a risk factor for thyroid cancer. EndocrPract 2011, 17:201–209.

27. Fiore E, Rago T, Scutari M, Ugolini C, Proietti A, Di Coscio G et al : Papillary thyroid cancer, although strongly associated with lymphocytic infiltration on histology, is only weakly predicted by serum thyroid auto-antibodies in patients with nodular thyroid diseases. J Endocrinol Invest 2009, 32:344–351.

28. Antonelli A, Mosca M, Fallahi P, Neri R, Ferrari SM, D'AscanioA et al. Thyroid cancer in systemic lupus erythematosus: a case–control study. J ClinEndocrinolMetab 2010 95:314–318.

29. Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK. The Bethesda system for reporting thyroid fine needle aspirates: A cytologic study with histologic follow-up. J Cytol. 2013;30(2):94–9.

30. Zarif HA, Ghandurah SE, Al-Garni MA, Binmahfooz SK, Alsaywid BS, Satti MB. Thyroid nodules cytopathology applying the bethesda system with histopathological correlation. Saudi J Med MedSci 2018;6:143-8.

31. A Safa, ZainabHarb, Aljufairi E and Ali SA. Classification of thyroid fine-needle aspiration cytology into Bethesda categories: An institutional experience and review of the literature. Cytojournal.2018; 15: 4.

32. Roe JH, Koether CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J Biol Chem. 1943; 147:399–40

33. Saleh HA, Bo Jin, John Barnwell, OpadaAlzohaili. Utility of immunohistochemical markers in differentiating benign from malignant follicular derived thyroid nodules. Diagnostic Pathology 2010 ; 5(9):1-11

34. Arcolia V, Journe F, Renaud F, Leteurtre E, Gabius HJ, RemmelinkM.Combination of Galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer 2017;14: 4183-89

35. Krampitz, G.W. and J.A. Norton, RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Cancer, 2014. 120(13): 1920-31.

36. Donis-Keller, H., et al., Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Human molecular genetics, 1993. 2(7): 851-56.

37. Machens, A. and H. Dralle, Biological relevance of medullary thyroid microcarcinoma. The Journal of Clinical Endocrinology & Metabolism, 2012. 97(5): 1547-53

38. Etit, D., et al., Histopathologic and clinical features of medullary microcarcinoma and C-cell hyperplasia in prophylactic thyroidectomies for medullary carcinoma: a study of 42 cases. Archives of pathology & laboratory medicine, 2008. 132(11): 1767-68.

39. Niccoli-Sire, P., et al., Early or prophylactic thyroidectomy in MEN 2/FMTC gene carriers: results in 71 thyroidectomized patients. The French Calcitonin Tumours Study Group (GETC). European journal of endocrinology, 1999. 141(5): 468-74

40. Alvandi, E., et al., Molecular analysis of the RET proto-oncogene key exons in patients with medullary thyroid carcinoma: a comprehensive study of the Iranian population. Thyroid, 2011. 21(4): 373-82.

41. Frank-Raue, K., et al., Long-term outcome in 46 gene carriers of hereditary medullary thyroid carcinoma after prophylactic thyroidectomy: impact of individual RET genotype. European Journal of Endocrinology, 2006. 155;2: 229-36.

CHAPTER VII

SUMMARY AND

CONSLUSION

SUMMARY

Total 165 cases were studied under this study period and observed that Anti TG and Anti TPO antibodies are markedly elevated in autoimmune thyroiditis and also raised in papillary carcinoma.

On Fine needle Aspiration study, total of 51%cases were benign and 20%cases were neoplastic lesions and 29% cases were falling under grey zone for which further evaluation were required.

Among these 165 cases, 71cases had histo-pathological correlation and found that 47 cases were noneoplastic and benign lesions combined and 24 cases were malignant lesions.

48 cases where histopathological diagnosis was difficult, those cases were subjected for IHC HBME1 and Galectin 3and noticed that HBME 1 is very sensitive for malignancies and Galectin 3 is very specific for papillary carcinoma of thyroid.

Study done for *ret* oncogene gene mutation in prophylactic cases observed commonest mutation in exon C634R and these thyroid tissue were also showing C cell hyperplasia on histopathology slides.

CONCLUSION

- Anti TPO is significantly raised in chronic thyroiditis and papillary carcinoma

- The Bethesda system of Reporting Thyroid Cytology (TBSRTC) is strongly recommended for thyroid cytopathology

- HBME 1 and Galectin 3 –are important immune marker for diagnosing malignancies

- Genetic analysis is recommended in high risk population

GRAPHICAL ABSTRACT

Increased incidence of autoimmune/chronic thyroiditis

↓

Anti TPO is significantly raised

↓

Carries high risk for papillary carcinoma

↓

Hence follow up required

↓

Prophylactic thyroidectomy recommended in high risk cases
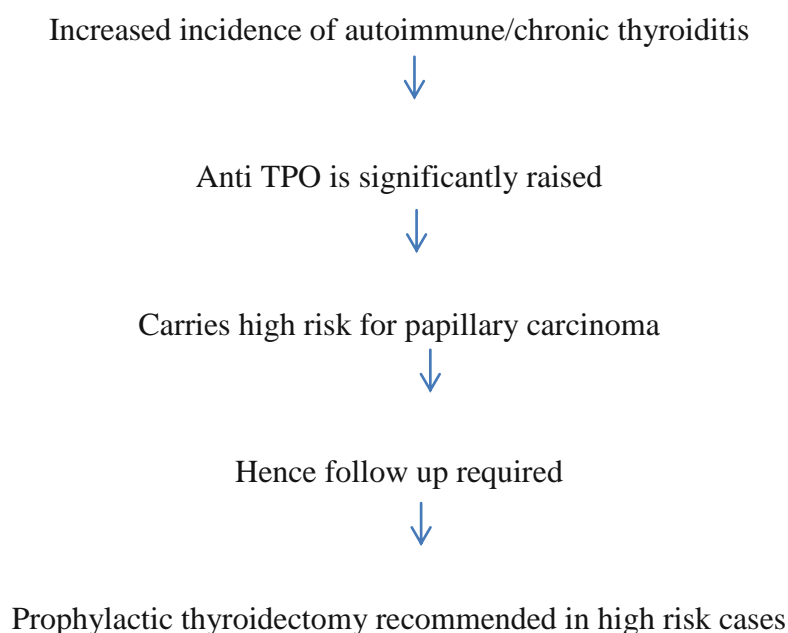
Immune marker HBME1 and Galectin 3 remain most reliable immune markers for confirmation of malignancy

ret oncogene genetic analysis recommended in individuals with familial medullary carcinoma

FUTURE DIRECTIONS AND LIMITATIONS

Limitations:

- Sample size is less
- Follow up serum biomarkers levels were not done due to loss of follow up ofcases
- Genetic analysis was not done in all cases due to financial constrain.

Future directions:

- Similar studies can be encouraged in large population as cohort study
- Follow up cases with complete molecular study of each case can be done and thus predictive and prognostic markers can beidentified and evaluated.

ANNEXURES

Index

1. **Informed consent form**

2. **Proforma for collection of sample**

3. **Plagiarism certificate**

4. **Ethical clearance certificate**

5. **Publications**

6. **Paper presentation and conference certificates**

PERFORMA FOR COLLECTION OF SAMPLE

Case No: Date :

Investigator :

TOPIC : Sensitivity and Specificity of Differential Diagnostic Biomarkers in Thyroid

Diseases: From Circulating Chemistry to Molecular Cytogenetics.

Particulars of the patient :

Name :   Hospital :

Age :   O.P.D/I.P No. :

Address

Occupation

Presenting complaints and duration :

Past history :

Family history :

Personal history :

Obstetric history:

General physical examination :

Systemic examination :

Clinical diagnosis :

Investigation :

Biochemical analysis

T3

T4

TSH

Thyroglobulin

Antithyroglubulin antibody

Anti thyroperoxidase antibody

HISTOPATHOLGICAL STUDY :

1. **Nature of the specimen**

2. **Gross**

3. **Microscopy**

   Impression :

Other investigation: IHC

HMBE -1 : positivity/negativity/intensity

E-cadherin : positivity/negativity/intensity

FINAL IMPRESSION :

NOTES IF ANY:

                                                          Signature of investigator

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT   **:**   Sensitivity and Specificity of Diagnostic

Biomarkers in Thyroid Diseases: From Circulating Chemistry to Molecular

Cytogenetics.

PRINCIPAL INVESTIGATOR   **:**Dr Anita P Javalgi

GUIDE : Dr B R Yelikar

BENEFITS:

Participation in the study may benefit in accurate diagnosis of various thyroid diseases

and hence usefulness of confirmatory tests in proper management of disease and

medical information produced by this study .

RISK AND DISCOMFORTS: I understand that, there is no risk involved in the

procedures performed.

They are also informed that there will not be any kind of financial burden on them

(patients).

 CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of

hospital record and will be subjected to confidentiality and privacy regulations of the

hospital. If the data is used for publications the identity of participants will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more information about the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore I agree to participate in the present study.

_____   _____

Participant / Guardian   Date:

     I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____   _____

Investigator   Date:

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No-114/2015-16   April 10, 2015.

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on 16ᵗʰ March 2015 at 11 AM to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate student / Faculty members of this University / college from ethical clearance point of view. After scrutiny the following original / corrected & revised version synopsis of the Thesis / Research project has been accorded Ethical Clearance.

Title "Sensitivity and specificity of diagnostic biomarkers in thyroid diseases : from circulating chemistry to molecular cytogenetics."

Name of Ph.D./ P. G. / U. G. Student / Faculty member. Dr. Anita P.Javalgi Department of Pathology.

Name of Guide : Dr.B.R.Yelikar. Professor, Department of Pathology.

<table>
<tr><td>

Dr. Sharada Metgud
Chairperson, I.E.C
BLDE University,
VIJAYAPUR – 586 103

</td><td>

Dr.G.V.Kulkarni
Secretary, I.E.C
BLDE University,
VIJAYAPUR – 586 103.

</td></tr>
</table>

<u>Note:-</u>**Kindly send Quarterly progress report to the Member Secreatary.** Member Secretary, Institutional Ethical Committee, BLDE University, BIJAPUR.

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant documents.

Smt. Bangaramma Sajjan Campus, Sholapur Road, Vijayapur – 586103, Karnataka, India.

University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeuniversity.ac.in, E-mail: office@bldeuniversity.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldeuniversity.ac.in, Emaibmpmc.principal@bldeuniversity.ac.in

BLDE (DEEMED TO BE UNIVERSITY)

PLAGIARISM VERIFICATION CERTIFICATE

1. **Name of the Student:** Anita P Javalgi**.**   **Reg No**: 14PhD001

2. **Title of the Thesis:** "*Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: from Circulating Chemistry to Molecular Cytogenetics.*"

3. **Department:** Department of Pathology.

4. **Name of the Guide & Designation:** Dr B R Yelikar$_{MBBS,MD}$. Professor

5. **Name of the Co Guide & Designation:** Prof Kusal K Das$_{PhD}$ Professor

The above thesis was verified for similarity detection. The report is as follows:

Software used: Urkund   **Date:**20.12.2019

 Similarity Index (%):8%(Eight)   **Total word Count:**14140

The report is attached for the review by the Student and Guide.

The plagiarism report of the above thesis has been reviewed by the undersigned.

The similarity index is below accepted norms.

The similarity index is above accepted norms, because of following reasons:

> Total 10% similarity out of which 2% is self-plagiarism

> So 10%-2% =8% similarity

The thesis may be considered for submission to the University. The software report is attached.

Signature of the Guide   Signature of Co-Guide   Signature of Student
Name & Designation   Name & Designation

Verified by (Signature)
Name & Designation

Autoantibodies and Immune Expression of HBME 1 and Galectin 3 in Thyroid Nodules

Anita P Javalgi[1], BR Yelikar[2], Kusal Das[3], Raga Sruthi[4], Rodrigues Lynda[5]

Abstract

Thyroid diseases are among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases. Since mortality and morbidity in thyroid cancer is often measured over decades, there is a paucity of prospective clinical studies that are capable of evaluating various tests and to find out the cost and time saving methods which may significantly reduce patient morbidity and unnecessary surgery in benign thyroid disease. Objectives of present study was to study anti thyroperoxidase (AntiTPO) antibody, anti-thyroglobulin (anti TG) antibody and immune expression of HBME1 and galacten 3 in various thyroid lesions. This is a prospective 2 year study from January 2015 to December 2016. All cases referred to cytology section were included and individual on hormone therapy or antithyroid drugs were excluded from study. FNAC was done in all cases. Thyroid function test, anti TPO and anti TG antibodies were measured. Histopathology correlation of cases was done of resected thyroid tissue and immune markers HBME 1 and galectin 3 was done where ever required. 165 cases had cytological diagnosis reframed under Bethesda reporting and serum biomarkers level obtained. 71 cases had histopathological correlation and observed nodular goiter commonest non neoplastic lesion followed by lymphocytic thyroiditis and papillary carcinoma. HBME 1 is specific to differentiate benign and malignant lesions and galectin 3 is highly specific for papillary carcinoma. Auto-antibodies are markedly raised in autoimmune thyroiditis and papillary carcinoma. To conclude autoantibodies level estimation helps in clinical diagnosis and management of thyroid lesions. Immunohistochemistry plays vital role in confirmation of malignant lesions.

Keywords: Anti thyroperoxidase/ anti thyroglobulin/HBME1/galectin 3.

Authors Affiliation: [1]Phd Scholar, Department of Pathology, [2]Professor, [3]Professor, [4,5]Consultant, Department of Physiology, Shri BM Patil Medical College, Hospital and Research Centre, BLDE Deemed to be University, Vijayapura, Karnataka 586103, India.

Corresponding Author: Anita P. Javalgi, Phd Scholar, Department of Pathology, Shri BM Patil Medical College, Hospital and Research Centre, BLDE Deemed to be University, Vijayapura, Karnataka 586103, India.

E-mail: anitajawalgi@gmail.com

Introduction

Thyroid diseases are among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases [1].

Thyroid nodules are a very frequent finding, and their prevalence steadily increases with age. Nodular thyroid disease refers to the presence of a solitary nodule or multiple nodules, solid to one or more cystic lesions. It is estimated that 5%–7% of adults have clinically detectable nodules in

the thyroid, and with the emergence of modern ultrasonographic (US) techniques detecting thyroid nodules of a few millimeters, the frequency of nodularity is estimated to be 16%–67% in unselected subjects. Most of the discovered nodules are benign; however, there are increasing incidence of cancers (2.4-fold increase), and this trend appears to be continuing. Recent population studies have shown that about 12% of adults have a palpable goitre. Autoimmune thyroid disease is probably commoner than iodine deficiency as a cause of goiter in areas that are now iodine sufficient [2,3].

Thyroid cancer is the most common endocrine malignancy and is the sixth most common cancer in women and the second most common cancer in women under 40 years of age [4].

Since mortality and morbidity in thyroid cancer is often measured over decades, there is a paucity of prospective clinical studies that are capable of evaluating various tests which may significantly reduce patient morbidity and unnecessary surgery in benign thyroid disease. The present study was taken to study the significance of FT3, FT4, TSH, anti TPO antibody, anti thyroglobulin antibody in various thyroid lesions. Cytological diagnosis of thyroid nodules, histopathological correlation and to study immune marker expression of HBME-1 and galectin 3 in the resected thyroid specimen in applicable cases.

Materials and Methods

This was a prospective 2 year study from January 2015 to December 2016. All patients with thyroid swelling from ENT & Surgery clinic being referred to Department of Pathology were included in the study with informed consent. Patients with thyroid swelling already on thyroid hormone therapy or antithyroid drugs were excluded from the study. Informed consent was obtained and then detailed clinical history was noted. Thorough clinical examination was carried out. 5 ml of venous blood sample was collected in plane vaccutainer and serum markers estimation which included thyroxine (T4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH), anti Thyroperoxidase (Anti TPO), Anti thyroglobulin antibody (Anti TG). Collected sample was run through Vidas biochemical analyser. Fine needle aspiration cytology (FNAC) was done and cytological diagnosis was given following The Bethesda Reporting System for Thyroid Cytology (TBRSTC). Resected thyroid tissue was grossed and processed FFPE as per standard protocol and stained with routine Hematoxylin and Eosin

(H&E) stain and histopathological diagnosis given and correlation between cytological diagnosis and histopathological diagnosis was also done in available cases. Immunohistochemistry for HBME1 and galectin 3 markers was carried out in required cases as per standard protocol.

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft® Excel for Mac 2011 programs. In the present study, descriptive statistics as well as 95% confidence interval for a single proportion, mean, P value and sensitivity and specificity of immune markers was calculated.

Results

Total samples included in the study were 165 cases, all cases serum biomarkers ie thyroid hormones and autoantibodies were measured and noted down. In present study females outnumbered males in thyroid disease with 81% and 19% male affected. Youngest patient was 12 yrs old (Table 1).

Serum biomarkers of thyroid function that is free T3, free T4 and thyroid stimulating hormone (TSH) were with in normal range. Anti thyroperoxidase (Anti TPO) and anti-thyroglobulin (Anti-TG) levels were raised in autoimmune thyroiditis and in few cases of papillary carcinoma (Table 2). P value was calculated and observed that P value was statistically significant for anti TPO and anti Tg levels in relation with thyroiditis and papillary carcinoma (Table 3).

The Bethesda Thyroid reporting was followed for cytological reporting and observed 83 cases (51%) were benign cases Bethesda category II, 9% cases were frank malignant remaining Bethesda category VI, 9.7% cases were follicular neoplasm category IV and 30.3% cases were falling in grey area Bethesda category V and category III. Histopathological diagnosis was correlated in available cases (Table 4). Seventy one cases had histopathological correlation. Commonest histopathological diagnosis was colloid goitre (21 cases), followed by 16 cases thyroiditis, 10 cases of follicular adenoma and 24 cases were thyroid malignancy.

Papillary carcinoma was commonest malignancy with 15 cases, 4 cases of follicular carcinoma, 2 cases of medullary carcinoma and 2 cases of metastatic carcinoma both were squamous cell carcinoma deposits. One rare case of mucoepidermoid carcinoma was diagnosed.

Forty eight (48) resected thyroid specimens with morphological similar features between

benign and malignancy, those cases were subjected immunohistochemistry. HBME 1 and Galectin 3 immune expression was studied. Gal-3 stained the majority of malignant cases (89%) in comparison to benign neoplasms the difference was statistically significant (p-value <0.0001). Gal-3 expression in thyroid papillary neoplasms was found to have a sensitivity of 88.2%, specificity of 89.12%, and positive predictive value of 91.22% and negative predictive value of 78.12%. HBME is more specific

Table 1: Age distribution

Sex/age	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Female	15	45	33	15	18	6	2	134
Male	3	6	11	6	2	3	-	31

Table 2: Thyroid Hormones and Antibodies Level in Various Thyroid Diseases

Thyroid lesion	TSH 0.4-4.0 muIU/ml	FT3 3.5-7.8 pmol/L	FT4 9 – 25 pmol/L	Anti TG <20 IU/ml	Anti TPO < 35 IU/ml
Goitre (colloid/nodular/toxic)	1.62+/-0.76	3.02+/-042	1.22+/-0.24	16.63+/-3.42	35.2+/-3.23
Lymphocytic thyroiditis	20.66+/-4.05	1.42+/-0.37	.53+/-0.16	43.25+/-7.46	63.26+/-5.96
Granulomatous thyroiditis	2.22+/-0.43	6.24+/-1.56	19.45+/-6.5	26+/-3.50	35.2+/-2.50
Graves disease	0.02+/-0.01	13.3+/-3.69	30+/-4.79	34.85+/-6.76	42.28+/-5.92
Follicular neopalsm Follicular adenoma	0.83+/-0.16	4+/-1.73	11.3+/-1.67	12.6+/-3.77	25+/-0
Follicular carcinoma	2+/-0	3.9+/-0.1	20+/-0	12+/-1.41	17.5+/-2.42
Papillary carcinoma	2.1 +/- 0.54	4.65+/-1.16	13.8+/-4.16	30.5+/-14.53	33.3+/-17.93
Medullary carcinoma	0.9	4	13	20	39

Table 3: p value of the serum Biomarkers

Thyroid lesion	TSH	FT3	FT4	Anti TG	Anti TPO
Goitre (colloid/nodular/toxic)	0.182	0.218	0.321	0.61	0.12
Lymphocytic thyroiditis	0.245	0.03	0.001	0.05	0.002
Granulomatous thyroiditis	0.215	0.04	0.02	0.06	0.001
Graves disease	0.05	0.04	0.003	0.015	0.025
Follicular neoplasm Follicular adenoma Follicular carcinoma	0.224	0.23	0.231	0.215	0.071
Papillary carcinoma	0.251	0.25	0.32	0.02	0.04
Medullary carcinoma	-	-	-	-	-

Table 4: Bethesda cytological reporting and histopathological correlation

Bethesda system	Includes	Cytology cases	HPR correlation Cases	Final diagnosis
I	Non diagnostic Non satisfactory	Acellular/ only fluid/ blood	24	-
II	Benign	83	24	Goitre (colloid/nodular/toxic) Lymphocytic thyroiditis Granulomatous disease Graves disease Adenomatoid nodule
III	Atypia of unknown significance (AUS)	32	13	
IV	Follicular neoplasm/ suspicion for follicular neoplasm	16	14	Follicular neopalsm; Follicular adenoma Follicular carcinoma
V	Suspicious for other malignancy	09	20	Papillary carcinoma Medullary carcinoma
VI	Malignancy	15		Metastatic carcinoma Mucoepidermoid carcinoma
Total	-	165	71	-

Forty cases had immunohistochemistry analysis with marker HBME 1 and Galectin 3.

Table 5: IHC HBME 1 & Galectin 3

HPR diagnosis	No of cases	HMBE 1	Galectin 3
Papillary carcinoma	15	Positive	Positive (15 case)
MNG with papillary like features	12	Weak positive	Positive (1case)
Lymphocytic thyroiditis	2	Negative	Negative
Graves disease	1	Negative	Positive
Follicular carcinoma	3	Weak positive	Negative

for thyroid malignancies compared to benign neoplasms with sensitivity of 91% and specificity of 95% (Table 5).

Discussion

In present study we had 165 thyroid lesion cases in which we observed female preponderance 79%, similar to study done by K Fariba et al. [5] with 66.8% females, WeiminXu et al. [6] and Howrah et al.

Cahoon KE et al. [7] had population based cohort study which measured serum Tg, urinary iodine, TSH, anti-thyroglobulin, anti- thyroid peroxidise levels and Ultrasound to assess the presence of nodules and estimate thyroid volume and concluded that serum Tg is significantly related to presence of thyroid abnormalities as well as indicators of thyroid function and iodine deficiency and, therefore, could be used to characterize the iodine status and thyroid function of individuals in the context of epidemiological study.

In spite of iodine sufficient belt the incidence of thyroid diseases are increasing, with the literature supporting with the autoimmune cause. Antibodies levels were raised in autoimmune thyroid disease and in known fact but levels were also high in papillary carcinoma and medullary carcinoma suggesting could play role in etiopathogenesis? Similar findings was also observed by Young Ah Cho et al. [8] in "Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study" Eun Sook Kim et al. [9], reported that TGAb was associated with an increased risk of thyroid cancer in thyroid nodules. Similarly, other studies also showed an analogous association with malignancy by considering positive thyroid autoantibodies as a whole, including TPOAb and TGAb. Present study also comments on auto-antibodies levels high in autoimmune thyroiditis and few cases of papillary carcinoma.

In present study, cytological diagnosis of all cases were categorised according to Bethesda reporting system thus signifying its importance for surgical management of thyroid nodules. Study done by Zarif, et al. [10]. demonstrates higher risks of malignancy in diagnostic categories (DC) I, DC II, DC III and DC IV than that of the original BSRTC definition, along with a higher specificity and positive predictive value for cancer diagnosis, and a lower sensitivity and negative predictive value.

Several immunohistochemical markers representing different components of the cell, such as the membrane, the cytoplasm, or the nucleus, have been studied in thyroid neoplasms. Some of the antibodies that have been examined include galectin-3, Hector Battifora mesothelial cell antibody (HBME-1), cytokeratin-19, RET, TTF-1, hTERT, telomerase, p27 and p53 to name a few. Two markers that have been extensively studied are galectin-3 and HMBE-1 [11].

Saleh HA et al. [12] studied immunohistochemical markers like galectin-3, HBME-1, CK19 and Ret oncoprotein to differentiate benign and thyroid nodules and concluded that immunomarkers are significantly more expressed in malignant tumours compared to benign lesions and may be of additional diagnostic value when combined with routine histology [13].

Study done by Arcolia et al. [14]: diagnostic performances of individual or combined thyroid markers demonstrated that gal1 is a useful immunohistochemical marker to discriminate malignant tumours from benign thyroid nodules. They further validate that gal3 is a sensitive marker for the diagnosis of thyroid malignancy, and add support for its combination with CK 19 and HBME 1 with the highest performance for the diagnosis of well differentiated thyroid cancer. Such combination of markers should be validated in a larger series of tissues including various subtypes of thyroid lesions [14].

Zhang et al. [15] observed in his study the potential of triple immunochemical staining to be used as an ancillary test to clarify cytologic diagnoses of indeterminate thyroid nodules.

Also demonstrated the diagnostic value of dual positive/colocalization of Galectin-3 and HBME-1 for thyroid malignancy [15]. Similarly present study highlights the importance of galectin 3 and HBME-1 immune markers in diagnosing thyroid malignancies.

Conclusion

Cumulative approach including thyroid serum markers and tissue immune marker study gives a complete diagnostic approach of thyroid lesions and Gal-3 is a promising marker in the diagnosis of PTC and its variants and HBME-1 differential expressions in thyroid carcinoma compared with benign neoplasms may also represent a promising target for therapy of thyroid cancers.

References

1. J. Larry Jameson and Anthony P. Weetman. Disorders of the Thyroid Gland (Endocrinology and Metabolsim) Harrison's principles of Internal Medicine. 18th edition McGraw Hill publications. 2012;16:2911-14.

2. Unnikrishnan AG and MNUsha. Thyroid disorders in India: An epidemiological perspective, Indian J EndocrinolMetab. Jul 2011;15(Suppl2):78–81.

3. Grogan RH, Mitmaker EJ, and Clark OH. The Evolution of Biomarkers in Thyroid Cancer- From Mass Screening to a Personalized Biosignature. Cancers. 2010;2:885-912.

4. Knudsen N, Bulow I, Jorgensen T, Perrild H, Ovesen L, Laurberg P. Serum Tg- A sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. J Clin Endocrinol Metab. 2001;86:3599–03.

5. Karimi F, Kalantarhormozi MR, Dabbaghmanesh MH, RanjbarOmrani G. Thyroid disorders and the prevalence of antithyroid antibodies in Shiraz population. Arch Iran Med. 2014;17(1):347–51.

6. Weimin Xu, Liangliang Huo, Zexin Chen, Yangmei Huang, Xingyi Jin, Jing Deng. The Relationship of TPOAb and TGAb with Risk of Thyroid Nodules: A Large Epidemiological Study. Int. J. Environ. Res. Public Health. 2017;14(723):1-22.

7. Elizabeth K Cahoon, Alexander Rozhko, Maureen Hatch, Olga Polyanskaya, Evgenia Ostroumova, MinTang, et al. Factors associated with serum thyroglobulin levels in a population living in Belarus. Clin Endocrinol (Oxf). 2013 July;79(1):1-16.

8. Cho et al. Biomarkers of thyroid function and autoimmunity for predicting high-risk groups of thyroid cancer: a nested case–control study. BMC Cancer. 2014;14:873.

9. Kim ES, Lim DJ, Baek KH, Lee JM, Kim MK, Kwon HS et al. thyroglobulin antibody is associated with increased cancer risk in thyroid nodules. Thyroid 2010;20(8):1-8.

10. Zarif HA, Ghandurah SE, Al-Garni MA, Binmahfooz SK, Alsaywid BS, Satti MB. Thyroid nodules cytopathology applying the bethesda system with histopathological correlation. Saudi J Med MedSci 2018;6:143-8.

11. Grogan RH, Mitmaker EJ, and Clark OH. The Evolution of Biomarkers in Thyroid Cancer- From Mass Screening to a Personalized Biosignature. Cancers. 2010;2:885-912.

12. Saleh HA, Bo Jin, John Barnwell, Opada Alzohaili. Utility of immunohistochemical markers in differentiating benign from malignant follicular derived thyroid nodules. Diagnostic Pathology. 2010;5(9):1-11.

13. Prasad ML, Pellegata NS, Huang Y, Nagaraja HN, de la Chapelle A, Kloos RT. Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunohistochemistry is useful for the differential diagnosis of thyroid tumours. Mod Pathol. 2005;18:48–57.

14. Arcolia V, Journe F, Renaud F, Leteurtre E, Gabius HJ, Remmelink M. Combination of Galectin3, CK19 and HBME1 immunostaining improves the diagnosis of thyroid cancer. 2017;14:4183-89.

15. Zhang L, Krausz T, and De May RM. A Pilot Study of Galectin-3, HBME-1, and p27 Triple Immunostaining Pattern for Diagnosis of Indeterminate Thyroid Nodules in Cytology With Correlation to Histology Appl Immunohistochem Mol Morphol. 2015;23:481–90.

Antioxidants and Antibodies in Structural Thyroid Diseases

Anita P Javalgi[1], B R Yelikar[2], Kusal Das[3]

[1]PhD Scholar, [2]Prof & Head, [3]Professor, Department of Pathology, BLDE University's Shri B M Patil Medical College, Vijayapura, Karnataka

ABSTRACT

Thyroid nodules are a very frequent finding, and their prevalence steadily increases with age. Routinely clinical factors such as age, gender, and radiation history are meaningful for predicting thyroid nodules. There are few studies exploring the association of serum indexes of thyroid hormones or autoantibodies with the risk of thyroid nodules. Hence present study was undertaken with following objectives. To measure the serum markers of FT3, FT4,TSH, anti TPO antibody, anti-thyroglobulin antibody and role of Vitamin E and vitamin C antioxidants in relation with thyroid serum markers in various thyroid diseases. Correlation of these serum markers with cytological diagnosis was done. Serum analysis of thyroid hormones, autoantibodies level and FNAC was done with patients having thyroid swelling.

It was noted in present study that prevalence of positive serum autoantibodies displays geographical heterogeneity, unrelated to goitre prevalence. Autoantibodies levels are raised in autoimmune thyroid diseases and in few variants of thyroid malignancy. And it was also observed anti-Oxidants (vitamin e and vitamin c) levels were variable in thyroid disorders. To conclude autoantibodies are markedly raised in thyroiditis condition and the incidence of autoimmune thyroiditis is increasing in iodine sufficient as well as iodine deficient geographical areas. In present study it was also observed that autoantibodies levels were raised in papillary carcinoma thyroid indicating role of anti-TPO and anti-TG in etiopathogenesis. Antioxidants levels were variable and low in most of thyroid diseases suggesting its role in etiopathogenesis.

Keywords: auto-antibodies, antioxidants, goitre, papillary carcinoma

Introduction

Thyroid diseases are among the commonest endocrine disorders worldwide. India too, is no exception. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases. Routinely clinical factors such as age, gender, and radiation history are meaningful for predicting thyroid nodules. There are few studies exploring the association of serum indexes of thyroid hormones or autoantibodies with the risk of thyroid nodules. Although thyroid hormones and autoantibodies are reported to be dependently associated with thyroid function and thyroid diseases, little attention has been paid to whether thyroid hormones and autoantibodies are associated with thyroid nodules[2]. Thyroid nodule, as an entity, is one of the most common diseases originating from the endocrine system. Thyroid nodules may be single, multiple, solid, or cystic and may or may not be functional.[3] Most thyroid nodules are benign tumours and 5% are reported as malignant.[4,5] Autoimmune thyroiditis (AT) is a common disorder of the thyroid gland. It is usually diagnosed when thyroid autoantibodies (TPOAbs/TGAbs) are detected in patients with hypothyroidism or goiter.[6]

Autoimmune diseases (AID) appear when the host immune system turns against its own antigens leading to dysfunction or destruction of tissues and organs. AID may develop in mechanisms involving immune deregulation, genetic predisposition and due to influence of environmental factors.[7] Thyroid autoimmune

Corresponding Author:
Dr Anita P Javalgi
Department of Pathology
BLDE University's Shri B M Patil Medical College
Vijayapura, Karnataka
Phone: 09590196666
Email: anitajawalgi@gmail.com

diseases like GD and Hashimoto thyroiditis (HT) affect the thyroid gland and are called autoimmune thyroid diseases (AITD).[8]

Oxidative reactions occur in all tissues and organs, thyroid gland being one, in which oxidative processes are indispensable for thyroid hormone synthesis. Both hyper- and hypothyroidism have been proven to promote cellular oxidative stress by influencing the intensity of oxygen reactions and have been shown to affect concentrations of the vitamins involved in scavenging of free radicals (usually decreasing their concentrations, although study results differ) i.e. vitamins A, C and E.[9]

Therefore, this study was undertaken to determine whether there is an association between thyroid nodules, thyroid hormones levels and thyroid autoantibodies and correlation of these markers with cytological diagnosis. Also an attempt to understand the role of Vitamin E and vitamin C antioxidants in relation in various thyroid diseases was made.

Methodology

This was prospective study carried out in 2016 at out tertiary care hospital. The study group includes patients with thyroid swelling referred to Department of Pathology for FNAC. Patients with thyroid swelling with thyroid hormone therapy or antithyroid drugs were excluded.

Early morning fasting 5ml of venous blood sample was collected in plane vaccutainer and the collected serum sample was run through Vidas biochemical analyzer based on the principal chemiluminescent immunoassay. Serum markers estimation included Free thyroxine(T4), Free tri-iodothyronine (T3), Thyroid stimulating hormone (TSH), Anti thyroglobulin antibody (AntiTG ab), Anti thyroperoxidase (anti TPO), Vitamin C and E levels (HPLC method).

Descriptive statistics as well as 95% confidence interval for a single proportion and a mean was calculated.

Results

In this one year cross sectional study total 54cases with thyroid swelling referred to cytology section were included and all cases we had serum biomarker level estimation. In present study females outnumbered males with 43 females (79%) and 11 males (21%), youngest being 12yrs and oldest age 72yrs.(TABLE I: AGE DISTRIBUTION)

Table I: Age distribution

Sex/age	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Female	5	14	10	5	6	2	1	43
Male	1	2	3	3	1	1	-	11

The commonest thyroid disease was colloid goiter followed by thyroiditis. Lymphocytic thyroiditis was common followed by granulomatous thyroiditis and other variants. Papillary carcinoma was commonest malignant lesion affecting females in 3rd and 4th decade.(Table II)

Table II: Various thyroid diseases with sex distribution of disease

Thyroid lesion	Females	Males	Total
Goitre (colloid/nodular/toxic)	13	3	16
Lymphocytic thyroiditis	14	1	15
Granulomatous thyroiditis	2	2	4
Graves disease	7	0	7
Follicular neoplasm			
Follicular adenoma	1	2	3
Follicular carcinoma	0	2	2
Papillary carcinoma	6	0	6
Medullary carcinoma	0	1	1
Total	43	11	54

Thyroid function test (free T3, free T4 and TSH) and autoantibodies level ie anti-TPO and anti-TG levels were measured and mean calculated with SD and observed that most of thyroid disorders were in euthyroid state and auto-antibodies level were raised in autoimmune thyroiditis and few cases of papillary carcinoma. (Table III).

Table III: Thyroid function test and auto-antibodies level in thyroid diseases

Thyroid lesion	Total	TSH 0.4-4.0 muIU/ml	FT3 3.5-7.8 pmol/L	FT4 9 – 25 pmol/L	Anti TG <20 IU/ml	Anti TPO < 35 IU/ml
Goitre (colloid/nodular/toxic)	16	1.62+/-0.76	3.02+/-042	1.22+/-0.24	16.63+/-3.42	35.2+/-3.23
Lymphocytic thyroiditis	15	20.66+/-4.05	1.42+/-0.37	.53+/-0.16	43.25+/-7.46	63.26+/-5.96
Granulomatous thyroiditis	4	2.22+/-0.43	6.24+/-1.56	19.45+/-6.5	26+/-3.50	35.2+/-2.50
Graves disease	7	0.02+/-0.01	13.3+/-3.69	30+/-4.79	34.85+/-6.76	42.28+/-5.92
Follicular neopalsm;						
Follicular adenoma	3	0.83+/-0.16	4+/-1.73	11.3+/-1.67	12.6+/-3.77	25+/-0
Follicular carcinoma	2	2+/-0	3.9+/-0.1	20+/-0	12+/-1.41	17.5+/-2.42
Papillary carcinoma	6	2.1 +/- 0.54	4.65+/-1.16	13.8+/-4.16	30.5+/-14.53	33.3+/-17.93
Medullary carcinoma	1	0.9	4	13	20	39

Also anti-oxidants levels i.e. Vitamin C and Vitamin E were measured and observed that the levels were affected in thyroid abnormality with markedly reduced level in malignancy followed by thyroiditis and then goiter. (Table IV)

Table IV: vitamin C & vitamin E measurements in various thyroid diseases

Thyroid lesion	Total	Vitamin C level 0.2–2.0 mg/dl	Vitamin E level 5–20 μg/ml
Goitre (colloid/nodular/toxic)	16	1+/-0.56	9+/-1.5
Lymphocytic thyroiditis	15	0.1+/-0.01	1.75+/-0.5
Granulomatous thyroiditis	4	0.45+/-0.5	4.5+/-1.2
Graves disease	7	0.5+/-0.06	1.62+/-0.04
Follicular neopalsm;			
Follicular adenoma	3	0.2+/-0.01	4.6+/-1.3
Follicular carcinoma	2	0.2+/-0.01	3+/-1.2
Papillary carcinoma	6	0.7+/- 0.04	2.18+/-0.56
Medullary carcinoma	1	0.4	2

Discussion

Thyroid nodule is one of the most common diseases originating from the endocrine system.. Thyroid swelling may or may not be associated with functional derangement. The thyroid epithelial cells, induced by random mutations or rearrangements, will grow from a normal state to an abnormal state. This induction of growth exacerbates cellular mutagenesis that generates the nodules. [10,11] Most thyroid nodules are benign tumours and 5% are reported as malignant.[4,5] Various studies like Weimin Xu et al, J Paweł et al, showed female preponderance over males in acquiring thyroid disease, which was also noticed in our study.[3,6]

Most of autoimmune thyroid diseases are accompanied by the presence of anti-thyroid peroxidase (TPO), anti-thyroglobulin (Tg), and anti-thyroid-stimulating hormone receptor (TSHR) antibodies. However autoantibodies association with thyroid malignancy is also noted in few papillary carcinoma of thyroid. Antibodies against thyroid antigens such as carbonic anhydrase, megalin, T3 and T4, sodium iodide symporter (NIS), and pendrin have also been detected, although rarely.[12,13]

Prevalence of the thyroid autoantibody positivity is relatively high worldwide. It is well-known that

in iodine-sufficient areas there is a higher rate of AT prevalence than in iodine-deficient ones.[14] Iodine intake is probably one of the most important factors that affects thyroid autoimmunity and the incidence of AT.[15]

TPOAb and TGAb are two important thyroid autoantibodies which are commonly found in patients with thyroid diseases. [16] As shown in some previous studies, TPOAb is correlated with the severity of lymphocytic infiltration and could induce antibody-dependent cell-mediated cytotoxicity. [17,18] Boelaert K. et al.[19] reported that TPOAb was dependently associated with thyroid diseases, but little attention has been paid to whether measuring other thyroid autoantibodies, in addition to TSH, could help predict thyroid nodules in human populations.[3]

Our results showed raised antiTPO and antiTG in lymphocytic thyroiditis and papillary carcinoma which were similar to the findings of M. Parham et al. [20] in Iran. They indicated that the different prevalence of thyroid autoantibodies might explain the wide range of the reported prevalence of thyroid nodules. In addition, Eun Sook Kim et al.[21] reported that TGAb was associated with an increased risk of thyroid cancer in thyroid nodules. Similarly, other studies[22,23] also showed an analogous association with malignancy by considering positive thyroid autoantibodies as a whole, including TPOAb and TGAb.[3]

Oxidative reactions occur in all tissues and organs, thyroid gland being one, in which oxidative processes are indispensable for thyroid hormone synthesis. Both hyper- and hypothyroidism have been proven to promote cellular oxidative stress by influencing the intensity of oxygen reactions and have been shown to affect concentrations of the vitamins involved in scavenging of free radicals (usually decreasing their concentrations, although study results differ) i.e. vitamins A, C and E[9]. A study done by Salwa H. N. Al-Rubae'i and Abass K. Al-Musawi observed that there are marked variations in vitamin A, E and C in both hypothyroidism as well as hyperthyroidism.[24]

Vitamin A is a potent antioxidant and acts as a scavenger of free radicals either independently or as a part of large enzyme system. Vitamin A deficiency (VAD) has multiple effects on thyroid function in animals.[25] Hyperthyroidism is a hyper metabolic state accompanied by an increase in the total consumption of oxygen, fostering formation of reactive oxygen species and other free radicals, or the occurrence of oxidative stress. [26]

Lowered Vitamin E level is presumably due to its use in preventing free radical damage that seems more extensive in thyroid dysfunction patients.[27] Mano et al found in their study patients with various thyroid disorders that they presented elevated Vitamin E levels in their thyroid tissue.[24] Researchers concluded that Vitamin E acts as a scavenger in thyroid follicular cell dysfunction. Additional studies have demonstrated that active oxygen radicals inhibit the activity of an enzyme responsible for the conversion of T4 to the active hormone T3 and that sufficient Vitamin E levels may mitigate that effect.[28] Present study also detects low vitamin E levels in thyroid disorders.

Vitamin C is considered the most powerful natural antioxidant[29] which is capable of ''scavenging'' reactive oxygen species by reducing free radicals to more stable species.[30] Present study were in good agreement with those obtained by Mohan et al.[31] and Alicigüzel et al.[32] as these studies described low levels of Vitamin C in hyperthyroidism and increase oxidative stress at the same time, it also indicate that antioxidant vitamin become oxidized and it is eventually consumed in exerting its antioxidant action.

Conclusion

Present study concludes that autoantibodies levels were raised in thyroiditis and papillary carcinoma thyroid indicating role of anti-TPO and anti-TG in etiopathogenesis. vitamin C and E levels in various thyroid diseases were variable.

Ethical Clearance: Taken from Institutional Ethical Committee (IEC)

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Unnikrishnan AG, Usha MN. Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol Metab. Jul 2011;15: 78–81.

2. Karimi F, Kalantarhormozi MR, Dabbaghmanesh MH, Ranjbar Omrani G. Thyroid disorders and

the prevalence of antithyroid antibodies in Shiraz population. Arch Iran Med. 2014; 17(1): 347 – 51.

3. Weimen X, Huo L, Chen Z, Huang H, Jin X, Deng J,et al. The Relationship of TPOAb and TGAb with Risk of Thyroid Nodules: A Large Epidemiological Study. Int. J. Environ. Res. Public Health 2017; 14(17): 7231-11.

4. Hegedus, L. Clinical practice. The thyroid nodule. N. Engl. J. Med. 2004;351: 1764–71.

5. Ezzat S, Sarti DA, Cain DR, Braunstein GD. Thyroid incidentalomas: Prevalence by palpation and ultrasonography. Arch. Intern. Med. 1994; 154: 1838–40.

6. Paweł J, Felicja L, Małgorzata SL, Marek M. Trends in the prevalence of autoimmune thyroiditis in the leading private health-care provider in Poland. Adv Clin Exp Med. 2017;26(3):497–03

7. Estienne V, Duthoit CH, Reichert M. Androgen-dependent expression of FcγRIIB2 by thyrocytes from patients with autoimmune Graves' disease: a possible molecular clue for sex dependence of autoimmune disease. FASEB J. 2002;16:1087–92.

8. Domoslawski P, Bartosz P, Lukienczuk T, Podhorska M, Piotr D. Expression of estrogen and progesterone receptors and ki-67 antigen in grave's disease and nodular goiter. Folia histochemica et cytobiologica. 2013; 51 (2):135–40

9. Sworczak KT, Wiśniewski P. The role of vitamins in the prevention and treatment of thyroid disorders. Journal of Endocrinology 2011; 62 (4):340-44.

10. Krohn K, Fuhrer D, Bayer Y, Eszlinger M, Brauer V, Neumann S et al. Molecular pathogenesis of euthyroid and toxic multinodular goiter. Endocr. Rev. 2005; 26: 504–24.

11. Erdogan MF, Gursoy A, Erdogan. Natural course of benign thyroid nodules in a moderately iodine-deficient area. Clin. Endocrinol. Oxf. 2006; 65: 767–71.

12. Marcocci C, Marino M. Thyroid-directed antibodies. In: Braverman LE, Utiger R, editors. Part II Laboratory Assessment of Thyroid Function. Philadelphia; Lippincott Williams and Wilkins (2005): 360–72.

13. Fröhlich E, Wahl R. Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Front. Immunol. 8:521.doi : 10.3389/fimmu. 2017. 00521

14. Lind P, Langsteger W, Molnar M, Gallowitsch HJ, Mikosch P, Gomez I. Epidemiology of thyroid diseases in iodine sufficiency. Thyroid. 1998;8(12):1179–83.

15. Aghini LF, Fiore E, Tonacchera M. The effect of voluntary iodine prophylaxis in a small rural community: The Pescopagano survey 15 years later. J Clin Endocrinol Metab. 2013;98(3):1031– 39.

16. Roberts CG, Ladenson PW. Hypothyroidism. Lancet. 2004; 363: 793–03.

17. Mitchell JD, Kirkham N, Machin D. Focal lymphocytic thyroiditis in Southampton. J. Pathol. 1984;144: 269–73.

18. Kasagi K, Kousaka T, Higuchi K, Iida Y,Misaki T, Alam MS,et al.Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: Comparison with histological findings. Thyroid 1996; 6: 445–50.

19. Boelaert, Horacek K, Holder J, Watkinson RL, Sheppard JC, Franklyn MCet al. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J. Clin. Endocrinol. Metab. 2006; 91: 4295–01.

20. Parham, Aminorroaya M, Amini A. Prevalence of palpable thyroid nodule in Isfahan, Iran, 2006: A population based study. Exp. Clin. Endocrinol. Diabetes 2009; 117: 209–13.

21. Kim ES, Lim DJ, Baek KH, Lee JM, Kim MK, Kwon HS, et al. Thyroglobulin antibody is associated with increased cancer risk in thyroid nodules. Thyroid 2010; 20: 885–91.

22. Rago, Di Coscio T, Ugolini G, Scutari C, Basolo M, Latrofa F, et al. Clinical features of thyroid autoimmunity are associated with thyroiditis on histology and are not predictive of malignancy in 570 patients with indeterminate nodules on cytology who had a thyroidectomy. Clin. Endocrinol. Oxf. 2007; 67: 363–69.

23. Fiore, Rago E, Provenzale T, Scutari MA, Ugolini M, Basolo C, et al. Lower levels of TSH are

associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: Thyroid autonomy may play a protective role. Endocr. Relat. Cancer 2009; 16: 1251–60.

24. Salwa HN, Al-Rubaei and Al-Musawi AB. An evaluation of antioxidants and oxidative stress in Iraqi patients with thyroid gland dysfunction. Afr. J. Biochem. Res.2011; Vol. 5(7): 188-96.

25. Arthur JR, Beckett M, Mitchell JH (1999). Interactions between selenium and iodine deficiencies in man and animals. Nutr. Res. Rev; 12:55- 73.

26. Abalovich M, Liesuy S, Gutierrez S, Repetto M. Peripheral parameters of oxidative stress in Graves's disease: The effect of methimazole and 131 iodine treatment. Clin. Endocrinol. 2003; 59(3):321-327.

27. Garein H, Higueret P. The thyroid hormones in vitamin A deficient rats: Effect of retinoic acid supplementation. Ann. Nutr. Metab. 1983; 27: 495-500.

28. Brzezinska-Slebodzinska E, Pietras B (1997). The protective role of some antioxidants and scavengers on the free radicals-induced inhibition of the liver iodothyronine 5'-monodeiodinase activity and thiols content. J. Physiol. Pharmacol.1997; 48(3): 451-59.

29. Weber P, Bendich A, Schalch W (1996). Vitamin C and human health are view of recent data relevant to human requirements. Int. J. Nutr. Res.1996; 66:19-30.

30. Gumuslu S, Korgun DK, Bilmen S, Yargilcoglu p, Agar A (2000). Effect of sulfur dioxide inhibition on plasma vitamin C and ceruloplasmin in ageing rats. Ind. Health,2000; 38; 319-22.

31. Mohan KM, Bobby Z, Selvaraj N, Kumar DA, Chandra KB, Sen SK, Ramesh R, Ranganathan P (2004). Possible link between glycated hemoglobin and lipid peroxidation in hyperthyroidism. Clin. Chim. Acta.2004; 342:187-92.

32. Aliciguzel Y, Ozdem SN, Ozdem SS, Karayalcin U, Siedlak SL, Perry G et al (2001). Erythrocyte, plasma, and serum antioxidant activities in untreated toxic multinodular goiter patients. Free Radic. Biol. Med.2006; 30(6):665-70.

3rd Annual Conference of Association of Physiologists of India

ASSOPICON 2016
14th-17th September 2016

Theme : Physiology Decodes Novelty of Vascular Science

CERTIFICATE

This is to certify that

Dr/Mr/Miss _Anita. P. Javalgi_

Bearing Reg No. _KMC/77656_ has participated as **Delegate/Chairperson/Jury/Resource person/Presented Oral /Poster**

Topic entitled _Cross talk between thyroid antibodies and thyroid hormones — a pilot study_

In ASSOPICON-2016 held from 15th-17th September 2016,

Organized by Department of Physiology,

BLDE University's Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

Karnataka Medical Council has granted 4 credit hours for Delegates/Faculty

vide letter No.: K.M.C./C.M.E./701/2016

Dr. Manjunatha Aithala
Organizing Secretary

Zonal Chairman
KMC CME Accreditation Committee

Dr. S. P. Guggarigoudar
Organizing Chairman

Dr. G. K.Pal
General Secretary, ASSOPI

3rd Annual Conference of Association of Physiologists of India

INDIAN ACADEMY OF CYTOLOGISTS

CYTOCON 2017

47th ANNUAL CONFERENCE

10TH - 13TH NOVEMBER, 2017

NORTH EASTERN INDIRA GANDHI REGIONAL INSTITUTE OF HEALTH AND MEDICAL SCIENCES, SHILLONG

Certificate

This is to certify that Dr /Mr/Ms .... **Anita. P. Jawalgi** .... participated as Delegate/Speaker/Chairperson in the CME/ Conference/Workshop of CYTOCON 2017 held from 10th – 13th November 2017, organized by the Department of Pathology, NEIGRIHMS, Shillong and co-hosted by North East Regional Chapter of Indian Association of Pathologists and Microbiologists(NERC-IAPM).

He/She presented an Oral paper/Poster titled .... " A cytological diagnosis to circulating chemistry in various thyroid diseases : a complete diagnostic approach " ....

The CME/Conference/Workshop has been accredited by the Assam Council of Medical Registration (ACMR).

Credit hours for Delegates
CME: 2, Conference: 4, Workshop: 2

Dr Mrinal K Baruah
President, NERC-IAPM

Dr Vandana Raphael
Organizing Chairperson

Dr. B. P. Chakravarty
Registrar, ACMR

Dr. Aruna Prayaga
President, IAC

Credit hours for Speakers
CME: 3, Conference: 6, Workshop: 3

Dr. Dev Prasoon
Secretary, IAC

Dr. Yookarin Khonglah
Organizing Secretary

CYTOCON 2018

48th Annual National Conference of Indian Academy of Cytologists

1st to 4th November 2018
Goa, India

Hosted by IAC Goa Chapter

Prizes

This is to certify that Dr. _Anita Savalgi_ of _SDMCMSH, Karnataka_ has won the _first_ prize in the _Oral paper presentation_ on _03/11/2018_ Session at the

48th Annual National Conference of the Indian Academy of Cytologists in Goa, India on the 2nd and 3rd of November 2018:

"_Sensitivity & Specificity of diagnostic biomarkers inthyroid diseases: from circulating chemistry to Molecular Cytogenetics._"

Dr. R. G. W. Pinto
President
Organising Committee

Dr. (Col) U. Dinesh
President
IAC

Dr. Dev Prasoon
Secretary
IAC

Dr. Peter Rodrigues
Secretary
Organising Committee