# DISSERTATION

*On*

# A STUDY ON IMMUNO HISTOCHEMICAL ANALYSIS OF CD56 AND GALECTIN-3 EXPRESSION IN FOLLICULAR NEOPLASM OF THYROID

*Submitted in partial fulfilment of the requirements for the degree of*

**DOCTOR OF MEDICINE (BRANCH-III)**

**M.D. PATHOLOGY**

**THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY**

**CHENNAI**


**TIRUNELVELI MEDICAL COLLEGE**

**TIRUNELVELI**

**MAY 2018**

# CERTIFICATE

This is to certify that the dissertation titled **"A STUDY ON IMMUNO HISTOCHEMICAL ANALYSIS OF CD56 AND GALECTIN-3 EXPRESSION IN FOLLICULAR NEOPLASM OF THYROID",** is a bonafide work done by **Dr.S.FATHIMA**, Post Graduate Student, Department of Pathology, Tirunelveli Medical College, Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of **MD DEGREE** in **PATHOLOGY BRANCH-III**, under my guidance and supervision, during the academic period from 2015 to 2018.

**Professor. SITHY ATHIYA MUNAVARAH, MD,**
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This is to certify that the dissertation titled **"A STUDY ON IMMUNO HISTOCHEMICAL ANALYSIS OF CD56 AND GALECTIN-3 EXPRESSION IN FOLLICULAR NEOPLASM OF THYROID",** is a bonafide work done by **Dr.S.FATHIMA**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2015 to 2017. This work has not formed the basis for previous award of any degree.

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# DECLARATION

I solemnly declare that the dissertation titled **"A STUDY ON IMMUNO HISTOCHEMICAL ANALYSIS OF CD56 AND GALECTIN-3 EXPRESSION IN FOLLICULAR NEOPLASM OF THYROID",** was done by me at Tirunelveli Medical College, Tirunelveli– 627011, during the period 2015 to 2017 under the guidance and supervision of **Prof.K.SHANTARAMAN, MD**, to be submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of **MD DEGREE in PATHOLOGY BRANCH-III.**

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PRINCIPAL INVESTIGATOR: Dr.S.FATHIMA, MBBS.,
DESIGNATION OF PRINCIPAL INVESTIGATOR: PG STUDENT
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*Dear, Dr.S.FATHIMA, MBBS.,, The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.2015.*

**THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED**

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2. Study Protocol ☐
3. Department Research Committee Approval ☐
4. Patient Information Document and Consent Form in English and Vernacular Language ☐
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6. Proposed Methods for Patient Accrual Proposed ☐
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8. Insurance /Compensation Policy ☐
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1. The approval is valid for a period of 2 year/s or duration of project whichever is later
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   d) If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e) Approval for amendment changes must be obtained prior to implementation of changes.
   f) The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g) Any deviation/violation/waiver in the protocol must be informed.

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# CERTIFICATE

This is to certify that the dissertation titled **"A STUDY ON IMMUNO HISTOCHEMICAL ANALYSIS OF CD56 AND GALECTIN-3 EXPRESSION IN FOLLICULAR NEOPLASM OF THYROID",** of the candidate **Dr.FATHIMA.S** with registration number **201513302** for the award of **M.D. DEGREE in PATHOLOGY BRANCH-III.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains the pages from introduction to conclusion and result shows **5 percentage** of plagiarism in the dissertation.

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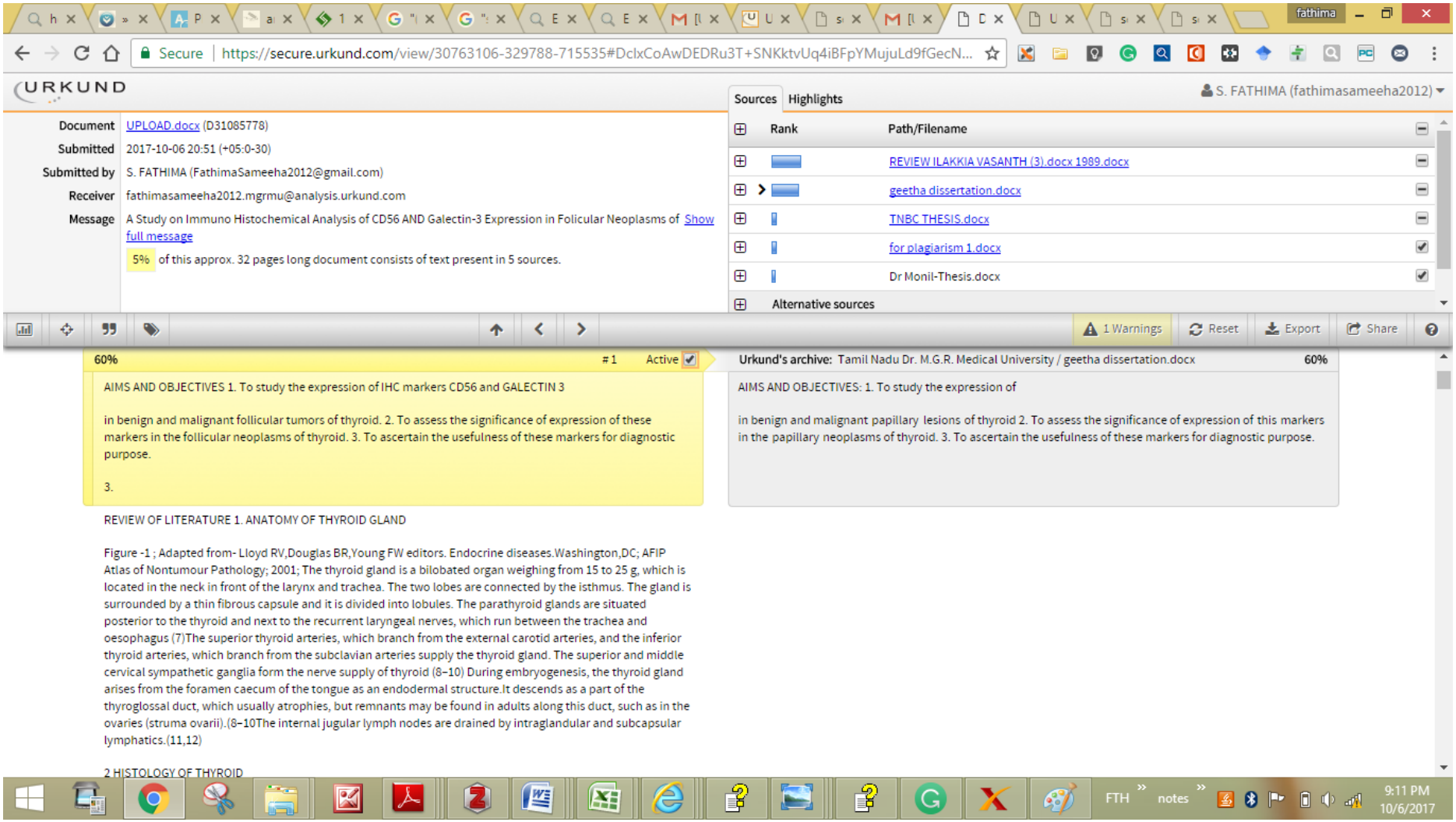
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REVIEW OF LITERATURE 1. ANATOMY OF THYROID GLAND
Figure -1 ; Adapted from- Lloyd RV,Douglas BR,Young FW editors. Endocrine diseases.Washington,DC; AFIP Atlas of Nontumour Pathology; 2001; The thyroid gland is a bilobated organ weighing from 15 to 25 g, which is located in the neck in front of the larynx and trachea. The two lobes are connected by the isthmus. The gland is surrounded by a thin fibrous capsule and it is divided into lobules. The parathyroid glands are situated posterior to the thyroid and next to the recurrent laryngeal nerves, which run between the trachea and oesophagus (7)The superior thyroid arteries, which branch from the external carotid arteries, and the inferior thyroid arteries, which branch from the subclavian arteries supply the thyroid gland. The superior and middle cervical sympathetic ganglia form the nerve supply of thyroid (8–10) During embryogenesis, the thyroid gland arises from the foramen caecum of the tongue as an endodermal structure.It descends as a part of the thyroglossal duct, which usually atrophies, but remnants may be found in adults along this duct, such as in the ovaries (struma ovarii).(8–10The internal jugular lymph nodes are drained by intraglandular and subcapsular lymphatics.(11,12)
2 HISTOLOGY OF THYROID
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## ACKNOWLEDGEMENT

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# ABBREVIATIONS

| | | |
|---|---|---|
| **ATC** | - | Anaplastic Thyroid Carcinoma |
| **CK-19** | - | Cytokeratin-19 |
| **EMA** | - | Epithelial Membrane Antigen |
| **FA** | - | Follicular Adenoma |
| **FTC** | - | Follicular Thyroid Carcinoma |
| **HTA** | - | Hyalinising Trabecular Adenoma |
| **HBME-1** | - | Hector Battifora Mesothelial -1 |
| **HCC** | - | Hurthle Cell Carcinoma |
| **IHC** | - | ImmunoHistoChemistry |
| **MIFC** | - | Minimally Invasive Follicular Carcinoma |
| **MoAB** | - | Monoclonal Antibody |
| **MTC** | - | Medullary Thyroid Carcinoma |
| **PAS** | - | Periodic Acid Schiff |
| **ROC** | - | Receiver Operating Curve |
| **TPO** | - | ThyroPeroxidase |
| **TRH** | - | Thyroid Releasing Hormone |
| **TSH** | - | Thyroid Secreting Hormone |
| **TTF -1** | - | Thyroid Transcription Factor -1 |
| **USG** | - | UltraSonoGram |
| **WIFC** | - | Widely Invasive Follicular Carcinoma |

# TABLE OF CONTENTS

# 1. INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, among which 95% of them originate from the thyroid follicular epithelial cells [(1)]. Worldwide, the incidence of thyroid cancer is roughly three to four times higher among women than men and it is ranked the sixth most common malignancy diagnosed in women[(2)]. Most of the tumors are common between the third to the sixth decade of life and they are rare in childhood[(2)] . Follicular adenoma and Follicular carcinoma of thyroid are the tumors of epithelial origin with follicular cell differentiation. Of them, the incidence of follicular adenoma was 3% and 4.3% as reported in two autopsy series[(3,4)] . Follicular Thyroid Cancer (FTC) is reported to be the second most common malignancy of the thyroid gland after Papillary Thyroid Carcinoma (PTC). Follicular neoplasm of the thyroid is classified into benign and malignant depending on the presence or absence of capsular and/or vascular permeation. However, evaluation of these features in histopathology is challenging, as the presence of an incomplete capsular invasion or equivocal vascular invasion due to processing or sectioning artefacts results in an inconclusive or inaccurate diagnosis[(5)].Distinguishing Follicular thyroid carcinoma(FTC) especially Minimally Invasive Follicular Carcinoma (MIFC) of thyroid from the most common neoplasm of the thyroid, follicular thyroid adenoma is very challenging, leading to avoidable surgery and exposing the patients to persuasive postoperative complications. Hence the question arises- Is histomorphology, the gold standard in the diagnosis of follicular thyroid lesions? This study aims at addressing the challenges regarding the diagnosis of follicular

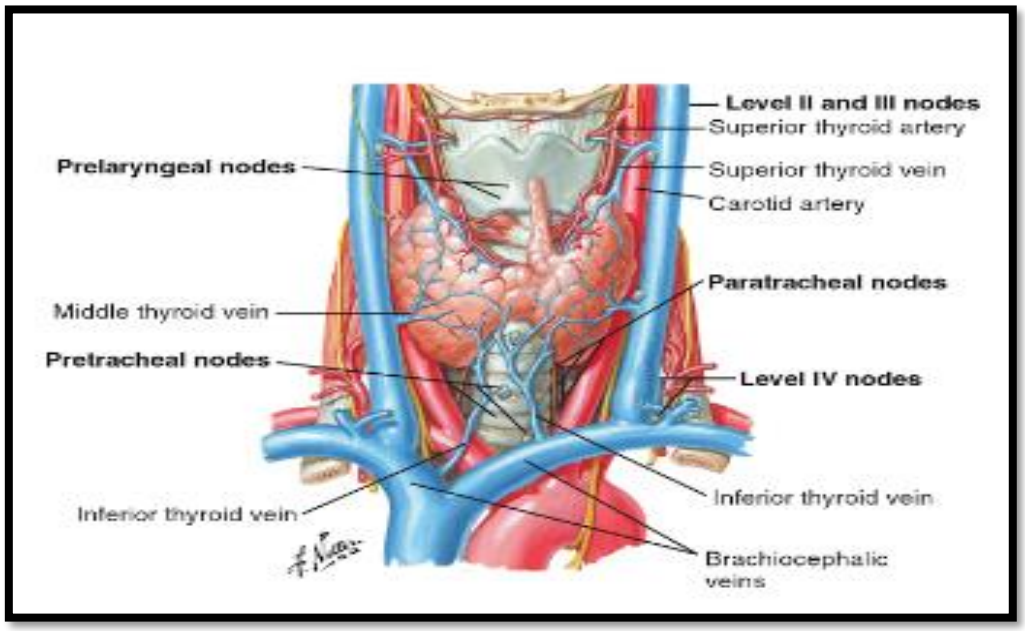
thyroid neoplasm. Recent studies have focused on identifying immunohistochemical markers that may help in differentiating benign from malignant follicular thyroid tumors[6].Estimation of the expression of CD56 and GALECTIN 3 may help to differentiate between malignant and benign follicular thyroid neoplasm.

# 2. AIMS AND OBJECTIVES

1. To study the expression of immunohistochemical markers CD56 and GALECTIN 3 in benign and malignant follicular tumors of thyroid.
2. To assess the significance of expression of these markers in follicular neoplasm of thyroid.
3. To ascertain the usefulness of these markers for diagnostic purpose.

# 3. REVIEW OF LITERATURE

## 3.1) Anatomy of Thyroid Gland

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The thyroid gland is a bilobated organ weighing from 15 to 25 g, which is located in the neck in front of the larynx and trachea. The two lobes are connected by the isthmus. The gland is surrounded by a thin fibrous capsule and it is divided into lobules. The parathyroid glands are situated posterior to the thyroid and next to the recurrent laryngeal nerves, which run between the trachea and oesophagus[7] The superior thyroid arteries, which branch from the external carotid arteries, and the inferior thyroid arteries, which branch from the subclavian arteries supply the thyroid gland. The superior and middle cervical sympathetic ganglia form the nerve supply of thyroid[8–10]

During embryogenesis, the thyroid gland arises from the foramen caecum of the tongue as an endodermal structure.It descends as a part of the thyroglossal duct, which usually atrophies, but remnants may be found in adults along this duct, such as in the ovaries (struma ovarii).[8–10]The internal jugular lymph nodes are drained by intraglandular and subcapsular lymphatics.[11,12]

### 3.2) Histology of thyroid

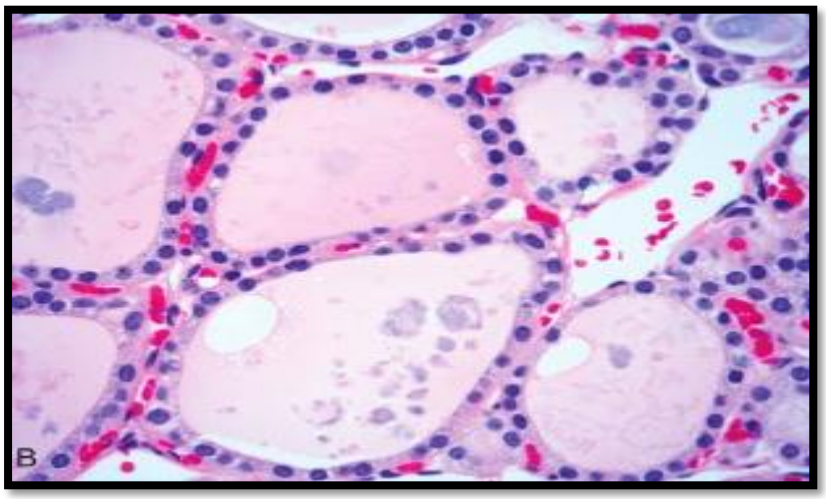



**Figure -2; Adapted from - Bruce M Wenig . Atlas of Head and Neck Pathology. Elsevier Health Sciences; 2015. 1803 p.**

- Section of a thyroid gland shows colloid filled follicles lined by endothelial cells, (H &E stain, under 40 x).

Ultrastructural studies of normal thyroid gland show that the follicular cells are variable in size, with an average diameter of 200 µm and they are arranged in a single layer around the central colloid [11] . Their nuclei are round containing diffuse chromatin. Colloid contains concentrated periodic acid-Schiff (PAS)-positive thyroglobulin.[12] .

### 3.3) Physiology and functions of thyroid

The thyroid gland is a part of the hypothalamus-pituitary-axis(HPA).A feedback system activating from the hypothalamus, producing Thyrotrophic Releasing Hormone( TRH) and activates the thyrotrophic cells in the anterior lobe of the pituitary gland to release( TSH) Thyrotrophic Secreting Hormone activates the thyroid follicular cells to produce thyroid hormones called triiodothyroxine (T3) and tetraiodothyronine or thyroxine (T4). As a negative feedback mechanism, the hormones (T3 and T4) control the secretion of both TSH and TRH. The thyroid hormones have numerous activities in body metabolism, such as oxygen consumption, cardiac output and heat production.[13]

### 3.4) Histochemistry of thyroid

Immunohistochemically, many markers have been documented in normal follicular cells, most of them are also being also expressed in well-differentiated tumors cells:

- Reactivity for thyroglobulin, thyroid peroxidase, triiodothyronine (T3), and thyroxine (T4) has been found both in the colloid and in the cytoplasm of the follicular cells.[14–16] Thyroglobulin is the most widely used of all these markers.[16]
- Thyroid transcription factor 1 (TTF-1) has developed as an extremely useful marker for thyroid origin and tumors composed of thyroid follicular cells.[17] The only other tissue that showed consistent

expression was the alveolar epithelium of the lung.[18] However, reports have recently appeared documenting its occasional presence in other sites, such as the normal and neoplastic female genital tract,[19,20] Wilms tumor,[21] and Merkel cell tumor.[22]

- Low molecular weight keratins: CK7, CK18, and (to a lesser degree) CK8 and CK19.[23]
- Epithelial membrane antigen (EMA).
- Vimentin.[23]
- Estrogen and progesterone receptors.[24]
- Blood group antigens.[25]
- Low molecular metal binding proteins such as ceruloplasmin, lactoferrin, transferrin, metallothionein.[26,27]
- Follicular cells are joined by tight junctions containing the known components of these structures, including occludin and the various claudins.[28]
- The follicles rest on a basement membrane that is immunoreactive for laminin and type IV collagen.[29]

- The second type of hormone-producing cell are the C cells, which accounts for only 0.1% of the thyroid mass. They may be found as small clusters in the middle to upper third of the lateral lobes, usually in an intrafollicular position. The C cells produce calcitonin, somatostatin and other peptides.

Immunohistochemically, they accumulate calcitonin, TTF-1, somatostatin and neuroendocrine markers, such as chromogranin A and synaptophysin.

- The third cell type of the normal thyroid gland are Solid cell nests, from which C cells are derived.

### 3.5) Neoplasm of thyroid

Numerous pathologic lesions affect thyroid gland with diverse morphologies. Despite the large number of lesions, it is convenient to divide them into two major types: one that shows a diffuse pattern and the other that produce nodules. Diffuse thyroid lesions are the lesions in which entire thyroid gland is affected, such as hyperplasias and thyroiditis. Nodular lesions are those ,which include both non neoplastic hyperplasias as well as benign and malignant tumors[30]. The thyroid tumors are of two major types; one is that arises from thyroid follicular cells such as papillary carcinoma of thyroid , follicular carcinoma of thyroid and anaplastic carcinoma of thyroid. Other is Medullary carcinoma of thyroid that arises from thyroid parafollicular C cells[2].

The majority of the thyroid tumors can be promptly diagnosed by characteristic histopathological features, but the distinction between follicular adenomas and follicular carcinomas are troublesome. Follicular thyroid tumors were categorised into benign or malignant depending upon the presence or absence of capsular and/or vascular invasion. On the other hand, assessment of these features can be very challenging due to the presence of incomplete capsular penetration or equivocal vascular permeation .This may be due to processing and sectioning artifacts. Atypical follicular adenoma with increased cellularity and

nuclear atypia are very difficult to differentiate from minimally invasive follicular carcinoma of thyroid. As some promising immunohistochemical markers including CD56, galectin 3 ,Hector Battifora Mesothelial (HBME-1), and CK19 may distinguish benign from malignant follicular thyroid tumors .[31]

### 3.6 ) WHO classification of thyroid tumors - (2017)[32]

1. Tumors of the thyroid gland
    a. Follicular adenoma
    b. Hyalinizing trabecular tumor
    c. Other encapsulated follicular patterned thyroid tumors
        i. Tumors of uncertain malignant potential
        ii. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
    d. Papillary thyroid carcinoma
    e. Follicular thyroid carcinoma
        i. Hürthle (oncocytic) cell tumors
    f. Poorly differentiated thyroid carcinoma
    g. Anaplastic thyroid carcinoma
    h. Squamous cell carcinoma
    i. Medullary thyroid carcinoma
    j. Mixed medullary and follicular thyroid carcinoma
    k. Mucoepidermoid carcinoma
    l. Sclerosing mucoepidermoid carcinoma with eosinophilia
    m. Mucinous carcinoma

n. Ectopic thymoma

o. Spindle epithelial tumor with thymus-like differentiation

p. Intrathyroid thymic carcinoma

q. Paraganglioma and mesenchymal / stromal tumors

    i. Paraganglioma

    ii. Peripheral nerve sheath tumors

    iii. Benign vascular tumors

    iv. Angiosarcoma

    v. Smooth muscle tumors

    vi. Solitary fibrous tumor

r. Hematolymphoid tumors

    i. Langerhans cell histiocytosis

    ii. Rosai-Dorfman disease

    iii. Follicular dendritic cell sarcoma

    iv. Primary thyroid lymphoma

s. Germ cell tumors

t. Secondary tumors

## 3.7) Benign follicular thyroid tumors

### 3.7.1 Follicular thyroid adenoma

Follicular adenoma is a benign encapsulated tumor with follicular epithelial cell differentiation. it is the most common neoplasm of the thyroid, with a evident of 15−20% proportion among thyroid nodules [33,34]. Follicuar

adenoma lacks: (i) the evidence of capsular, vascular or any other type of invasion; and (ii) the nuclear features of the papillary family of neoplasms.

a) Clinical presentation

Follicular adenomas are five-fold more common in females than in males with a wide age range ,but most common in the 5th-6th decades of life[35]. Adenomas are mainly nonfunctional. A small proportion shows autonomous hyperfunction (toxic adenoma), causing thyrotoxicosis. Tumors are usually found by palpation and may sometimes cause pressure effects, such as dysphagia and dyspnoea.[35]

b) Etiology

Radiation, iodine deficiency and history of nodular goiter are the major risk factors for follicular thyroid neoplasia [35] Some genetic alterations are shown in toxic adenomas, such as mutations in RAS, phosphotidylinositol 3-kinase, catalytic alpha polypeptide (PI3KCA) and TSH receptor gene [35].

c) Features of Fine Needle Aspiration Biopsy:[36]

Features of a follicular neoplasm [38]in contrast to an adenomatoid nodule are that, they have moderate to high cellularity, arranged in syncytial groups with prominent micro follicular pattern of clusters that may contain a drop of colloid. The cells are uniform , forming repetitive micro follicles with round nuclei . The nuclear chromatin may be coarsely granular, and it is usually evenly distributed with inconspicuous nucleoli. Absence of nuclear features for papillary thyroid carcinoma.

d) Macroscopic appearance of follicular adenoma**;**

Follicular thyroid adenoma is almost solitary and confined to one lobe of thyroid .Macroscopically, an adenoma is surrounded by a well-demarcated and intact capsule, the capsule varies in thickness but usually it is thin. If a thick capsule is present, suspicion of carcinoma should be thought of.[37]. Adenomas vary in size but generally measure < 3 cm. Larger tumors measuring more than 10 cm can also be seen. Follicular adenomas are usually nodular with firm consistency, tan to light brown in color. [9,10] (except in the presence of secondary [degenerative] changes); Secondary changes include hemorrhage, fibrosis, cyst formation, calcification.

e) Microscopic appearance of follicular adenoma;

Microscopically, in follicular adenoma the cells are arranged in a normo follicular(simple), macro follicular (colloid), micro follicular (fetal), and trabecular /solid (embryonal) pattern with uniform nuclear and cellular morphology and with rare mitotic cells.

f) Follicular Thyroid Adenoma -Histologic Types;

- Oncocytic (Hürthle) cell
- Signet ring cell
- Clear cell
- Follicular adenoma with:
    - spindle cells
    - mucinous stroma
    - mesenchymal components

- Hyalinizing Trabecular Adenoma
- Atypical Follicular Adenoma.

Other rare type of follicular adenoma are those

- with clear cell changes ( the signet ring, mucin-producing, and lipid-rich types);
- with adipose metaplasia of the stroma (so-called adenolipomas)[38],
- with cartilaginous metaplasia (so-called adenochonromas),[39]
- spindle cell adenomas (some vaguely resembling meningiomas),[40]
- with massive deposition of cytoplasmic black pigment following minocycline therapy (so-called black adenomas).[41].

f) Differential diagnosis of follicular adenoma;

The differential diagnosis of follicular thyroid adenoma are dominant nodule of nodular hyperplasia, follicular thyroid carcinoma with minimal invasion, and the follicular variant of papillary thyroid carcinoma. Some follicular adenomas are highly vascular so this may be confused with vascular tumors. Atypical follicular adenoma which have high cellularity and atypical nuclei may also difficult to diagnose.

### 3.7.2 Atypical follicular adenoma

**Synonyms**: Atypical follicular neoplasm; follicular tumor of uncertain malignant potential(FT-UMP); well- differentiated (follicular) tumor of uncertain malignant potential(WDT-UMP)

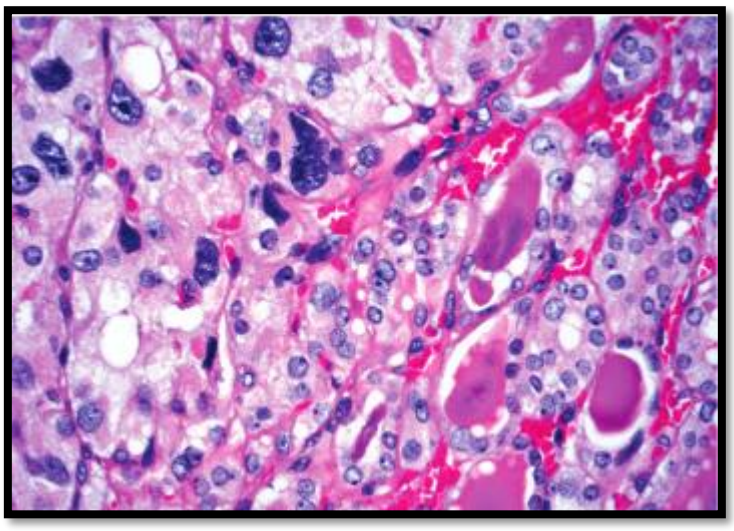


**Figure -3 ; Adapted from- Bruce M Wenig. Atlas of Head and Neck Pathology. Elsevier Health Sciences; 2015.**

- Section shows focal areas of bizarre hyper chromatic and pleomorphic nuclei lacking the features of malignancy such as mitosis and necrosis.

Atypical follicular adenoma is diagnosed, when any encapsulated follicular tumors have histological features suggestive of a more aggressive neoplasm, but does not show any evidence of either capsular or vascular invasion.[42] Atypical histological features include thickened capsule, unusual cellularity particularly along the peripheral aspects of the tumor that is along the tumor-capsular interface, increased mitoses, and atypical with prominent nucleoli , spontaneous necrosis and infarction . Follow-up studies have shown that atypical follicular adenoma behave like benign tumors[43–45]. Such tumors represent a "grey zone" and termed as follicular tumors of uncertain malignant potential. Immunohistochemistry using CD56 helps in distinguishing benign (particularly atypical follicular thyroid adenoma) from malignant tumors[31] . p27 is other reliable marker that may distinguish atypical follicular adenoma

from follicular thyroid carcinoma .GALECTIN 3 is a most sensitive marker in differentiating Follicular adenoma of thyroid from Follicular carcinoma of thyroid.

### 3.7.3 Hyalinising Trabecular Adenoma (HTA)

Benign encapsulated tumor with evidence of follicular epithelial cell differentiation showing trabecular and organoid growth patterns. Hyalinising Trabecular Adenoma (HTA) have extracellular hyalinization and elongated cells arranged around blood vessels. The pattern of growth may simulate paraganglioma and medullary carcinoma of thyroid .The presence of nuclear grooves and psammoma bodies may suggest papillary carcinoma, particularly in material from fine needle aspiration.[46] Another distinct morphologic feature of HTA is the so-called cytoplasmic yellow body, which is a round, pale yellow cytoplasmic inclusion body in a paranuclear location having a refractile quality and detectable both in tissue sections and in fine needle aspiration smears.[47,48]. Immunohistochemically hyalinising trabecular adenoma shows consistent positivity for Cytokeratins, thyroglobulin, and TTF-1. It shows reactivity for Galectin 3in about half of the cases [49] and focal inconstant reactivity for neuroendocrine markers such as NSE and neurotensin.[50]. HBME-1 is consistently negative in hyalinising trabecular adenoma. [51]

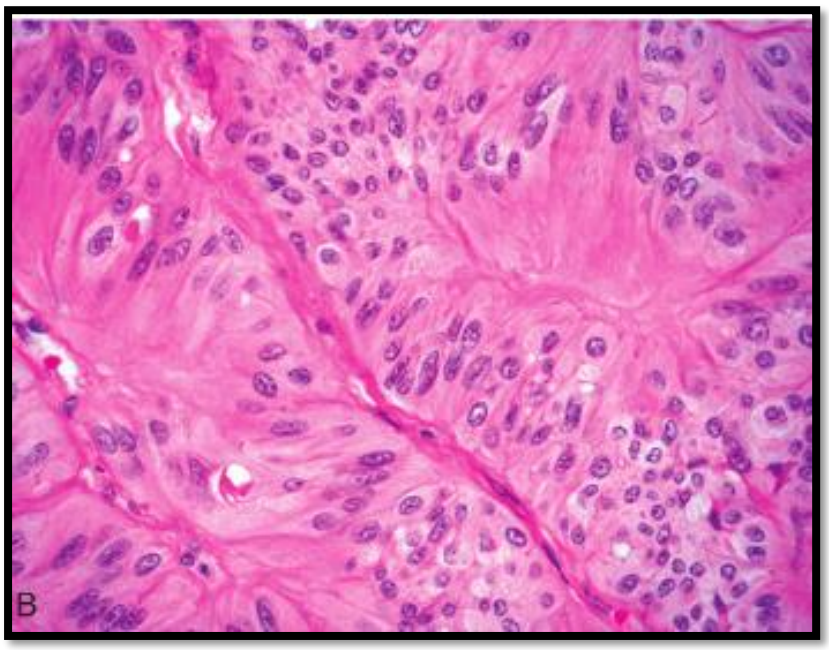



**Figure -4; Adapted from - Carney JA. Hyalinizing trabecular tumors of the thyroid gland:. Am J Surg Pathol. 2008 Apr;32(4):622–34.**

- Section shows elongated cells that may be oriented perpendicular to fibro vascular cores with oval and elongated nuclei ,fine dispersed chromatin and nuclear grooves.

## 3.8) Malignant follicular thyroid tumors

### 3.8.1 Follicular Thyroid Carcinoma (FTC)

a) Definition;

Follicular cell differentiated thyroid neoplasm, have the evidence of capsular and/or vascular invasion) and/or metastatic disease and it does not have the nuclear features of papillary thyroid carcinoma**.**

a) Epidemiology of thyroid cancer;

The National Cancer Institute reports that thyroid cancer is the most common endocrine malignancy ,with incidence of 64,330 new cases in 2016. Thyroid cancer constitutes approximately 3.8% of all new cancer cases [(52).]

Papillary carcinoma of thyroid comprises 85-90% of all thyroid cancer cases, followed by follicular thyroid carcinoma of (5-10% ) and medullary thyroid Cancer (about 2%), anaplastic thyroid carcinoma accounts for less than 2% of thyroid cancers[53].

The second most common malignancy of thyroid is Follicular carcinoma of thyroid [54,55]. The reported incidence of Follicular Thyroid Carcinoma ranges from 10% to 32% of all differentiated thyroid carcinomas [56]. .This wide range in the reported incidence could be due to an inter observer variability in the histo pathological diagnosis of follicular carcinoma of thyroid .[56]

According to the previous studies the incidence of follicular carcinoma of thyroid has been decreased recently. [57,58]. This decrease in incidence may be due to more liberal approach in the histopathological diagnosis (e.g. diagnosis of follicular variant of papillary carcinoma of thyroid) and iodine supplementation programs. A 5-year survival of follicular thyroid cancer patients is approximately 85−95% .[59]

c) Tumor genesis of follicular carcinoma;

The incidence of Follicular thyroid carcinoma is higher in endemic areas of iodine deficiency, because addition of iodine supplement to the diet has been associated with a decline in the incidence of follicular thyroid carcinoma in geographic areas[60]. Rarely, follicular thyroid carcinoma may occur from a pre-existing follicular adenoma.[61] Dyshormonogenesis and irradiation may leads to the development of follicular carcinoma of thyroid.

d) Cytogenetic and molecular features of Follicular Thyroid Carcinoma;

Activating point mutations of RAS gene occur in 20% to 40% of follicular adenomas and 30%to 50% of follicular carcinomas, suggesting that RAS mutation may represent an early event in tumor genesis · PAX8-PPARγ gene fusion - results in the production of fusion protein (PPFP) .PPFP is an oncoprotein and it is seen in follicular thyroid adenoma and carcinoma and papillary thyroid carcinomas- Follicular Variant , but not in conventional papillary thyroid carcinomas [(62).]

Genetic cancer susceptibility is more common among endocrine tumors than any other human tumors.[(63)] 6% of Familial Non Medullary Thyroid Cancers (FNMTCs) are believed to be Familial Follicular Thyroid Cancers (FFTCs), may be associated with syndromes such as Cowden or Werner syndromes or the Carney complex[(64)].

Phosphatase Tensin (PTEN) tumor suppressor gene mutation causes Cowden syndrome with greater susceptibility of breast, endometrial, thyroid, kidney and colorectal cancers, including dermatological , gastrointestinal and neurological features. Up to 10% of patients with Cowden syndrome develops thyroid cancer, usually a follicuar thyroid carcinoma.[(65)]

Werner syndrome which is also known as adult progeria, caused by the mutations in Werner syndrome, RecQ helicaselike (WRN) gene, causing premature aging of patients and related with an increased risk of carcinoma including follicular carcinoma of thyroid[(66).] The Carney complex is caused by the mutation in Protein kinase, cyclic Adenosine Mono Phosphate (cAMP)-

dependent, Regulatory, type 1,Aalpha (PRKAR1A) gene leads to the tumors in the heart, skin, endocrine organs such as the thyroid, and pigmentations of the skin .[13].

e) Clinical presentation of Follicular Thyroid Carcinoma(FTC);

Follicular thyroid carcinoma presents as an asymptomatic slowly growing solitary mass (usually measuring >2 cm) in the thyroid[67]. Pain may occur later in the disease course. The most important criteria for the diagnosis of follicular thyroid carcinoma is the demonstration of capsular invasion and/or vascular invasion. Therefore histological examination of the tumor- capsule interface is more important than examination of central portion of the tumor. Follicular carcinoma spreads hematogenously and characteristically metastases to bone, lung, brain and liver[68]. Follicular carcinoma of thyroid do not invade lymphatics. Oxyphilic variant of follicular carcinoma typically recurs in the neck and metastasizes more than the conventional type .[59]

f) Features of Fine Needle Aspiration Biopsy (FNAB) ;

In the diagnosis of follicular carcinoma of thyroid the use of Fine Needle Aspiration Biopsy is limited ,because it cannot provide the details about capsule and/ or vascular invasion. Cytological features in follicular adenoma and follicular carcinoma are similar with cellular smears composed of syncytial clusters of crowded cells and repetitive follicles with lumen having scant colloid. The cells are monomorphic and larger than non-neoplastic follicular epithelial cells. Nuclei is uniformly enlarged with inconspicuous nucleoli and shows nuclear overlapping.

g) Macroscopic appearance of Follicular Thyroid Carcinoma;

Follicular thyroid carcinoma are typically round or oval in shape and have thick capsule. Macroscopically, the tumors are light brown to pink in cut section, with areas of haemorrhage, necrosis and fibrosis.[13]. Kuru et al [69] found that nodule size ≥4 cm was associated with increased risk of malignancy compared with nodule size <4 cm.

e) Microscopic appearance of Follicular Thyroid Carcinoma;

Microscopically, these carcinomas are quite uniform and hyper cellular, showing follicle formation with minimal colloid, while a widely invasive subtype often shows a solid or trabecular growth pattern. The tumor cells lack the nuclear features of papillary carcinoma of thyroid.The hurtle cell variant of follicular thyroid carcinoma is composed of oxyphilic cells with a typical appearance of abundant granular and eosinophilic cytoplasm filled with swollen mitochondria with scant or absent colloid. Clear cell change may be prominent in oncocytic neoplasia .[63]

The presence of vascular and/or capsular invasion is the only feature that can distinguishes a follicular carcinoma from follicular adenoma, which means that distinction between the two requires thorough inspection of the tumor-capsule interface.[70]

i) Follicular carcinoma invasion ;

Histological confirmation of capsular invasion and /or vascular invasion is necessary for the diagnosis of Follicular Thyroid Carcinoma.

**Capsular invasion**

Capsular invasion remains a controversial issue in diagnosis of follicular carcinoma of thyroid . Complete transgression of the fibrous capsule (the tumor has to penetrate the entire thickness of the capsule) should be considered as an unequivocal evidence of capsular invasion.[71,72]

Lang et al and Franssila et al [45,68]noticed that , nest of tumor cells found in the capsule could be due to fibrosis rather than invasion. According to Iida's opinion[73], tumor cells invading the capsule focally or finding a few follicles in the capsule without an evidence of reactive fibrosis is insufficient to diagnose follicular carcinoma of thyroid .So deeper sectioning of these foci is most important in these cases, it may show the tumor cells traversing the entire capsular thickness .But the invading tumor nests should show a connection with the main tumor mass .

Problematic features relative to diagnostic interpretation include:

- Irregular contour(s) of the tumor,
- Tangential sectioning,
- Free-floating tumor nests in the capsule may indicate entrapment due to preoperative Fine Needle Aspiration or tumor degeneration.
- Tumor cells seen outside the capsule without any connection to the main tumor should not be considered as the foci of invasion .

- Minimally Invasive Follicular Carcinoma exhibited only focal invasion either to the capsule or a vessel, whereas widely invasive follicular thyroid carcinomas showed widespread infiltration into adjacent thyroid tissue or into the vessels .

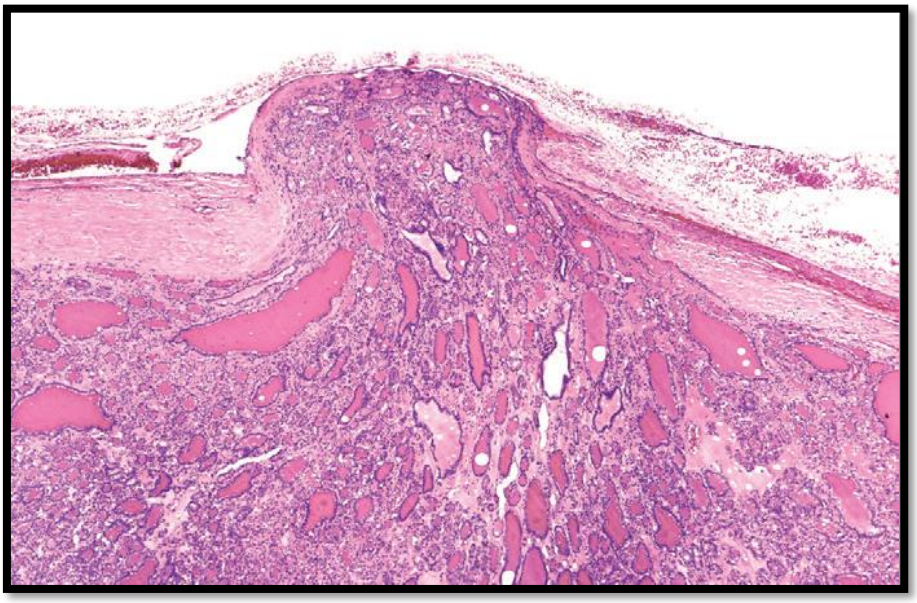


**Figure –5; Adapted from Christopher D M Fletcher, diagnostic histopathology of tumors ;Elsilver volume 2 page 1203**

-Section shows the tumor bud has actively penetrated and reaching beyond the external contour of the capsule like a mushroom fashion.

**Vascular invasion**

Vascular invasion is defined as the presence of an endothelialised tumor cell nests (located within or beyond the fibrous capsule) in the capsular vessels adherent to the vessel wall .

The histological criteria for diagnosis of vascular invasion are defined as follows:[30]

1. The invasive tumor should form a plug in sub endothelial location;

2. The tumor thrombus should be lined by endothelial cells.

3. The endothelialised tumor embolus should be attached to the vessel wall.

4. Tumor cell nests seen freely in a vessel lumen without endothelial covering may indicate artefacts.

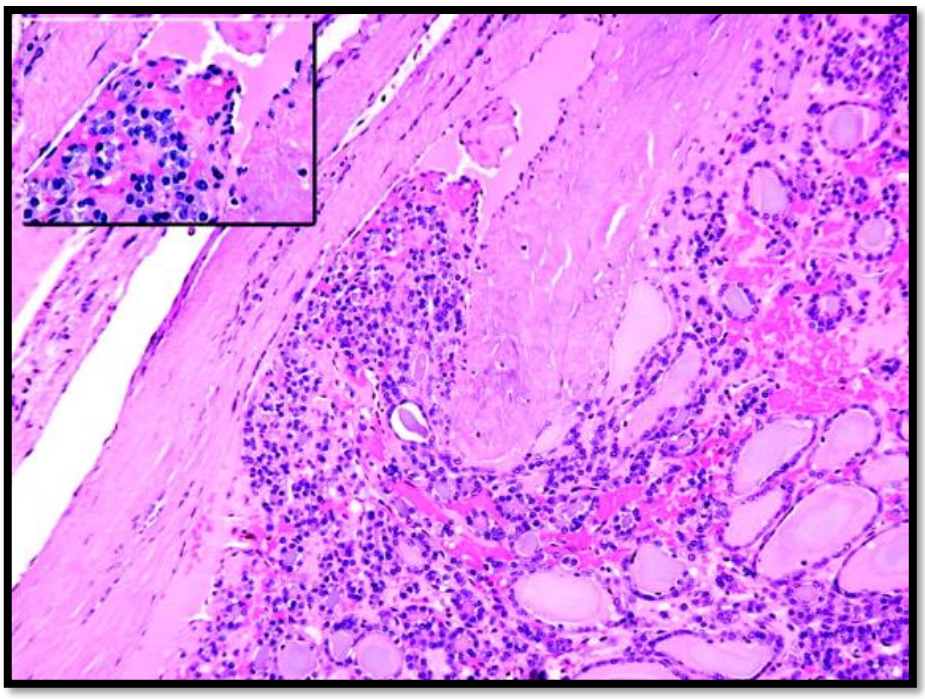


**Figure -6;Adapted from ; Baloch ZW, LiVolsi VA. Our approach to follicular-patterned lesions of the thyroid. J Clin Pathol. 2007 Mar;60(3):244–50.**

- Section shows vascular invasion in follicular thyroid carcinoma showing tumour cells invading a capsular vessel; the invading tumour nest is lined by endothelial cells (inset).

Vascular invasion is the more consistent feature of malignancy than capsular invasion. Mete and Asa recently redefined vascular invasion as “tumor cells invading into a vessel wall which is covered by endothelium, and associated with main tumor adherent to intravascular tumor thrombus .[74] such criteria are too strict for use in diagnosis of follicular carcinoma.Deeper sections and/or

elastic stains or trichrome may demonstrate the presence of endothelial lining or elastic membranes. because a continuous smooth muscle layer may not be present; so these stains usually are of only limited help. Stains for endothelial markers such as CD31 ,CD34 Factor VIII related antigen and Ulex Europaeus agglutinin I, are of limited use.[36].

**Follicular Carcinoma Categorization**[75]

According to the extent of the invasive component, two types of follicular thyroid carcinoma are recognized which differs in their biologic behavior and in their treatment:

**1. Minimally invasive (low-grade) follicular carcinoma,**

which in turn can be Subdivided into: -

- Have only capsular invasion, without angioinvasion
- **With angioinvasion**[76] **–**

  limited angioinvasion, including less than 4 vascular spaces

  Extensive angioinvasion,- including 4 or more than 4 vascular spaces.

**2. Widely invasive follicular carcinoma**

- shows widespread infiltration of the tumor into the thyroid parenchyma and into the blood vessels
- often lack complete encapsulation
- Penetration of entire thickness of the capsule by the tumor with or without vascular permeation.

- **Follicular Carcinoma, Minimally Invasive ;Treatment and prognosis;**

Baloch and LiVolsi reported that encapsulated follicular tumors showing only capsular invasion as minimally invasive follicular carcinoma and these tumors were believed to have a low risk of recurrence or metastases.[30]

Treatment options of minimally invasive follicular carcinoma include conservative treatment versus more radical approaches[77]

- Conservative therapy includes limited resection (lobectomy or subtotal thyroidectomy) without radioactive iodine.
- Radical therapeutic intervention includes total thyroidectomy followed by administration of radioactive iodine.

Conservative modalities are utilised only for the tumors with limited invasion and the absence of metastases. In the evidence of any metastasis, the patients should be treated with radioactive iodine. D'Avanzo[55] reported that 10-year survival of patients with minimally invasive follicular carcinoma of thyroid is 98%.But the prognosis mainly depends on extent of capsular and vascular permeation. The long term prognosis for the tumors with only capsular permeation is excellent and the risk of metastasis is very low approximately 0.1%[55].Prognosis is also considered good for the tumors with limited angioinvasion. But the prognosis for tumors with extensive angioinvasion is guarded.

**Follicular Carcinoma, Widely Invasive Treatment and Prognosis ;**

For Widely Invasive Follicular thyroid Carcinoma - more aggressive treatment is required which includes total thyroidectomy and radioactive iodine

therapy . Prognosis is generally poor, but it is variable. These tumors shows hematogenous spread with metastasis to bone, lungs , brain and skin. Metastatic disease may be identified at an early stage of the disease and radioactive iodine should be given at this stage . The metastatic foci appears histologically similar to the primary tumor. A 10 year survival of patients with angioinvasive follicular carcinoma with or without capsular invasion is 80% and 38% with extensive capsular invasion of the tumor and the adjacent thyroid parenchyma [55]. The cause of death is due to distant metastases .[78]

**Adverse prognostic factors of follicular thyroid carcinoma include**[79]:

- presence of extraglandular spread into adjacent soft tissues;
- presence of distant metastasis;
- older age of the patient (over 40 years);
- male gender may be associated with a worse prognosis;
- extensive intrathyroidal invasion;
- presence of intravascular invasion;
- **tumor size**: tumors size more than 4 cm have a worse prognosis;
- **Histologic type-** solid/trabecular pattern-worse prognosis

f) **Follicular Carcinoma is further classified based on the tissue pattern as follows;**

- ❖ Oncocytic (Hürthle) cell variant
- ❖ Signet ring cell variant

- ❖ Clear cell variant
- ❖ Mucinous variant
- ❖ Hyalinising trabecular carcinoma.

### 3.8.2) Follicular carcinoma oncocytic type (Hurthle cell variant)

**HURTHLE CELL CARCINOMA- ANGIOINVASIVE**

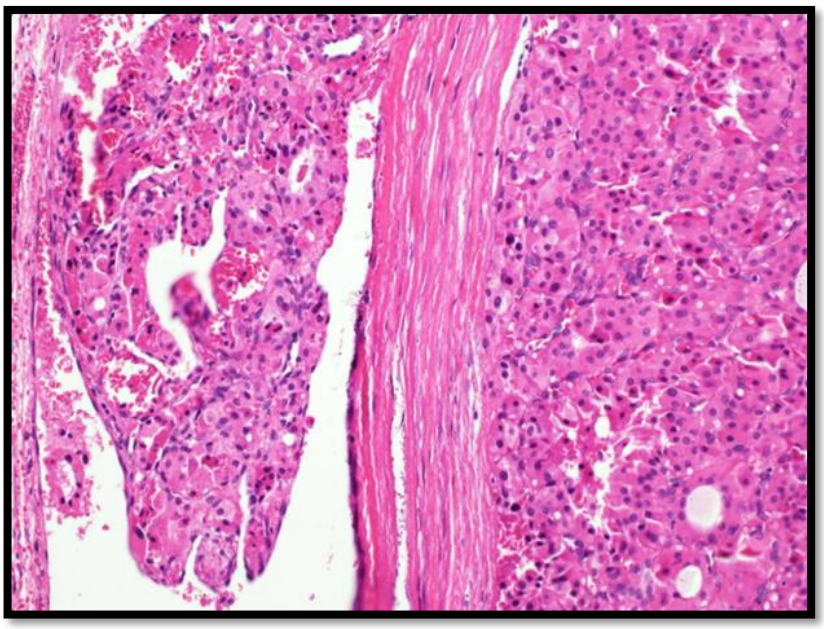


**Figure – 7;Adapted from Rosai J, Carangui ML, DeLellis RA (1992) Atlas of tumor pathology: tumors of the thyroid gland. Armed Forces Institute of Pathology, Washington, DC**

-Section shows endothelialized tumor embolus in the vascular space,tumor proper on the right (H&E 10x)

- o Frequency of malignancy is higher in older age group (35%)[80]
- o higher prevalence of aggressive behavior
- o larger size (≥4cm strongly correlated to malignancy)
- o higher frequency of extrathyroidal extension,

- greater inclination to regional node and distant metastases
- lower survival rate

### 3.9) Role of immunohistochemistry in follicular neoplasm of thyroid

The histopathological diagnosis of follicular neoplasm of thyroid remains the cornerstone in the classification of follicular thyroid lesions. Recent studies have concentrated on identifying immunohistochemical markers which are used to distinguish benign from malignant follicular thyroid tumors, (follicular adenoma and follicular carcinoma and to differentiate follicular variant of papillary thyroid carcinoma from follicular carcinoma and adenoma of thyroid) . In this view number of growing immunomarkers have been tested, and some of them are found to be promising like CD56, Hector Battifora Mesothelial 1 (HBME-1), Galectin - 3 and Cytokeratin -19 [81,82].

Nevertheless, there is no single immunomarker sensitive enough to provide a definitive malignant diagnosis. As proposed by many investigators a panel of immunohistochemical markers are recommended in follicular tumors to improve diagnostic accuracy. Galectin 3 and CD56 made significant difference between follicular adenoma and follicular carcinoma of thyroid. Galectin 3 was the only marker which made statistically significant difference between adenoma and carcinoma of Hurthle cells. Galectin- 3 is a sensitive marker for malignancy, while the negative expression of CD56 is very specific for malignancy. But CD56 and Galectin-3 could not be able to differentiate between follicular variant of papillary carcinoma and follicular carcinoma of thyroid . The combination panel of 2 or 3 markers also given promising results.

-Various other antibodies have also been studied for their potential value in the distinction between follicular carcinoma and adenoma of thyroid, but none so far has been shown to be foolproof.

- Immune staining for endothelial markers has not been very successful for detecting vascular invasion, because the staining may be erratic.[83]
- Although some antibodies such as tissue polypeptide antigen, dipeptidyl amino peptidase IV, Leu-7, thyroperoxidase (MoAb47), matrix metalloproteinase-2, matrixmetalloproteinase-7, and cyclooxygenase-2 are reported to show differential staining of follicular adenomas and follicular carcinomas[15,84,85]

### 3.10) Immunohistochemical markers

#### 3.10.1 GALECTIN 3

GALECTIN- 3 (31 kD) is a beta-galactosidase binding lectin. It belongs to the agglutinin family. It is a kind of multipeptide consisting of a short amino-terminal sequence, which is a repetitive collagen-like sequence that is rich in proline, glycine, and tyrosine and a Carboxy terminal.[86] Galectins are localized on the cell surface, within the extracellular matrix, as well as in the cytoplasm and the nucleus of the cells. It may serve as the receptors for extracellular matrix proteins, such as laminin and fibronectin [87]. Galectin-3 is able to make cross links with cell membrane glycoproteins, thus forming new network involved in cellular signaling of receptors. Galectins are expressed in thyroid , breast, colon and as well as in macrophages and activated endothelial cells, and they play a vital role in number of biological processes such as cell growth, differentiation,

apoptosis, inflammation, and angiogenesis, tumor progression.[88] Galectin-3 is known to mediate and inhibit cell adhesion and participate in neutrophil and macrophage activation. Galectin-3 works as redundant pre-mRNA splicing factor, regulates the cell cycle and apoptosis, being either anti-apoptotic or proapoptotic. According to a recent study [88] serum gal3 increased significantly in patients with breast, gastrointestinal, lung and ovarian cancer, as well as in patients with melanoma and non-Hodgkin's lymphoma. These authors also reported that the level of serum gal3 of patients with metastatic disease was higher than in patients with a localized tumor. This proves that circulating gal3 may have arole in tumor progression and, based on this, assay of gal3 carried out in an early stage could predict the metastatic potential of the tumor.

Several studies have analyzed galectin-1 and galectin-3 expression in benign and malignant thyroid tissue. Several studies have recently challenged galectin-3 as a tumour marker for follicular carcinoma of thyroid . Galectin-3 is also a fairly reliable marker in distinguishing follicular adenomas and carcinomas; however, it should be combined with other markers to increase its accuracy. . Galectin 3 is a marker of early malignant transformation and minimally invasive carcinoma.[89] .Several authors emphasize that the galectin-3positive and morphologically suspect follicular adenomas (with cellular atypia) can be considered potentially as early carcinomas, in which capsular and vascular invasion cannot be observed yet, but transformation at the molecular level may have been already occurred ,[90]

Recent studies, found difference in expression of Galectin 3 between follicular thyroid carcinoma and follicular adenoma . Galectin 3 was the only marker able to make a difference between Hurthle cell carcinoma and adenoma, which is also confirmed by the study .[91]

STANINIG PATTERN OF GALECTIN 3;

With reference to the study [92] if more than 5% of the tumour cells shows cytoplasmic staining for Galectin-3, then they were scored as positive regardless of their intensity. Distribution and intensity of Galectin -3 interpretation was based on the study followed by Weber KB et al. and Hermann ME et al., [92,93]The staining intensity was graded on a scale of 0 to 3 where 0, 1+, 2+, and 3+ denote no staining, weak/ slight staining, moderate staining and intense staining respectively, and the proportion of stained cells were interpreted as 1+ (less than 5% of cells), 2+ (5% to 50% of cells) and 3+ (more than 50% of cells) [93]

**Intensity of staining of cells;**

1 = weak

2 =moderate

3 = strong

**Proportion of cells showing positive expression for Galectin - 3;**

1+ → (less than 5% of cells)

2+ → (5% to 50% of cells)

3+ → (more than50% of cells)

Cytoplasmic galectin-3 expression was found only in carcinomas, and therefore it may be considered an evidence of malignancy. Cytoplasmic predominance of galectin-3expression has been related to the progression of normal tissue to adenoma and carcinoma.It was studied in the colon carcinoma model .Galectin-3 may play an important role in malignant transformation, especially in cell adherence and metastasis. [94]

3.10.2) **CD56**

CD56 (NCAM)- Neural cell adhesion molecule, Leu19) is a homophilic binding membrane glycoprotein of Ig-superfamily that has an important role in cell-to-cell adhesion.It is expressed normally in Natural Killer cells (NK cells), activated T cells, large granular lymphocytes, brain tissue and specific endocrines[95] thyroid follicular cells Previous studies have demonstrated that CD56 is present in the normal follicular cells of the thyroid also in benign thyroid lesions (thyroid follicular adenomas, chronic autoimmune thyroiditis, nodular goiter or Basedow disease), but its expression is low or absent when malignant transformation occurs.[96]

Reduced expression of CD56 has been implicated with tumor progression of the patient with cancer. Loss of CD56 expression was associated with metastatic potential and a poor prognosis in some malignant tumors. A study by Artuset al showed increased expression of CD56 in follicular adenomas than the surrounding thyroid tissue and decreased CD56 expression in follicular thyroid carcinoma than the benign thyroid tissue[82]. Its expression is reduced, or totally lost, in cases of papillary carcinoma, follicular carcinoma and anaplastic

carcinoma The most specific combination of markers CD56andGAL 3 reduces sensitivity and increases specificity for malignancy. Cases were considered CD56 positive if they showed cytoplasmic ± membranous staining in ≥10% of the cells .[97]

The immunostained slides were evaluated for the

**Extent(percentage) of positive cells** ;

0: <10% (For CD56, focal cytoplasmic and membranous reactivity of up to 10% was considered negative).

1+: 10-25%

2+: >25-50%,

3+: >50 %

**Intensity of staining reaction;**

1 = weak

2 =moderate

3 = strong

A total score was calculated by summation of the forementioned extent and intensity scores.

### 3.10.3) HBME-1

HBME1 (Hector Battifora Mesothelial 1), is a monoclonal antibody which reacts with uncharacterized antibody in microvilli of mesothelial cells, tracheal epithelium, and adenocarcinomas of the pancreas, lung, and breast [98].Hector Battifora mesothelial-1 (HBME-1)has been noticed as an important thyroid

marker of follicular origin, with greater affinity to malignant tumors when compared to benign lesions.

HBME-1 has been studied in many thyroid tumors with the aim to differentiate benign from malignant lesions. Follicular variant of papillary carcinoma has significantly higher expression of this marker than follicular adenoma or follicular carcinoma..The results of a few other studies were also showing higher expression of HBME-1 in follicular carcinoma than in follicular adenoma. The staining pattern is membranous ± cytoplasmatic immunoreactivity for HBME- 1. [99]

**3.10.4) CK 19**

Cytokeratin 19 belongs to intermediate filaments of cytokeratins family. Normally it is expressed in simple and glandular epithelium, basal layer of stratified epithelium, and in hair follicles . Normal thyroid follicular cells do not produce this protein, so upregulation of CK19 is connected with neoplastic transformation. Strong and diffuse membranous ± cytoplasmatic immunoreactivity of CK19 is most often related to papillary thyroid carcinoma .[100] Cytokeratin-19 (CK-19) expression in thyroid nodules is intense and diffuse in papillary carcinoma and variable expression in follicular thyroid carcinoma and in follicular adenoma, with nil or low expression in other benign lesions. Due to its low specificity, CK19 is not suitable for use in the differential diagnosis of suspected follicular-patterned lesions.

**3.10.5) p27**

The CDK inhibitor p27( p27/KIP 1) which is located on chromosome12p13 and encoded by CDKN1B gene, regulates cell proliferation, cell motility, and apoptosis. Phosphorylation regulates p27 inhibition of cyclin-CDK complexes, its localization, and its ubiquitin-mediated proteolysis during G0 and early G1 phases of the cell cycle.Thus preventing entry to S phase through activation of retinoblastoma protein. Normal epithelia of breast, prostate, ovary, lung, and other sites express high levels of nuclear p27[(42)].

Normal thyroid follicular epithelium shows strong nuclear immune reactivity for p27. The significant difference of p27 expression is found between follicular thyroid adenoma and follicular-derived thyroid carcinoma. Loss of p27 is an early event in follicular tumor genesis, which starts at minimally invasive carcinomas .Some investigators have reported a higher immune reactivity for p27 in the follicular variant of papillary thyroid carcinoma, in comparison with classical Papillary Thyroid Carcinoma (PTC). A variable loss of p27 protein has been shown in many human tumors. p27 is reduced in premalignant and noninvasive cancerous lesions, including ductal carcinoma in situ of the breast, aggressive ovarian cancers and oral dysplasia .It is the prognostic for subsequent development of oral squamous carcinoma.

Reduced p27 expression was also found in normal oral mucosa adjacent to tumor tissue ,benign prostatic hypertrophy, a hyperplastic premalignant prostatic neoplasm, and in ovarian tumors of low malignant potential .

### 3.10.6) CD44v6

CD44, also known as extracellular matrix receptor III, hyaluronate receptor, and heparan sulfate proteoglycan, belongs to a family of immunologically related integral membrane glycoproteins that bind hyaluronic acid. CD44 has affinity for hyaluronic acid and the ligands, such as osteopontin, collagens, and matrix metalloproteinases and mediates cell-cell and cell-matrix interactions. Adhesion with hyaluronic acid plays.an vital role in cell migration, tumor growth, and progression. The CD44 gene, located on chromosome 11, consists of at least 19 exons [(101)].Isoforms sharing CD44 variant exon 6 are known to cause tumor invasiveness and metastatic potential in malignant rat cell line. CD44v6 has been analysed on thyroid lesions by immunohistochemistry in surgical specimens and fine-needle aspirates. Intense membrane staining has been demonstrated in well-differentiated papillary and follicular carcinomas show the highest immunoreactivity for CD44v6(75%–90% and 90%–100%, respectively).[(102)] Its expression also been detected in poorly differentiated and anaplastic carcinomas, medullary carcinomas ,oncocytic variant follicular and papillary carcinomas of thyroid and also in benign lesions including hyperplastic nodules (40%) and follicular adenomas (30%–43%).[(103)]. From these findings it was suggested that CD44v6 may be correlated with deregulation of follicular proliferation, rather than malignant transformation, so it should not be used as a single marker to discriminate benign from malignant thyroid lesions.

### 3.10.7) Thyroid Transcription Factor 1 (TTF-1)

Thyroid Transcription Factor-1(TTF-1) is a nuclear tissue-specific protein which interacts with Thyroglobulin(TG) gene.TTF-1 controls the gene expression in thyroid, lungs, and diencephalon during embryogenesis. TTF-1 in association with PAX8 regulates the expression of Thyroglobulin(TG) gene , ThyroPeroxidase (TPO), thyrotrophic receptor in the thyroid. In the lung, TTF-1 regulates the expression of surfactant proteins[18]. Since TTF-1 expression is highly specific for thyroid and lung tumors, it has been widely used to detect the primary site of tumor origin in patients with metastatic disease of unknown origin. TTF-1 immunoreactivity is identified in pulmonary tumors, including neuroendocrine tumors, and rarely in small cell carcinomas from other sites.[104] In the thyroid, nuclear reactivity for TTF-1 is present in benign and malignant follicular lesions and medullary thyroid carcinomas. Poorly differentiated carcinomas usually show weak and focal staining for TTF-1.Most anaplastic carcinomas lack TTF-1 reactivity.[105] When used in combination with thyroglobulin, TTF-1 is an effective marker for thyroid origin. Lack of TTF-1 immunoreactivity in a thyroid tumor should warrant the investigation for other differential diagnoses, such as parathyroid tumors, paragangliomas, and metastatic tumors.

### 3.10.8) Thyroperoxidase(TPO)

Thyroperoxidase is a thyroid-specific enzyme (monoclonal antibody 47) reflecting normal thyroid function. Thyroperoxidase expression is demonstrated in normal thyroid follicular epithelial cells, usually in diffuse fashion[106]. During

thyroid cell dedifferentiation, the expression of TPO is lost. Therefore, lack of TPO expression is regarded as a marker of malignancy. Several studies [107] investigated the diagnostic utility of Thyroperoxidase(TPO) in thyroid lesions, yielding a sensitivity of 90% for Papillary Thyroid Carcinoma( PTC )and 76% for Follicular Thyroid Carcinoma( FTC) and a specificity of 88%.. The earlier studies produced more promising results than recent ones. Thyroperoxidase appears to be highly sensitive for Papillary Thyroid Carcinoma but borderline to poorly sensitive for Follicular Thyroid Carcinoma. The staining patterns of (TPO) follicular thyroid carcinoma and follicular adenoma showed a certain degree of overlapping without meaningful discriminatory value. A couple of recent studies[108] used a combination of TPO and GALECTIN -3 to improve diagnostic accuracy

**3.10.9) CYCLIN D1**

Cell cycle related genes include promoters and inhibitors. Promoters are are Cyclin D and E and inhibitors are p27, p53 and retinoblastoma (RB) protein. The promoters are considered oncogenes and the inhibitors are tumor suppressor genes. The most studied promoter of the cell cycle is Cyclin D.It is a member of the family of cyclins, is a 36-kD nuclear protein that functions as the regulatory subunit of cyclin-dependent kinases (CDK4 and CDK6). It is a key regulator of the G1/S transition through the cell cycle by inactivating RB protein. [109] Cyclin D1, encoded by human CCND1 gene, which is one of the most frequently amplified genes in human cancers. Deregulation of cyclin D expression results in the loss of control of normal cell growth and oncogenesis. Overexpression of

cyclin D1 has been demonstrated in various human cancers, including thyroid carcinomas.[110] Normal thyrocytes are immunohistochemically negative for cyclin D1. Wang et al [111] investigated cyclin D1 expression in 34 conventional papillary thyroid carcinomas, 10 minimally invasive follicular thyroid carcinomas, and 32 aggressive thyroid carcinomas. Their study demonstrated overexpression of cyclin D1 is found in most of the aggressive thyroid carcinomas

**3.10.10) E-cadherin**

E-cadherin is a calcium-dependent transmembrane cell adhesion molecule, is required for normal epithelial function. Down-regulation of E-cadherin expression has been observed in various carcinomas and it is usually associated with an advanced stage of tumour and tumour progression.[112] In normal and benign thyroid lesions, high expression of Ecadherin was demonstrated .In thyroid carcinomas, E-cadherin expression was reduced in poorly differentiated carcinomas, lost in undifferentiated carcinomas, and preserved in most minimally invasive carcinomas of thyroid. The studies suggest that loss of E-cadherin expression is the crucial event in dedifferentiation, progression, and metastatic spread of thyroid carcinomas.[113]

**3.10.11) Fibronectins (FNs)**

Fibronectins are extracellular matrix proteins produced by fibroblasts, are involved in cell adhesion, migration, and tumor progression. Oncofetal FNs, isoforms of fibronectin , are highly expressed in fetal and neoplastic tissues, especially in papillary thyroid carcinomas. Fibronectin-1 (FN-1) is upregulated in thyroid carcinomas as compared with normal thyroid tissue[114]. In the study of

Prasad and colleagues,[137] fibronectin was present in 91% of papillary carcinomas, 50% of follicular carcinomas,100% of anaplastic carcinomas, 75% hurthle cell carcinomas, 5% of follicular adenomas, and 7% of nodular goiters.[115]

**3.10.12) Beta-catenin**,

Beta-catenin is a 92-kD multifunctional protein, plays an important role in cell adhesion and signal transduction and serves as a downstream effector in the Wnt signaling pathway. The CTNNB1 gene encodes β-catenin. In normal resting cells, beta-catenin forms cytoplasmic/membranous-bound complexes with E-cadherin. Upon activation, beta-catenin translocates to the nucleus, promoting tumor growth through activation of the Wnt signaling pathway. Normal thyroid follicular cells display a strong membranous immunoreactivity for beta catenin. Along with E-cadherin, loss of membranous beta -catenin immunostaining is an indicator for loss of differentiation and adverse prognosis[116].The association of aberrant nuclear beta-catenin expression and poor prognosis was observed by some investigators. [117]. Point mutations of CTNNB1 occur in 25% and 66% of poorly differentiated and undifferentiated thyroid carcinomas, respectively.[118] The membrane expression of β-catenin is reduced in follicular cell adenomas and carcinomas.

**3.10.13) p53**

p53 is a tumor suppressor gene that plays an important role in normal cell growth. Mutations of the P53 gene lead to accumulation of p53, which can be detected by immunohistochemistry. p53 is one of the highest associative genes found in human cancers so far. In the process of gene mutation, function of p53

is also changed, losing the function of inhibiting tumor genesis. Recently, it has been discovered that p53 mutation is related to different types of thyroid carcinoma. In the series reported by Soares and coworkers, p53 was absent from 14 cases of goiter and adenoma and from 12 cases of papillary thyroid carcinoma. [119]p53 was present in 20% of follicular carcinomas (predominantly of the widely invasive type), 16% of poorly differentiated carcinomas, and 67% of undifferentiated carcinomas. In the series reported by Holm and Nesland, 6 of 32 (19%) papillary carcinomas, 5 of 29 (17%) follicular carcinomas, and 18 of 24 (75%) undifferentiated carcinomas were positive for p53[120]. The p53 mutation has an effect on infiltration, lymphatic metastasis and prognosis of thyroid carcinoma. Mutations and over expression of the p53 gene are common in head and neck squamous cell carcinoma, bladder carcinomas, anaplastic ependymomas and pancreatic intraepithelial neoplasia -3(PIN-3).

### 3.10.14) Telomerase

Telomerase is a kind of reverse transcriptase, that can replicate on the template of its own RNA. It can sustain the length of telomere , in order to escape apolexis, death and obtain cell immortalization. Therefore telomerase aberrant activation leads to the infinite multiplication of tumor cells. Umbricht[121] reported that telomerase is helpful in discriminating follicular thyroid adenoma from follicular thyroid carcinoma. In follicular thyroid carcinoma, telomerase expression is 100% whereas it is only 19% in the case of follicular thyroid adenoma. Saji[122] reported hTERT expression in the samples of thyroid nodule and in normal thyroid tissues. And it was observed that, hTERT expression in

follicular carcinoma of thyroid is higher (100%), 69.2% in Papillary Thyroid Carcinoma, 28% in the benign thyroid nodules, and normal tissues showed no expression. Therefore, telomerase- reverse transcriptase expression is significant in understanding thyroid carcinoma pathogenesis, diagnosis, therapy and prognosis.

**3.10.15) Matrix Metallo Proteinase (MMP) -1, 9**

Matrix Metallo Proteinase is a group of zinc ions dependent proteinase family, that can cause degradation of the matrix membrane skeleton . It has been known that invasion of blood vessel by the tumor is necessary to distinguish thyroid follicular cancer from thyroid adenoma. This infiltration is achieved by degrading collagen in the basement membrane located under blood vessel endothelium, which forms the mechanism of follicular cancer haematogenous metastasis. MMP-1 is one of MMP family members, it is a interstitial collagenase and may degrade type I, II, III collagen. MMP-9 (gelatinase B) is the other member of MMP family, and it mainly acts to gelatin and IV/V type collagen. The MMP-9 expression was more in follicular thyroid cancer, than the follicular adenoma.[123]

**Given below is a table of common IHC markers used in tumors of thyroid gland**

| NO. | NAME OF ANTIBODIES FOR IMMUNOHISTOCHEMISTRY | TYPE OF MOLECULE | ANTIGEN LOCALISATION | PATHOGENESIS. | EXPRESSION IN NORMAL CELLS AND TISSUES | POSITIVE EXPRESSION | LOW OR NEGATIVE EXPRESSION IN |
|---|---|---|---|---|---|---|---|
| 1 | GALECTIN -3 | Beta galactosidase binding lectin | Cytoplasmic | cell growth, differentiation, anti-apoptotic or proapoptotic, inflammation, and angiogenesis ,tumor progression | Mainly epithelial cells,lymphocytes,mesenchymalcells,macrophages and endothelial cells. | Papillary carcinoma,follicular ,oncocytic rcinoma,oncocytic variant of papillary carcinoma | Benign thyroid lesions(adenoma,nodular goiter),normal thyroid tissue. |
| 2 | CD56 | (NCAM)Neural cell adhesion molecule(Leu-19) | Membranous + cytoplasmic | role in cell-to-cell adhesion, Reduced expression of CD56 associated with tumor progression | Natural Killer cells (NK cells), activated T cells, large granular lymphocytes, brain tissue and specific endocrines | Benign thyroid lesions(follicular adenoma,nodular goitre,basedows disease),pheochromocytoma | Follicular thyroid carcinoma(FTC), Papillary carcinoma (PTC),anaplastic carcinoma(AC) of thyroid |
| 3 | HBME-1 | Monoclonal antibody against antigens of mesothelioma Cells | Membranous | Targets an unknown antigen of mesothelial microvilli | HBME-1 is stronglyexpressed in mesothelium, bronchial epithelium, endocervical epithelium and cartilage | Papillary thyroid carcinoma, Follicular carcinoma of thyroid., Adenocarcinoma of lung, pancreas,mesothelioma | Benign thyroid nodules |
| 4 | CK 19 | Smallest member of cytokeratin family of intermediate filaments | Membranous + Cytoplasmic | Act through the interaction of the cytokeratin with the actin binding domain of dystrophin | simple and glandular epithelia, basal layer of stratified epithelium, and in hair follicles, | Papillary thyroid carcinoma, heterogenous lebelling in Follicular carcinoma of thyroid | Nil or reduced in other benign lesions of thyroid |
| 5 | P27 | CDK inhibitor | Cytoplasmic | Regulates cell proliferation, cell motility, and apoptosis | Normal epithelia of breast, prostate, ovary, lung, | Follicular adenoma and normal thyroid follicular epithelial cells. | Follicular carcinoma of thyroid.,Follicular variant of papillary carcinoma,DCIS of breast, oral dysplasia ,BPH |

| NO. | NAME OF ANTIBODIES FOR IMMUNOHISTOCHEMISTRY | TYPE OF MOLECULE | ANTIGEN LOCALISATION | PATHOGENESIS | EXPRESSION IN NORMAL CELLS AND TISSUES | POSITIVE EXPRESSION | LOW OR NEGATIVE EXPRESSION IN |
|---|---|---|---|---|---|---|---|
| 6 | CD44 v6 | ECM receptor III (hyaluronate receptor ),heparan sulfate proteoglycan | Membranous | mediates cell-cell and cell-matrix interactions, Adhesion with hyaluronic acid plays.an important role in cell migration, tumor growth, and | langerhans cells and dendritic cellsand in normal pancreas and thyroid. | Papillary thyroid carcinoma, Follicular carcinoma of thyroid.,and oncocytic variant of follicular carcinoma | Benign lesions of thyroid |
| 7 | Thyroperoxidase | Monoclonal antibody - 47 Thyroid specific enzyme | Cytoplasmic | Enzyme involved in thyroid harmone synthesis | Thyroid | Folicular adenoma, nodular Goiter . | Papillary thyroid carcinoma, Follicular carcinoma of thyroid. |
| 8 | Beta Catenin | Transcriptional activator in WnT signalling pathway | Membranous + nuclear | Binds to E-CADHERIN in cell adhesion and anchoring cytoskeleton | Urothelium ,breast,colon,esophagus,stomach,thyroid | Papillary thyroid carcinoma ,Poorly differentiated cancers. Abberant nuclear localisation in deep fibromatosis and most of the cancers,and in solid pseudopapillary tumors of pancreas | Follicular adenomaand follicular carcinoma of thyroid. |
| 9 | E-Cadherin | Transmembrane cell adhesion molecule | Membranous + nuclear | Binds with catenin for cell polarisation ,glandular differentiationand stratification | Epithelial cells | Positive in nomal and benign tumors of thyroid | Poorly differentiated cancers, and in metastasis |
| 10 | CYCLIN D1 (oncogenes) | Promoter of cell cycle | Nuclear | Cyclin -Regulating cell cycle with CDK complexes during G1 phase of cell cycle and | Cycling cells | Metastatic Papillary thyroid carcinoma ,,Anaplastic thyroid carcinoma | Well differentiated cancers. ,benign tumors and normal thyrocytes |

| NO. | NAME OF ANTIBODIES FOR IMMUNOHISTOCHEMISTRY | TYPE OF MOLECULE | ANTIGEN LOCALISATION | PATHOGENESIS | EXPRESSION IN NORMAL CELLS AND TISSUES | POSITIVE EXPRESSION | LOW OR NEGATIVE EXPRESSION IN |
|---|---|---|---|---|---|---|---|
| 11 | FIBRONECTINS (FNs) | Glycoproteins in Extracellular matrix | Cytoplasmic + nuclear | Cell adhesion, migration, and tumor progression | Found in basement membranes and extra cellular matrix | Papillary thyroid carcinoma, Anaplastic thyroid carcinoma ,,Hurthle cell carcinoma,50% in Follicular carcinoma of thyroid. | Adenomas and nodular goiters |
| 12 | TTF-1 | Nuclear tissue specific protein | Nuclear | Transcription factor for thyroglobulin, TPO,and surfactant proteins | Thyroid,lung and some brain tissues | Papillary thyroid carcinoma ,Follicular carcinoma of thyroid., Insular carcinoma of thyroid and adenocarcinoma of lung | Poorly differentiated cancers, Anaplastic thyroid carcinoma ,Parathyroid tumors and paraganglioma and metastasis |
| 13 | P53 | Tumor suppressor gene and oncoprotein | nuclear | Normal cell growth regulator.But when mutated it losses the function. | Absent in benign tumors and normal tissues. | Most of the human cancars | Absent in benign tumors of thyroid and goiters. |
| 14 | TELOMERASE (Oncoproteins) | A kind of reverse transcriptase | Nuclear | telomere, escape apolexis, death and obtain immortalization. Therefore telomerase aberrant activation focuses on the malignancy | In normal cells especially keratinocytes,lymphocytes and endothelial cells. | Follicular thyroid cancer and Papillary thyroid cancer | Follicular adenoms and other benign lesions of thyroid.but negative in normal thyroid tissues |
| 15 | Matrix Metallo proteinase 1,9 | Group of Zinc ions dependent proteinase | cytoplasmic | Causes degradation of matrix membrane skeleton | Inflammatory cells ,endothelial cells and stromal cells | Increased in cancers particularly in metastasis,follicular thyroid carcinoma. | Benign follicular adenomas |

# 4. MATERIALS AND METHODS

This retrospective study material include the cases of benign and malignant follicular tumors of thyroid that were diagnosed using Hematoxylin and Eosin stain in the Department of Pathology, Tirunelveli Medical College and from the Department of Pathology, Thoothukudi Medical College during a period of June 2014 to June 2017

## 4.1) Study design

Retrospective study

## 4.2) Inclusion criteria

All the cases that were reported as follicular adenoma and its variants, follicular carcinoma of thyroid and its variants using Hematoxylin and Eosin stain (H &E) are included.

The blocks and slides of the respective cases are collected.

## 4.3) Exclusion criteria

a) The thyroid tumors other than follicular tumors of thyroid

b) Autolysed specimens

c) Poorly processed material

d) Cases with dense tissue necrosis

## 4.4 ) Sample size

A total number of 45 cases of surgically resected follicular thyroid tumors that include both benign and malignant follicular thyroid tumors were collected. Out of 45 cases 33 cases were benign follicular tumors that includes (n= 32)

Follicular adenoma and (n=1) Hyalinising Trabecular Adenoma and12 cases were malignant follicular tumors of thyroid that includes (Widely invasive Follicular Carcinoma (n=4), Minimally Invasive Follicular Carcinoma (n=7), Hurthle Cell Carcinoma( n=1) were collected.

**4.5 ) Materials required**

(1) Donor blocks which contains formalin fixed paraffin embedded tissue obtained from all the cases of follicular tumors of thyroid.

(2) Hematoxylin and eosin stained tissue sections made from the donor blocks.

(3) Postively charged slides for holding tissue sections for IHC

(4) Chemicals for preparing antigen retrieval solutions and for wash buffers

(5) Pressure cooker for antigen retrieval.

(6) Kits for performing immunohistochemistry which includes primary antibodies (CD56 and GALECTIN 3) and universal kit.

(7) Microscope used for grading of IHC slides

**4.6 Methodology**

COLLECTION OF DONOR BLOCKS AND SLIDES

The details of the patient such as age, gender, thyroid status, ultrasonogram findings, previous thyroid surgeries, FNAC report were collected from the clinical case sheets and from the Medical Records Department of Tirunelveli Medical College Hospital and Thoothukudi Medical College Hospital. Gross findings regarding size of the lesion, capsular (thick or thin) details, histopathological diagnosis were collected from the general surgical

report register, who were operated between the period of June 2014 to June 2017.

The following cases were selected in histological sections –

a) the diagnosis of follicular adenoma was made based on the presence of encapsulated mass with homogenous follicular proliferation ,lack of papillary thyroid carcinomas features and absence of vascular and /or capsular invasion.

b) Follicular carcinoma of thyroid was diagnosed based on tumor with thick capsule , full capsular and/or vascular invasion by the tumor cells , and atypical hyperchromatic nuclei that lacked features of Papillary Thyroid Carcinoma .

Either an endothelium-surrounded tumor cells as a thrombus or a penetrating mass into a vessel indicate a vascular invasion. Capsular invasion was comprehended as the penetration of the tumor tissue through the capsule.. Minimally invasive follicular thyroid carcinoma exhibited only focal invasion either to the capsule into a vessel, whereas widely invasive follicular thyroid carcinoma showed widespread infiltration into adjacent thyroid tissue or into the vessels.

COLLECTION OF CONTROL BLOCKS

Control tissues included in this study were , mucosa of appendix (crypts positive to Gal-3), muscular layer of appendix (nerve fibers and ganglion cells positive for CD56).

PREPARATION OF HAEMATOXYLIN AND EOSIN SLIDES

The sections which were prepared from 10% formalin fixed paraffin embedded blocks of all cases of both benign and malignant follicular thyroid tumors were collected. A section of 4 microns thickness were made. Slides were stained with routine H&E stains. The archival tumors were initially fixed into formalin in order to preserve the structure of the tissue and stop metabolism of the cells. Paraffn embedding was performed, by washing and dehydration with ascending series of alcohol solutions and diluting in an organic solvent, in order to harden the tissue to be cut into thin slides with a microtome.Archived paraffin blocks were cut with a microtome and paraffn was dissolved with a solvent and rehydrated with descending alcohol series.

Haematoxylin stain was performed in water and counterstained with eosin in alcohol after dehydration. Finally, the stained slides were mounted with a non-aqueous medium and a coverslip. Haematoxylin stains the cytoplasm of the cell into pinkish colour whereas eosin stains the nuclei as blue (HE stain) .

SLIDE PREPARATION FOR IHC

Section cutting;

Sections were taken at 5microns thickness on the surface of the APES (3-aminopropyltriethoxysilane) coated slides. This was followed by incubation of slides at 60-70C for 1 hour .

Antigen retrieval solution;

We used antigen retrieval solution and a wash buffer as prescribed by the manufacturer (PATH INSITU).

1. Tris EDTA buffer at a $P_H$ of 9 .

2. Tris wash buffer at $P_H$ of 7.6.

BUFFER PREPARATION

TRIS EDTA BUFFER

Tris –6.05gm

EDTA--O.744gm

1 N HCL—4ml

Distilled water—1litre

TRIS WASH BUFFER

Tris -6.05gm

Sodium chloride -8gm

1N HCL-4ml

Distilled water-1 litre

PRECAUTIONS

a) The glassware of immunohistochemistry must be clean and dry
b) The buffer used should be prepared fresh and adequate pH must be achieved by adjusting accordingly.
c) During the process of immunohistochemistry , the slide should never be allowed to dry, and so a humidity chamber is used during staining and incubation.
d) DAB chromogen should be handled carefully as it is potentially carcinogenic.

e) All reagents –primary antibody ,DAB chromogen, Peroxidase block should be stored at ideal 4- 6 degree celcius

f) Every batch of slide should accompanied by positive and negative controls which ensures quality of the procedure.

PROCEDURE

Antigen retrieval;

Many methods have been used for antigen retrieval which includes Microwave method, and water bath, autoclave, proteolytic enzyme and pressure cooker method. In our institution we followed antigen retrieval by using pressure cooker method ,as it produces even heating with lesser disadvantages as compared to other methods.

Procedure for immunohistochemistry as given by manufacturer;

1. Section cutting and incubation is followed by Xylene wash (3 changes) for 15minutes each.
2. Rehydrated in graded alchohol containing 100%, 80%, 70% for five minutes each.
3. Rinsed in distilled water for 2minutes each.
4. Antigen retrieval for 15-20 minutes in Tris-EDTA buffer and ph of the retrievl buffer adjusted to 9.
5. washed in distilled water, two changes, 2 minutes each.
6. Washed in TRIS wash buffer- 3changes 5minutes each.
7. Do endogeneous peroxidase blocking by adding H2O2 on the section, for 7-10minutes.

8. Washed in TRIS wash buffer- 3changes 10minutes each.
9. Application of primary antibody (CD 56 and GALECTIN 3) – 30 mins in moist chamber.
10. Washed in TRIS wash buffer- 3changes 10minutes each.
11. Add polyexcel Target binder for 15 mins
12. Washed in TRIS wash buffer- 3changes 10minutes each
13. Application of HRP POLYMERASE for 15 mins.
14. Washed in TBS wash buffer- 3changes 10minutes each.
15. Application of Diamino-benzidine tetrachloride(DAB) chromogen (1 drop) and DAB buffer (1ml) for 5 mins.
16. Washed in distilled water – 2 changes.
17. Counterstaining with Harris Hematoxylin – 1dip/30seconds to impart background staining.
18. Wash in running tap water.
19. Dehydrate (70%,80%,100%) 5minutes
20. Clear with xylene – 2 changes 5 minutes each.
21. Mount the section with Dextrene Phthalate Xylene
22. Observation and grading under light microscope.

ANTIBODIES USED

| Antibodies | Clone | Dilution | Manufacturer | Antigen Retrival buffer | TRIS Buffer pH | Visualisation kit manufacturer |
|---|---|---|---|---|---|---|
| Cd 56 | 123c3 | Prediluted | Pathin situ | Tris EDTA | 9 | Pathin situ |
| Galectin 3 | b2c10 | Prediluted | Pathin situ | Tris EDTA | 9 | Pathin situ |

**4.7 GRADING OF IHC STAINIG;**

IHC Grading of GALECTIN 3;

Cytoplasmatic ± nuclear immunoreactivity for Gal-3, more than 5 % of cells was considered as positive staining without regard to intensity of staining.

**Intensity of staining of cells;**

1 = weak

2 =moderate

3 = strong

**Propotion of cells stained**

1+ (< 5% of cells)

2+ (5% to 50% of cells)

3+ (>50% of cells)

IHC GRADING OF CD56

Strong and complete membranous expression with or without cytoplasmic staining of the cells qualified the case as positive for CD56.

**Extent (percentage) of positive cells**

0 : <10%

1+: 10-25%

2+: >25-50%,

3+: >50 %

**Intensity of staining reaction;**

1 = weak

2 =moderate

3 = strong

A total score was calculated by summation of the fore mentioned (Extent + intensity) scores.

PROBLEM TROBULESHOOTING IN IHC STAINING OF CD56 AND GALECTIN 3 IN THIS STUDY.

Inspite of the strong and diffuse staining of imunohistochemical markers CD56 and Galectin 3, in few sections we could not differentiate between membranous and cytoplasmic staining of the cells. This is due to the intense background staining of colloid in immunohistochemistry.

BLOCKING BACKGROUND STAINIG

Background staining is the unwanted staining of cells in the section. The common causes for background staining in immunohistochemistry are either due to non specific antibody binding to endogenous Fc receptors or due to hydrophobic - ionic interactions and endogenous enzyme activity. Background

staining may be specific or non specific. Specific background staining occurs due to an apparent affinity of certain tissue components to the applied antibodies.Non specific background staining may due to the presence of charged sites in the tissue section.

Background staining can be minimised by adding a innocuous protein solution to the section s before applying the primary antibody. The applied protein should saturate and neutralise the charged sites thus enabling the primary antibody to bind to the antigenic site only. Blocking of endogenous enzymatic activity should be done before addition of enzyme labelled secondary reagent to prevent the inactivation of enzyme.

## 5. OBSERVATION AND RESULTS

This retrospective study includes totally 45 cases of follicular thyroid tumors. Of them, 33 cases were benign (n=32,Follicular adenoma and n=1,Hyalinising trabecular adenoma) constituting 73% and the remaining 12 cases are malignant (Follicular thyroid carcinoma) constituting 27%.

**TABLE -1. DISTRIBUTION OF CASES**

| | NO. OF CASES | PERCENTAGE |
|---|---|---|
| BENIGN | n=33 | 73% |
| MALIGNANT | n=12 | 27% |

**CHART- 1**

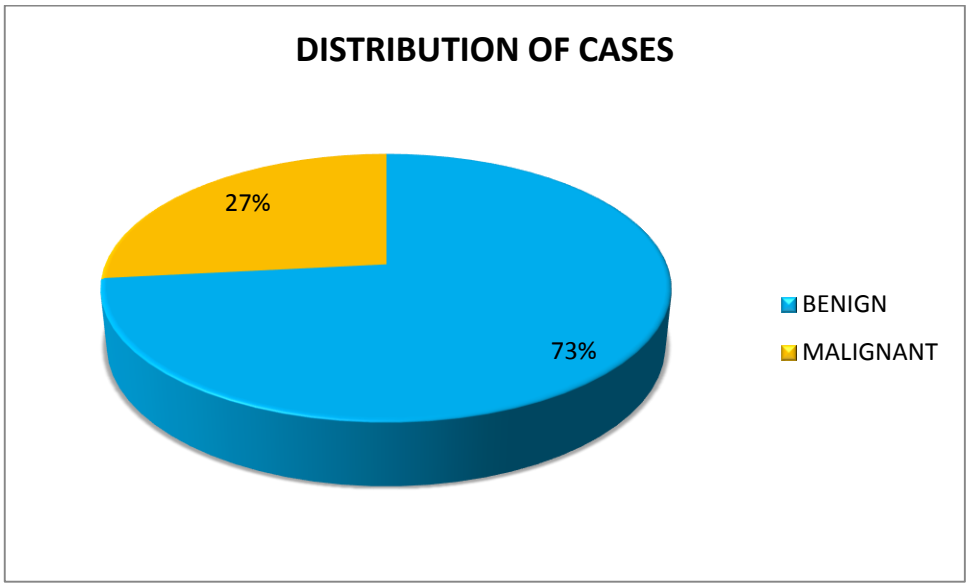



**TABLE - 2. DISTRIBUTION OF BENIGN CASES**

| CASES | NUMBER |
|---|---|
| FOLLICULAR ADENOMA | 32 |
| HYALINISING TRABECULAR ADENOMA | 1 |

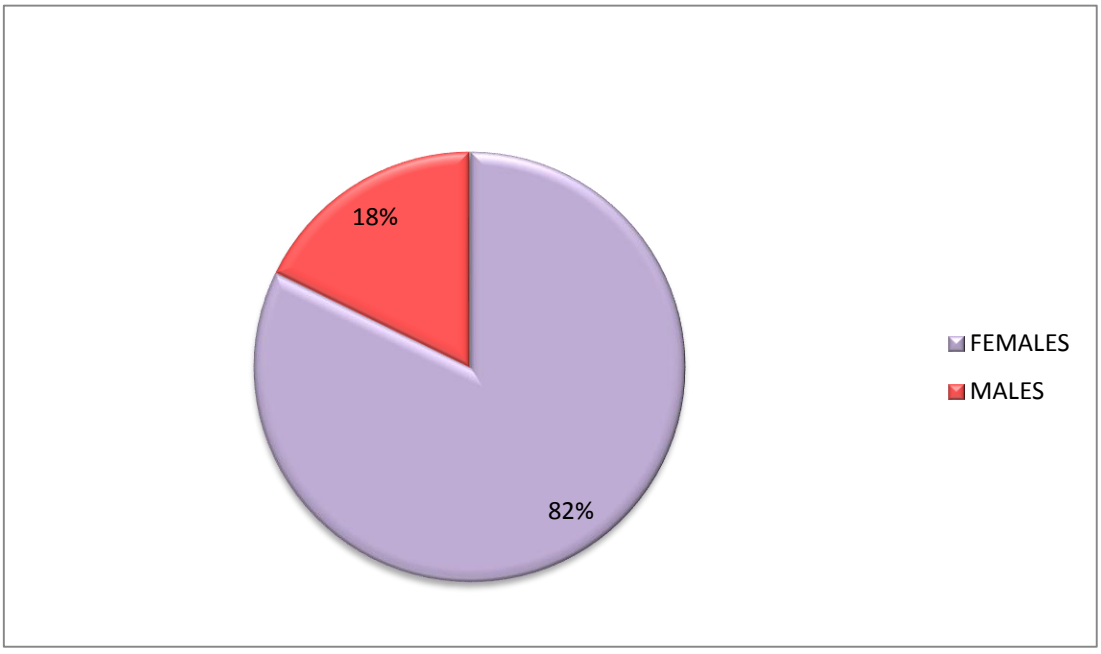
**TABLE -3. DISTRIBUTION OF MALIGNANT CASES**

| CASES | NUMBER |
|---|---|
| WIDELY INVASIVE FOLLICULAR CARCINOMA (WIFC) | 4 |
| MINIMALLY INVASIVE FOLLICULAR CARCINOMA (MIFC) | 7 |
| HURTHLE CELL CARCINOMA (HCC) | 1 |

**TABLE -4. DISTRIBUTION OF SAMPLE BASED ON GENDER**

| GENDER | NO. OF CASES | PERCENTAGE |
|---|---|---|
| FEMALES | 37 | 82% |
| MALES | 8 | 18% |

**CHART - 2 . DISTRIBUTION OF SAMPLE BASED ON GENDER**

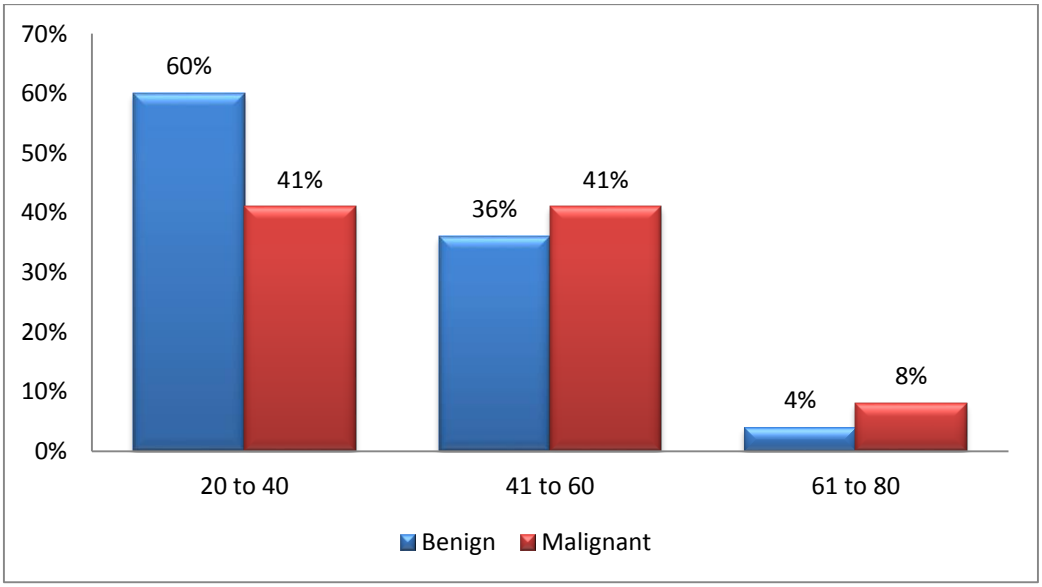


According to the study[2], females were affected more common than males in follicular neoplasm of thyroid. In our study also females (82%) were more affected than males(18%) in both follicular adenoma and follicular carcinoma of thyroid.

**TABLE -5. AGE WISE DISTRIBUTION OF SAMPLE**

| AGE | BENIGN | MALIGNANT |
|---|---|---|
| 20 to 40 years | 60% | 41% |
| 41 to 60 years | 36% | 41% |
| 61 to 80 years | 4% | 8% |

**CHART - 3. AGEWISE DISTRIBUTION OF SAMPLE**



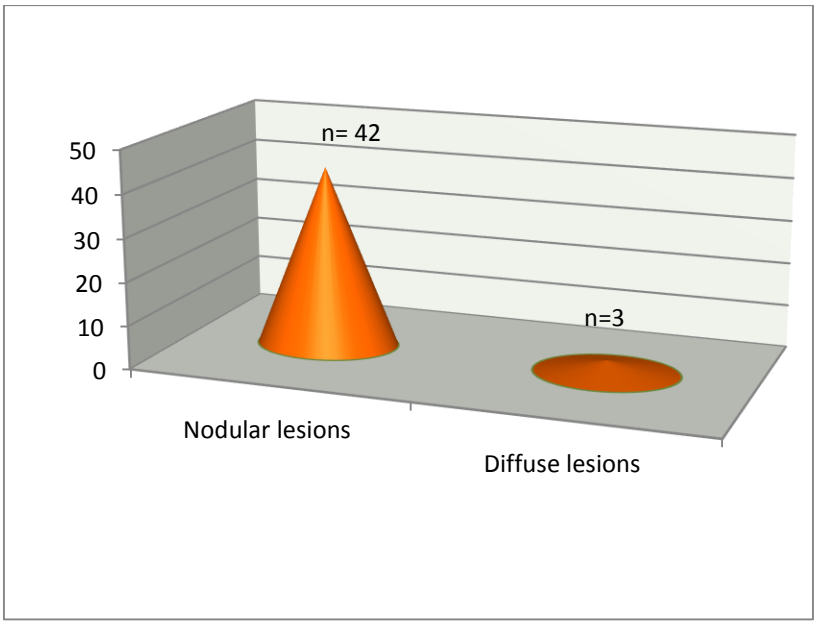

In this study, the age group commonly affected by the benign follicular tumors (Follicular adenoma) were between 20 to 63 years, median age 37 +9.47

SD. The age group commonly affected by malignant tumors (Follicular carcinoma) were between 30 to 70 years, median age 42 +14.2 SD. These data was found to correlate with the study [2], that most of the follicular tumors were common during third to sixth decades of life.

**TABLE- 6. DISTRIBUTION OF SAMPLE BASED ON NATURE OF LESIONS**

| | NO.OF CASES | PERCENTAGE |
|---|---|---|
| NODULAR LESIONS | 42 | 93% |
| DIFFUSE LESIONS | 3 | 7% |

**CHART- 4. DISTRIBUTION OF SAMPLE BASED ON NATURE OF LESIONS**



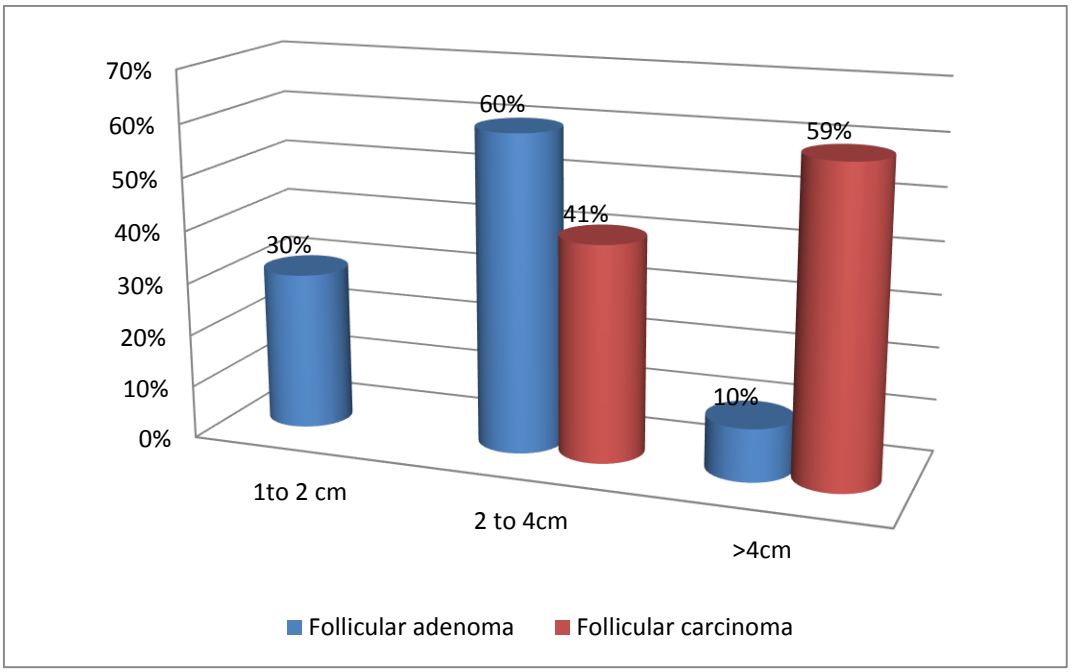

In this study, nodular lesions were found to be 93% (n=42) and diffuse lesions were only7%(n=3). According to Christopher most of the patients with a follicular adenoma or a follicular carcinoma usually presents with a thyroid

nodule that is palpable on physical examination or identified on an imaging study. Palpable thyroid nodules occur in 4%–7% of the population and up to 60%–70% will have a nonpalpable nodule that can be identified by imaging the thyroid gland with ultrasound[124]

**TABLE -7. SAMPLES BASED ON SIZE OF NODULE**

| SIZE OF LESION | FOLLICULAR ADENOMA | FOLLICULAR CARCINOMA |
|---|---|---|
| 1 to 2cm | 30% (n=10) | NIL |
| 2 to 4cm | 60% (n=20) | 41% (n=5) |
| >4cm | 10% (n=3) | 59% (n=7) |

**CHART - 5. SAMPLES BASED ON SIZE OF NODULE**



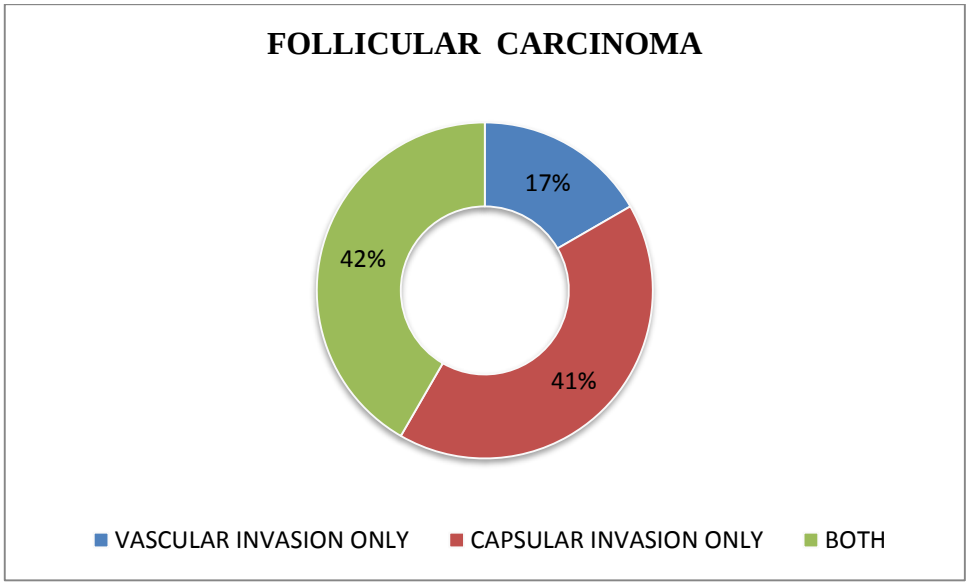

In this study 7 out of 12 cases ( 59 % )of follicular carcinoma, 3 out of 33 cases (10% ) of follicular adenoma had the size of the nodule more than 4 cm**.** Kuru et al. [69] found that nodule size ≥4 cm was associated with increased risk of malignancy compared with nodule size <4 cm. Kim et al. [125] performed a

retrospective study of 263 patients, who underwent thyroidectomy for nodules ≥4 cm. A significant proportion of the nodules (58.6%) were malignant on final histopathology.

**TABLE -8; DISTRIBUTION OF FOLLICULAR THYROID CARCINOMA CASES BASED ON CAPSULAR AND VASCULAR INVASION**

| INVASION IN HISTOPATHOLOGY | NO.OF FOLLICULAR THYROID CARCINOMA CASES | PERCENTAGE |
|---|---|---|
| VASCULAR INVASION ONLY | n=2 | 17% |
| CAPSULAR INVASION ONLY | n=5 | 41% |
| BOTH | n=5 | 42% |

**CHART 6. DISTRIBUTION OF FOLLICULAR THYROID CARCINOMA CASES BASED ON CAPSULAR AND VASCULAR INVASION**



## RESULTS OF IMMUNOHISTOCHEMISTRY

**TABLE 9. EXPRESSION OF CD56 AND GALECTIN 3 IN BENIGN AND MALIGNANT FOLLICULAR TUMORS OF THYROID**

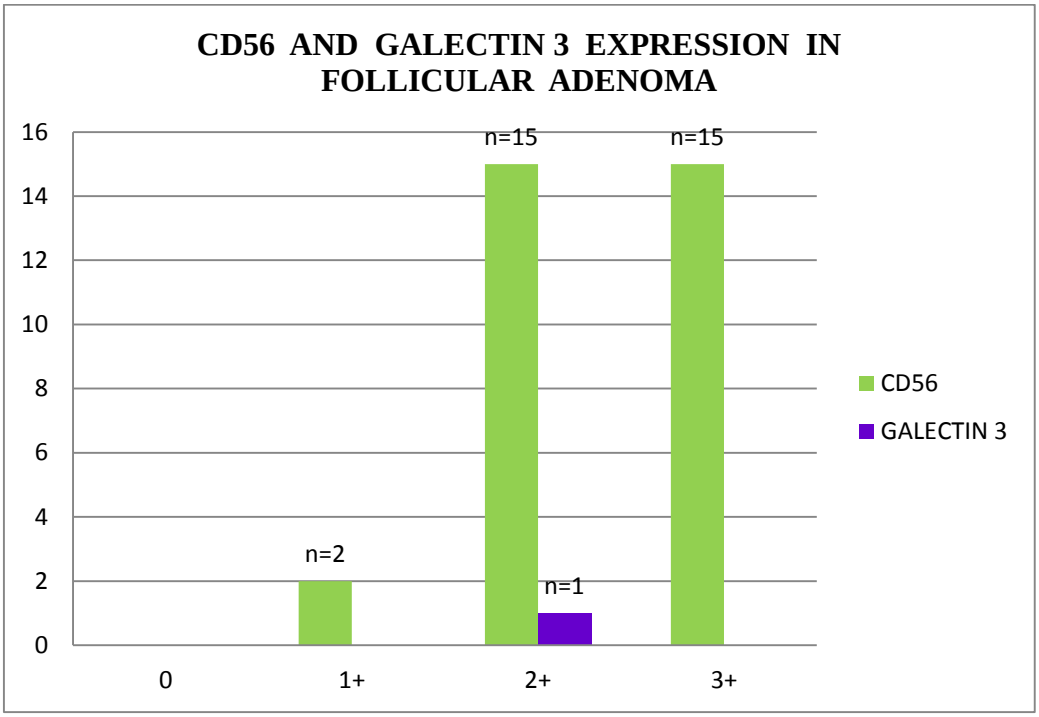
| | CD56 EXPRESSION | | GAL3 EXPRESSION | |
|---|---|---|---|---|
| CASES | NO. | PERCENTAGE | NO. | PERCETAGE |
| FOLLICULAR ADENOMA | 32 | 96.60% | 1 | 3.03% |
| FOLLICULAR CARCINOMA | 2 | 16.60% | 10 | 83.30% |

In this study CD56 expression was higher in follicular adenoma ( 96.6% ) and CD56 expression in follicular thyroid carcinoma was only 16.6%. GALECTIN 3 expression was higher in follicular thyroid carcinoma (83.3%) and its expression in follicular adenoma was only 3.03%.

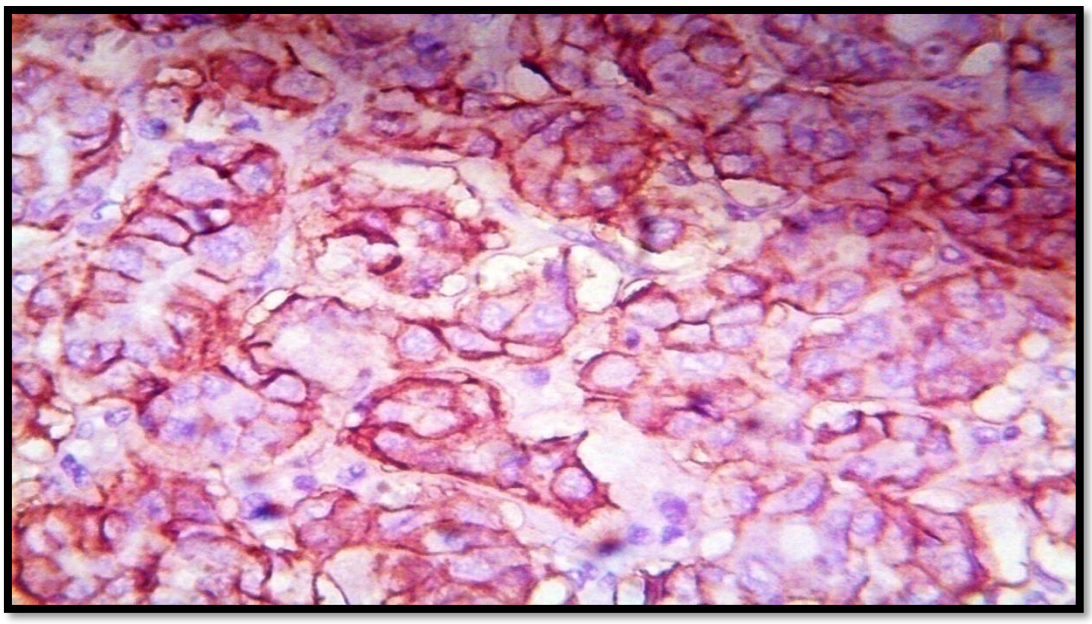
**TABLE-10. IMMUNOHISTOCHEMICAL SCORING OF CD 56 AND GALECTIN 3 EXPRESSION IN FOLLICULAR ADENOMA OF THYROID**

| **MARKERS** | **IMMUNOHISTOCHEMICAL SCORING IN FOLLICULAR ADENOMA** | | | |
|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ |
| CD56 | | n=2 | n=15 | n=15 |
| GALECTIN 3 | | | n=1 | |

## CHART 7




In follicular adenoma out of 33 cases , 2 cases showed 1+ ,15 cases showed 2+, and 15 cases showed 3+ positivity for CD56. Only 1 case showed 1+ positivity for GALECTIN 3.

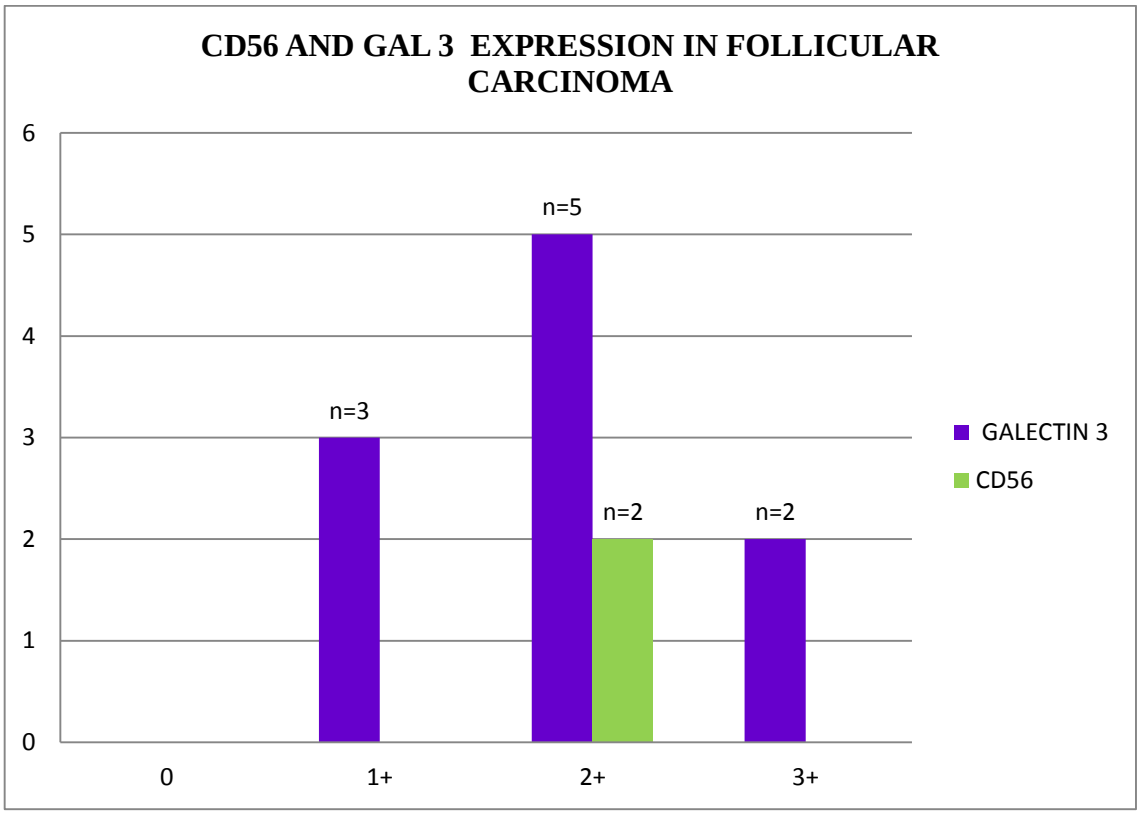


A case of follicular adenoma showing CD 56 (3+)Membranous positivity(40x)

## TABLE-11. IMMUNOHISTOCHEMICAL SCORING OF CD56 AND GALECTIN 3 EXPRESSION IN FOLLICULAR CARCINOMA OF THYROID

| MARKERS | IMMUNOHISTOCHEMICAL SCORING IN FOLLICUALR THYROID CARCINOMA | | | |
|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ |
| CD56 (n=2) | | | n=2 | |
| GALECTIN 3(n=10) | | n=3 | n=5 | n= 2 |

## CHART 8



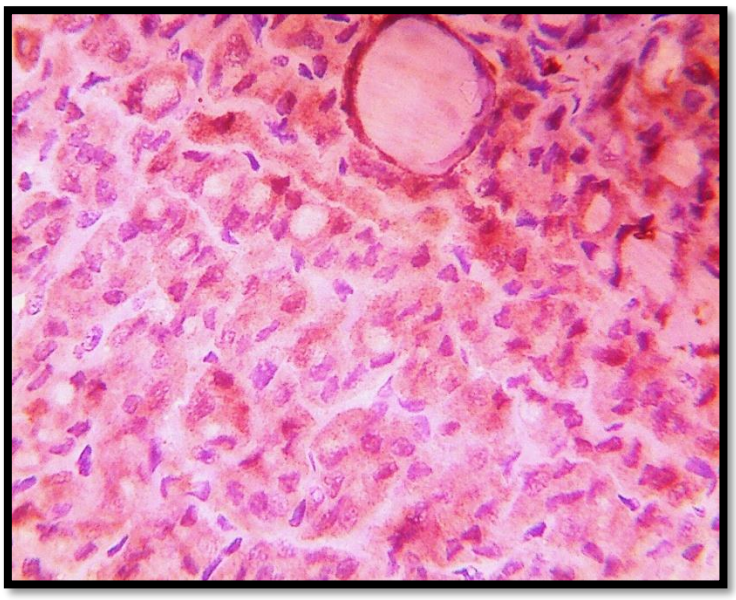
**CD56 EXPRESSION IN FOLLICULAR THYROID CARCINOMA**

| | IMMUNOHISTOCHEMICAL SCORING OF CD56 | | | |
|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ |
| MINIMALLY INVASIVE FOLLICULAR CARCINOMA | | | n=1 | |
| HURTHLE CELL CARCINOMA | | | n=1 | |

**GALECTIN 3 EXPRESSION IN FOLLICULAR THYROID CARCINOMA**

| | IMMUNOHISTOCHEMICAL SCORING OF GALECTIN 3 | | | |
|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ |
| MINIMALLY INVASIVE FOLLICULAR CARCINOMA | | n=3 | n=3 | n=1 |
| WIDELY INVASIVE FOLLICULAR CARCINOMA | | | n=2 | n=1 |

In Follicular Thyroid Carcinoma out of 12 cases, 3 cases (Minimally invasive follicular carcinoma) showed 1+, 5 cases (3 Minimally invasive follicular carcinoma ,2 Widely invasive follicular carcinoma) showed 2+, and 2 cases (1 Minimally invasive follicular carcinoma , 1 Widely invasive follicular carcinoma ) showed 3+ positivity for GALECTIN 3. Only 2cases of (1 Minimally invasive follicular carcinoma,1 Hurthle cell carcinoma) showed 2+ positivity for CD 56.



**A case of follicular carcinoma with capsular invasion shows GALECTIN 3 (3+)-Cytoplasmic positivity(40x)**

**Statistical Analysis**:

Statistical analysis was done using SSPS 11 software . PEARSON Chi-square test with 2x2 contingency table was used to calculate p-value to ascertain statistical significance. Probability (p) values less than 0.05 were considered statistically significant.

**TABLE 12**

| **FOR BENIGN TUMORS** | **CD56** | **GALECTIN 3** |
|---|---|---|
| SENSITIVITY | 97% | 3% |
| SPECIFICITY | 83% | 17% |
| POSITIVE PREDICTIVE VALUE | 94% | 9% |
| NEGATIVE PREDICTIVE VALUE | 91% | 6% |
| POSITIVE LIKELIHOOD RATIO | 5.70 | 0.036 |
| NEGATIVE LIKELIHOOD RATIO | 0.036 | 5.70 |
| 95% CONFIDENCE INTERVAL | 13.09 – 1955.68 | 0.005-0.076 |
| ODDS RATIO | 160 | 0.006 |
| p VALUE | <0.0001 | <0.0001 |

**TABLE 13**

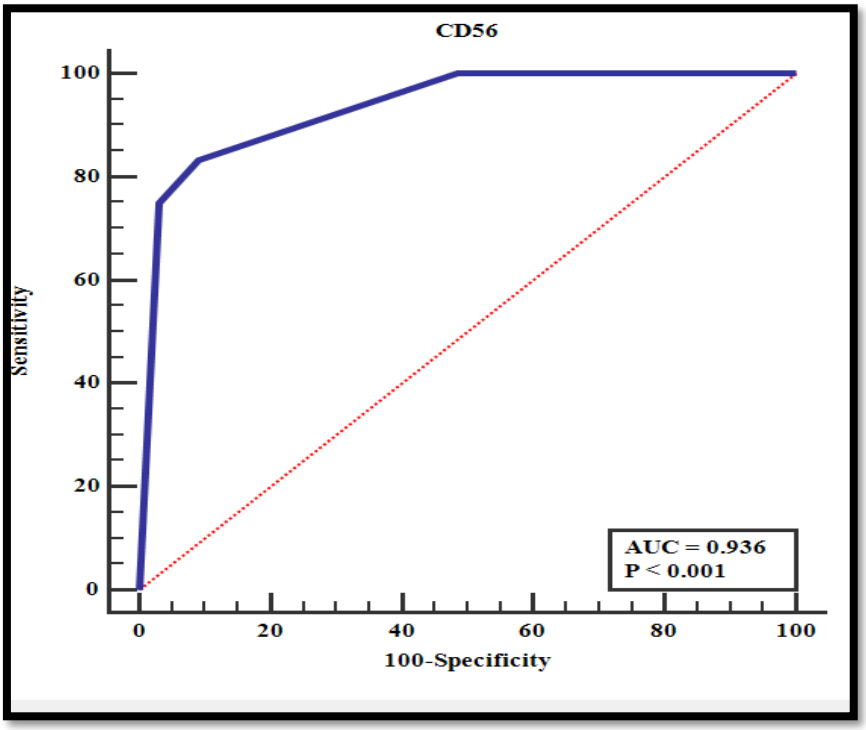
| **FOR MALIGNANT TUMORS** | **CD56** | **GALECTIN 3** |
|---|---|---|
| SENSITIVITY | 17% | 83 % |
| SPECIFICITY | 3% | 97% |
| POSITIVE PREDICTIVE VALUE | 6% | 91% |
| NEGATIVE PREDICTIVE VALUE | 9% | 94% |
| POSITIVE LIKELIHOOD RATIO | 0.175 | 27.6 |
| NEGATIVE LIKELIHOOD RATIO | 27.6 | 0.175 |
| 95% CONFIDENCE INTERVAL | 0.005-0.076 | 13.09 – 1955.68 |
| ODDS RATIO | 0.006 | 160 |
| p VALUE | <0.0001 | <0.0001 |

With objective to compare markers Receiver Operating Curve(ROC) was constructed .

Receiver operating characteristic curve (ROC) was generated by placing sensitivity (true positive fraction or rate) on the y axis and 100-specificity (false positive rate or fraction) on the x axis, and drawing a curve along the dots using online software (http://www.MEDCALC). Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular cutoff. Quantitative measures of accuracy, for example,the Area Under the ROC curve (AUC) was also calculated by the software. The sensitivity, specificity, PPV, NPV, and corresponding 95% confidence interval and odds ratio were calculated using online software (http://vassarstats.net/index.htm).

## RECEIVER OPERATING CURVE (ROC) FOR CD56 AND GALECTIN 3 EXPRESSION IN MALIGNANCY

### CHART 9



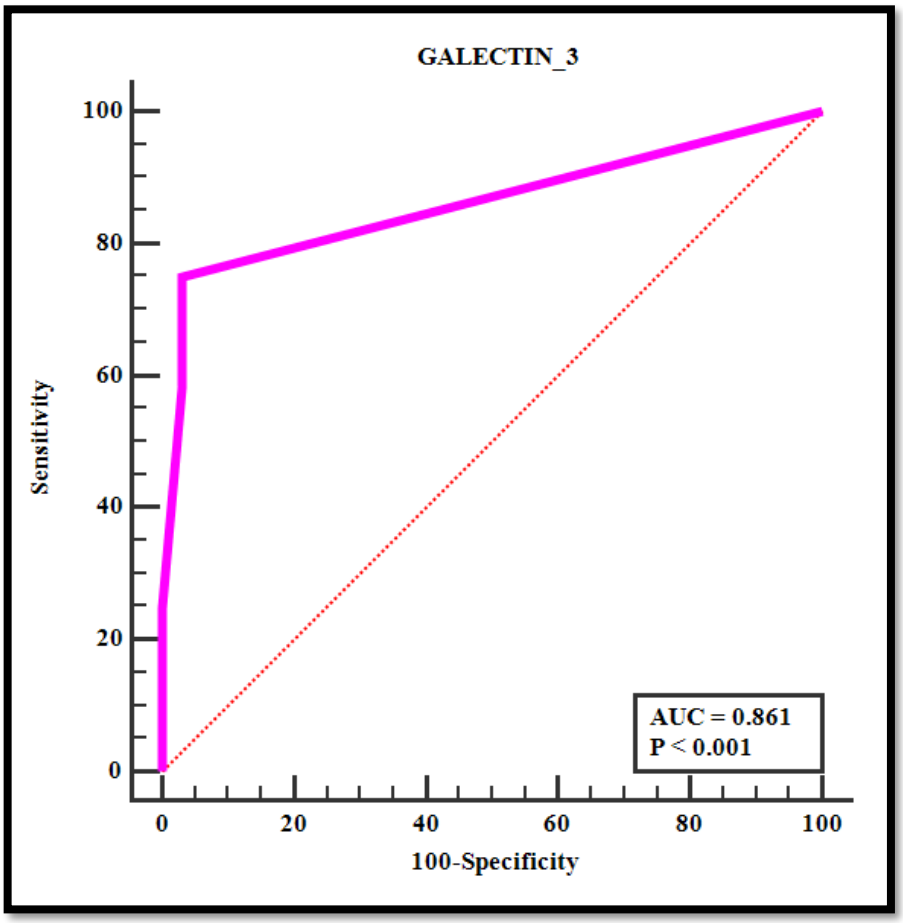

Area under the ROC curve (AUC)

| | |
|---|---|
| Area under the ROC curve (AUC) | 0.936 |
| Standard Error[a] | 0.0365 |
| 95% Confidence interval[b] | 0.820 to 0.987 |
| z statistic | 11.944 |
| Significance level P (Area=0.5) | <0.0001 |

DeLong et al., 1988 [a]

Binomial exact [b]

## CHART 10




Area under the ROC curve (AUC)

| | |
|---|---|
| Area under the ROC curve (AUC) | 0.861 |
| Standard Error[a] | 0.0670 |
| 95% Confidence interval[b] | 0.725 to 0.946 |
| z statistic | 5.388 |
| Significance level P (Area=0.5) | <0.0001 |

DeLong et al., 1988 [a]

Binomial exact [b]

| Variable | AUC | SE[a] | 95% CI[b] |
|---|---|---|---|
| CD56 | 0.936 | 0.0365 | 0.820 to 0.987 |
| GALECTIN_3 | 0.861 | 0.0670 | 0.725 to 0.946 |

[a] DeLong et al., 1988

[b] Binomial exact

Receiver operating curve (ROC) for individual marker and combination of markers were obtained. Estimated binormal ROC curves, with lower and upper bounds of the asymmetric 95% confidence interval for true-positive fraction at a variety of false-positive fraction were demonstrated.

Area Unrder Curve (AUC) is used as a quantitative measure of accuracy for each marker. AUC<0.75 was not clinically useful and AUC=1 is perfect.(126) Inthis study Area Under Curve for CD56 as a negative marker was 0.936 with 95 % confidence interval(0.820 to0.987) and odds ratio was 160. So AUC for CD56( as a negative marker) found to be a good parameter in detecting malignancies with a p value of< 0.0001.

Area Under Curve for Galectin 3 marker was 0.861 with 95 % confidence interval(0.725 to0.946) and odds ratio is160. So AUC for galectin 3 found to be a good parameter in detecting malignancies with a p value of< 0.0001.

In this study CD56 showed high sensitivity for follicular adenoma of thyroid (97%)and Galectin 3 showed high sensitivity for follicular carcinoma of

thyroid (83%).As a negative marker CD56 showed high specificity for follicular thyroid carcinoma(97%) .

When we combined two markers CD56 and GALECTIN 3 , specificity increased for malignancy.

## 6. ANNEXURE-I ; COLOR PLATES

## FOLLICULAR ADENOMA

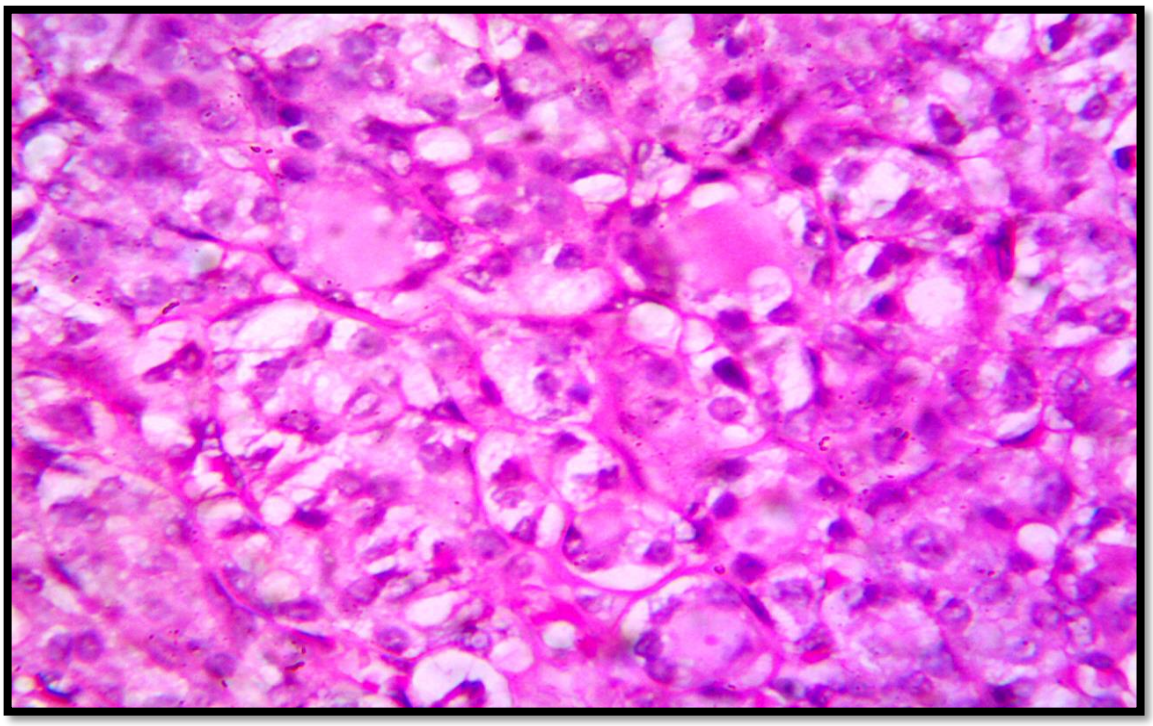


**Figure 1:** Follicular adenoma of thyroid H &E stain ( under 40x)

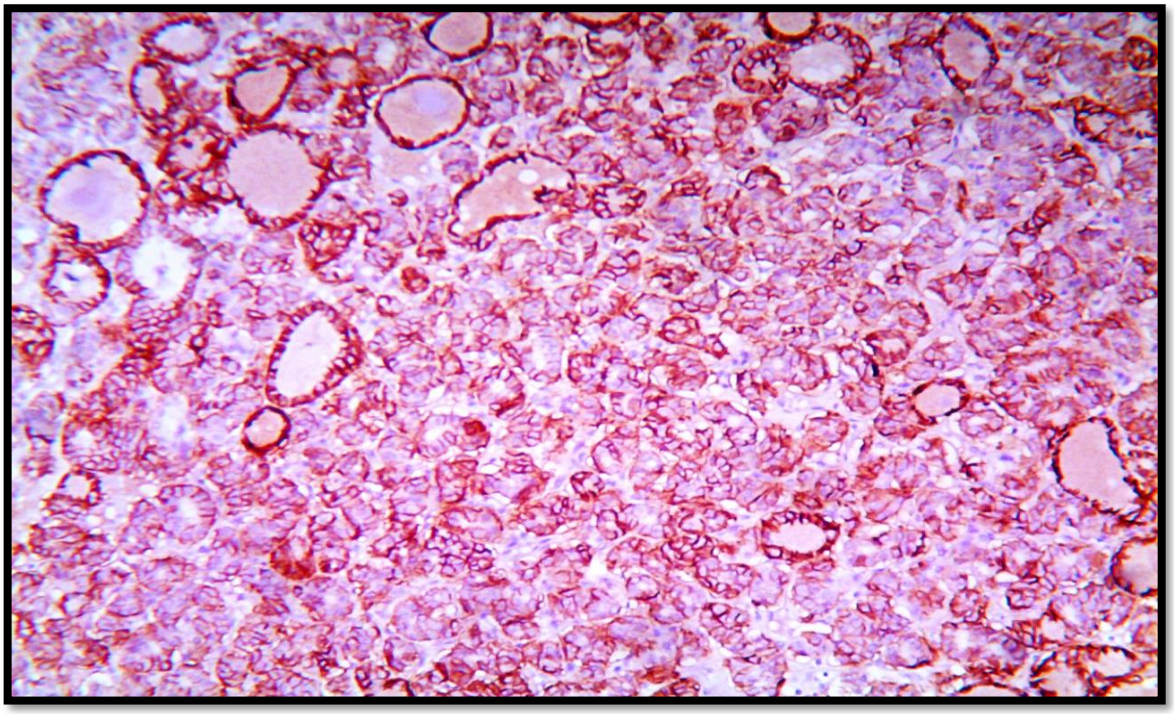


**Figure 2: CD56 expression Follicular adenoma of thyroid shows 3+ membraneous positivity ( under 10x)**

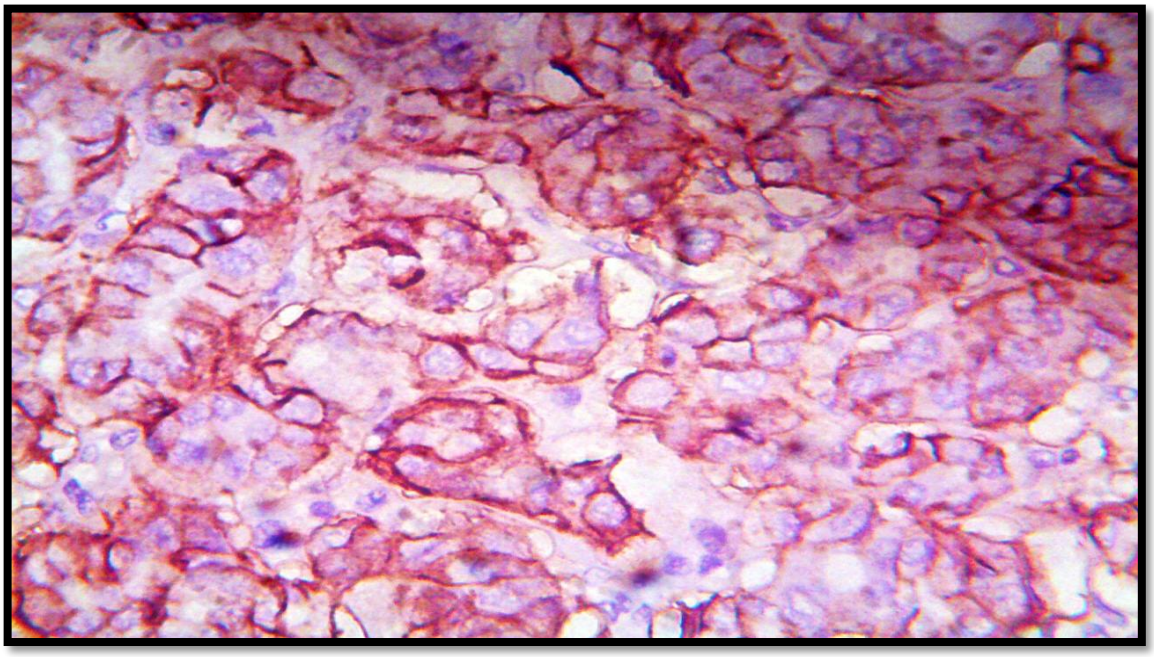


**Figure 3:**CD56 expression in Follicular adenoma of thyroid shows membranous positivity (3+) under (40x)

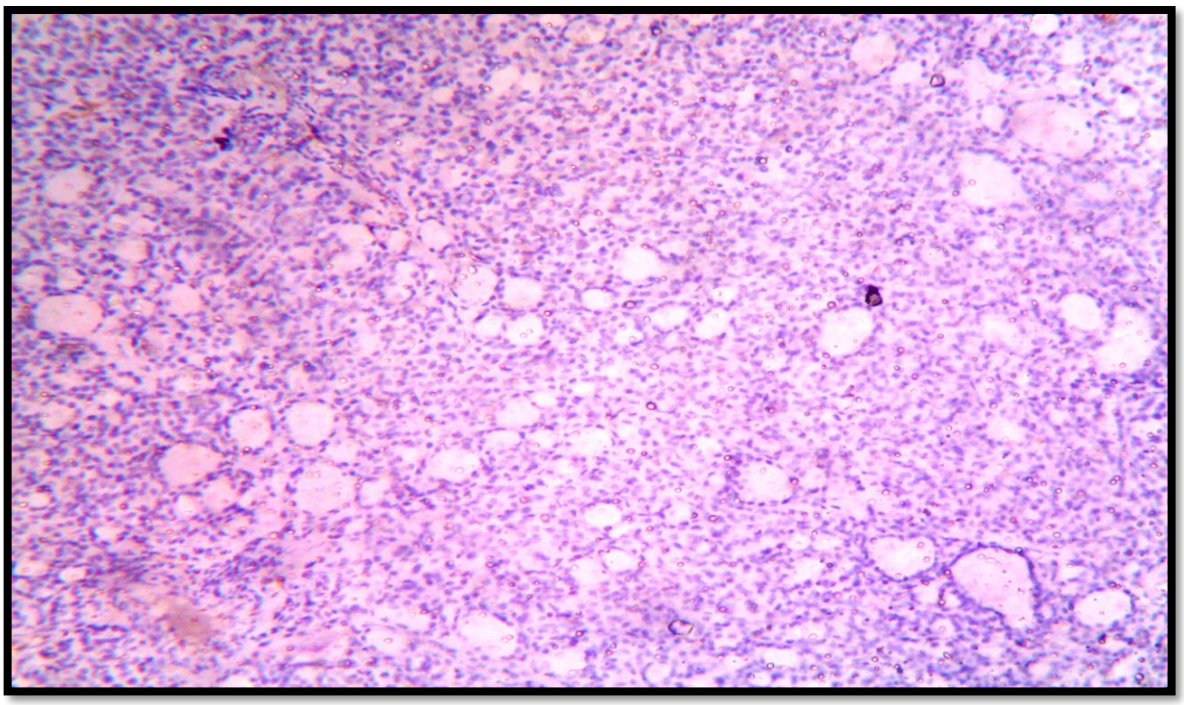


**Figure 4:** GALECTIN 3 shows negative expression in Follicular adenoma of thyroid under 10x

**HYALINISING TRABECULAR ADENOMA**

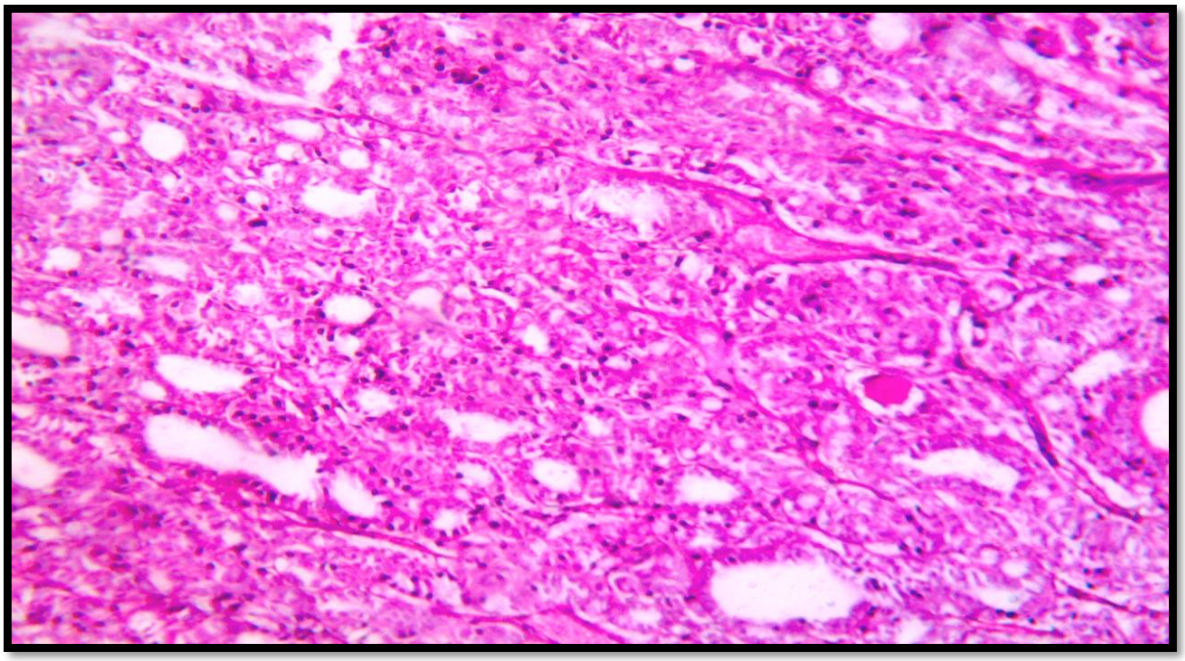


**Figure 5:** Hyalinising Trabecular Adenoma H&E Stain under(x10)

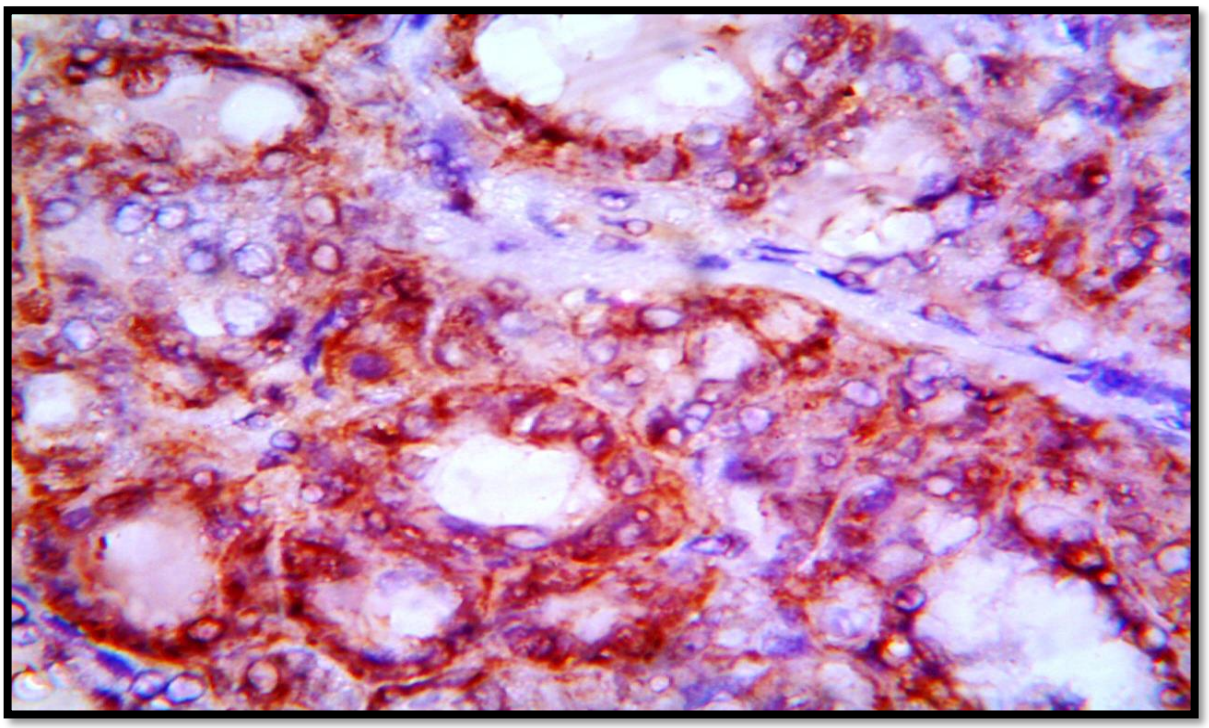


**Figure 6:** Hyalinising Trabecular Adenoma shows CD56 membranous positivity (3+) under( x40)

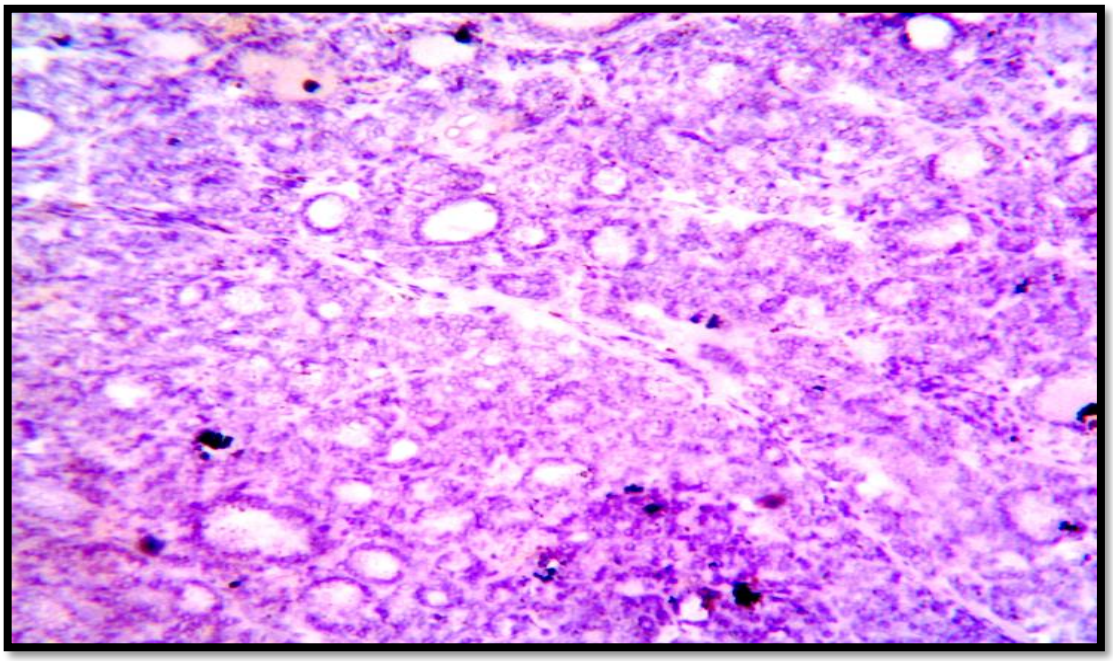


**Figure 7:**Hyalinising Trabecular Adenoma shows Galectin - 3 Negativity under( 40x)

## FOLLICULAR THYROID CARCINOMA

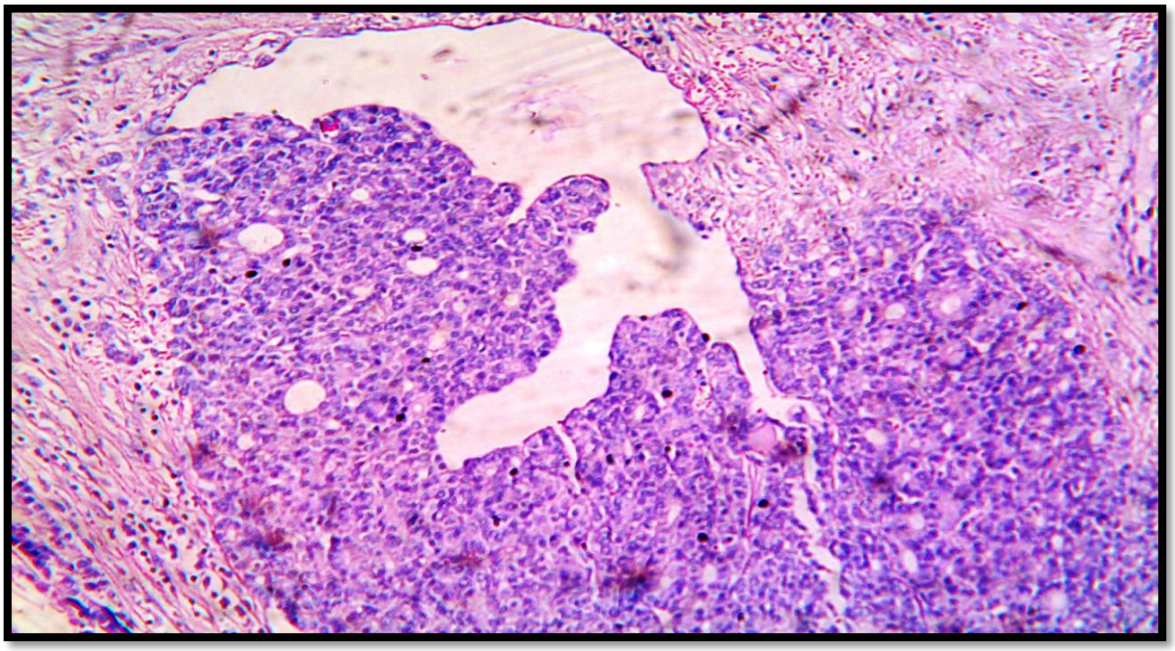


**Figure 8:** Follicular Thyroid Carcinoma with angioinvasion , H&E stain under ( 40x)

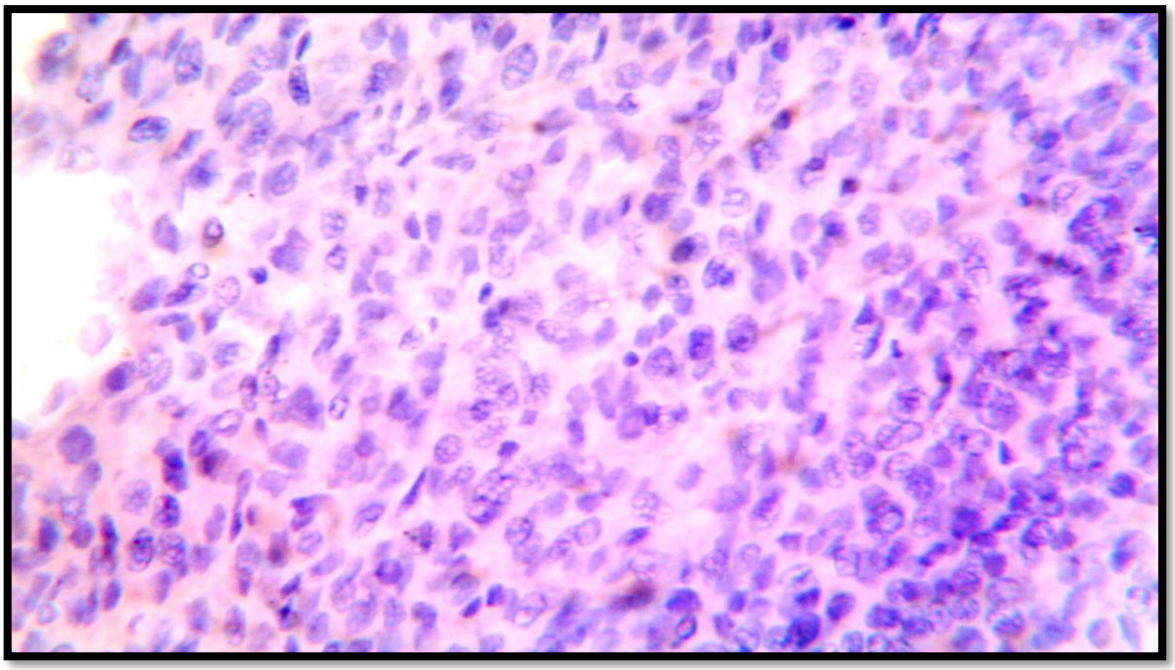


**Figure 9:** CD56 shows negative expression in Follicular Carcinoma of Thyroid,under( 10x)

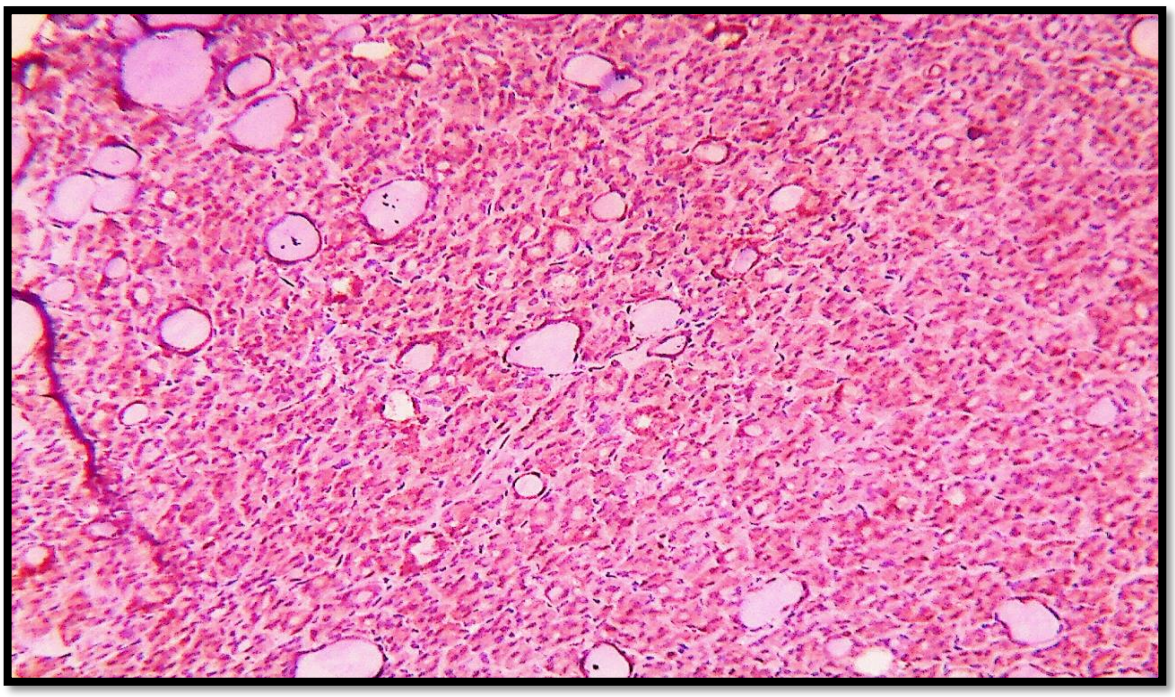


**Figure 10: GALECTIN** 3 expression in Follicular Thyroid Carcinoma shows cytoplasmic positivity( 3+) under 10x

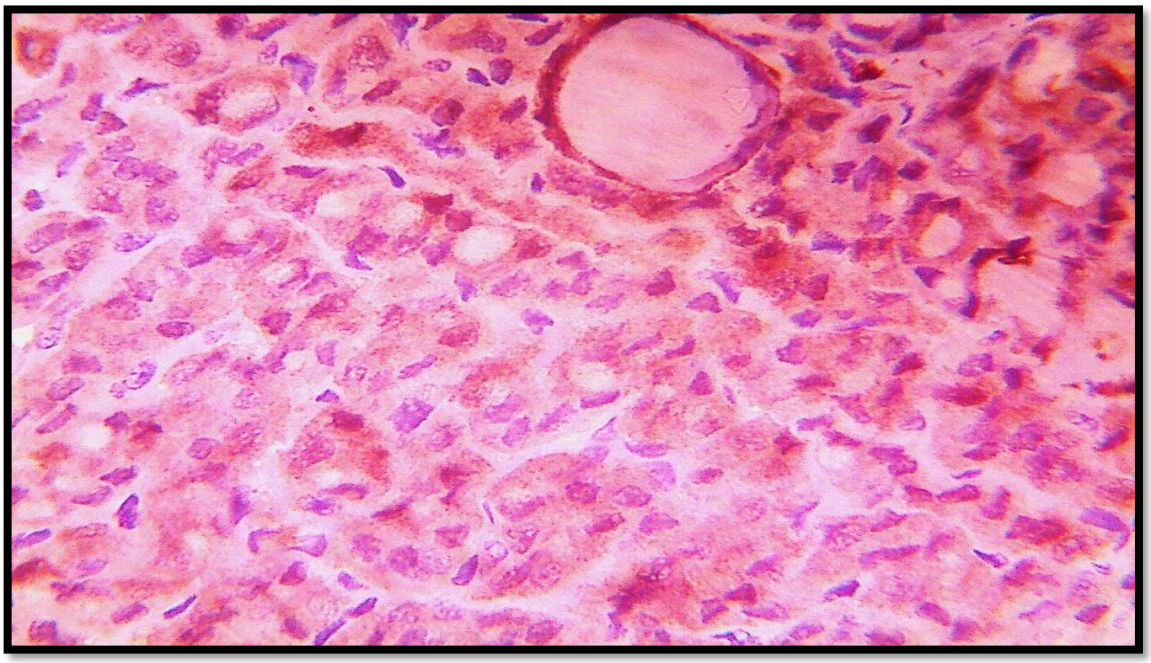


**Figure 11 :** GALECTIN 3 expression in Follicular Thyroid Carcinoma shows cytoplasmic positivity( 3+) under (40x)

## HURTHLE CELL CARCINOMA

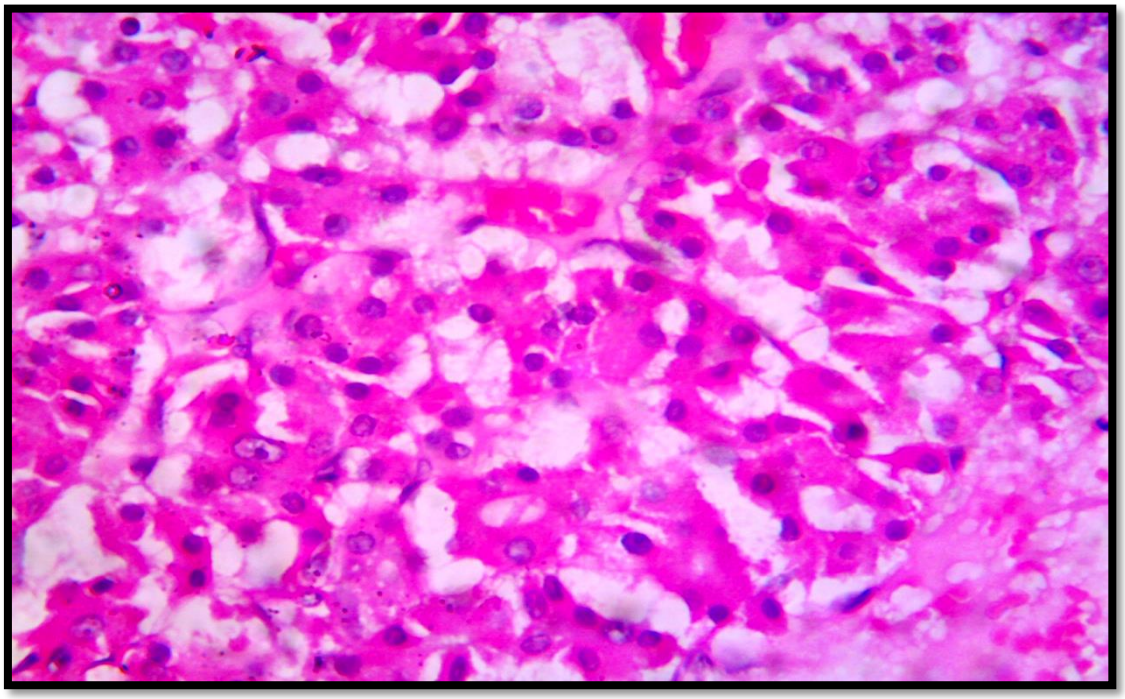


**Figure 12 :** HURTHLE CELL CARCINOMA H&E STAIN UNDER ( 10x)

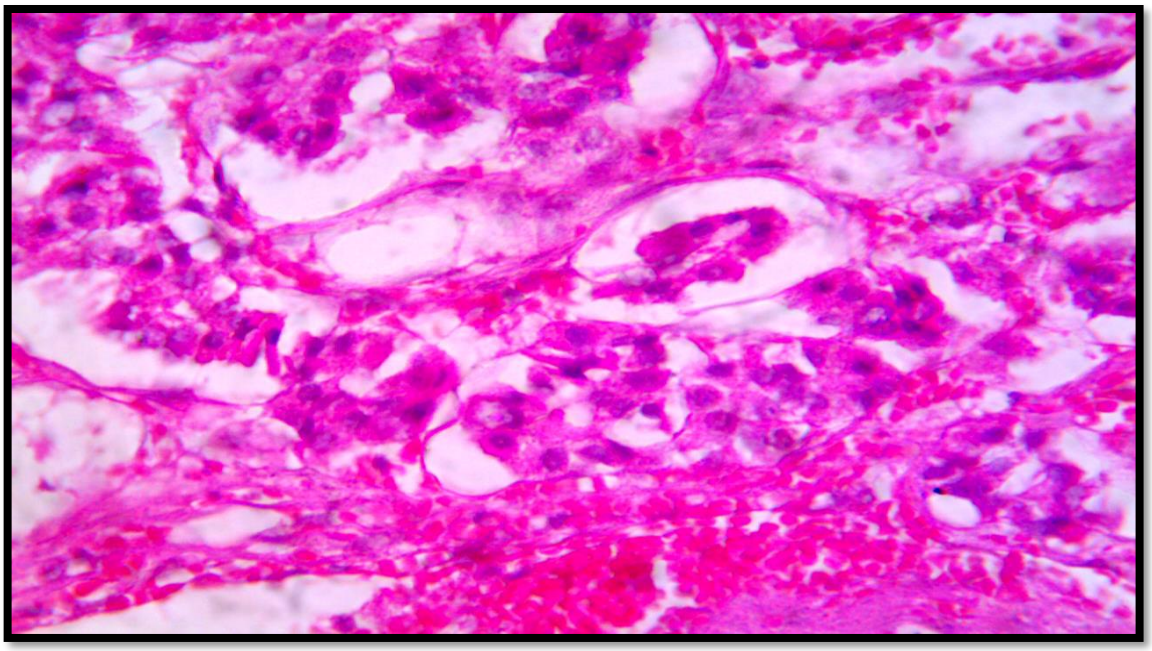


**Figure 13 :** HURTHLE CELL CARCINOMA H&E STAIN UNDER ( 40 x)

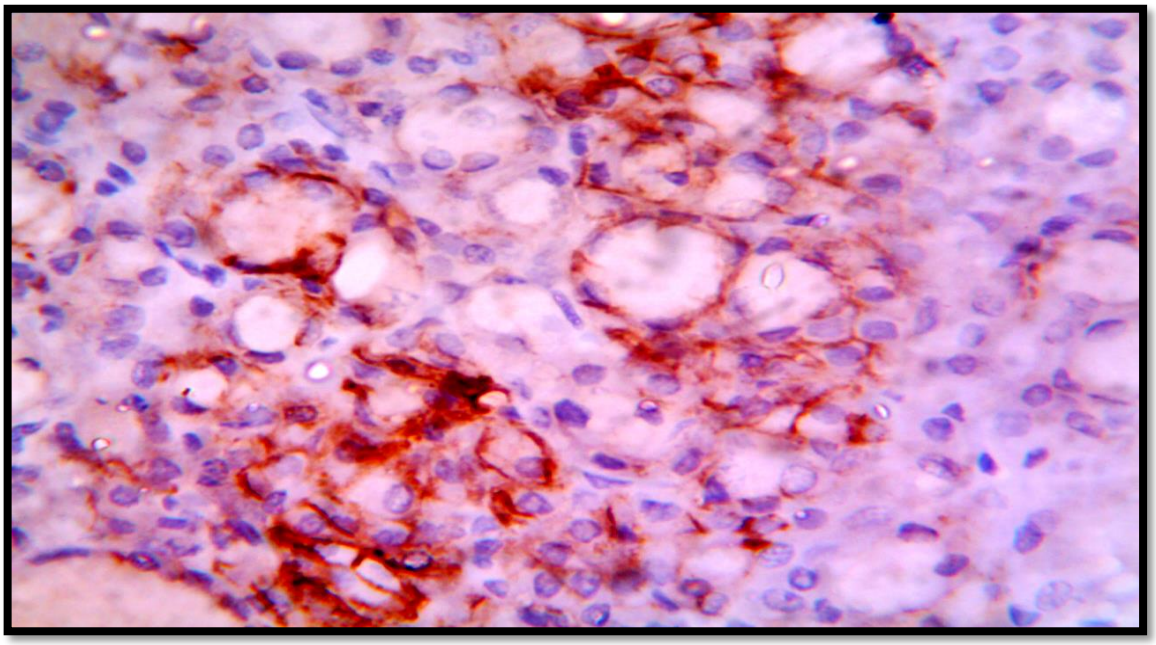


**Figure 14: CD** 56 expression in Hurthle Cell Carcinoma of thyroid - Shows membranous positivity ( 2+) under (40x)

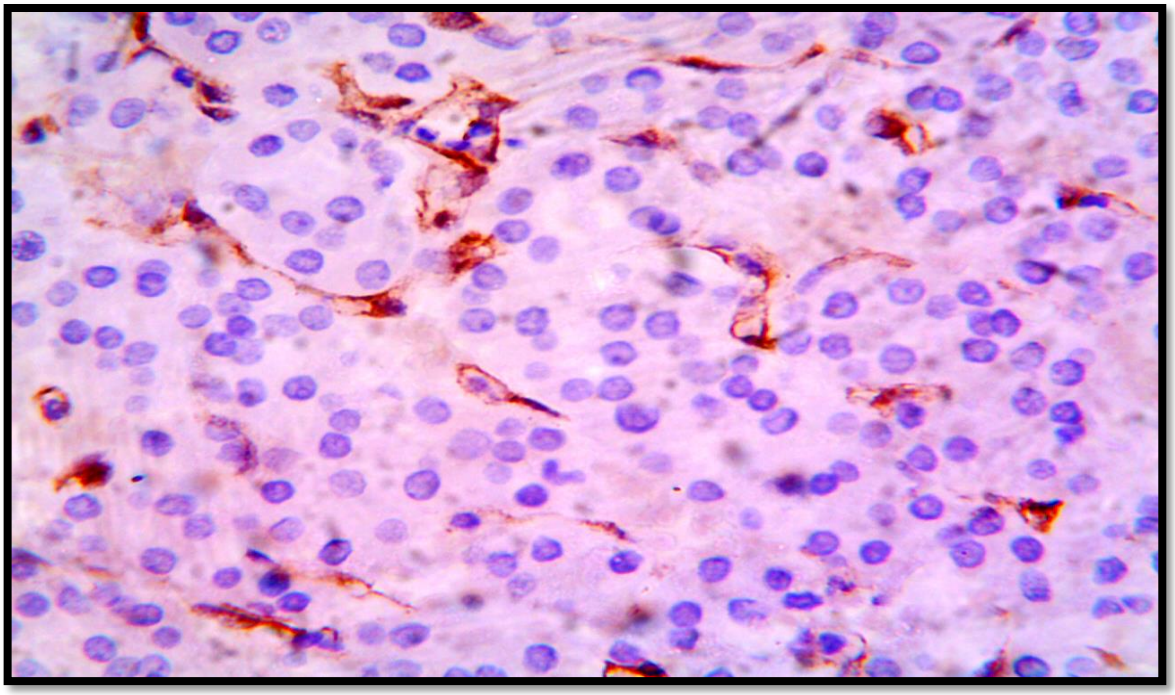


**Figure 15 :** Galectin 3 shows negative expression in Hurthle cell carcinoma under (40x)

# 7. DISCUSSION

Thyroid tumors represent a wide spectrum of tumors with different biological behaviours. The majority of the tumors can be easily diagnosed by distinctive histopathological features, but the separation between follicular thyroid adenoma and follicular thyroid carcinoma (particularly minimally invasive type) is very difficult. The difference between follicular adenoma of thyroid and minimally invasive follicular carcinoma of thyroid warrants histological demonstration of full thickness capsular permeation and/or vessel invasion by the tumor thrombus . The important finding which distinguishes benign tumor from malignant follicular thyroid tumor is the presence or absence of unequivocal capsular and/or vascular invasion .But identification of this finding is very challenging due to incomplete capsular penetration, or due to some technical difficulties. For these reasons investigators have focused on finding IHC markers that are differentially expressed in benign versus malignant follicular tumors.

In this study we analysed the expression of CD56 and GALECTIN 3 between follicular adenoma and follicular carcinoma of thyroid

CD56 is a neural adhesion molecule (NCAM) which is an antigen related to the follicular epithelial differentiation. Many previous studies revealed CD56 expression was high in normal thyroid follicular cells and benign follicular lesions as follicular adenomas and nodular hyperplasias .Reduced or loss of expression of CD56 is interrelated with tumor progression of patients. In the

cases of papillary thyroid carcinoma, follicular thyroid carcinoma and anaplastic thyroid carcinoma expression of CD56 is reduced,or totally lost.

**CD56 EXPRESSION IN PREVIOUS STUDIES**

Shin et al. reported that 100% of Follicular adenoma and 58.3% of Hurthle cell adenoma cases showed immunopositivity for CD56.[127] Park et al. [128] found that 93.3% of Follicular adenoma (FA) and 90.5% of Hurthle cell adenoma( HN) cases cases were positive for CD56.

Abd El Atti and Shash [129] reported that 91.7% of Follicular adenoma (FA and 87.5% of cases of Hurthle cell adenoma (HN) and cases showed CD56 expression. The studies of both El Demellawy [130] et al. and[131]et al. mentioned that expression of CD56 was found 100% in the studied cases of Hurthle cell adenoma (HN) and Follicular adenoma.

Using CD56 as a negative marker, the sensitivity and the specificity of CD56 were very impressive in distinguishing Follicular Thyroid Carcinoma from Follicular adenoma and also in distinguishing papillary carcinoma - Follicular Variant (FVPC) from Follicular adenoma of thyroid(FA). Conversely, Dina et al agreed with the results, that the highest specificity was attained in differentiating Follicular Thyroid Carcinoma( FTC ) from Follicular Tumor with Undetermined Malignant Potential( FT-UMP) [130]

**CD56 EXPRESSION IN PRESENT STUDY**

In agreement with those studies, this study reported a high positive expression of CD56 in follicular adenoma cases compared to follicular thyroid carcinoma cases.

In this present study CD56 showed positive membranous expression in (96.6% )32 out of 33 benign (n=31 , follicular adenoma and n=1, HTA) cases with sensitivity of 97% and specificity of 83% , with Positive Predictive Value (PPV) 94% and Negative Predictive Value( NPV) 91% in diagnosing benign tumors. CD56 expression is only 16.6% (n=2 out of 12) in Follicular Thyroid Carcinoma (FTC) cases. But CD56 distinguished Follicular Adenoma (FA) from Follicular Thyroid Carcinoma (FTC) with significant difference (p value is < 0.0001).

CD56 expression is absent in 83.3% (10 out of 12) of Follicular Thyroid Carcinoma cases with sensitivity of 83% and specificity of 97%, with Positive and Negative Predictive Values (PPV and NPV) are 91% and 94% respectively in diagnosing malignant tumors and its p value is less than 0.0001.

The results of our study showed that CD56 had lost its expression or expressed less in malignant tumors than benign tumors. In follicular thyroid carcinoma the specificity of CD56 (as a negative marker) was highly remarkable in distinguishing benign from malignant thyroid tumors(Follicular adenoma from Follicular Carcinoma).

Variation among studies in respect to sensitivity and specificity of CD56was substantial [96,128,129] Sensitivity for carcinoma varied from 58 to 100 % with a median value of 84 % (recalculated average value for all studies is 80 %). Specificity values ranged from 46 to 100 %, with a median value of 92 %.

Galectin-3 is a [beta]-galactoside-binding lectin with a molecular weight of 31 KD which plays a significant role in a number of biologic processes. It has

a role in regulating cell-cell and cell-matrix interaction, adhesion, migration, and damaged cell repair. It has a role in inflammation and neoplastic transformation. This marker has been implicated in regulation of normal cellular proliferation ,apoptosis, malignant transformation, and the metastasis of cancer cells.

**GALECTIN 3 EXPRESSION IN PREVIOUS STUDIES**

Many researchers have found that galectin-3 is useful in discriminating benign from malignant thyroid lesions.[132,133]

Saleh et al [89]have noticed the results recording that galectin-3 showed 85% sensitivity for immunohistochemical separation between carcinomas and benign nodules (positive in 27.5 % of benign vs. 85.1% of malignant nodules). However, the specificity was only 72.4%. They also observed that benign follicular lesions (both neoplastic and nonneoplastic) showed lower expression than malignant tumors.

Kovacs et al found that cytoplasmic expression of Galectin-3 is an useful marker in the differential diagnosis of solitary encapsulated follicular thyroid tumors, especially the minimally invasive follicular carcinoma of thyroid [134]. Galectin-3 can aid in distinguishing minimally invasive follicular carcinoma from follicular adenoma.

Gasbarri et al. [103] in his study observed that galectin-3 is never expressed in benign thyroid lesions.

Saggiorato et al. [86] observed only 4 out of 52 cases of follicular adenoma cases showing Galectin -3 immunopositivity. Whereas investigators analyzed that Galectin 3 was positive in all other thyroid cancers .

In the same way, Orlandi et al[135] reported that,even though all the thyroid cancers that they analyzed were Galectin -3immunopositive, only 3/29 FA exhibited such positivity.Some authors considered true Galectin -3-positive Follicular adenoma as an indication of potentially early or incipient carcinoma, in which the capsular and/or vascular invasion not been observed yet histologically. [134]

In fact,it is possible that Galectin-3 nuclear localization is related only to cellular proliferation, whereas its cytoplasmic accumulation would represent the a sign of malignant cell transformation. This observation showed agreement with other studies .([136]

In this background, the presence of Galectin- 3 positive follicular adenomas should be considered as an a marker of malignant transformation. As this lectin is early expressed during the process of malignant transformation, it could play a main role in cell growth, cell cycle, and antiapoptotic mechanisms [86]. Some authors reported Galectin-3 as a very sensitive marker of follicular malignancy [137]. Beesley et al. found this lectin expression in all follicular thyroid carcinomas , in 1 out of 20 adenomas, and in three out of eight nodular goiters. [6]

Aratake et al. found galectin-3 expression in five out of six follicular thyroid carcinomas and in two out of 14 follicular adenomas [138]. Similarly, Nascimento et al. reported galectin-3 positivity in 11 out of 14 carcinomas and 1 out of 9 adenomas [139].

The reason for the contradictory results was explained by Park et al [140] who explained the discrepancies in the frequency of galectin-3 immunoreactivity

in benign lesions .He stated that this controversy may be due to the different antibody detection systems and the cutoff values for positive and negative staining.

In addition, Herrmann et al have found that galectin-3 was also expressed focally in reactive follicular epithelial cells and in entrapped follicles in chronic lymphocytic thyroiditis. If a biotin-detection system used in galectin 3 immunostaining, variety of thyroid lesions might show prominent endogenous biotin like activity, which could cause bias in interpretation[93]Finally, they concluded that for galectin-3 immunostaining, biotin-free detection systems could be useful. Galectin -3 was also expressed in places where the follicular cells were in large number in a highly inflamed area and both in cells without cytological atypia or with hurthle-cell transformation. The most likely explanation for the galectin-3 expression of non-neoplastic follicular cells in an inflamed area may be due to the cytokines secreted by inflammatory cells .[28]

According to this explanation, making the distinction between follicular adenomas and carcinomas based on galectin 3 positivity only may be very dangerous. So the combination of galectin 3 with other markers increases the sensitivity and specificity in deciding whether the lesion is benign or malignant.

The best combination of immunohistochemical markers for detecting malignancy were CD56 with Galectin 3 and Cytokeratin -19 with Galectin-3 respectively. In accordance with the previous studies ,CD56 and Galectin -3 was the best combination in distinguishing follicular carcinoma from follicular adenoma of thyroid.

## GALECTIN 3 EXPRESSION IN PRESENT STUDY

In present study galectin 3 showed positive cytoplasmic expression in 83.3 %(10 out of 12) FTC cases and in FA its expression is only 3.03% (1 out of 33) cases with sensitivity of 83% and specificity of 97%, with PPV and NPV are 91% and 94% respectively in diagnosing malignant tumors. CD56 expression is only 16.6% (n=2 out of 12) in FTC (n=1 MIFC ,n=1 HCC) cases. Galectin 3 distinguished follicular adenoma from follicular carcinoma with significant difference( p value is < 0.0001). Hence in our study Galectin 3 expression is higher in malignant (FTC) tumors than in benign( FA)tumors.

The results in this study clearly explained the cytoplasmic expression of Galectin-3 made significant difference between follicular thyroid adenoma and follicular carcinoma minimally invasive type.

In this study galectin-3 was specifically expressed by malignant thyroid tumors, showing that this molecule could be used as an adequate marker for malignant thyroid follicular cells.

In diagnosing follicular thyroid carcinoma,the sensitive marker in our study was Galectin 3, and the specific marker was CD56. When we combined two markers, we did not reach 100%specificity for malignancy, on the other hand values were increased when compared to expression of single marker.

# 8. SUMMARY

This study was conducted in the Department Of Pathology, Tirunelveli Medical College .The slides were prepared from the cases that were diagnosed as follicular adenoma and follicular carcinoma of thyroid**.** Totally out of 45 cases, 33 cases were benign (follicular adenoma)and 12 were malignant (follicular thyroid caarcinoma). Immunohistochemistry was performed using the markers CD56 and GALECTIN 3 for all the 45 cases (follicular adenoma and follicular carcinoma). Among them CD56 showed positive expression for 32 out of 33 cases of follicular adenoma and 2 out of 12 cases of follicular thyroid carcinoma .GALECTIN -3 showed positive expression for 10 out of 12 cases of Follicular Thyroid Carcinoma ,and 1 out of 33 cases of follicular adenoma.

In this study, high rates (96.6%) of diffuse, strong, positive staining for CD56 were observed in follicular adenoma, whereas there were high rates (83.3%) of CD56 negativity in follicular thyroid carcinoma. Consistent with this, CD56 was found to be the more specific marker for follicular thyroid carcinoma. Galectin-3 is also a reliable sensitive marker in distinguishing follicular adenomas from follicular thyroid carcinomas.

Furthermore, the present study demonstrated that when a positive marker(Galectin -3) was added to the (CD56 )negative marker, the sensitivity and specificity of the two markers CD56 and galectin-3, were increased (83% and 97%,). Hence in the differentiation of benign from malignant follicular thyroid tumors a panel of combination of two markers(CD56 and Galectin -3) achieved a more statistical significant difference with a *p* value of less than 0.0001.

In this study ,the marker showing higher sensitivity for follicular carcinoma of thyroid was GALECTIN- 3 (83%) and the marker with higher specificity was CD56 (97%) .

# 9. CONCLUSION

We conclude the following

1. GALECTIN -3 is a sensitive marker for Follicular Thyroid Carcinoma(FTC). (83%).
2. Loss of expression of CD56 is found to be very specific for Follicular Thyroid Carcinoma (97%).
3. A combination of the two markers CD56 and galectin 3 is found more specific for Follicular Carcinoma of Thyroid .
4. Immunoexpression of CD 56 and GALECTIN 3 are hence postulated to be important ancillary tests in the diagnosis of follicular thyroid neoplasms, albeit it does not replace the conventional histopathological examination.
5. Therefore, we conclude that a combination panel of CD56 and GALECTIN 3 is an useful tool to increase the chance of detecting Follicular Thyroid Carcinoma.

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## 11. ANNEXURE - II-MASTER CHART

| S. NO | PATH NO | AGE | SEX | IP NO | CLINICAL DIAGNOSIS | USG FINDINGS | SIZE OF LESION | T3(60-200) ng | T4(4-12.5) | TSH (0.3-5.5) mIu/ml | PREVIOUS FNAC REPORT | TYPE OF SURGERY | HPE REPORT | CAPSULAR INVASION | VASCULAR INVASION | CD56 | GALECTIN 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2515/14 | 37 | F | 42304 | multi Nodular goiter | right lobe nodular lesion 3x2cm ,left lobe tiny nodular lesion | 3.5 cm | 71 | 6.5 | 2 | Follicular Neoplasm | Total Thyroidectomy | Mixed Folicular Adenoma in a background of nodular Goiter | No | No | 3+ | Negative |
| 2 | 3641/14 | 46 | M | 65911 | Papillary carcinoma thyroid | 1X1cm nodule in right lobe with colloid areas | 1cm | 91 | 12 | 2.5 | Papillary carcinoma | Total Thyroidectomy | Micro Folicular Adenoma in a background of nodular Goiter | No | No | 2+ | Negative |
| 3 | 1170/14 | 34 | F | 30621 | Nontoxic multinodular goiter | bothlobes enlarged largest nodule 2.5x2cm | 2.5cm | 210 | 16 | 0.2 | nodular goitre | Total Thyroidectomy | Micro Folicular Adenoma in a background of hashimotos thyroiditis and features of toxic goitre | no | no | negative | 2+ |
| 4 | 1624/14 | 42 | F | 29022 | Solitary nodule left lobe of thyroid | Solitary nodular lesion 4x3cm left lobe | 4.5 | 2O4 | 15 | O.1 | Colloid Goiter | Total Thyroidectomy | Folicular Adenoma in a background of Adenomatous Hyperplasia | No | No | 2+ | Negative |
| 5 | 3442/14 | 37 | F | 61707 | multi Nodular goiter | both lobes enlarged with multiple nodules | 3.5cm | 61 | 5.6 | 4.5 | Colloid Goiter | Total Thyroidectomy | Folicular Adenoma in a background of nodular Goiter | No | No | 1+ | Negative |
| 6 | 679/14 | 35 | F | 2343 | solitary nodule right lobe of thyroid | Solitary nodular lesion 3x3cm right lobe of thyroid | 3cm | 79 | 6.4 | 2 | Follicular Neoplasam | Hemithyroidectomy | Folicular Adenoma thyroid | No | No | 2+ | Negative |
| 7 | 3438/14 | 40 | M | 62522 | solitary nodule right lobe of thyroid | right lobe nodular lesion 2x2cm ,left lobe normal | 2cm | 21 | 2.5 | 6 | Nodular Goiter | Hemithyroidectomy | Micro Folicular Adenoma in a background of nodular Goiter | No | No | 3+ | Negative |
| 8 | 444/14 | 20 | F | 4131 | colloid goitre | multiple nodules with both lobes enlarged largest nodule 3.5x2cm | 3.5cm | 256 | 21 | 0.2 | Nodular Goiter | totalthyroidectomy | Follicular Adenoma in a background of Colloid Goiter | No | No | 2+ | Negative |

| S. NO | PATH NO | AGE | SEX | IP NO | CLINICAL DIAGNOSIS | USG FINDINGS | SIZE OF LESION | T3(60-200) ng | T4(4-12.5) | TSH (0.3-5.5) mIu/ml | PREVIOUS FNAC REPORT | TYPE OF SURGERY | HPE REPORT | CAPSULAR INVASION | VASCULAR INVASION | CD56 | GALECTIN 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 1164/14 | 35 | F | 20482 | Multinodular goitre | both lobes enlarged with multiple nodules | 3,8cm | 88 | 7.8 | 3.8 | Follicular Neoplasm | totalthyroidectomy | widely invasive follicular carcinoma | yes | Yes | negative | 3+ |
| 10 | 87/14 | 30 | F | 1145 | Solitary nodule left lobe of thyroid | largest solitary nodule 5x3.5cm left lobe of thyroid | 5cm | 76 | 8.4 | 2.5 | Nodular Goiter | Hemithyroidectomy | Minimally invasive Follicular Carcinoma | Yes | No | negative | 3+ |
| 11 | 2995/14 | 54 | F | 51551 | Papillary carcinoma thyroid | multiple nodules with altered echoes with foci of cystic degeneration | 3cm | 16 | 1.5 | 12 | Follicular Neoplasam | totalthyroidectomy | Widely invasive Follicular Carcinoma Thyroid. | Yes | Yes | negative | 2+ |
| 12 | 1416/14 | 58 | M | 32556 | follicular carcinoma thyroid | both lobes enlarged with nodules altered echoes | 4.5cm | 70 | 3.8 | 3 | Follicular Neoplasam | totalthyroidectomy | minimally invasive Follicular Carcinoma Thyroid. | Yes | no | negative | 2+ |
| 13 | 782/14 | 30 | F | 17147 | multinodular goitre | both lobes enlarged with multiple nodules | 6cm | 90 | 6.7 | 3.5 | Follicular Neoplasam | totalthyroidectomy | minimally invasive Follicular Carcinoma Thyroid. | no | yes | negative | 1+ |
| 14 | 4050/15 | 44 | F | 73997 | colloid goitre | both lobes enlarged with multiple nodules largest 2.5x2cm | 2cm | 100 | 3.5 | 2.6 | Colloid Goiter | Hemithyroidectomy | Folicular Adenoma in a background of nodular Goiter | No | No | 2+ | Negative |
| 15 | 4014/15 | 28 | M | 74953 | Solitary nodule left lobe of thyroid | Solitary nodular lesion 3x3cm right lobe of thyroid | 3cm | 120 | 7.5 | 2.5 | Nodular Goiter | totalthyroidectomy | Mixed Folicular Adenoma with a cystic degeneration | No | No | 2+ | Negative |
| 16 | 3024/15 | 31 | F | 52858 | multinodular goitre | nodular goire with cystic degeneration | 5cm | 78 | 6.4 | 3.5 | Nodular Goiter with Cystic degeneration | totalthyroidectomy | hyalinising trabecular adenoma | No | No | 3+ | Negative |

| S. NO | PATH NO | AGE | SEX | IP NO | CLINICAL DIAGNOSIS | USG FINDINGS | SIZE OF LESION | T3(60-200) ng | T4(4-12.5) | TSH (0.3-5.5) mIu/ml | PREVIOUS FNAC REPORT | TYPE OF SURGERY | HPE REPORT | CAPSULAR INVASION | VASCULAR INVASION | CD56 | GALECTIN 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 2483/15 | 36 | F | 45615 | non toxic goitre | nodular colloid goitre | 3cm | 68 | 8.4 | 2.5 | Nodular Goiter | totalthyroidectomy | Folicular Adenoma with a cystic degeneration | No | No | 3+ | Negative |
| 18 | 4021/15 | 45 | F | 73663 | multinodular goitre | right lobe thyroid nodule ,possibility of mixed adenoma | 2cm | 44 | 3.3 | 20 | Follicular Neoplasam | totalthyroidectomy | Folicular Adenoma in a background of nodular Goiter | No | No | 3+ | Negative |
| 19 | 613/15 | 42 | F | 14792 | diffuse goitre | diffuse thyromegaly | 3.5cm | 92 | 6.8 | 2 | Nodular Goiter | Hemithyroidectomy | Folicular Adenoma | No | No | 3+ | Negative |
| 20 | 4054/15 | 45 | M | 73887 | multinodular goitre | enlarged with right lobe 5x3cm associated with areas of degeneration and | 5cm | 65 | 9.7 | 6 | Follicular Neoplasm | totalthyroidectomy | Minimally invasive Folicular Carcinoma | one foci + | No | 1+ | Negative |
| 21 | 3857/15 | 65 | F | 72046 | diffuse goitre | diffuse thyromegaly | 3.5cm | 77 | 5.8 | 1.5 | Follicular Neoplasm | totalthyroidectomy | Minimally invasive Folicular Carcinoma | yes | No | 2+ | Negative |
| 22 | 2649/16 | 35 | F | 48470 | adenamatous nodule | nodular goire with cystic degeneration | 3cm | 99 | 6.9 | 2 | Nodular Goiter with Cystic degeneration | totalthyroidectomy | Mixed Folicular Adenoma in a background of Colloid Goiter | No | No | 2+ | Negative |
| 23 | 2722/16 | 53 | F | 48925 | solitary nodule right lobe of thyroid | Solitary nodular lesion 4x3cm right lobe of thyroid | 4.2cm | 87 | 11 | 1.5 | Nodular Goiter | totalthyroidectomy | Folicular Adenoma | No | No | 3+ | Negative |
| 24 | 1950/16 | 40 | F | 41075 | nodular goitre | largest nodule right lobe3x3cm | 3cm | 69 | 6 | 3 | Follicular Neoplasm | Hemithyroidectomy | Folicular Adenoma | No | No | 2+ | Negative |
| 25 | 766/16 | 24 | f | 1043 | diffuse goitre | diffuse thyromegaly | 3.5cm | 80 | 5.9 | 1.6 | Follicular Neoplasm | Hemithyroidectomy | Folicular Adenoma | no | no | 2+ | Negative |
| 26 | 920/16 | 28 | F | 16475 | diffuse goitre | diffuse thyromegaly | 2cm | 78 | 5.4 | 2.8 | Follicular Neoplasm | Hemithyroidectomy | Micro Folicular Adenoma | No | No | 3+ | Negative |

| S. NO | PATH NO | AGE | SEX | IP NO | CLINICAL DIAGNOSIS | USG FINDINGS | SIZE OF LESION | T3(60-200) ng | T4(4-12.5) | TSH (0.3-5.5) mIu/ml | PREVIOUS FNAC REPORT | TYPE OF SURGERY | HPE REPORT | CAPSULAR INVASION | VASCULAR INVASION | CD56 | GALECTIN 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 1149/16 | 48 | F | 22589 | multinodular goitre | both lobes enlarged ,largest nodule right lobe3cm | 3.5cm | 83 | 9.5 | 2.5 | Nodular Goiter | Hemithyroidectomy | Micro Folicular Adenoma | No | No | 2+ | Negative |
| 28 | 1871/16 | 63 | F | 34604 | follicular adenoma thyroid | right lobe follicular adenoma | 3cm | 66 | 7.4 | 1.2 | Follicular Neoplasm | totalthyroidectomy | Folicular Adenoma in a background of Nodular Goiter | No | No | 2+ | Negative |
| 29 | 1287/16 | 28 | m | 23012 | solitary nodule right lobe of thyroid | solitary nodule right lobe | 2cm | 76 | 8.3 | 3 | nodular goiter | Hemithyroidectomy | follicular adenoma right lobe of thyroid | no | No | 3+ | Negative |
| 30 | 2477/16 | 43 | f | 43466 | multinodular goitre | large mixed echoeic lesion right lobe left lobe tiny nodule | 6cm | 90 | 5.6 | 2.4 | Follicular Neoplasam | totalthyroidectomy | Widely invasive Follicular Carcinoma Thyroid. | yes | yes | negative | 2+ |
| 31 | 2831/16 | 42 | f | 49841 | follicular carcinoma thyroid | both lobes enlarged with altered echoes | 2.5cm | 73 | 6.8 | 1.8 | Follicular Neoplasm | totalthyroidectomy | Minimally invasive follicular carcinoma thyroid | Yes | No | negative | 2+ |
| 32 | 2005/16 | 35 | F | 42861 | solitary nodule thyroid | solitary nodule right lobe | 5cm | 66 | 7.2 | 2.6 | Follicular Neoplasm | Hemithyroidectomy | Widely invasive Follicular Carcinoma Thyroid. | Yes | Yes | negative | 3+ |
| 33 | 3886/16 | 70 | F | 70941 | multinodular goitre | hperechoeic nodule right lobe,colloid nodule left lobe | 3.5cm | 43 | 3.8 | 8 | Nodular Goiter with Cystic degeneration | Hemithyroidectomy | Hurthle cell carcinoma | Yes | Yes | 2+ | Negative |
| 34 | 3925/16 | 30 | F | 71751 | multinodular goitre | both lobes multiple nodules ,largest nodule in right lobe 5.5 x3cm | 5cm | 55 | 3.4 | 5.8 | Hashimotos thyroiditis | totalthyroidectomy | Minimally invasive Folicular Carcinoma with cystic degeneration | no | yes | negative | 1+ |
| 35 | 2/17 | 41 | M | 83393 | nodular goiter | multiple nodules | 3cm | 65 | 7.8 | 1.5 | Nodular Goiter | totalthyroidectomy | Folicular Adenoma in a background of Colloid Goiter | No | No | 3+ | Negative |

| S. NO | PATH NO | AGE | SEX | IP NO | CLINICAL DIAGNOSIS | USG FINDINGS | SIZE OF LESION | T3(60-200) ng | T4(4-12.5) | TSH (0.3-5.5) mIu/ml | PREVIOUS FNAC REPORT | TYPE OF SURGERY | HPE REPORT | CAPSULAR INVASION | VASCULAR INVASION | CD56 | GALECTIN 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 852/17 | 32 | F | 14462 | multinodular goitre | both lobes enlarged withnodular lesion with areas of degeneration | 3cm | 76 | 5.5 | 1 | Nodular Goiter | totalthyroidectomy | Micro Folicular Adenoma in a background of Nodular Goiter | No | No | 2+ | Negative |
| 37 | 853/17 | 33 | F | 14485 | adenamatous nodule | hyperechoeic nodule right lobe with colloid filled areas | 2cm | 89 | 6.5 | 1.2 | Adenomatous Goiter | Hemithyroidectomy | Micro Folicular Adenoma in a background of Colloid Goiter | No | No | 3+ | Negative |
| 38 | 1594/17 | 28 | F | 31130 | solitary nodule thyroid right lobe | solitary nodule right lobe | 1cm | 99 | 5.4 | 2.6 | Nodular Goiter | Hemithyroidectomy | Mixed Folicular Adenoma in a background of Colloid Goiter | No | No | 2+ | Negative |
| 39 | 1625/17 | 45 | F | 1E+05 | non toxic goitre nodular goiter | nodular goitre with cystic degeneration | 2cm | 76 | 5.8 | 1.3 | Nodular Goiter | Hemithyroidectomy | Folicular Adenoma Fetal type | No | No | 3+ | Negative |
| 40 | 1636/17 | 28 | F | 1E+05 | Solitary nodule left lobe of thyroid | left lobe adenomatous nodule | 2cm | 67 | 6 | 1.5 | Nodular Goiter | Hemithyroidectomy | Folicular Adenoma | No | No | 1+ | Negative |
| 41 | 1697/17 | 55 | M | 26953 | Papillary carcinoma thyroid | both lobes complex lesion close to carotid with foci of calcification | 2.5cm | 86 | 9.4 | 1.6 | Papillary carcinoma thyroid | totalthyroidectomy | Folicular Adenoma in a background of Nodular Goiter | No | No | 3+ | Negative |
| 42 | 1770/17 | 39 | F | 32902 | Solitary nodule left lobe of thyroid | left lobe nodular lesion 3x2cm | 3cm | 99 | 6.8 | 1.2 | Nodular Goiter | Hemithyroidectomy | Folicular Adenoma (micro and macro) | No | No | 2+ | Negative |
| 43 | 1899/17 | 44 | F | 36503 | Solitary nodule left lobe of thyroid | nodular goitre | 3.5cm | 78 | 5.5 | 2.6 | Nodular Goiter | Hemithyroidectomy | Mixed Folicular Adenoma | No | No | 2+ | Negative |
| 44 | 2284/17 | 25 | F | 41305 | Follicular neoplasm | solitary nodule right lobe | 3cm | 30 | 2.5 | 11 | Follicular Neoplasam | totalthyroidectomy | Micro Folicular Adenoma in a background of Graves disease | No | No | 3+ | Negative |
| 45 | 2331/17 | 29 | F | 43560 | Solitary nodule left lobe of thyroid | solitary nodule left lobe | 3cm | 66 | 7.8 | 3.5 | Follicular Neoplasam | totalthyroidectomy | Folicular Adenoma Fetal type | No | No | 3+ | Negative |