"EVALUATION OF DIAGNOSTIC SIGNIFICANCE OF TROPHOBLAST CELL SURFACE ANTIGEN-2 EXPRESSION IN THYROID NEOPLASMS- A STUDY IN TERTIARY CARE CENTRE OF BELAGAVI."

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DR. Ranjit Kangle MD,
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Date:
Place: Belagavi.

DR.(Mrs) N. S. Mahantashetti MD (Paed).
Principal
J. N. Medical College,
Belagavi, Karnataka.

Date:
Place: Belagavi.

PLAGIARISM ACCEPTANCE LETTER

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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

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Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BN0119006.
Postgraduate Student,
2019-20 Batch,
Department of Pathology,
J. N. Medical College, Belagavi

ABSTRACT

"EVALUATION OF DIAGNOSTIC SIGNIFICANCE OF TROPHOBLAST CELL SURFACE ANTIGEN-2 EXPRESSION IN THYROID NEOPLASMS- A STUDY IN TERTIARY CARE CENTRE OF BELAGAVI."

BACKGROUND:

Thyroid cancer is the commonest endocrine malignancy and incidence continues to increase worldwide from past few decases.[12,13]Although differentiation of benign and malignant lesions can be made with hematoxylin & eosin (H&E) stained histopathological slides, challenges are being faced when there is absence of characteristic nuclear features of papillary thyroid carcinoma(PTC), encapsulated follicular lesions with a suspicious capsular & vascular invasion, neoplasm of thyroid follicular cells with follicular pattern and nuclear features of PTC.[12,21,22] Hence immunohistochemistry(IHC) markers are helpful in definitive diagnosis of morphologically overlapping cases. Trophoblast cell surface antigen 2 is a novel IHC marker and stated to be more sensitive and specific in diagnosing PTC.

OBJECTIVE:

Primary objective:- To evaluate the diagnostic significance of TROP-2 expression in thyroid neoplasms.

Secondary objective:- To correlate clinical diagnosis with histopathological findings of thyroid lesions.

METHODOLOGY:

It is a retrospective and prospective study done in a tertiary care hospital. It includes 45 patients who have undergone partial or total thyroidectomy. All benign and malignant thyroid lesions along with multinodular goiter were included in the study . Slides were stained with IHC marker TROP-2 and then evaluated. The data was obtained using the SPSS software and regression and correlation analysis was done.

RESULTS:

The cases comprised of 41 females and 4 males with malignancy generally seen in 4th decade. Thyroid lesions are most commonly found in right lobe(46.1%) with localized

swelling(82.3%) as a most common chief complaint. Most of the malignant cases were having euthyroid profile(89.9%). Duration of symptom found to be less than a year in most of the cases(53.4%). Expression of TROP 2 in Classical variant of PTC showed 88.23% sensitivity, 100% specificity, 100% PPV and 88.88% NPV, with high overall specificity for PTC.

CONCLUSION:

The present study found that TROP 2 is a sensitive and specific marker in diagnosisof classical variant of PTC with high overall specificity for PTC. However, it is not much useful in distinguishing follicular variant of PTC from follicular neoplasms.

KEYWORDS:

Immunohistochemistry, TROP-2, Thyroid carcinoma

LIST OF ABBREVIATIONS USED

PTC	Papillary thyroid carcinoma
FTC	Follicular thyroid carcinoma
MTC	Medullary thyroid carcinoma
ATC	Anaplastic thyroid carcinoma
FV- PTC	Follicular variant of Papillary thyroid carcinoma
CV-PTC	Classical variant of Papillary thyroid carcinoma
FA	Follicular Adenoma
MNG	Multinodular goiter
TROP 2	Trophoblast cell surface antigen 2
Tacstd-2	Tumor associated calcium signal transducer-2
MMP2	matrix metalloproteinase 2
ERK	extracellular receptor kinase
JNK	c-Jun N-terminal kinase
HBME 1	Hector battifora mesothelial 1
TNM	Tumor size, Nodal status, Metastasis
STN	Solitary thyroid nodule
H&E	Haematoxylin and Eosin
IHC	Immunohistochemistry
LNM	Lymph Node Metastatsis
LVI	Lymphovascular Invasion
WHO	World Health Organisation
NPV	Negative predictive value
PPV	Positive predictive value
TFT	Thyroid Function test
MEN	Multiple endocrine neoplasia
FAP	Familial adenomatous polyposis
HPE	Histopathological examination
V/S	Versus

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INTRODUCTION

Thyroid cancer is the commonest endocrine malignancy and incidence continues to increase worldwide from past few decades.[12.13] Thyroid cancer incidence has risen in most developed countries since the 1990s, and has doubled. According to the cancer registrations in 2018, out of 18,078,957 new cases of overall cancers showed 9,555,027 mortalities, of which 567,223 new cases (3.31%) and 41,071 (0.46%) died due to thyroid cancer. In the Asia continent, highest thyroid cancer incidence along with the highest mortality rate was found which were 340,245 (60%) cases and 23,847 cases (58.1%) respectively. In worldwide survey the standardised incidence and mortality rate of thyroid cancer in 2012 were 4 and 0.5 per 100,000, respectively.[14]

Thyroid cancer is most commonly seen in younger age groups. Age at onset appears as a bell-shaped curve across the literatures, with the highest incidence in the second, third, and fourth decades of life, however, there has been a rise in the incidence of thyroid cancer during the fourth and fifth decades of life within the past 2 decades.[15] Thyroid cancer initially manifests as single or multiple thyroid nodules, lymphadenopathy around the neck and voice violence. Age, Gender (female), weight, obesity, height and body mass index, diabetes mellitus, exposure to ionising radiation, tumour size, pregnancy, lifestyle, weather conditions,[16,17] genetic factors, underlying thyroid disease, hormonal factors (more prevalence in female) and nutritional factors(especially iodine), use of levothyroxine play an important role in thyroid cancer pathogenesis.[18.19] The age-adjusted incidence rates of thyroid cancer is 1/100,000 for men and 1.8/100000 for females as per the Mumbai Cancer Registry.[20]

Several pathological lesions affect thyroid gland which can be divided into two major groups-

a) Thyroid lesions showing diffuse pattern e.g. Hyperplastic lesion and Thyroiditis.

b) Thyroid lesions producing nodules- includes disorders producing a clinical nodule and consist of non neoplastic hyperplasia as well as benign and malignant tumors. Of all malignant cases, incidence of papillary thyroid carcinoma(PTC) is >85%, follicular thyroid carcinoma(FTC) is 5-10%, medullary thyroid carcinoma(MTC) is nearly 5% and anaplastic thyroid carcinoma(ATC) is <5%.[6]

The diagnosis of thyroid tumor on H and E stained slide becomes challenging when there is absence of characteristic nuclear features of PTC, When there is an encapsulated follicular lesions with a suspicious capsular & vascular invasion and neoplasm of thyroid follicular cells, with follicular pattern and nuclear features of PTC.[12,21,22]

Hence immunohistochemistry(IHC) markers are helpful in definitive diagnosis of morphologically overlapping cases. [12] None of the routinely used IHC markers including Hector battifora mesothelial-1 (HBME 1), galectin-3, Cytokeratin-19 give 100% differential diagnosis as they are expressed in both benign and malignant lesions.[12] However, use of these markers have several difficulties, such as low specificity, variability of sensitivity and appearance of background staining . Thus we need highly sensitive and specific marker to differentiate benign from malignant lesions and to improve the diagnosis of PTC.

Trophoblast cell surface antigen-2 (TROP-2) is a type 1 transmembranous glycoprotein encoded by tumor associated calcium signal transducer-2(Tacstd-2) located

on chromosome 1p32.[12,13,22] It was 1st identified in human trophoblast and choriocarcinoma cell lines.[12,22] Later it was found to be overexpressed in colorectal, breast, gastric, endometrial, ovarian cancers and very rarely in normal tissue.[21,22] Molecular profiling study of thyroid tumors found that TROP-2 is overexpressed in thyroid malignancy and enhances invasion by inducing matrix metalloproteinase 2 (MMP2) through extracellular receptor kinase(ERK) and c-Jun N-terminal kinase pathway(JNK).[13]

Recently conducted studies have shown expression of TROP-2 in thyroid malignancy. TROP-2 is useful in differentiating benign and malignant thyroid lesions in both cytological and surgical specimens.[22,102] They have concluded high sensitivity and specificity of TROP-2 in diagnosis of PTC.[12,21,22]

So present study aims at analyzing TROP-2 as a novel diagnostic marker in thyroid neoplasms.

OBJECTIVES

Primary objective:- To evaluate the diagnostic significance of TROP-2 expression in thyroid neoplasms.

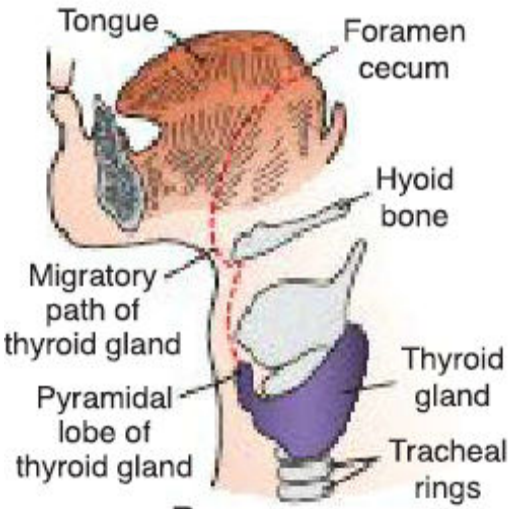
Secondary objective:- To correlate clinical diagnosis with histopathological findings of thyroid lesions.

REVIEW OF LITERATURE

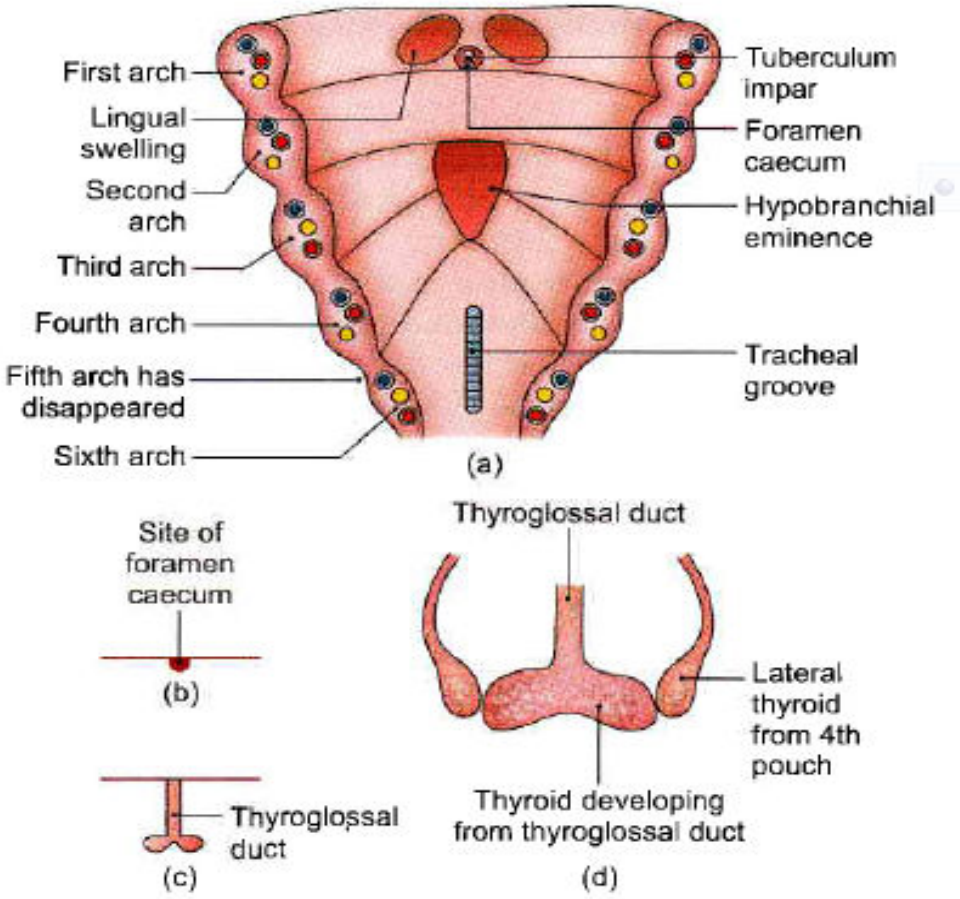
Embryology-

Development of thyroid gland strats between 2nd and 3rd week of gestation, is completed by 11th week.(1) The thyroid gland appears as an epithelial proliferation in the floor of the pharynx between the tuberculum impar and the copula at a point later indicated by the foramen cecum. Subsequently, the thyroid descends in front of the pharyngeal gut as a bilobed diverticulum. During this migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct. This duct later disappears. With further development, the thyroid gland descends in front of the hyoid bone and the laryngeal cartilages. It reaches its final position in front of the trachea in the seventh week. By then, it has acquired a small median isthmus and two lateral lobes. The thyroid begins to function at approximately the end of the third month, at which time the first follicles containing colloid become visible. Follicular cells produce the colloid that serves as a source of thyroxine(T4) and triiodothyronine(T3). Parafollicular cells, or C cells, derived from the ultimobranchial body serve as a source of calcitonin.(2)

The remnant of thyroglossal duct may form thyroglossal cyst or thyroglossal fistula. Thyroid tissue can develop at abnormal sites along the course of the duct resulting in ligual or retrosternal thyroid. Acessory thyroid may be present.(3)



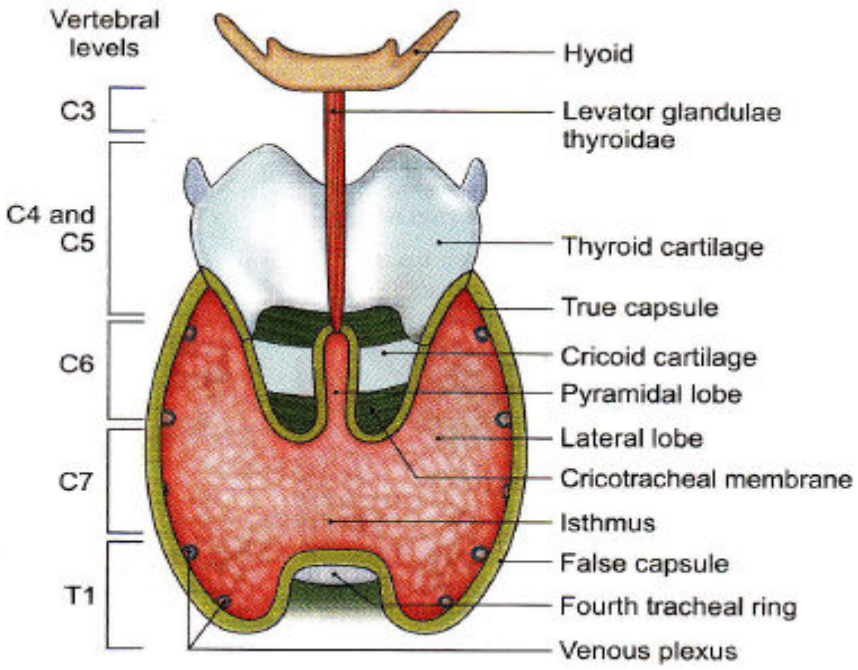
Position of the thyroid gland in the adult. Broken line denotes the path of migration



Development of thyroid gland

Anatomy

The thyroid is a butterfly-shaped endocrine gland located in the lower part of the front and sides of the neck. The normal adult thyroid consists of right and left lobes which are joined by isthmus. Sometimes a third, pyramidal lobe may project upwards from the isthmus. Each lobe of thyroid measures about 5cmx 2.5cmx 2.5cm and isthmus 1.2cmx 1.2cm. The gland approximately weighs about 25g. Size of gland in female is generally larger and it further increases during menstruation and pregnancy. The gland lies in front of C5- C7 and T1 vertebrae and encases upper part of trachea.[3]



Location and subdivisions of the thyroid gland including the false capsule

Thyroid has two capsules, 1.True capsule which is peripheral condensation of the connective tissue of gland and 2. False capsule which is derived from pretracheal layer of deep cervical fascia. A dense capillary plexus is present deep to the true capsule so to avoid haemorrhage during operations, thyroid is removed along with the true capsule.[3]

The lobes of thyroid are conical and have-

a)An apex- directed upwards and is related to superior thyroid artery and external laryngeal nerve.[3]

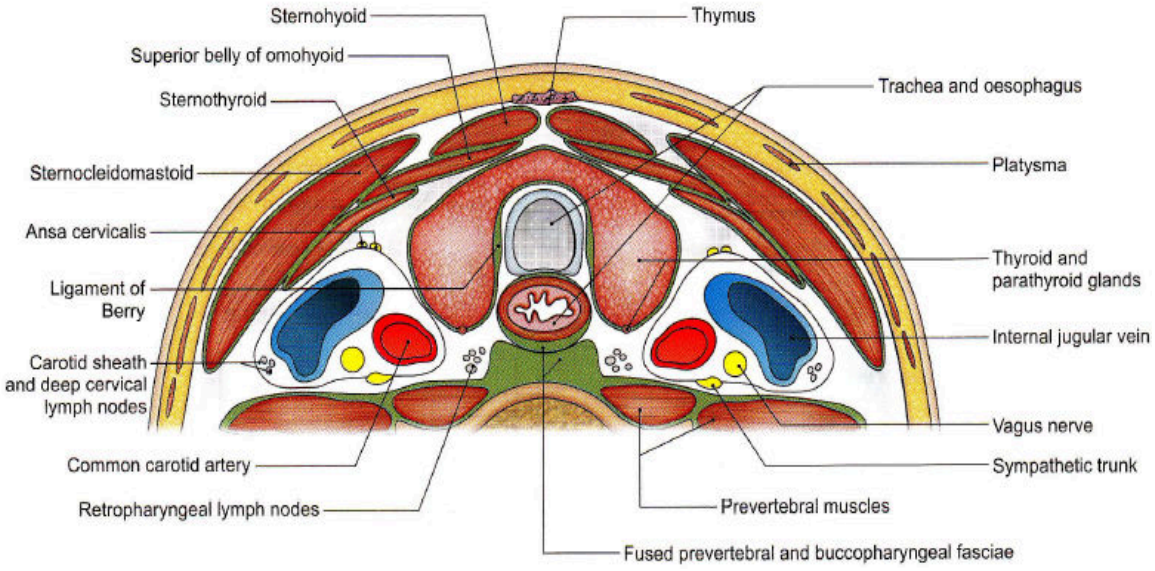
b)A base- at the level of 4th or 5th tracheal ring and related to inferior thyroid artery and recurrent laryngeal nerve.[3]

c)Three surfaces-

- Lateral/ Superficial surface- is convex and is covered by sternohyoid, superior belly of omohyoid, sternothyroid, anterior border of sternocleidomastoid
- Medial surface- is related to trachea, oesophagus, inferior constrictor and cricothyroid, external laryngeal and recurrent laryngeal nerve.
- Posterolateral/ Posterior surface- is related to carotid sheath[3]

d)Two borders-

- Anterior- is thin and related to anterior branch of superior thyroid artery
- Posterior- is thick and rounded which separates medial and posterior surfaces and is related to parathyroid glands, inferior thyroid artery, left sided thoracic duct.[3]



Transverse section of neck at the level of the isthmus of the thyroid gland

ARTERIAL SUPPLY-

The gland is mainly supplied by superior thyroid artery and inferior thyroid artey.[3]

VENOUS DRAINAGE-

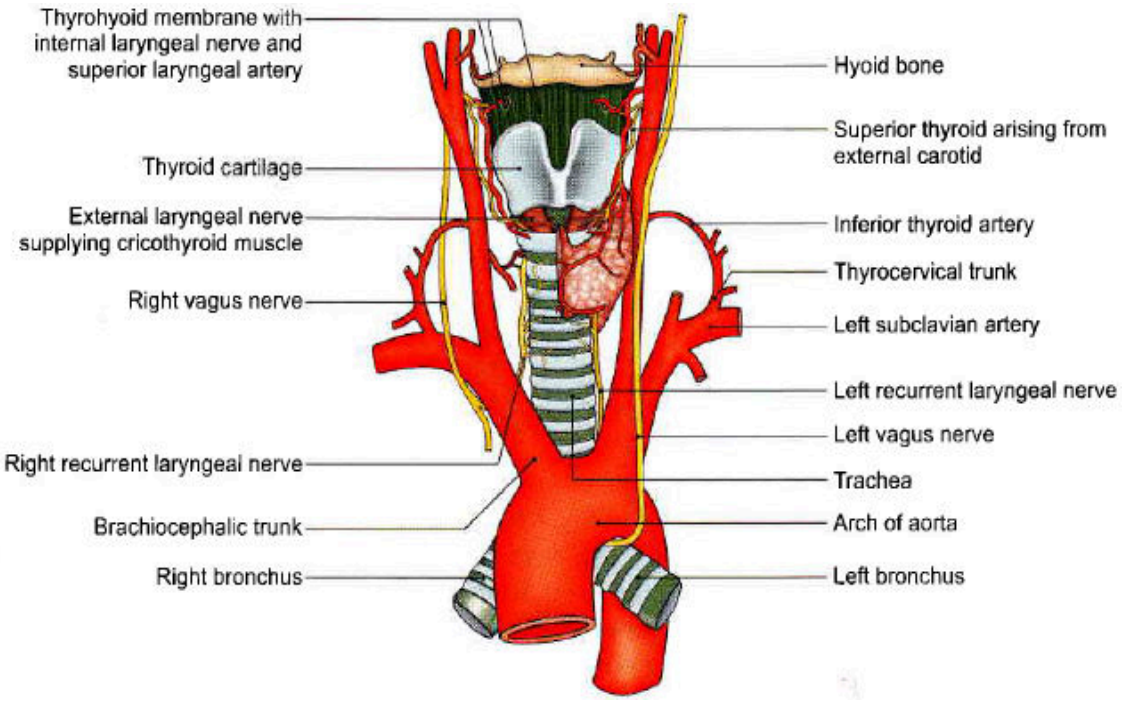
The gland is drained by superior, middle and inferior thyroid veins.[3]

LYMPHATIC DRAINAGE-

From the upper part of gland reaches to upper deep cervical lymph nodes and from lower part of gland, lymph drains to lower deep cervical lymph nodes directly or through the pretracheal and paratracheal nodes.[3]

NERVE SUPPLY-

Mainly from middle cervical ganglion and partly from superior and inferior cervical ganglia. These are vasoconstrictor.[3]

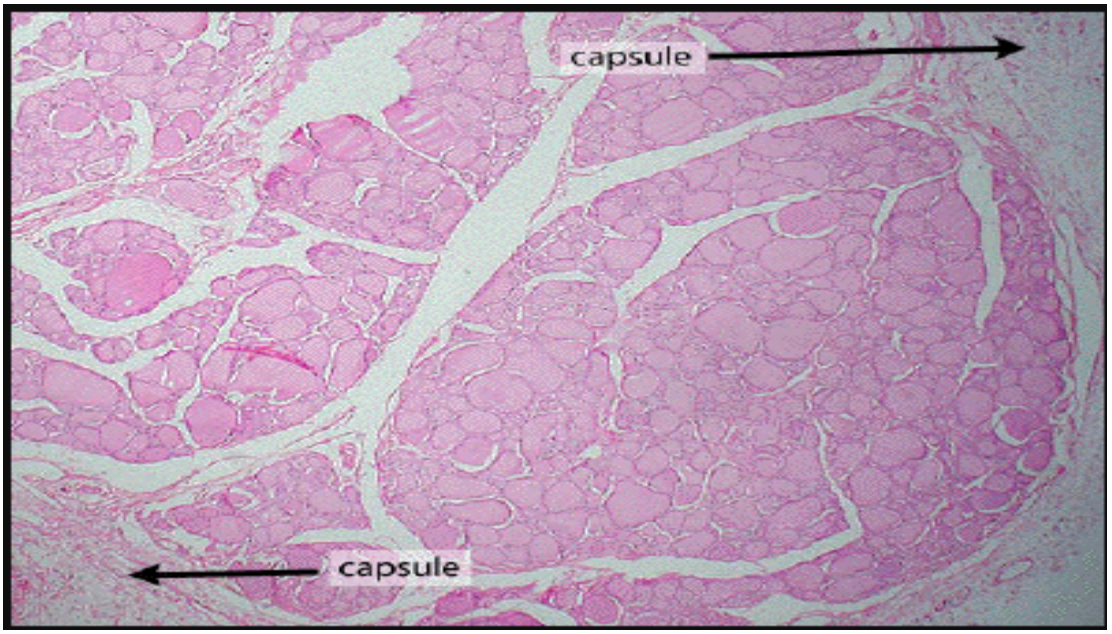


Arterial supply of anterior aspect of Thyroid gland

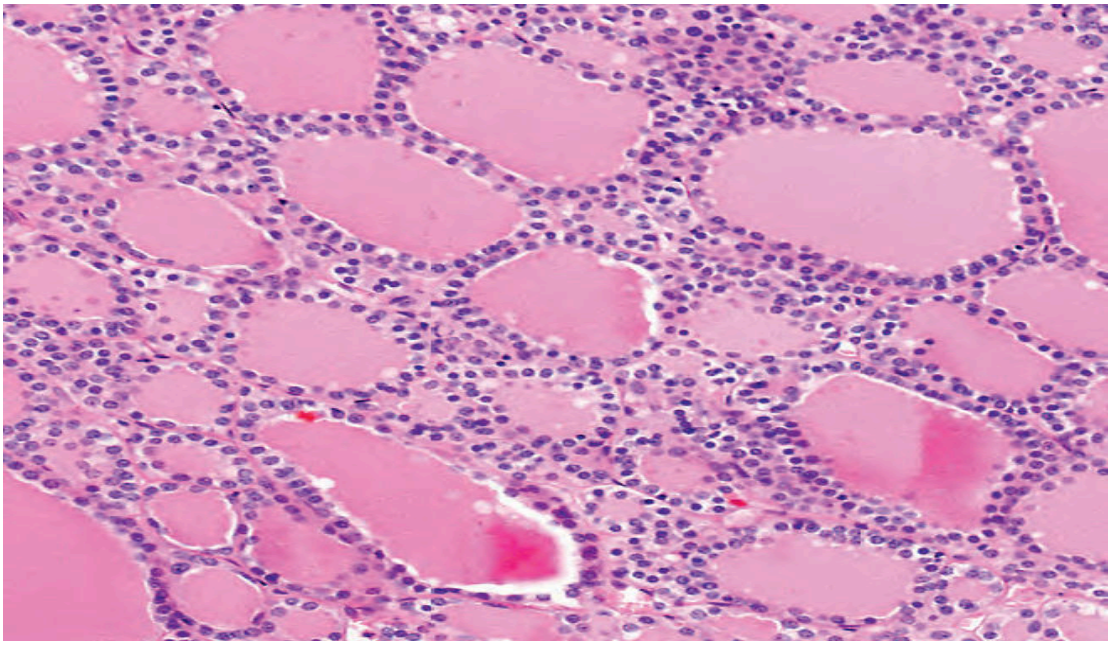
Histology-

The functional units of the thyroid gland are the thyroid follicles, these are spheroidal structures composed of a single layer of cuboidal epithelial cells bounded by a basement membrane. In normal thyroid these follicles are of variable sizes and contain a homogeneous colloid. The thyroid gland is enveloped by a fibrous capsule from which fine collagenous septae extend into the gland dividing it into lobules. The septa convey a rich blood supply, together with lymphatics and nerves. Tiny capillaries percolate through the thyroid tissue and surround the follicles and, although these are difficult to see in an H&E preparation, they can be highlighted using an immunohistochemical(IHC) method for an endothelial marker(CD34).[4]

When inactive thyroid epithelial cells are simple flat or cuboidal cells. In contrast when epithelial cells are actively synthesising or secreting thyroid hormone; they are tall and columnar. A second type of endocrine cell with the ultrastructural characteristics of neuroendocrine cells is the C cell or parafollicular cell C which is found in the thyroid gland as individual scattered cells in the follicle lining or as small clumps in the interstices between follicles. They are less prominent and can usually only be identified ultrastructurally and by immunohistochemical methods. These cells secrete calcitonin which is a physiological antagonist to parathyroid hormone and therefore lowers blood calcium levels by suppressing the osteoclastic resorption of bone.[4]



The thyroid gland is enveloped by a fibrous capsule from which fine collagenous septa extend into the gland, dividing it into lobules

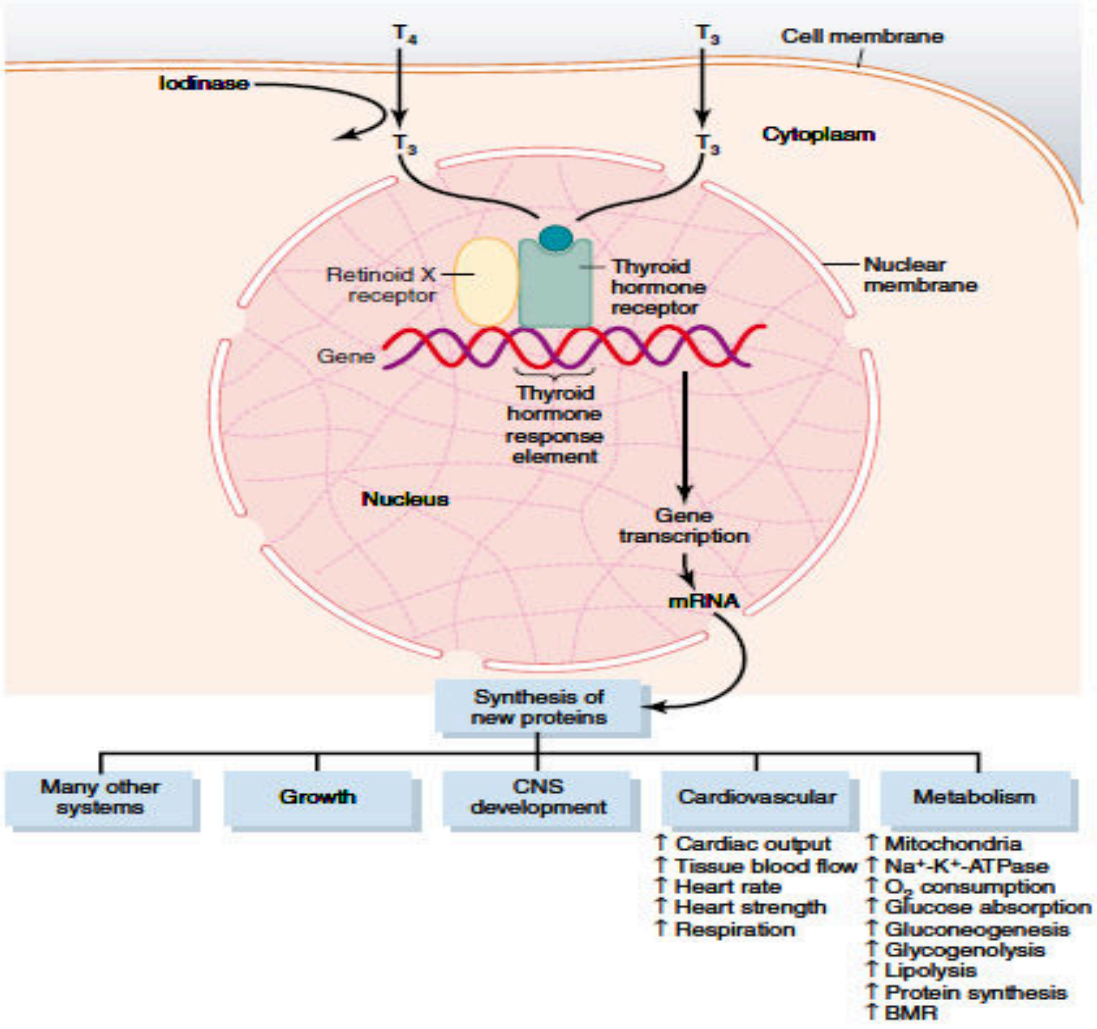


Follicles are variable in size which contain a homogeneous colloid and are lined by cuboidal cells

Physiology-

The thyroid gland secretes two major hormones- thyroxine and triiodothyronine which are commonly called *T4* and *T3* ,respectively. Both of these hormones profoundly increase the metabolic rate of the body. Complete lack of thyroid secretion usually causes the basal metabolic rate to fall 40 to 50 percent below normal, and extreme excesses of thyroid secretion can increase the basal metabolic rate to 60 to 100 percent above normal. Thyroid secretion is controlled primarily by thyroid-stimulating hormone (TSH) secreted by the anterior pituitary gland.(5)

The thyroid gland also secretes calcitonin, an important hormone for calcium metabolism.(5)



Thyroid hormone activation of target cells

Functions

Thyroid hormone has diverse cellular effects, including up-regulation of carbohydrate and lipid catabolism and stimulation of protein synthesis in a wide range of cells. The net result of these processes is an increase in the basal metabolic rate. One of the most important functions of thyroid hormone is its critical role in brain development in the fetus and neonate.(6)

The major function of the thyroid gland is to regulate metabolism which is done by means of iodine – containing thyroid hormones, thyroxine (T4) and triiodothyronine (T3). The synthesis and release of T3 and T4 are regulated by hypophyseal thyroid–stimulating hormone (TSH) and involves the following steps. (7)

1. Iodine Trapping- by thyroid cells involves uptake of iodine from the blood and concentrating it more than twenty fold.

2. Oxidation- of the iodine takes place within the cells by thyroid peroxides.

3. Iodination- occurs at the microvilli level between the oxidized iodine and the tyrosine residues of thyroglobulin so as to form mono-iodotyrosine (MIT) and diiodotyrosine (DIT).

4. Coupling- of MIT and DIT in the presence of thyroid peroxidase forms triiodothyronine (T3) and thyroxine (T4)**.**

The thyroid hormones so formed are released by endocytosis of colloid and proteolysis of thyroglobulin within the follicular cells resulting in discharge of (T3) and (T4) into circulation where they are bound to thyroxine – binding globulin.

Biochemical tests:

1. **Thyroid function tests (TFT):** (TSH, free T4, free T3)

   Measurement of TSH is the most useful initial study. Thyroid function tests are not beneficial in distinguishing benign from malignant lesions as most patients with thyroid cancer are generally euthyroid. Benign conditions like autonomously functioning adenoma or Hashimoto's thyroiditis are more often associated with hypothyroidism except for Hashitoxicosis. Hyperfunctioning solitary nodules carry a low risk of malignancy (at least in adults)[8]. It has recently been reported that the risk of malignancy in a thyroid nodule increases proportionally to serum TSH concentrations at presentation, even within the normal range and thus TSH was proposed as a novel independent predictor of the presence of thyroid malignancy[9].

2. **Serum thyroglobulin level:**

   It cannot distinguish a benign from a malignant solitary thyroid nodule unless the level is markedly increased. It is indicated for monitoring patients with thyroid cancer post total thyroidectomy. Serum thyroglobulin level is increased more in follicular carcinoma but may sometimes be increased in benign thyroid disorders. Therefore, it is not routinely recommended in the evaluation of solitary thyroid nodule (STN).[8]

3. **Serum calcitonin levels:**

   It is increased in patients with medullary thyroid carcinoma (MTC) or multiple endocrine neoplasia type II (MEN-II)[8]

4. **Serum carcinoembryonic antigen (CEA) level:**

   It is increased in MTC[8].

Other Investigations:

1. Ultrasonography (USG) of thyroid-

USG was first used to diagnose thyroid nodules in 1967 by Fujimoto[9]. It is a non expensive imaging method of choice to know whether a thyroid nodule is solid, cystic or a mixture of the two. Even a 1mm size non palpable nodule within the thyroid tissue can be detected with high resolution ultrasonography. Solid or mixed lesions can be either a benign or malignant tumor. A solid thyroid nodule may be isoechoic, hypoechoic or hyperechoic compared to the surrounding thyroid tissue. Positive predictors of malignancy include solid hypoechoic nodules, presence of calcification, irregular shape and cystic elements. Cystic nodules larger than 2cm in diameter, with haemorrhagic content and nodules that reappear after evacuating by FNAC are more suspicious for malignancy. The main limitation of this technique is its failure to definitely differentiate malignant thyroid nodules from benign ones. While main indication is accurate measurement of the size of a nodule and also acts as a guide for FNAC.[8,9,10]

2. Computed tomography scan (CT SCAN) and magnetic resonance imaging (MRI):

These imaging modalities have only limited role in the evaluation of a thyroid lesions. However, they are both most useful for evaluating the extent of large substernal goiters which may be compressing nearby structures and also detect tracheal involvement either by tumor invasion or compression, extension into the mediastinum (retrosternal lesions), or recurrent disease. MRI is more accuate than CT scan in distinguishing recurrent or persistent thyroid tumor from post-operative fibrosis[8,10].

3. Fine needle aspiration cytology (FNAC):

FNAC is the most important investigation in the thyroid lesions diagnosis, as cytology is the key determinant in whether thyroidectomy is to be indicated. It has

become the investigation of choice because of its safety, cost effectiveness and accuracy. The technique is very simple to perform, and has minimal complications.[8,10,11]

Detailed cytologic assesement of FNAC smears in Solitary nodular goiter are helpful to differentiate between various types of goiters. Follicular lesions, one of the most common diagnoses may suggest a hyperplastic nodule, FA, Follicular carcinoma or a FV-PTC. The diagnoses of follicular carcinoma requires the identification of capsular or vascular invasion which is not possible with FNAC.[8,10,11]

In cases of cystic thyroid nodules, the accuracy is increased if aspiration is done from the margin of the nodule rather than from the cystic fluid and debris in the centre. The overall sensitivity, specificity, accuracy of FNAC is 83%, 92% and 95% respectively[8,10,11].

4. Radio-isotope scanning:

It can be used to determine if a thyroid nodule is functioning and radioisotopes that have been used are technetium(^{99}mTc), ^{123}I, ^{131}I.

Depending upon the ability of the thyroid to take up radioactive isotope, thyroid nodules are further classified into cold, warm and hot. Cold nodules are hypofunctional while warm nodules are normal and hot nodules are occasionally hyperfunctional.[9]

About 85% of nodules on scanning are cold, and these lesions have a 10-25% chances of malignancy. Of the 5% nodules shown to be hot on scanning, about 1% are malignant.[9]

The major drawback of this imaging is its failure to differentiate between benign and malignant lesions with great accuracy. Its usefulness in the routine evaluation of all

patients with thyroid nodules is questionable according to some cost effectiveness studies[9].

I. NON – NEOPLASTIC LESIONS-

1. Inflammatory lesions.

a) Acute thyroiditis.

b) Subacute (granulomatous) thyroiditis.

c) Other granulomatous inflammations

i. Palpation thyroiditis

ii. Tubercular thyroiditis.

iii. Sarcoidosis

iv. Mycoses

v. Postoperative necrotizing granulomas

d) Autoimmune thyroiditis

i. Hashimoto thyroiditis

ii. Non-specific lymphocytic thyroiditis

e) Reidel thyroiditis.

f) Multifocal sclerosing thyroiditis

2. Metabolic diseases.

a) Diffuse non-toxic (simple) goiter or colloid goiter.

i. Endemic goiter.

ii. Sporadic goiter.

b) Multinodular goiter.

c) Diffuse toxic goiter or Graves disease.

d) Amyloid goiter

II. NEOPLASTIC LESIONS-

Thyroid cancer is fairly common; the annual incidence ranges from 0.5 to 10 cases per 1,00,000 population. [23] Papillary carcinoma is the commonest histologic type. With the exception of angiosarcoma, the tumors are 2-4 times more frequent in women than in men. [24] The female gender is usually associated with a slightly better prognosis. The better differentiated tumors generally occur in younger patients, while the less differentiated tumors occur in older patients. The mean ages for the low, intermediate, and high-grade tumors are the forties, fifties, and sixties, respectively. For the same tumor type, younger patients below the age of 40 years generally fare considerably better than older patients. [25]

There are many factors that determine outcome, including age of the patient, size of the primary tumor, presence of gross extrathyroid extension, presence of regional lymph nodes, and the presence of distant metastatic disease.[26] The 5 year survival figures for stages 1,2, 3, and 4 are 100%, 100%, 93% and 51% respectively. [27]

WHO classification of tumors of thyroid[43]-

Follicular adenoma

Hyallnizing trabecular tumour

Other encapsulated follicular-patterned thyroid tumours

- Follicular tumour of uncertain malignant potential
- Well-differentiated tumour of uncertain malignant potential
- Non-invasive follicular thyroid neoplasm with papillary-like nuclear features

Papillary thyroid carcinoma (PTC)

- Papillary carcinoma

- Follicular variant of PTC
- Encapsulated variant of PTC
- Papillary microcarcinoma
- Columnar cell variant of PTC
- Oncocytic variant of PTC

Follicular thyroid carcinoma (FTC), NOS

- FTC, minimally invasive
- FTC, encapsulated angioinvasive
- FTC, widely invasive

Hurthle (oncocytic) cell tumours

- Hurthle cell adenoma
- Hurthle cell carcinoma

Poorly differentiated thyroid carcinoma

Anaplastic thyroid carcinoma

Squamous cell carcinoma

Medullary thyroid carcinoma

Mixed medullary and follicular thyroid carcinoma

Mucoepldermoid carcinoma

Sclerosing mucoepidermold carcinoma with eosinophilla

Mucinous Carcinoma

Ectopic thymoma

Spindle epithelial tumour with thymus-like differentiation

lntrathyroid thymic carcinoma

Paraganglioma and mesenchymal / stromal tumours

- Paraganglioma
- Peripheral nerve sheath tumours (PNSTs)

  Schwannoma

  Malignant PNST
- Benign vascular tumours

  Haemangioma

  Cavernous haemangioma

  Lymphangioma
- Angiosarcoma
- Smooth muscle tumours

  Leiomyoma

  Leiomyosarcoma
- Solitary fibrous tumour

Haematolymphoid tumours

- Langerhans cell histiocytosis
- Rosai-Dorfman disease
- Follicular dendritic cell sarcoma
- Primary thyroid lymphoma

Germ cell tumours

- Benign teratoma (grade O or 1)
- Immature teratoma (grade 2)
- Malignant teratoma (grade 3)
- **Secondary tumors**

I. NEOPLASTIC LESIONS

Papillary carcinomas

Papillary carcinoma is defined as a "malignant epithelial tumor showing evidence of follicular cell differentiation, and characterized by distinctive nuclear features". That is, the key to diagnosis is the nuclear characteristics, and demonstration of invasive in growth is not required.[23]

The gross appearance of papillary thyroid cancer is quite variable. [1] The lesions may appear anywhere within the gland. By definition, typical papillary carcinomas are greater than 1.0 to 1.5 cm, often averaging 2 to 3 cm, although lesions may be quite large. The lesions are firm and usually white in color with an invasive appearance. Lesional calcification is a common feature. Because of extensive sclerosis, the lesion may grossly resemble a scar. In addition, cyst formation may be observed. In fact, some lesions may rarely be completely cystic, making diagnosis difficult. [1] Necrosis (in the absence of a prior needle biopsy) is not a feature of typical papillary carcinoma and suggests a higher grade lesion. [27]

Microscopically, most of these tumors will be composed predominantly or focally of papillary areas. The neoplastic papillae contain a central core of fibrovascular (occasionally just fibrous) tissue lined by single to multiple layers of cuboidal epithelial cells with crowded oval nuclei.[28]

The tumor cell nuclei of papillary cancer have been described as clear, ground-glass, empty, or Orphan Annie-eyed . These nuclei are larger and more oval than normal follicular nuclei and contain hypodense chromatin. [28] These nuclei often overlap one another. Although cleared nuclei are characteristic of papillary carcinoma, autoimmune

thyroiditis, particularly Hashimoto disease, often shows similar nuclear changes.[1] Intranuclear inclusions of cytoplasm are often found.[29]

Another characteristic of the papillary cancer nucleus is the nuclear groove. [28] Nuclear grooves may be seen in other thyroid lesions including Hashimoto disease, adenomatous hyperplasia, and diffuse hyperplasia, as well as in follicular adenomas (particularly hyalinizing trabecular neoplasm). [29] Clear nuclei are found in more than 80% of such lesions, intranuclear inclusions are found in approximately 80% to 85%, and nuclear grooves are seen in almost all cases. Mitoses are exceptional in usual papillary carcinoma.[1]

Psammoma bodies are found in approximately 40% to 50% of cases, but their presence in thyroid tissue indicates that a papillary carcinoma is most likely present somewhere in the gland.[1] These are basophilic structures showing concentric laminations stain for mucin, calcium, and iron, and appear to arise from necrosis of individual tumor cells, which occasionally may be seen at their very center, and which represent the nidus for their formation, very much the way a grain of sand acts as the nidus for the formation of a pearl in an oyster.[30] Psammoma bodies are usually present within the cores of papillae or in the tumor stroma and should be distinguished from other forms of calcification and from the intraluminal foci of inspissated secretion often seen in Hürthle cell tumors.[30] The finding of psammoma bodies in a cervical lymph node is strong evidence of a papillary cancer in the thyroid . [31]

SUBTYPES OF PAPILLARY CARCINOMA

a) Papillary Microcarcinoma (Occult Papillary Carcinoma)

According to the World Health Organization (WHO), papillary microcarcinoma is defined as a tumor measuring 1 cm or less; however, some experts have also defined tumors measuring up to 1.5 cm as microcarcinomas. [32] Other terms for these lesions have included occult papillary carcinoma, occult sclerosing carcinoma, small papillary carcinoma, and nonencapsulated sclerosing tumor. These lesions are quite common as incidental findings at autopsy or in thyroidectomy for benign disease or in completion thyroidectomies in patients with a history of carcinoma involving the opposite thyroid lobe. The small papillary carcinoma is a nonencapsulated, sclerotic, white to tan nodule often located subcapsularly.[33]

Histologically, these tumors may be totally follicular or show papillary areas as well. Sclerosis may be prominent.[33]

b) Follicular Variant of Papillary thyroid Cancer

The incidence of this variant is difficult to determine because, in the past, some of these lesions have been classified as follicular carcinomas or adenomas.

Grossly and histologically, the tumor may appear encapsulated.[1] The follicles vary in size and shape, but are often elongated or irregular shaped, with abortive papillary formation, and the colloid is usually deep staining and scalloped.[23] Psammoma bodies and desmoplastic response at invasive areas may be present. The diagnosis rests on identification of the typical nuclear features of papillary carcinoma.[4,26] Immunohistochemical staining shows the presence of low- and high-molecular-weight

cytokeratins and HBME-1, which may aid in differentiating this lesion from follicular adenomas and carcinomas.[1]

Two distinct types of follicular variants include the diffuse follicular variant and the encapsulated follicular variant.[1] In the diffuse follicular variant, the gland is diffusely replaced by tumor. Lymph node and distant metastases are common in these patients. The encapsulated follicular variant refers to the follicular variant, that is characterized by the presence of a capsule around the lesion. It is also referred to as 'Lindsay tumor' .These lesions are associated with an excellent prognosis. In some cases, the diagnosis of this particular variant of papillary carcinoma can be difficult because of the presence of multifocal rather than diffuse distribution of nuclear features of papillary thyroid carcinoma. [34,35] Because of this peculiar morphologic presentation, these tumors can be misdiagnosed as adenomatoid nodule or follicular adenoma.[34] Some authors have suggested that these tumors be classified as "tumors of undetermined malignant potential" as a result of the excellent prognosis ; however, others have shown that some cases belonging in this category can lead to distant metastasis. [1]

c) Tall-Cell Variant

Tall-cell variant make up approximately 10% of the papillary cancers. [1] The tumor tends to occur in older adult patients. The tumor is large (>6 cm), extends extrathyroidally, and shows mitotic activity and vascular invasion more often than usual papillary cancer.[1] The tall-cell variant is three times as tall as it is wide, and its cytoplasm is often eosinophilic.[32] Tall cells should represent 50% or more of the papillary carcinoma cells to make the diagnosis of tall-cell variant. [36] The tumors show an extensive papillary pattern, often with a heavy lymphocytic infiltration present in or around the papillae. Some tall-cell tumors arise in glands with extensive histologic

evidence of chronic thyroiditis. Dedifferentiation to squamous cell carcinoma has been described. [1]

d) Columnar Cell Variant

The columnar cell variant is a rare form of papillary carcinoma. The tumor needs to be distinguished from other papillary carcinomas because this lesion is associated with an extremely poor outcome, with most deaths occurring within 5 years of diagnosis.[37] Grossly, the tumors often measure more than 6 cm. The tumor is characterized microscopically by papillary growth. Tall columnar cells line the papillae.[37]

The nuclear features are usually not those of typical papillary carcinomas. The nuclei are hyperchromatic with a punctate chromatin; nuclear stratification is a prominent feature. The cells usually have scant cytoplasm, which can be clear. Mitoses are frequently seen. Psammoma bodies are rare. Extrathyroidal extension is common, as are distant metastases.[37] Encapsulated variants, which may have a better prognosis, have been described.[1]

e) Warthin-Like Variant

By light microscopy, these tumors resemble a "Warthin tumor" of the salivary gland. These tumors usually arise in a background of lymphocytic thyroiditis and show papillary architecture. Tumor cells with abundant eosinophilic cytoplasm line the papillae, and the papillary cores contain a brisk lymphoplasmacytic infiltrate. Some tumors may show transition to tall-cell variant, which usually occurs at the invasive edge of tumor.[38]

f) Diffuse Sclerosis Variant

The diffuse sclerosis variant of papillary carcinoma is rare, representing only approximately 3% of all papillary carcinomas.[1] The tumor, which most often affects children and young adults, may present as bilateral goiter. The tumor permeates the gland outlining the intraglandular lymphatics. Tumor papillae have associated areas of squamous metaplasia. Numerous psammoma bodies are found. Lymphocytic infiltrate is found around the tumor foci.[1]

g) Solid Variant

When the solid growth represents greater than 50% of the tumor mass, a diagnosis of solid variant of papillary carcinoma may be made. The solid variant is most commonly seen in children and has been reported in more than 30% of patients with papillary carcinoma after the Chernobyl nuclear accident. The nuclear features are those of papillary carcinoma. These tumors are widely invasive throughout the thyroid. Almost half of these cases are associated with history of external radiation to the head and neck region. [1]

h) Encapsulated Variant

The encapsulated variant presents grossly as an adenoma and comprises from 8% to 13% of papillary cancers. Microscopically, such lesions usually show total encapsulation; however, there are cytologic features of papillary cancer, including nuclear changes and psammoma bodies. Some of these lesions will show focal invasion into the capsule.[1] Cases composed entirely of follicles are difficult to distinguish from follicular adenoma/carcinoma. The patients tend to be younger, compression symptoms are most uncommon, and the frequency of lymph node metastasis (12-38%) is lower

compared with classical papillary carcinoma. The prognosis is excellent, with all patients remaining disease free after treatment.[23]

i) Oncocytic (oxyphilic) variant.

In this variant, the nuclear features remain those of papillary carcinoma but the cytoplasm is abundant and has a granular oxyphilic quality. The pattern of growth may be papillary or follicular, and the tumor may be encapsulated or invasive, this resulting in a bewildering number of possible combinations: oncocytic, encapsulated oncocytic, oncocytic follicular, and encapsulated oncocytic follicular variants.[30]

j) Cribriform–morular variant.

This variant is characterized by the presence of a cribriform pattern of growth and morular formations. Nuclear clearing can be seen, shown at the ultrastructural level to be due to the accumulation of microfilaments (apparently made up of biotin) and therefore different from that seen in conventional papillary carcinoma.[30] The thyroid carcinomas that occur in patients with familial adenomatous polyposis (FAP) commonly exhibit this histologic pattern.[23]

k) Papillary carcinoma with exuberant nodular fasciitis-like stroma.

In this variant, the prominence of the stromal reaction of the tumor may obscure the neoplastic epithelial component. As a result, a biopsy may be misinterpreted as nodular fasciitis, fibromatosis, or some other proliferative stromal condition.[30]

l) Other Variants

Rare variants of papillary cancer for which prognostic data are not well established include the spindle cell variant, the clear-cell variant, papillary carcinoma with lipomatous stroma, myxoid variant.[1]

Follicular carcinomas

Follicular carcinoma comprises approximately 5% of thyroid cancers; however, in iodide-deficient areas, this tumor is more prevalent, making up 25% to 40% of thyroid cancers. Follicular carcinoma has a marked propensity for vascular invasion (not lymphatics).[1,39]

Grossly, the minimally invasive follicular carcinoma resembles a follicular adenoma; the lesion is well encapsulated. The thickness of the capsule is prominent and is often thicker than that seen in follicular adenoma.[1,39]

Microscopically, the tumor demonstrates a microfollicular or trabecular pattern with regular, small round follicles. [1, 39] Hemorrhage, necrosis, or even tumor infarction may be noted, and significant mitotic activity is often found. In a follicular neoplasm lacking the cytoarchitectural features of papillary carcinoma, the only feature that distinguishes a carcinoma from an adenoma is the presence of vascular and/or capsular invasion in the former, which means that a reliable distinction between the two requires thorough examination of the tumor thyroid interface .[23]

The criterion for vascular invasion applies solely and strictly to veins in or beyond the capsule because tumor plugs within capillaries in the substance tumor have no apparent diagnostic and prognostic importance .[34,36] The vessel should contain one or more clusters of tumor cells attached to the wall and protruding into the lumen. Often, the intravascular tumor masses are covered by endothelium, in a fashion similar to that of an ordinary thrombus. [30]

Interruption of the capsule must be full thickness for the process to qualify as capsular invasion. After it has violated a narrow segment of the capsule, it is common for

the tumor to expand in a mushroom-like fashion in the adjacent area. As a result, a tangential section might show a tumor nodule outside the main mass, separated from it by an intact capsule, deeper sections being nccessary to demonstrate the focus of capsular rupture.(5)

Another interesting phenomenon is the occasional formation of a second (or even a third) capsule in the advancing edge of the tumor that has already violated the original capsule. (5) Foci of capsular invasion should be distinguished from capsular rupture resulting from a fine needle aspiration procedure. The latter should be suspected when the area in question has a fissure-like quality, contains foci of recent or old hemorrhage, and exhibits florid stromal reparative changes.(30)

A mimic of vascular invasion is represented by the sometimes extremely florid proliferation of endothelial and smooth muscle cells of capsular vessels, which may look like a papillary endothelial hyperplasia (the 'Masson lesion') or may even acquire a Kaposi-like appearance. (30) Depending on its degree of invasiveness, follicular carcinoma has been subdivided into a minimally invasive and a widely invasive form.(30)

Minimally invasive follicular carcinomas are grossly encapsulated tumors showing focal capsular/vascular invasion that is usually apparent only on histologic examination.(23) The pattern of growth usually resembles that of an adenoma of embryonal or fetal type. These are further classified as follows(23)

-With capsular (but no vascular) invasion

-With limited (<4) vascular invasion (with or without capsular invasion)

-With extensive (≥4) vascular invasion (with or without capsular invasion)

Widely invasive follicular carcinoma is the high-risk counterpart of the minimally invasive subtype. It shows widespread infiltration of blood vessels and/or adjacent thyroid tissue. It often lacks encapsulation altogether. It has been suggested that grossly encapsulated tumors showing extensive blood vessel invasion (four vessels or more) should be placed in this category because of their similar natural history.[30]

MEDULLARY THYROID CARCINOMA

Medullary thyroid carcinoma is rare and comprises less than 10% of all thyroid malignancies. This tumor is of great diagnostic importance because of its aggressiveness, its close association with multiple endocrine neoplasia (MEN) syndromes (MEN2A and MEN2B), and a relationship to a C-cell hyperplasia as the probable precursor lesion. Although the majority of medullary carcinomas are sporadic, approximately 10% to 20% are famillial. Medullary carcinoma can affect patients of any age; however, most affected individuals are adults with an average age of approximately 50 years.[1]

Medullary carcinoma is usually located in the area of highest C-cell concentration (i.e., the lateral upper two-thirds of the gland). In familial cases, multiple small nodules may be detected grossly, and rarely, lesions may be found in the isthmus. The tumors range in size from barely visible to several centimeters.[1,36]

Many medullary carcinomas are grossly circumscribed, but some show infiltrative borders. Some tumors show gross necrosis and hemorrhage.[1, 36]

Microscopically, the classic presentation is represented by a solid proliferation of round to polygonal cells of granular amphophilic cytoplasm and medium-sized nucleus, separated by a highly vascular stroma, hyalinized collagen, and amyloid. [30] The nuclei are uniform; the nuclear-to-cytoplasmic ratio is low. Intranuclear cytoplasmic inclusions

are commonly noted. Mitoses can be seen.[4] Coarse calcification is common and can be prominent enough to be detected radiographically.[30]

Several medullary carcinoma variants have been described.

In the **papillary variant**, a papillary or pseudopapillary growth pattern is identified. The pseudopapillary variant is more common and probably results from fixation artifact. The true papillary variant is extremely rare and needs to be differentiated from typical papillary thyroid carcinoma; nuclear morphology is the most important distinguishing feature. [1]

The **follicular variant** is characterized by the presence of follicles, glands, or tubules. Care must be rendered to determine that the follicular structures are not just entrapped normal thyroid within the lesion. [1]

Some medullary carcinomas are grossly and microscopically encapsulated. The follow-up in **encapsulated medullary carcinomas** indicates that they have a more benign prognosis than usual medullary tumors. The histologic differential diagnosis for the encapsulated variant includes hyalinizing trabecular adenoma. Immunohistochemistry for calcitonin is positive in the medullary carcinoma but not within the hyalinizing trabecular adenoma. [1]

The **small-cell variant of medullary carcinoma** has also been described. These tumors look like pulmonary small-cell carcinoma, from which they need to be distinguished, if possible. The prognosis is worse than for typical medullary carcinoma. [1]

In **Oxyphilic variant**, the accumulation of mitochondria in the cytoplasm of medullary carcinoma can result in a strikingly oxyphilic (oncocytic) appearance, which can be either a focal or diffuse phenomenon .The occasional presence of a solid,

trabecular and follicular growth pattern may further heighten its histologic mimicry of Hurthle cell adenoma/ carcinoma. Clues that should alert one to the possibility of medullary carcinoma are the prominence of fibrovascular septa and the presence of foci of conventional medullary carcinoma (not always found). [23]

The **giant-cell (anaplastic) variant** is rare and is characterized by large atypical cells admixed with areas of typical medullary carcinoma. Because of the presence of large atypical cells, this variant needs to be differentiated from anaplastic thyroid carcinoma, a tumor with a worse prognosis when compared with medullary carcinoma. [1]

The **clear-cell variant** is a rare form of medullary carcinoma and is characterized by cells with abundant clear cytoplasm. Differential diagnostic consideration for this variant includes follicular-derived neoplasms with clear-cell cytoplasm as well as metastatic renal cell carcinoma.[1]

The **Spindle cell variant**- Spindle cells are not uncommon in medullary carcinoma, but some medullary carcinomas are composed almost exclusively of plump or slender spindly cells arranged in intersecting fascicles, whorls and packets, mimicking mesenchymal neoplasms for differential diagnosis.[26]

The Pigmented variant.- Rare cases of medullary carcinoma show melanin pigmentation. [23]

The Squamous variant- Exceptionally, medullary carcinoma can show focal squamous differentiation.[23]

Neuroblastoma-like- Sometimes there can be fibrillary matrix and rosette, resembling neuroblastoma.[23]

Carcinoid-like variant- Some medullary carcinomas show histologic features resembling foregut, midgut or hindgut carcinoid, with tumor islands, trabeculae or glands separated by fibrohyaline stroma. There can be a component of classic medullary carcinoma. Such cases can potentially be mistaken for follicular cell neoplasm or metastatic carcinoid.[23]

Paraganglioma-like- The tumor has a nested architecture, mimicking paraganglioma. S-100 protein-positive sustentacular like dendritic cells are interspersed.[23]

Medullary microcarcinoma (latent carcinoma)- Rarely sporadic medullary carcinomas measuring less than 1 cm are discovered incidentally in thyroidectomy specimens or at autopsy. The prognosis is excellent except when there are clinical symptoms.[23]

UNDIFFERENTIATED (ANAPLASTIC) CARCINOMAS

Anaplastic carcinomas are a group of high-grade thyroid carcinomas that are usually undifferentiated histologically and have an extremely poor prognosis, with many patients surviving less than 6 months following diagnosis. Synonyms for anaplastic carcinoma include undifferentiated, dedifferentiated, and sarcomatoid carcinoma.[1] These tumors represent approximately 5% of thyroid malignancies. The tumor is more commonly seen in older adult women who present with a rapidly enlarging mass, which often results in dyspnea.[1]

Grossly, the tumors are widely invasive, with variable extent of involvement of the thyroid parenchyma, adjacent soft tissues, and adjacent structures such as the larynx,

trachea, pharynx and esophagus. They are fleshy and white-tan, with frequent necrosis and haemorrhage. [23]

Microscopically, the term undifferentiated or anaplastic carcinoma is used in the thyroid gland in connection with two major categories that sometimes coexist. The first is undifferentiated in the sense that it does not make follicles, papillae, or even trabeculae or nests, but the tumor still retains an unmistakable epithelial appearance on morphologic and immunohistochemical grounds.[23]

The second category is actually composed of two patterns which are often seen together and which are sometimes grouped under the qualifier of sarcomatoid: **spindle cell** and **giant cell**. They may exhibit a fascicular or storiform pattern of growth, heavy neutrophilic infiltration, prominent vascularization, and divergent differentiation into bone, cartilage, and skeletal muscle. As a result, their appearance may closely simulate a large variety of soft tissue sarcomas. Another variation on the theme of the spindle cell form of undifferentiated carcinoma is the *paucicellular variant*, which mimics Riedel thyroiditis because of the extreme degree of fibrosis and hyalinization, and which is recognized because of the scattered atypia, areas of necrosis, vascular invasion, vascular permeation, and positivity for epithelial markers. [23]

THYROID ADENOMA AND RELATED TUMOURS

Follicular adenoma

Follicular adenoma is a benign, encapsulated tumour of the thyroid showing evidence of follicular cell differentiation. Adenomas are common in iodine deficient areas, usually as part of a nodular goiter.[40]

Grossly, the tumor is usually a solitary, round or oval, nodule measuring around 1-3 cms in diameter and surrounded by a thin capsule. The cut surfaces show grey-white, tan or brown fleshy tumour. Generally, those tumours with a grey-white colour have a solid or trabecular growth pattern while tan to brown tumours show evidence of follicle formation with colloid deposition. Secondary changes such as haemorrhage and cystic degeneration may be present. Occasionally, follicular adenomas can arise in a background of nodular hyperplasia, and the tumour is distinguishable from the background nodules (colloid or hyperplastic) by the encapsulation and the fleshy appearance.[40]

Microscopically, trabeculae or solid sheets; rarely, a focal papillary pattern can be present. The tumor cells are cuboidal or low columnar, and possess dark- .staining or pale-staining round nuclei with inconspicuous nucleoli, although some tumors can exhibit nuclear pleomorphism. Mitoses generally are uncommon. The cytoplasm can be eosinophilic, oxyphilic or clear. In an individual case, the histologic pattern can be uniform throughout, or the pattern can vary from area to area. Neoplasms composed predominantly of large follicles are termed "macrofollicular", those of normalsized follicles "normofollicular", and those of small follicles "microfollicular" or "fetal". Neoplasms showing a trabecular/solid pattern are termed "trabecular" or "embryonal" .Within the tumor, delicate capillaries are present between the follicles and cell islands, but they are usually inconspicuous in routine histologic section. Secondary changes such as hemorrhage, hemosiderin deposition, sclerosis, edema, necrosis and cystic change are not uncommon.[23]

Variants follicular adenoma

1. Oncocytic adenoma

These solitary, well-delineated and encapsulated tumours are characterized by a distinct mahogany brown appearance, often with central areas of scarring.(40)

Microscopically, the tumour is composed of cells with abundant granular eosinophillic cytoplasm and large open nuclei with prominent nucleoli, although rarely the nuclei can be hyperchromatic. Adenomatoid oncocytic nodules often occur in association with Hashimoto thyroiditis and are difficult to distinguish from true adenomas.(40)

2. Follicular adenoma with papillary hyperplasia

Also known as papillary variant of follicular adenoma, this subtype is usually encapsulated and partially cystic. It comprises broad or delicate branching papillae as well as follicles, lined by columnar cells with uniform, round and hyperchromatic nuclei, regularly aligned at the base. By definition, nuclear features of papillary thyroid carcinoma should be absent. This tumour occurs predominantly in children and adolescents and may be multiple.(40)

3. Fetal adenoma

This variant is characterized by a microfollicular/trabecular structure in an oedematous stroma, particularly in the center of the tumour.(40)

4. Signet-ring cell follicular adenoma

This lesion is characterized by signet ring tumour cells with a discrete cytoplasmic vacuole displacing the nucleus to the periphery. The vacuoles are immunoreactive for thyroglobulin, and often stain for mucosubstances.(40)

5. Mucinous follicular adenoma

This variant characterized by accumulation of abundant extracellular mucin, often accompanied by a microcystic, reticular or multicystic growth pattern .Typical features of follicular neoplasm are often evident in some areas of the tumour in addition, there can be signet ring cell change. [23]

6. Lipoadenoma

This is a follicular adenoma with mature adipose cells interspersed throughout the tumour.[40]

7. Clear cell follicular adenoma

This variant is characterized by cytoplasmic clearing of the tumour cells which can result from ballooning of mitochondria, accumulation of lipid or glycogen, or deposition of intracellular thyroglobulin. [40]

8. Toxic (hyperfunctioning) adenoma

This is a follicular adenoma associated with symptoms of hyperthyroidism due to autonomous production of thyroxine. Histologically, the follicles are lined by tall cells, often showing showing papillary projections within the lumina, similar to the follicles seen in Graves disease. The tumour appears as a "hot" nodule on radioactive iodine scan.[40]

9. Atypical adenoma

The term "atypical adenoma 'has been variably used to refer to follicular neoplasms exhibiting high cellularity, nuclear atypia, or unusual histologic patterns (such as spindle cell fascicles), but lacking vascular and capsular invasion on thorough

sampling. Despite the histologically worrisome appearance, this tumour pursues a benign course. [40]

10. Follicular adenoma with bizarre nuclei

A variant characterized by the presence of isolated or small groups of monstrous tumour cells with enlarged hyperchromatic nuclei within an otherwise typical follicular adenoma. [40]

Hyalinizing trabecular tumour

Hyalinizing trabecular tumour is a rare tumour of follicular cell origin with a trabecular pattern of growth and marked intratrabecular hyalinization. This tumour is synonymously known as hyalinizing trabecular adenoma, paraganglioma-like adenoma; hyaline cell tumour with massive accumulation of cytoplasmic microfilaments; hyalinizing trabecular adenoma-like lesion; papillary carcinoma ; hyalinizing trabecular variant.[41]

Grossly, the tumour is usually a single, solid, encapsulated or circumscribed and of medium size, measuring 2.5 cm or less in diameter. The cut surface is homogenous, delicately lobulated, with a yellow tinge and occasionally marked with off white flecks and streaks, and gaping vessels.[23,41]

Microscopically, the tumour is a solid epithelial neoplasm that is circumscribed and may be surrounded by a thin capsule. It features a trabecular-alveolar growth pattern of medium to large-sized cells with finely granular, acidophilic, amphophilic or clear cytoplasm, intratrabecular hyaline (PAS- positive basement membrane material), polygonal and fusiform cells, nuclei with prominent grooves and cytoplasmic pseudoinclusions, and small nucleoli, occasional mitoses, round paranuclear cytoplasmic

bodies with a slight yellow tinge, and occasional mitotic figures. Psammoma bodies may be present. The cells are arranged in sinous or straight trabeculae supported by a delicate fibrovascular stroma.colloid is scant or absent.[23, 41]

Multinodular goiter

With time, recurrent episodes of hyperplasia and involution combine to produce a more irregular enlargement of the thyroid, termed *multinodular goiter* or *adenomatous goiter*. Because they derive from simple goiter, they occur in both sporadic and endemic forms, having the same female-to-male distribution and presumably the same origins but affecting older individuals because they are late complications.[6]

Morphologically multinodular goiters are multilobulated, asymmetrically enlarged glands that can reach weights of more than 2000 gm. In some instances one nodule may so stand out as to impart the clinical appearance of a solitary nodule. On cut section, irregular nodules containing variable amounts of brown, gelatinous colloid are present. Older lesions have areas of hemorrhage, fibrosis, calcification, and cystic change.[6]

Microscopically, there is a wide range of appearances. Some nodules are composed of huge follicles lined by flattened epithelium, others are extremely cellular and hyperplastic, and still others are composed predominantly or exclusively of Hürthle cells. Some of the dilated follicles have a conglomerate of small active follicles at one pole (so-called Sanderson polsters). Others have papillary projections facing the lumen of a cystic follicle, a feature that may lead to confusion with papillary carcinoma.[103]

In contrast to follicular neoplasms, a prominent capsule between the hyperplastic nodules and residual compressed thyroid parenchyma is not present.[6] The adenoma is usually single, is totally surrounded by a capsule, is dissimilar from the remaining parenchyma, compresses the adjacent tissue, and is composed mainly of follicles that are smaller than those of the normal gland. The lesion of nodular hyperplasia is almost always one of many nodules, its encapsulation is incomplete, the follicular size is variable, some or all of the follicles are larger than those in the surrounding gland, and there is no compression of the adjacent parenchyma. In some cases, the distinction becomes impossible, inasmuch as lesions with the morphologic features of adenoma may be multiple and/or occur in a setting of nodular hyperplasia.[30]

STAGING OF THYROID CANCER

TNM STAGING PRIMARY TUMOR (T)[42]

T – Primary Tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

T1 Tumour 2 cm or less in greatest dimension, limited to the thyroidThyroid Gland

T1a Tumour 1 cm or less in greatest dimension, limited to the thyroid

T1b Tumour more than 1 cm but not more than 2 cm in greatest dimension, limited to the thyroid

T2 Tumour more than 2 cm but not more than 4 cm in greatest dimension, limited to the thyroid

T3 Tumour more than 4 cm in greatest dimension, limited to the thyroid or any tumour with minimal extrathyroid extension (e.g., extension to sternothyroid muscle or perithyroid soft tissues)

T4a Tumour extends beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues, larynx, trachea, oesophagus, recurrent laryngeal nerve

T4b Tumour invades prevertebral fascia, mediastinal vessels, or encases carotid artery

All anaplastic carcinomas are considered T4 tumours

T4a* (anaplastic carcinoma only) Tumour (any size) limited to the thyroid

T4b* (anaplastic carcinoma only) Tumour (any size) extends beyond the thyroid capsules

N – Regional Lymph Nodes

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Regional lymph node metastasis

N1a Metastasis in Level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)

N1b Metastasis in other unilateral, bilateral or contralateral cervical (Levels I, II II, IV, or V) or retropharyngeal or superior mediastinal lymph nodes

M – Distant Metastasis

M0 No distant metastasis M1 Distant metastasis

STAGE GROUPING

Separate stage groupings are recommended for papillary and follicular (differentiated), medullary, and anaplastic (undifferentiated) carcinomas:

Papillary or Follicular			
Under 45 years			
Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1
Papillary or Follicular *45 years and older*			
Stage I	T1a, T1b	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1a	M0
Stage IVA	T1, T2, T3	N1b	M0
	T4a	N0, N1	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1
Medullary			
Stage I	T1a, T1b	N0	M0
Stage II	T2, T3	N0	M0
Stage III	T1, T2, T3	N1a	M0
Stage IVA	T1, T2, T3	N1b	M0
	T4a	Any N	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Anaplastic Carcinoma All anaplastic carcinoma are stage IV			
Stage IVA	T4a	Any N	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Grading of differentiated papillary and follicular thyroid carcinomas:

Histologic grade may be done based on nuclear atypia, tumor necrosis and vascular invasion.[44,45]

Another study considered presence/absence of nuclear atypia, presence/absence of tumor necrosis, mitotic frequency on 10 areas with 40x microscopic field (MF), presence/absence of vascular invasion, presence/absence of capsular invasion and the presence/absence of extrathyroidal extension for histological grading of tumors.[46]

Grade 1 or low grade is considered when none of the histological features were present and Grade 2 or high grade when one or more of the above mentioned features were present. Various studies found histologic grading of thyroid carcinomas to be a strong and independent prognostic factor.[44,45,46]

Immunohistochemistry-

The prognosis and management of thyroid nodules depends on the diagnosis. The main diagnostic gold standard is pathologic evaluation with routine hematoxylin and eosin stained slides. (H and E).[47,48]

There are often morphologic similarities between benign and malignant lesions and follicular and papillary architectures may be seen in both benign and malignant

lesions.[47,49] Some important features of malignancy, like pale nuclei, vesicular nucleus for papillary thyroid carcinoma are open to subjective interpretations and there are disagreements among pathologists.(47,48)

Papillary thyroid carcinoma (PTC) consitutes the majority of all thyroid malignant neoplasms and the follicular variant (FV-TPC) is the most common among the subtypes. Histopathology is characterized by follicular growth patterned and nuclear features identical to usual type.(50,51) Some cases of FV-TPC are surrounded by fibrous capsule.(51) There are diagnostic problems when the characteristic nuclear features are not diffusely distributed throughout the lesion.(48,50,5)] Such lesions can be diagnosed as benign follicular nodule or if they show capsular or vascular invasion as in follicular carcinoma.[50,51,52]

The alternative methods like immunohistochemistry and genetic mutation analysis has become available for distinguishing these lesions. Different studies have previously evaluated the utility of several markers, such as CK19, galectin3, Hector Battifora mesothelial-1 (HBME-1), CD44, CD57, Cyclin D1, P27, and trophoblastic cell surface antigen-2 (TROP-2). (21)

TROP 2

The “gold standard” in the diagnosis of thyroid nodules is histomorphological evaluation of H and E slides. However, morphologic overlap between various papillary and follicular lesions especially the follicular variant of papillary thyroid carcinoma (FVPTC) is common.

Thus, immunohistochemical and molecular methods were investigated to aid in the diagnosis of these challenging cases. Several IHC markers such as Galectin 3, Cytokeratin-19, CD 56, HBME-1, Ki67 and p53 have been recommended to help in the discrimination between these controversial thyroid nodules. Nonetheless up till now

there is no agreed consensus about an IHC panel that would reliably overcome such diagnostic obstacles.[53,54]

Discovery of TROP-2

Although first described almost 40 years ago as a cell surface marker of trophoblast cells [55], TROP-2 (trophoblast cell-surface antigen 2) was rediscovered in ensuing years as tumor-associated calcium signal transducer 2 (TACSTD2), membrane component chromosome 1 surface marker 1 (M1S1), gastrointestinal antigen 733-1 (GA733-1), and epithelial glycoprotein-1 (EGP-1). [56,57]

The expression, role, and function of TROP- 2 became of interest in about 1990, where a monoclonal antibody was developed that reacted with a glycoprotein expressed by many different cancer types. In initial reports it was referred as EGP 1. [(8-61] The recognition that this antibody recognized a unique marker of trophoblast and neoplastic cells, [renamed TROP-2 once it was identified as the same antigen called by different designations. [58,59,60]], was fortuitous, because it was developed in the search of a marker of non-small-cell lung cancer (NSCLC).[58,59] The murine monoclonal antibody, designated RS7-3G11 (later shortened to RS7), was developed by immunizing mice with a cell membrane preparation isolated from a surgical specimen of a squamous NSCLC. RS7 bound strongly to lung, breast, and prostate cancer cell lines, weakly to colon cancer cell lines, and was absent in a lung fibroblast cell line, as well as granulocytes, monocytes and lymphocytes. Immunohistology of fresh frozen tissues showed that RS7 bound to breast, colon, renal, lung, and prostate cancers (33/40 [83%] positive, with 22/33 [67%] staining strongly positive).[59]

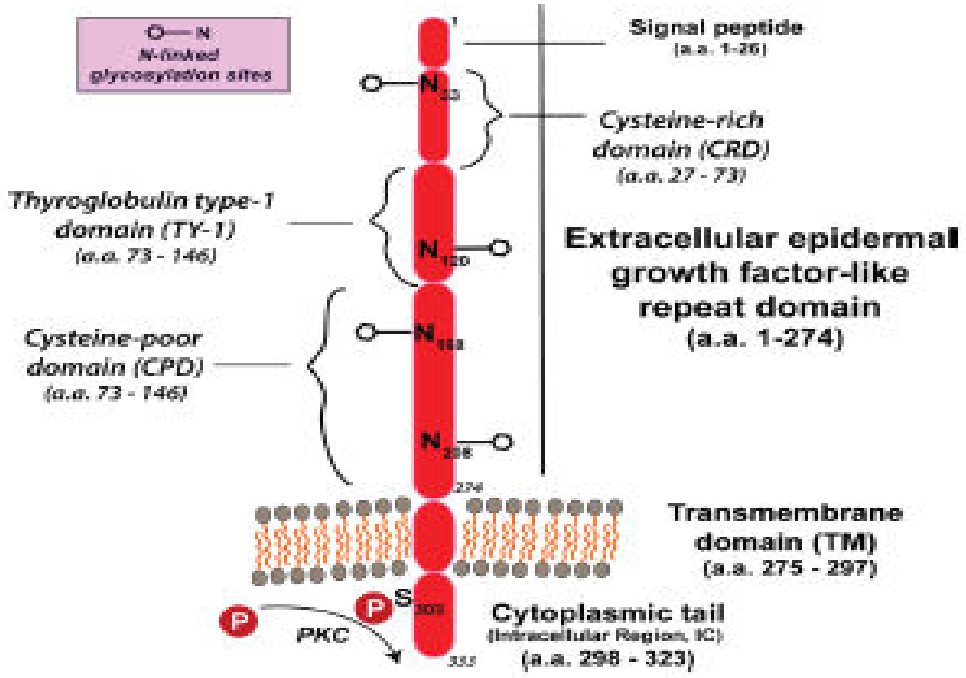
The molecular properties of the antigen bound by RS7 were identified in 1993[59], when the EGP-1 antigen was described as a 46-kDa glycoprotein (35 kDa when deglycosylated) [60,61,62] that was phosphorylated by protein kinase C (PKC), this

occurring specifically on serine 303 in the cytoplasmic domain. [62] This suggested that TROP-2 has a role in signal transduction across the cell membrane. Particularly interesting was the observation that 50% the RS7 antibody bound to the cell surface internalized within ~1 h. [59,63]

Early studies recognized that TROP-2 is involved in regulating cancer growth and invasion.[55,64,65] The gene, *TACSTD2*, was mapped on chromosome 1p32.[66] The 36-kDa nascent polypeptide, which is post-translationally modified by N-linked glycosylation, forms a type-1 transmembrane protein that is distinct from its sister molecule, epithelial cell adhesion molecule (EpCAM or EGP-2). [59,60,61]

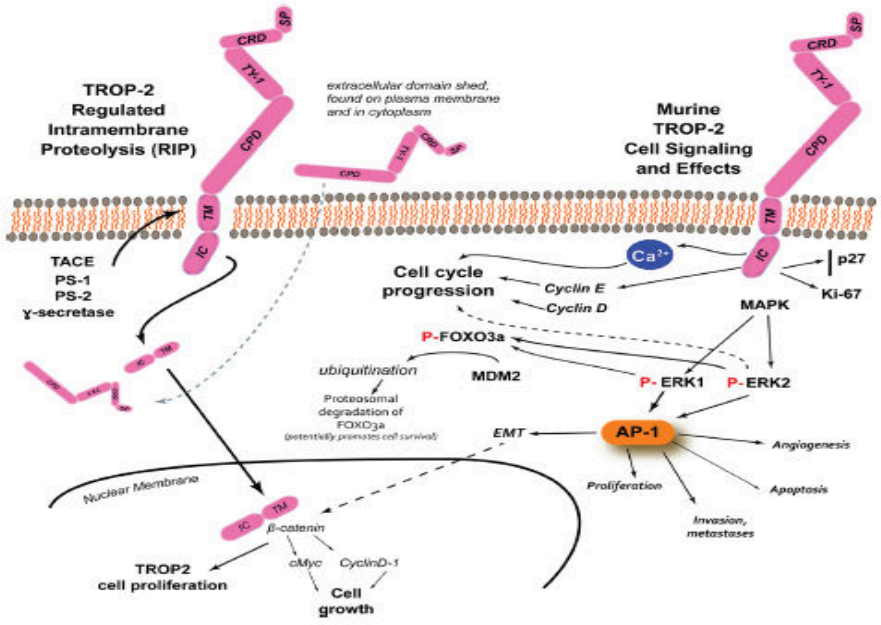
TROP-2 properties and functions-

The *TROP-2/TACSTD2* gene has been sequenced in several mammalian species.[67,68,69] Its intronless gene encodes a 35–46-kDa protein having 323 amino acids, comprised of a large extracellular domain, a single transmembrane domain, and a short intracellular, or cytoplasmic tail. [62, 64, 70] It encodes a transmembrane Ca++-signal transducer. [62, 71]



TROP-2 can affect signaling by insulin-like growth factor-1 (IGF-1) [72], and by interaction with neuregulin 1, inhibits ErbB3 (HER3) in head and neck squamous cell cancer.[73] Having a HIKE domain and a PIP2 binding site, as well as the serine phosphorylated by PKC, indicate that TROP-2 is involved in calcium signaling. This Ca2+ release is thought to induce mitogen-activated protein kinase (MAPK) signaling and advance the cell cycle.[74] The phosphorylation of TROP-2 by increased PKC could in turn activate the Raf and NF-κB pathways .[56]

TROP-2 also activates the ERK1/2 (extracellular signal regulated kinase)-MAPK pathways, contributing to cell progression [72], and could play a role in deregulating stem cell functions via Notch, Hedgehog and Wnt pathways.[74] As mentioned, the MAPK pathway is also stimulated when Ca2+ is increased. Thus, TROP-2 generally increases the levels of phosphorylated MAPK, affecting cell cycle progression. Further, activation of ERK has been reported in several tumor types that overexpress TROP-2, and this ERK1/2 activation is thought to promote tumor survival by having anti-apoptotic effects.[74]



Trop2-mediated signaling pathways

Trop2 Expression in Normal Tissues

Trop2 is expressed in a number of normal tissues, which is important to note when considering the targeting of Trop2 expressing cancer tissues. In fetal rat lungs, Trop2 is mainly expressed in type II alveolar epithelial cells (AECs), interstitial fibroblasts, smooth muscle cells, myofibroblasts, and airway epithelial cells. [75] Membrane localized expression of Trop2 has been noted in stratified squamous, cuboidal, and columnar epithelial cells. Trop2 has been expressed, albeit at different levels, in the following normal (non-cancerous) tissues: the epithelial barrier/lining of the stratum basale epidermis, breast, cervix, cornea, the epithelial secretory tissue of the endocrine and exocrine glands, esophagus, heart, kidneys (distal convoluted tubules and collecting ducts), larynx, lung, liver, pancreas, prostate, salivary gland, skin, thymus, tonsils, trachea, trophoblast cells, urothelium, and uterus. [76]

TROP 2 in cancer

TROP-2 has been studied in embryonic and fetal development[68], but most studies have focused on its role in cancer. As mentioned, with rare exceptions, it has been linked to increased tumor growth and enhanced proliferation, cell migration and anchorage-independent growth, and is overexpressed in most human solid epithelial cancers, such as oral, head-and-neck, thyroid, lung, esophageal, gastric, colorectal, pancreatic, breast, renal, uterine, cervical, ovarian cancers, and glioma.[77,78] The ectopic production of TROP-2 in cancer cells in culture has been shown to transform murine fibroblasts when injected into mice, suggesting at first that *TROP-2* is an oncogene.[79] Further studies modified this view [80], so that although the level of TROP-2 generally influences malignancy, it may not by itself be a true oncogene.

Tumor growth in mice is related to levels of *TROP- 2* mRNA, where high expression of TROP-2 is found in the largest tumors.[81] Inhibition of TROP-2 with anti-TROP-2 antibodies decreases migration of colon and breast cancer cells *in vitro* [79,82], while overexpression increases the migration of pancreatic cancer cells .[74] High TROP-2 expression is also correlated with increased metastasis in patients with different cancer types (oral squamous, thyroid, some esophageal, gastric, colorectal, pancreatic, ovarian, uterine, cervical, prostate, and urinary bladder).[57, 77, 83, 84]

MAPK (mitogen-activated protein kinase) signaling pathway is a highly conserved intracellular pathway that plays vital roles in transmission of signals to cell nucleus, where they transcriptionally regulate genes that are involved in various cellular processes.[63,64] The fundamental role of MAPK signaling pathway has been well demonstrated in human tumorigenesis, particularly for PTC.[65] TROP 2 regulates the expression of MMP2 and It has been well demonstrated that matrix metalloproteinases (MMPs) play important role in tumor progression. Many MMP family members, such as MMP2, MMP7, MMP9, MMP11, and MMP13, have been implicated to be associated with the development and progression of thyroid cancer.[72] Molecular profiling study of thyroid tumors found that TROP-2 is overexpressed in thyroid malignancy and enhances invasion by inducing matrix metalloproteinase 2 (MMP2) through extracellular receptor kinase(ERK) and c-Jun N-terminal kinase pathway(JNK) pathways.[13]

Summarizing, the increased expression of TROP-2 is reported to be "necessary and sufficient" for stimulation of cancer growth, while a bicistronic cyclin D1/TROP- 2 mRNA chimera is an oncogene.[81,85] Importantly, elevated expression is associated with more aggressive disease and a poor prognosis in several cancer types.[56,57, 68,77,78, 87] This elevated tumor expression of TROP-2 does not appear to circulate in the blood, yet there is a report that some esophageal cancer patients have

circulating antibody to this biomarker .[88] There are at least six major signaling pathways involving TROP-2 in cell proliferation, but its precise role in these and which pathway(s) are critical in different cancers and in different therapeutic approaches remain to be elucidated.

Several IHC markers have been used previously in thyroid neoplasms which include CK19, galectin-3, HBME-1, FN1, CD44v6, PPAR-g, E-cadherin, p27, and cyclin D1. However, there is no single marker accurate enough to produce a reliable diagnosis of malignancy. It is known that CK19 and galectin-3 stain in 1–5% of cells in benign thyroids. TROP-2 is free of this drawback. Blood and colloid, both of which are common sources of prominent background in HBME-1 immunostaining, were negative or weakly positive for TROP-2. To summarise, TROP-2 has advantage over the classic three marker panel (CK19, galectin-3, and HBME-1), since it doesn't produce false-positives in non-neoplastic thyroid tissue.[102]

MATERIALS AND METHODS

The present study is a prospective as well as a retrospective study conducted at the Dept. of pathology of KAHER's JN Medical college, and Dr Prabhakar Kore Hopsital, and Research Centre, Belagavi. The surgically resected specimens of 45 patients were collected from Jan 2017 to year Dec 2020 from histopathology laboratory.

Study Design: This is a observational study

Inclusion criteria:

1. All benign lesions of thyroid including multinodular goiter (MNG).

2. All malignant lesions of thyroid.

Exclusion criteria:

1. Colloid goitre.

2. Inflammatory lesions of thyroid.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human subjects Research.

(Ref.: MDC/DOME/03) (Annexure II)

Sampling Procedure: Universal sampling.

Case Selection: For retrospective cases data as well as tissue blocks were retrieved from the storage. The clinical data required was collected from medical records after obtaining permission from the concerned authorities. This clinical data of 45 patients was analysed for age, sex, size of the lesion, Duration of symptoms, lymph node metastases and tumor stage. Data regarding ultrasonography findings and other laboratory findings, if available

were also collected and included in the study. The specimens were adequately fixed using 10% neutral buffered formalin. Sections of 4-5 micron thickness were cut from formalin fixed, paraffin embedded (FFPE) blocks. A section from each of these blocks was taken and stained with Haematoxylin and Eosin (H &E). (Annexure IV)

The H& E stained slides were evaluated for

- Diagnosis of lesion
- Pattern of growth
- Invasion

Immunohistochemistry: All cases were studied for the expression of TROP 2. For IHC analysis, tissue sections of 3-4 micron thickness were prepared from FFPE blocks, on saline coated slides. Slides were air dried for 2 hrs at 58 degree C. Slides were deparafinised, dehydrated and rehydrated. The rehydrated slides were subjected to antigen retrieval using heat in a decloaking chamber. Slides were incubated for 15 mins on high heat after adding distilled water. After 15 min, the chamber was opened and slides were immediately transferred to room temperature. Slides were then washed with IHC wash buffer. The sections were then stained according to IHC procedure. Incubation of these slides was carried out with respective optimized primary antibody for 60 min. A brown precipitate is produced on addition of substrate chromogen. Slides were removed. Appropriate controls were stained with each batch of the study slides. (Annexure V)

Antibody used in IHC: TROP 2 by Bio SB company

Assesment of expression of TROP 2:

The reactivity of TROP 2 is observed as membranous staining with or without cytoplasmic staining, regardless of intensity. All non specific cytoplasmic staining was ignored.

Scoring of Immunostaining: for each case a score was assiagned.

0 : Negative or <5% positively stained cells

1 : 6%-25% positively stained cells

2 : 25%-50% positively stained cells

3 : 50%-75% positively stained cells

4 : 75%-100% positively stained cells

Statistical Analysis:

Data is coded and entered in Microsoft excel. Analysis is done using a SPSS 20 software. Descriptive statistics is used to evaluate association of data. Categorical data were expressed in terms of rates , ratios and percentages.

Sensitivity and specificity were calculated to assesss the diagnostic accuracy of TROP 2.

Microsoft excel was used for the formulation of graphs.

RESULTS

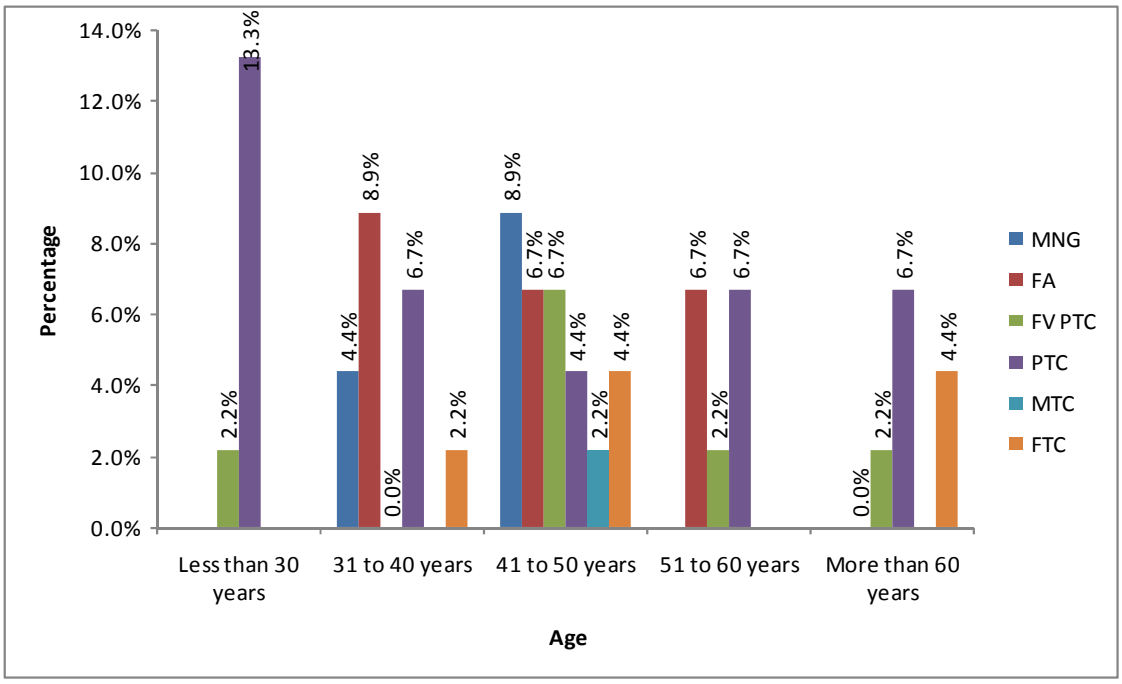
Table 1: Distribution of subjects according to their Age

Age of Subjects		Histopathological Examination Findings						Total
		Non Neoplastic Lesion	Neoplastic Lesions					
			Benign	Malignant				
				Papillary		Others		
		MNG	FA	FV PTC	CV PTC	MTC	FTC	
Less than 30 years	n	0	0	1	6	0	0	7
	%	0.0%	0.0%	2.2%	13.3%	0.0%	0.0%	15.6%
31 to 40 years	n	2	4	0	3	0	1	10
	%	4.4%	8.9%	0.0%	6.7%	0.0%	2.2%	22.2%
41 to 50 years	n	4	3	3	2	1	2	15
	%	8.9%	6.7%	6.7%	4.4%	2.2%	4.4%	33.3%
51 to 60 years	n	0	3	1	3	0	0	7
	%	0.0%	6.7%	2.2%	6.7%	0.0%	0.0%	15.6%
More than 60 years	n	0	0	1	3	0	2	6
	%	0.0%	0.0%	2.2%	6.7%	0.0%	4.4%	13.3%
TOTAL	N	6	10	6	17	1	5	45
	%	13.3%	22.3%	13.3%	37.8%	2.2%	11.1%	100.0%

MNG – Multinodular Goitre, FA- Follicular Adenoma, FV PTC – Follicular Variant of Papillary Thyroid carcinoma, PTC – Papillary Thyroid Carcinoma, MTC – Medullary Thyroid Carcinoma, FTC – Follicular Thyroid Carcinoma, n – number of subjects

In this study all 45 cases were in the age group of 22 to 80 years which means youngest patient is 22 years old while oldest is 80yrs old. Overall thyroid lesions are seen more common in 41 to 50 years of age. It contributes about 33.3% of the total study cases. However peak incidence of malignancy is found in 4th decade (i.e. 41- 50 years age group).

Graph 1: Distribution of subjects according to their Age

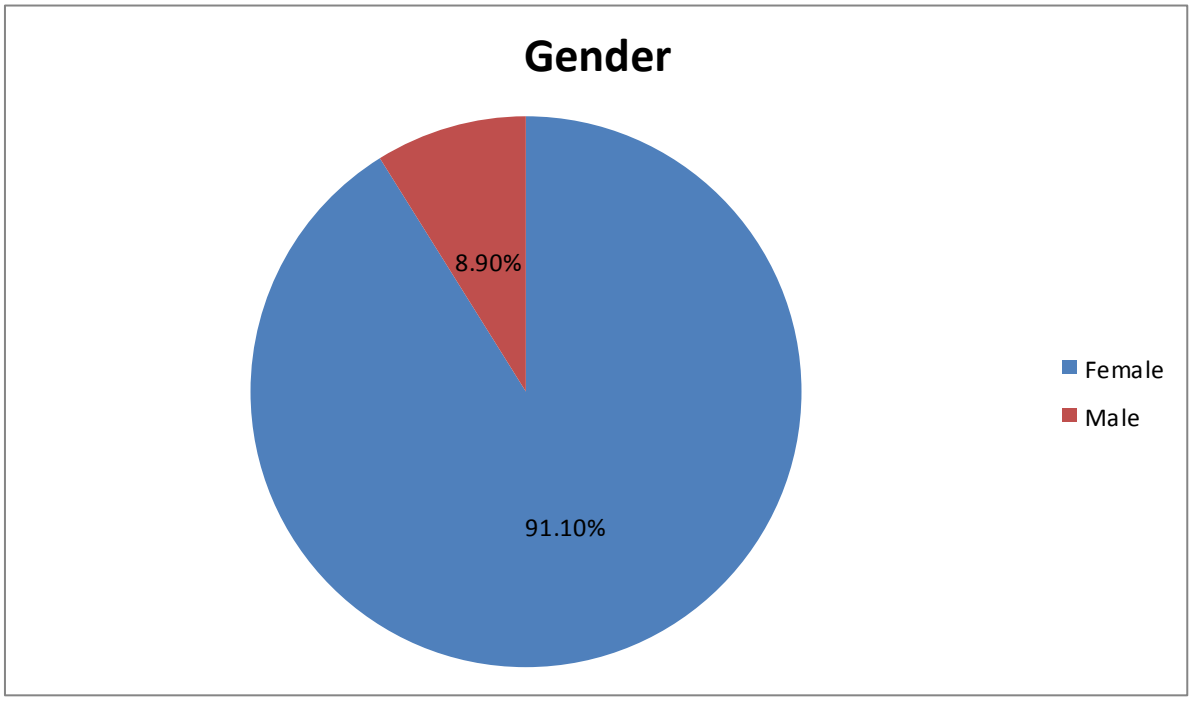


The peak incidence of PTC(13.3%) is seen in <30 years of age whereas peak incidence of FTC(4.4%) is seen in 4th as well as in 6th decade. Most of FA cases are seen in 31-40 years age group.

Table 2: Distribution of subjects according to their Gender

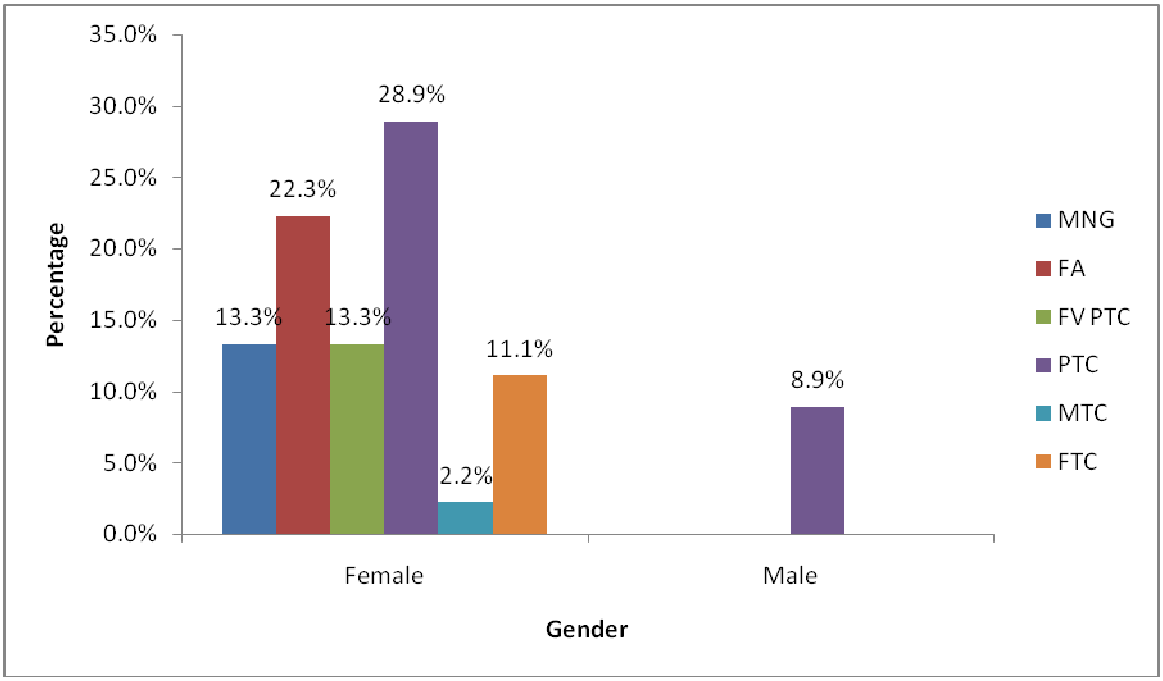
Gender		Histopathological Examination Findings						Total
		Non Neoplastic Lesion	Neoplastic Lesions					
			Benign	Malignant				
				Papillary		Others		
		MNG	FA	FV PTC	PTC	MTC	FTC	
Female	n	6	10	6	13	1	5	41
	%	13.3%	22.3%	13.3%	28.9%	2.2%	11.1%	91.1%
Male	n	0	0	0	4	0	0	4
	%	0.0%	0.0%	0.0%	8.9%	0.0%	0.0%	8.9%
TOTAL	N	6	10	6	17	1	5	45
	%	13.3%	22.3%	13.3%	37.8%	2.2%	11.1%	100.0%

MNG – Multinodular Goitre, FA- Follicular Adenoma, FV PTC – Follicular Variant of Papillary Thyroid carcinoma, PTC – Papillary Thyroid Carcinoma, MTC – Medullary Thyroid Carcinoma, FTC – Follicular Thyroid Carcinoma, n – number of subjects



This study comprised of 91.10 % females and 8.90% males. Overall it is found that thyroid lesions are 10 times more common in females than in males.

Graph 2: Distribution of subjects according to their Gender

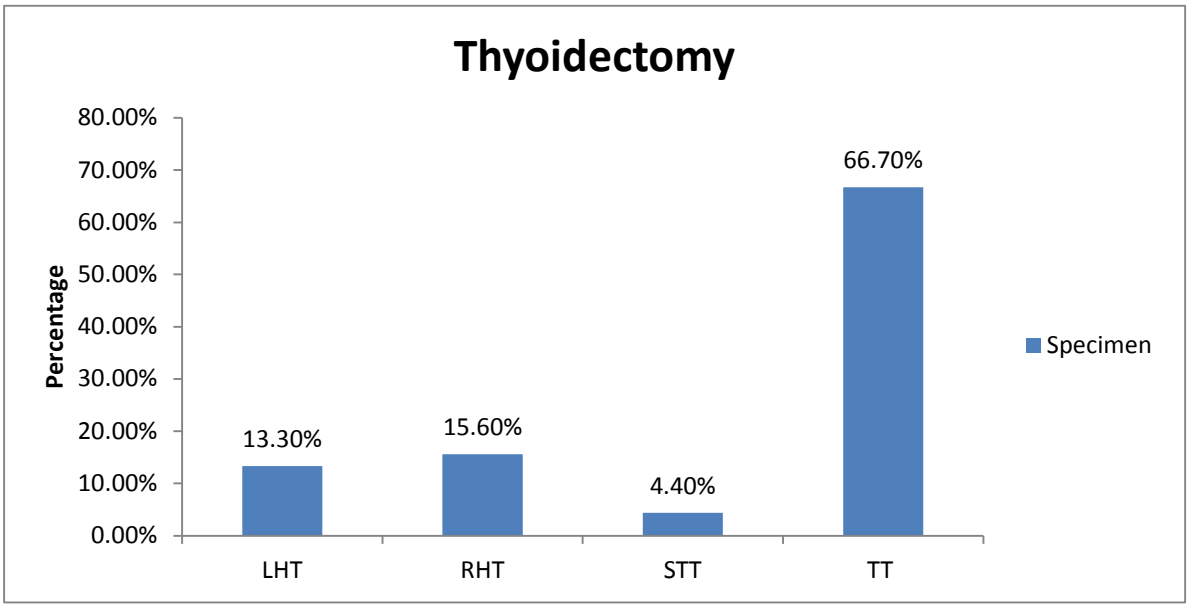


All the subgroups of thyroid lesions showed female predominance. All 4 male cases included in this study belong to PTC. So M:F ratio in PTC is found to be 1 :4.75 that means PTC is five times more common in females than in males

Table 3: Distribution of Subjects according to the Type of Thyroidectomy

Thyroid Specimen	No of Patients	Percent
Left Hemithyroidectomy (LHT)	6	13.3%
Right Hemithyroidectomy (RHT)	7	15.6%
Subtotal Thyroidectomy (STT)	2	4.4%
Total Thyroidectomy (TT)	30	66.7%
Total	45	100.0%

Graph 3: Distribution of Subjects according to the Type of Thyrroidectomy

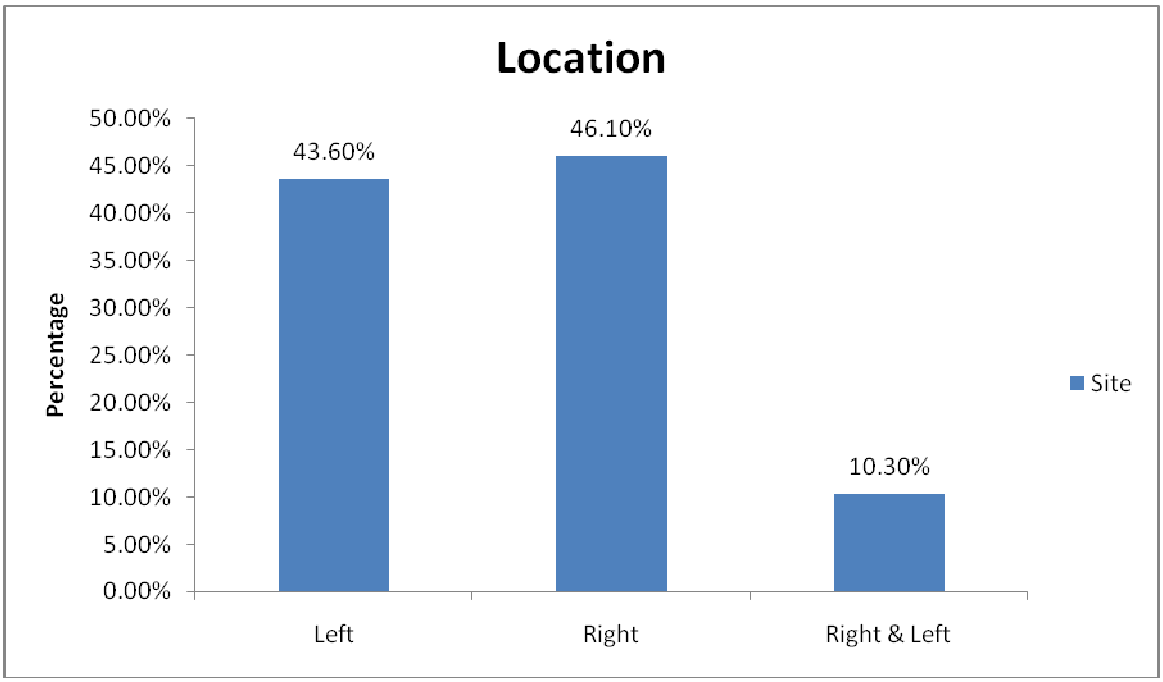


The commonest operation done in study population is total thyroidectomy.

Table 4: Distribution of subjects according to the Location of thyroid lesion in neoplastic cases

Site	No of Patients	Percent
Left lobe	17	43.6%
Right lobe	18	46.1%
Right & Left lobe	4	10.3%
Total	39	100.0%

Graph 4: Distribution of subjects according to the Location of thyroid lesion in neoplastic cases

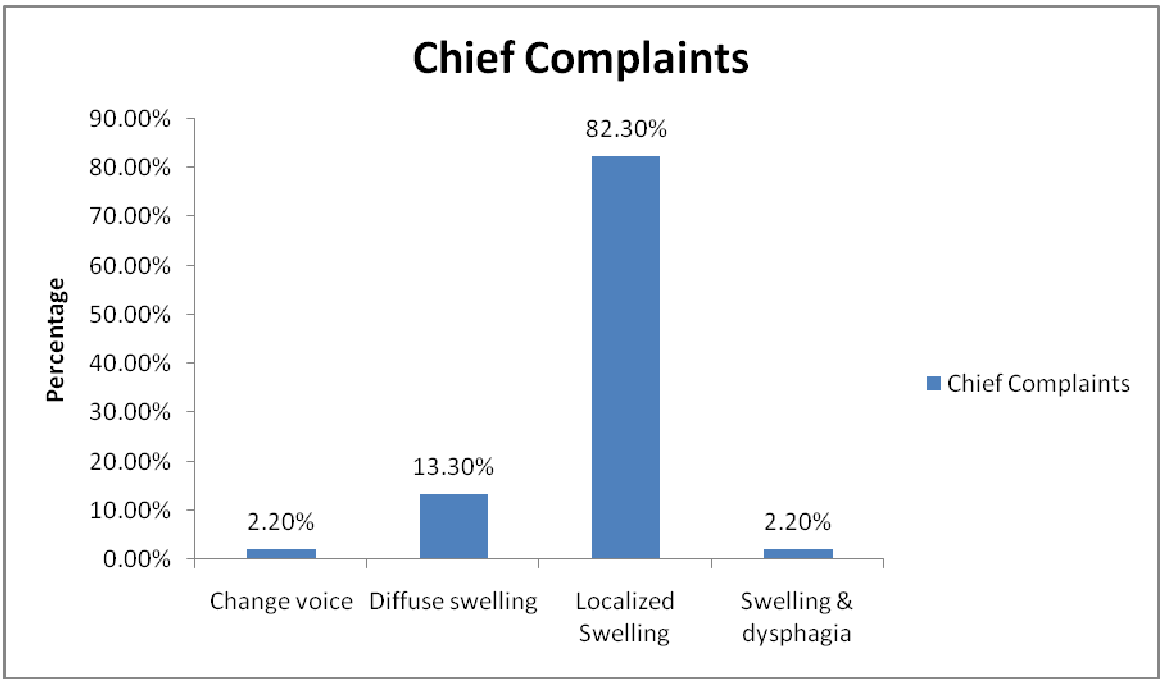


Most of the thyroid lesions in this study involve right lobe(46.1%) followed by left lobe(43.6%) and very few cases found to involve both the lobes(4%).

Table 5: Distribution of Subjects according to the Chief Complaints

Chief Complaints	Number of Patients	Percent
Change voice	1	2.2%
Diffuse swelling	6	13.3%
Localized Swelling	37	82.3%
Swelling & dysphagia	1	2.2%
Total	45	100.0%

Graph 5: Distribution of Subjects according to the Chief Complaints

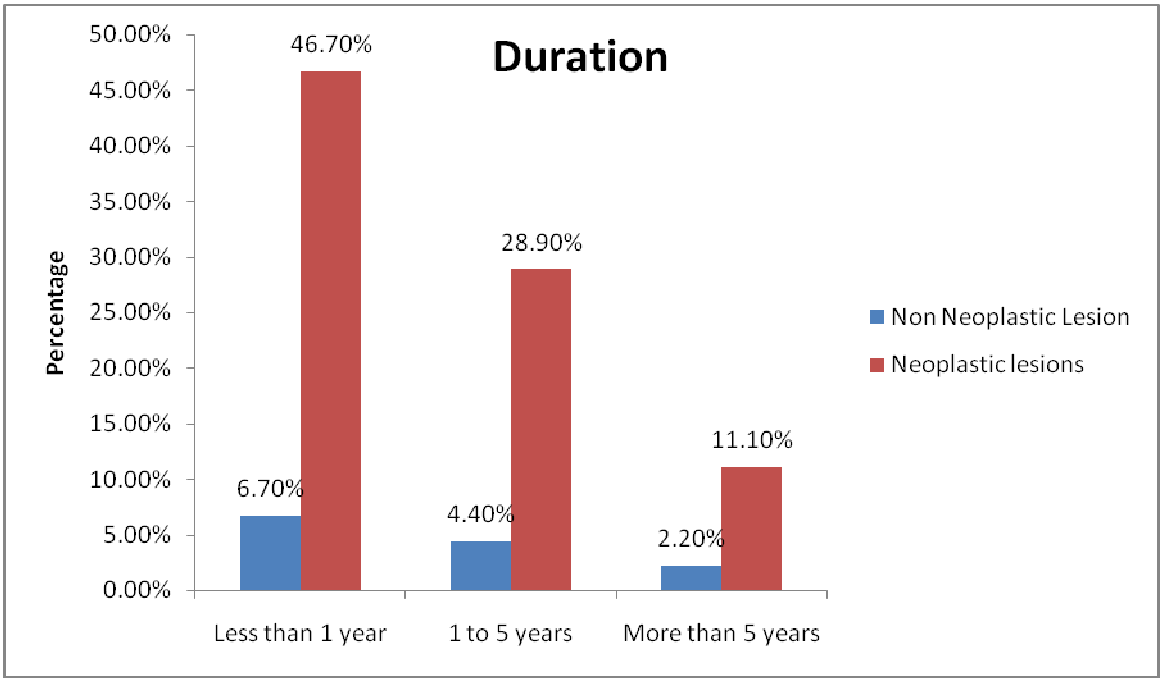


Most common complaint in study population is found to be a localized swelling which is most common finding in malignancy of thyroid.

Table 6: Distribution of Subjects according to the Duration of symptoms

Duration	Non Neoplastic Lesions		Neoplastic Lesions		Total	
	n	%	n	%	N	%
Less than 1 year	3	6.7%	21	46.7%	24	53.4%
1 to 5 years	2	4.4%	13	28.9%	15	33.3%
More than 5 years	1	2.2%	5	11.1%	6	13.3%
Total	6	13.3%	39	86.7%	45	100.0%

Graph 6: Distribution of Subjects according to the Duration of symptoms

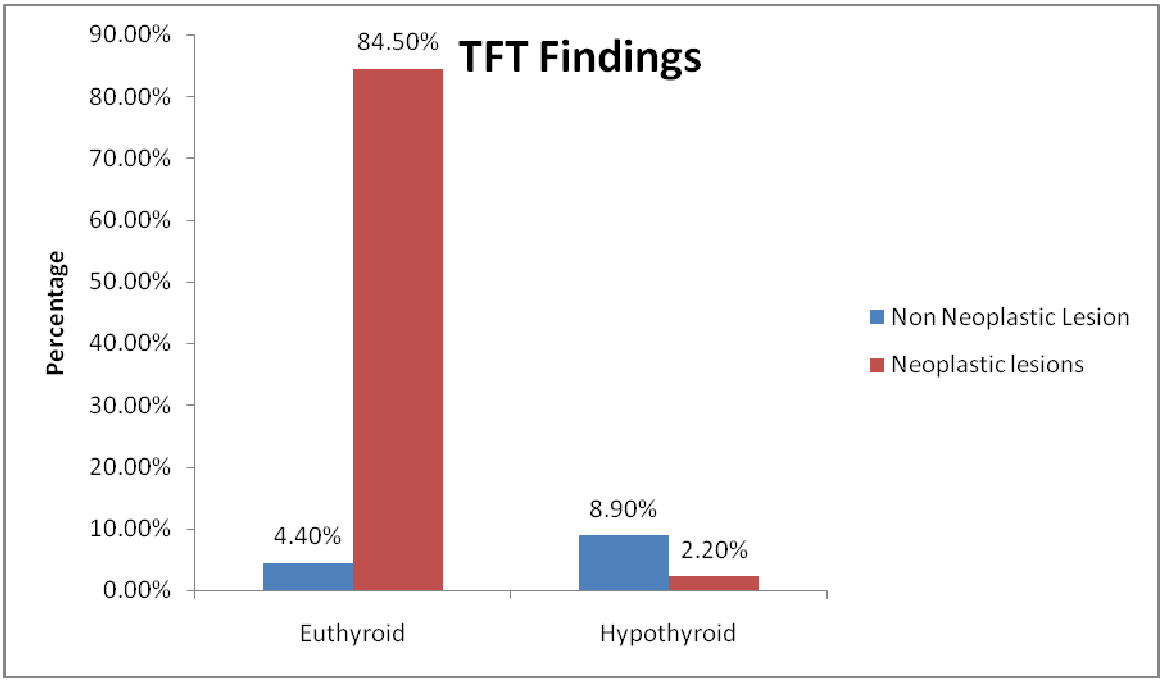


Overall duration of symptoms found in study population in most of the cases(53.4%) is less than a year. For neoplastic cases also duration of symptoms in most of the cases is less than a year followed by 1-5 years followed by more than 5 years

Table7: Distribution of Subjects according to the Thyroid Function Test

TFT Findings	Non Neoplastic Lesions		Neoplastic Lesions		Total	
	n	%	n	%	N	%
Euthyroid	2	4.4%	38	84.5%	40	89.9%
Hypothyroid	4	8.9%	1	2.2%	5	11.1%
Total	6	13.3%	39	86.7%	45	100.0%

Graph 7: Distribution of Subjects according to the Thyroid Function Test



Majority of cases were Euthyroid followed by hypothyroid TFT. In hypothyroid cases 4 belonged to non neoplastic lesions and 1 belonged to neoplastic lesion

Table 8: Correlation of Clinical Diagnosis with Histopathological Findings

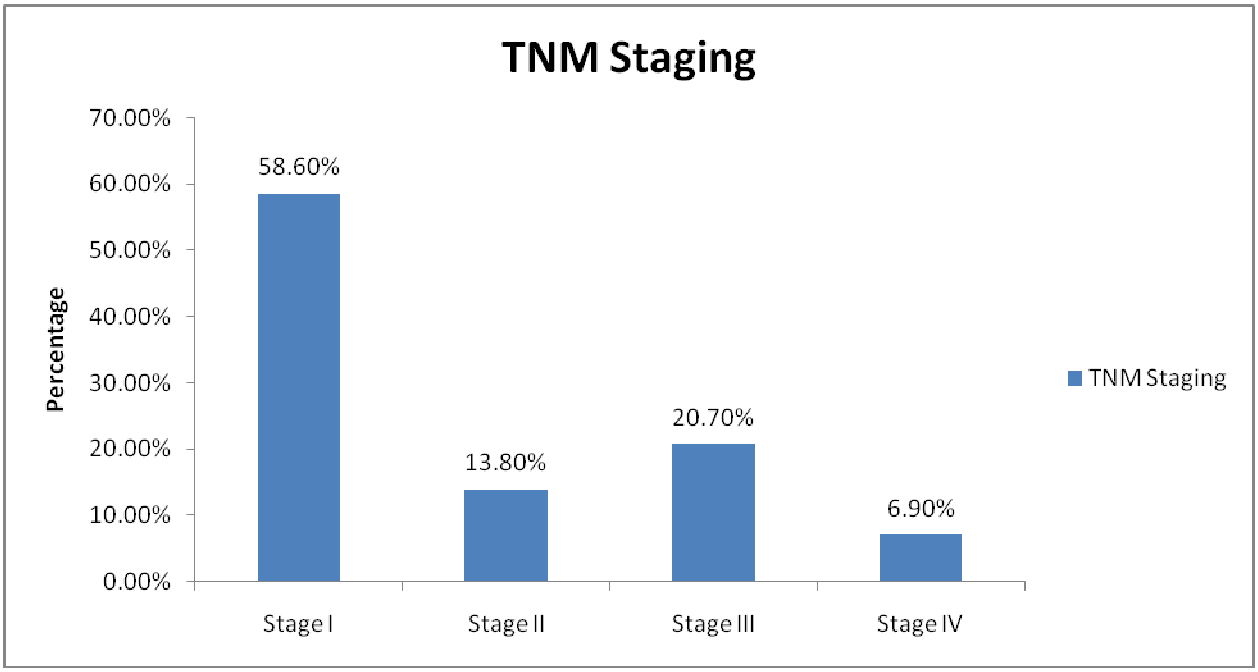
Parameters	Provisional clinical diagnosis correlated with HPE	Differed on HPE
1.MNG(n=6)	04 cases (8.89%)	02 cases (4.44%) (Colloid G, ?Carcinoma)
2.FA (n=10)	03 cases (6.66%)	07 cases (15.56%) (4 Colloid G, 3 Carcinoma)
3.PTC (n=23)	14 cases (31.11%)	09 cases (20%) (4 MNG, 5 Colloid G)
4.FTC (n=5)	04 cases (8.89%)	01 case (2.23%) (Graves disease)
5.MTC (n=1)	01 case (2.22%)	0 (0%)
Total (n=45)	26 (57.77%)	19 (42.23%)

In present study clinical diagnosis is correlating with histopathological diagnosis in 57.77% of cases. However, in 42.23% of cases histopathological diagnosis differ from clinical diagnosis.

Table 9: Distribution of malignant cases according to the TNM staging

TNM Staging	No of Patients	Percent
Stage I	17	58.6%
Stage II	4	13.8%
Stage III	6	20.7%
Stage IV	2	6.9%
Total	29	100.0%

Graph 8: Distribution of malignant cases according to the TNM staging

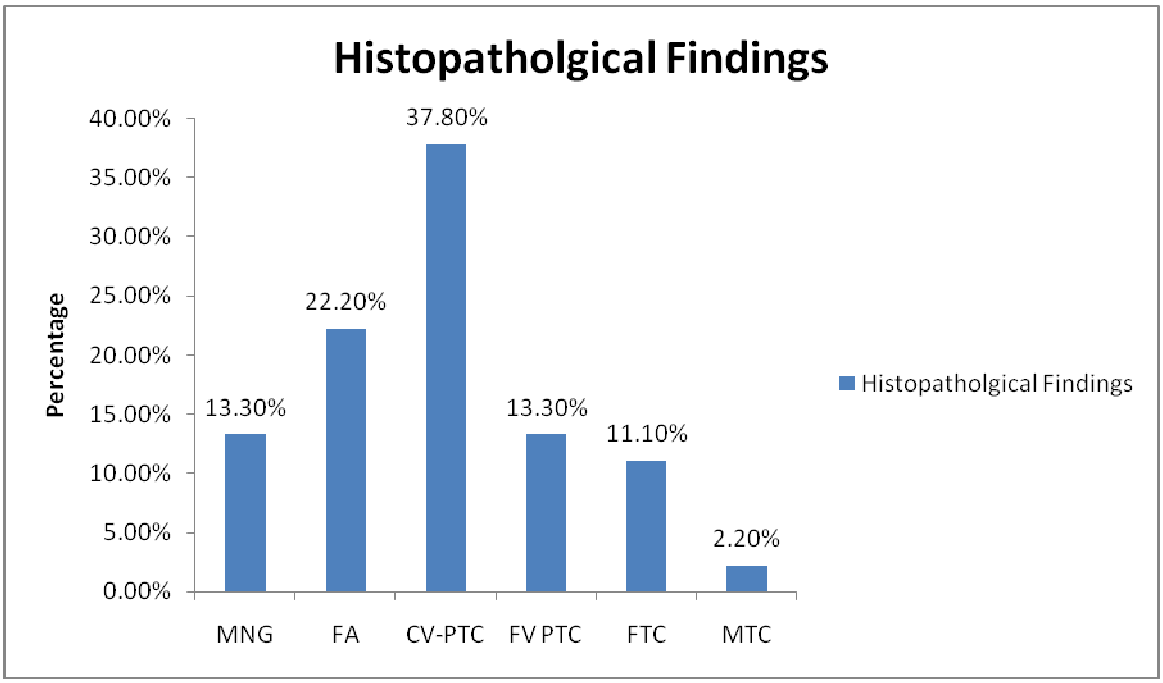


Most of the malignant cases (58.6%) were seen at stage I at the time of diagnosis followed by stage III, Stage II, Stage IV.

Table 10: Distribution of cases according to the Histopatholgical Findings

Histopatholgical Findings	No of Patients	Percent
Multinodular Goitre (MNG)	06	13.3%
Follicular Adenoma (FA)	10	22.2%
Classical Variant of Papillary Thyroid Carcinoma (CV - PTC)	17	37.8%
Follicular Variant of Papillary Thyroid Carcinoma (FV – PTC)	06	13.3%
Follicular Thyroid Carcinoma (FTC)	05	11.1%
Medullary Thyroid Carcinoma (MTC)	01	2.2%
Total	45	100.0%

Graph 9: Distribution of cases according to the Histopathological Findings

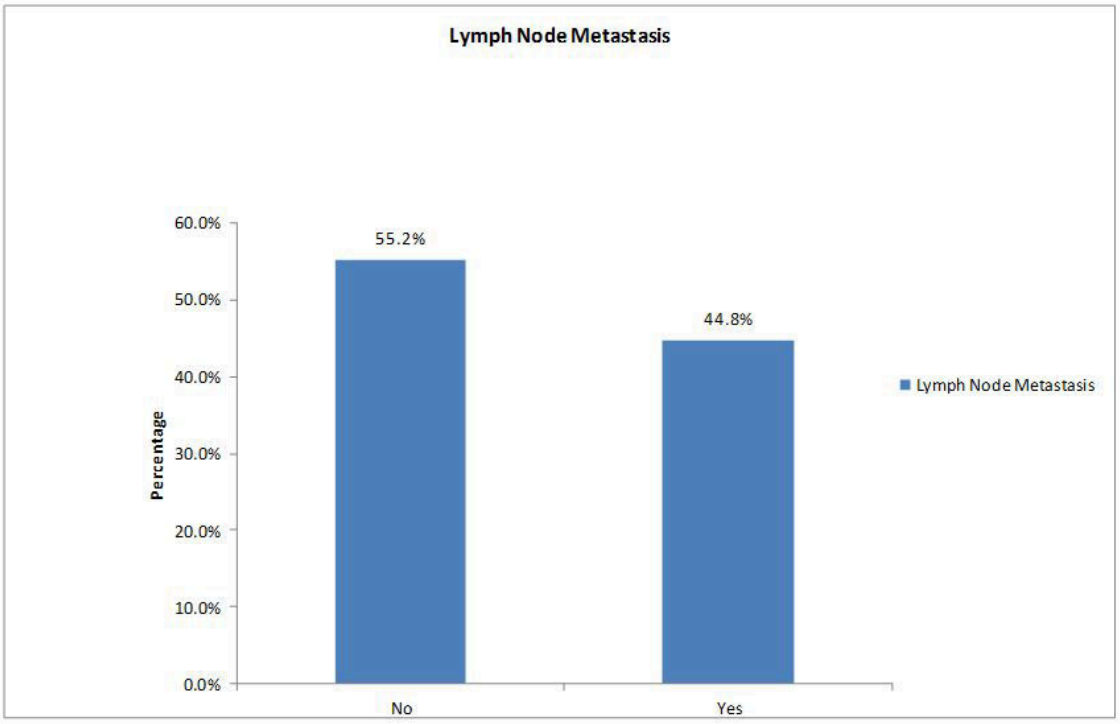


Out of 45 cases taken into this study, 16 were non neoplastic lesions including 10 cases of follicular adenoma and 6 cases of MNG. Total malignant cases were 29 and amongst this most common histopathological diagnosis is CV-PTC (37.80%) followed by FV PTC (13.30%), FTC (11.10%), MTC (2.20%)

Table 11 : Distribution of Malignant cases based on Lymph node metastasis

Lymph node metastais	No of Patients	Percent
No	16	55.2%
Yes	13	44.8%
Total	29	100.0

Graph 10: Distribution of Malignant cases based on Lymph node metstasis

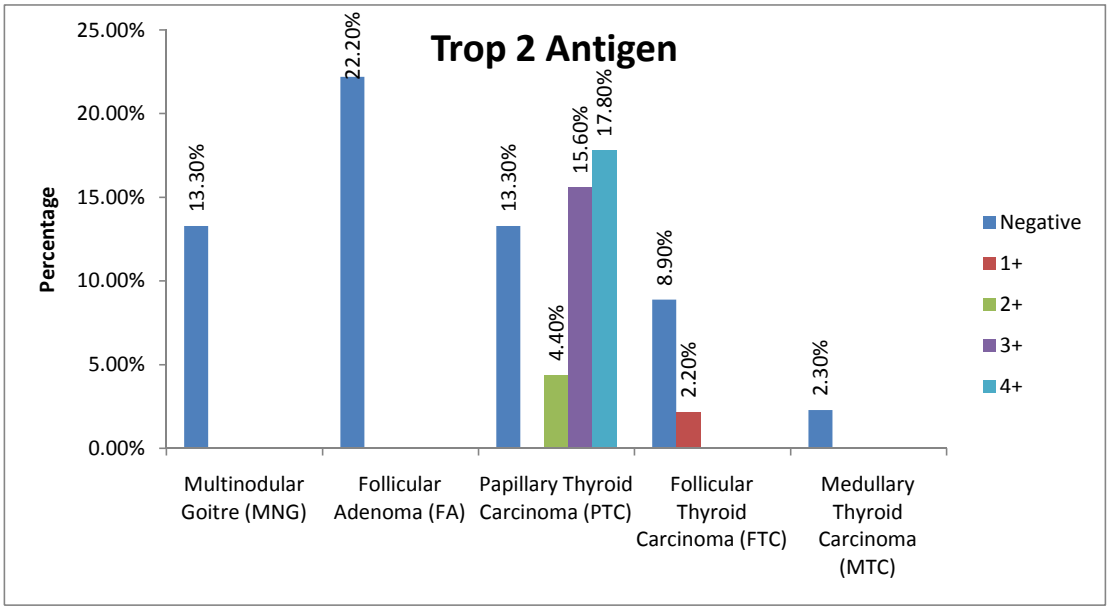


Amongst malignant cases, 44.82% cases showed LNM.

Table 12: Scoring of TROP 2 in Thyroid lesions

Histopatholgical Findings	Trop 2 antigen					
	N &%	Negative	Positive			
			1+	2+	3+	4+
Multinodular Goitre (MNG) (n=06)	n	6	0	0	0	0
	%	13.3%	0%	0%	0%	0%
Follicular Adenoma (FA) (n=10)	n	10	0	0	0	0
	%	22.2%	0%	0%	0%	0%
Papillary Thyroid Carcinoma(PTC) (n=23)	n	6	0	3	7	7
	%	13.3%	0	4.4%	15.6%	17.8%
Follicular Thyroid Carcinoma(FTC) (n=05)	n	4	1	0	0	0
	%	8.9%	2.2%	0%	0%	0%
Medullary Thyroid Carcinoma(MTC) (n=01)	n	1	0	0	0	0
	%	2.3%	0%	0%	0%	0%
Total (n=45)	n	27	1	2	7	8
	%	60%	2.2%	4.4%	15.6%	17.8%

Graph 11: Scoring of TROP 2 in Thyroid lesions

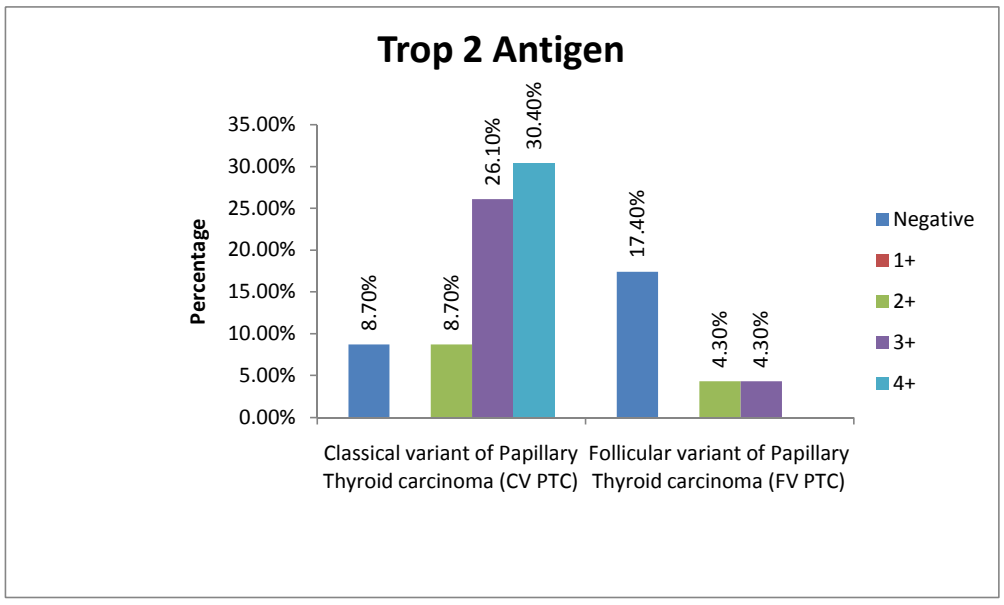


The cellular localization of TROP 2 IHC is similar in all positive cases seen.(i.e They all showed cell membrane positivity with or without cytoplasmic positivity). In PTC cases strong and diffuse reactivity (3+ & 4+) is seen in 15 cases while 2+ immunoreactivity observed in 2 cases. Six cases of PTC were negative which showed characteristic nuclear features of PTC throughout the tumor on routinely stained sections. One out of five FTC showed weak positivity for TROP 2. MTC was negative for TROP 2. All FA and MNG cases are negative for trop 2.

Table 13: Trop 2 expression in subtypes of Papillary Thyroid Carcinoma (PTC)

Histopatholgical Findings	Trop 2 antigen					
	N &%	Negative	Positive			
			1+	2+	3+	4+
Classical variant of Papillary Thyroid carcinoma (CV PTC=17)	n	2	0	2	6	7
	%	8.7%	0%	8.7%	26.1%	30.4%
Follicular variant of Papillary Thyroid carcinoma (FV PTC=06)	n	4	0	1	1	0
	%	17.4%	0%	4.3%	4.3%	0%
Total (n=23)	n	6	0	3	7	7
	%	26.1	0%	13%	30.4%	30.4%

Graph 12: Trop 2 expression in subtypes of Papillary Thyroid Carcinoma (PTC)



Amongst subtypes of PTC most of positive cases (i.e. 65.2%) belonged to CV of PTC. Out of 6 cases of FV of PTC 4 cases (66.66%) didn't show any immunoreactivity for TROP 2.

Table 14 : TROP 2 expression in Thyroid lesions

SL no	Histopathological Diagnosis {total n (% total)}	TROP 2 Negative n (%)	TROP 2 Positive n (%)
1	Mutinodular Goitre- 6 (100%)	6 (100%)	0(0%)
2	Follicular Adenoma- 10 (100%)	10 (100%)	0(0%)
3	Classical Variant of Papillary Thyroid Carcinoma - 17 (100%)	2 (11.8%)	15 (88.2%)
4	Follicular Variant of Papillary Thyroid Carcinoma - 6 (100%)	4 (66.7%)	2 (33.3%)
5	Follicular Thyroid Carcinoma- 5 (100%)	4 (80.0%)	1 (20%)
6	Medullary Thyroid Carcinoma- 1 (100%)	1 (100%)	0 (0%)

Graph 13: TROP 2 expression in Thyroid lesions

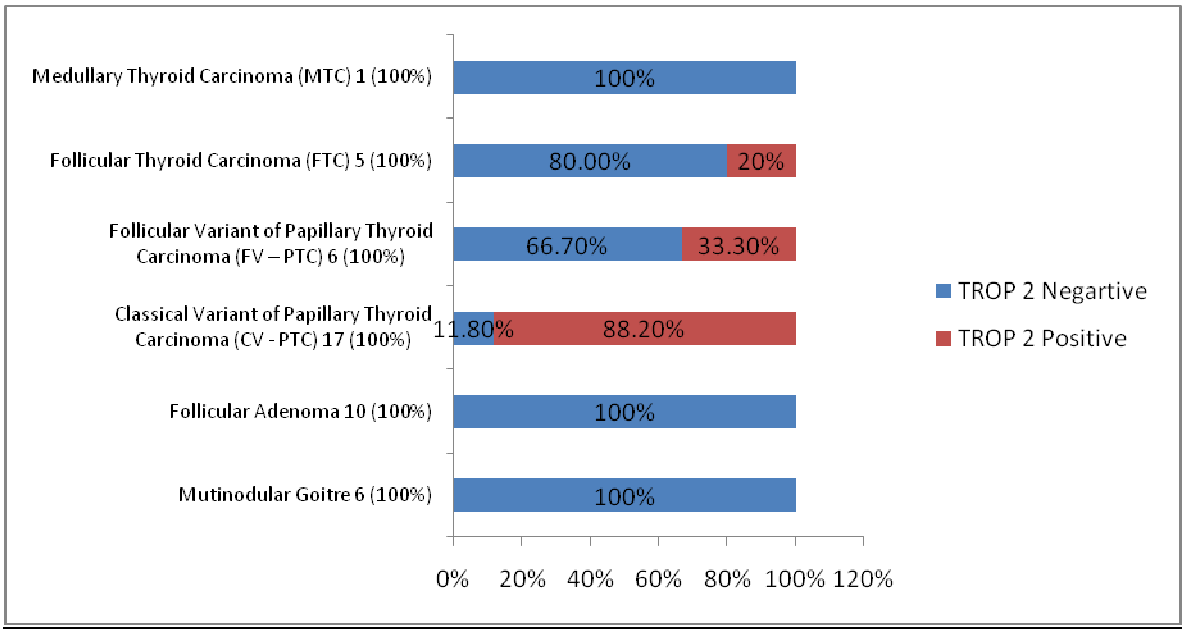


Table 15: TROP 2 expression in CV PTC v/s Benign lesions

TROP 2	**CV PTC**	**Benign lesions**	**Total**
Positive	15	0	15
negative	02	16	18
Total	17	16	33

Table 16: TROP 2 expression in PTC v/s Benign lesions

TROP 2	**PTC**	**Benign lesions**	**Total**
Positive	17	0	17
negative	06	16	22
Total	23	16	39

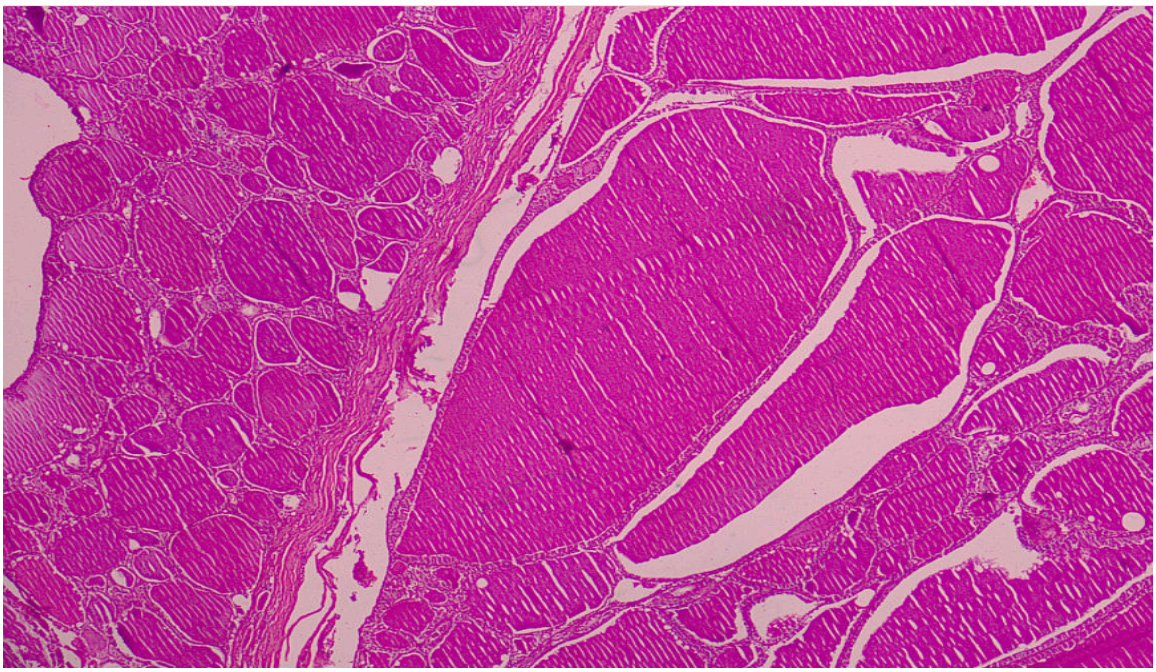
From present study it was found that-

- TROP 2 showed 88.23% sensitivity,100% specificity in distinguishing CV PTC from other benign lesions. The PPV and NPV was 100% and 88.88%, respectively.

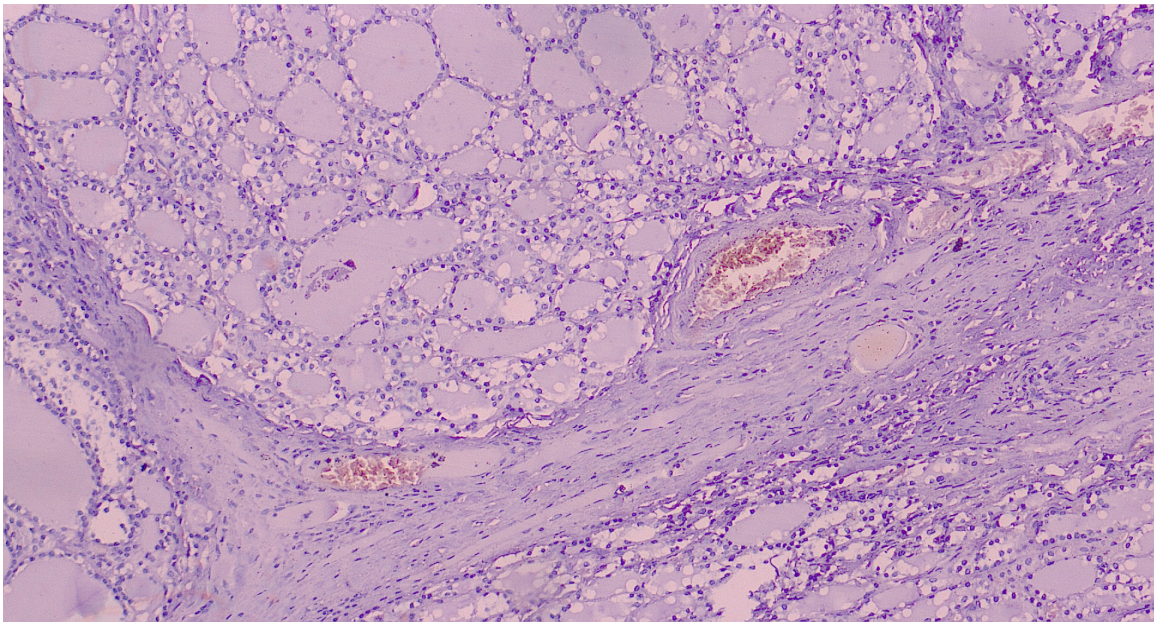
- TROP 2 showed 73.91% sensitivity,100% specificity in distinguishing all variants of PTC from other benign lesions. The PPV and NPV was 100% and 72.72%, respectively.

- TROP 2 showed 33.33% sensitivity, in distinguishing FV PTC from follicular neoplasms.

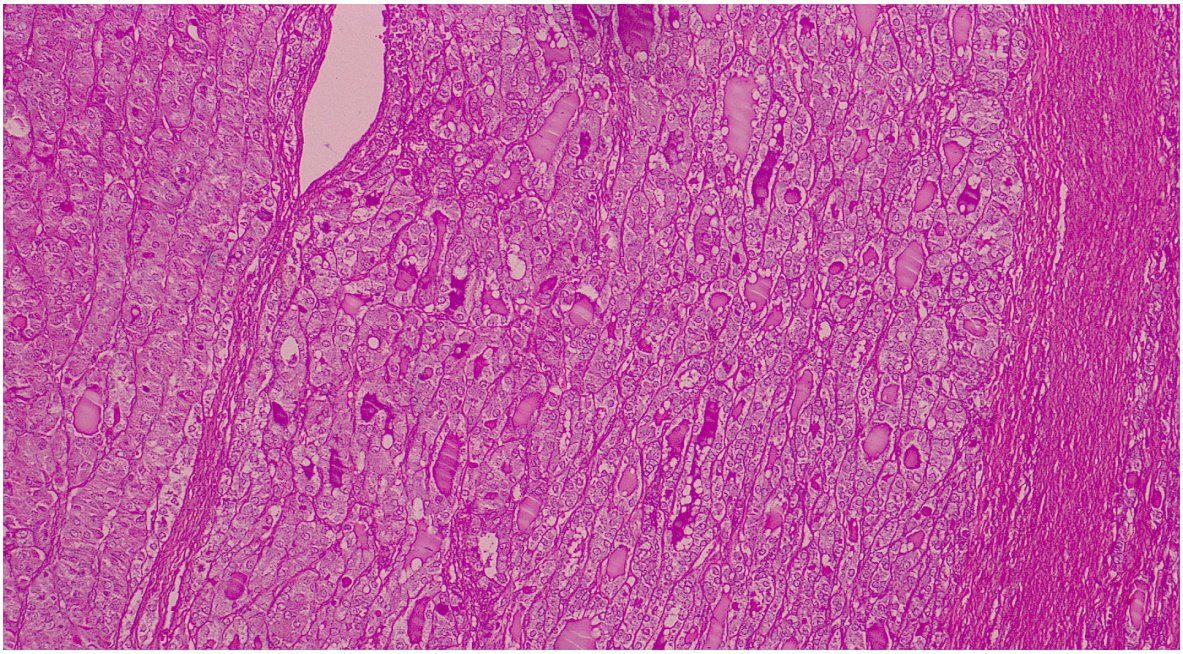
PICTOMICROGRAPHS



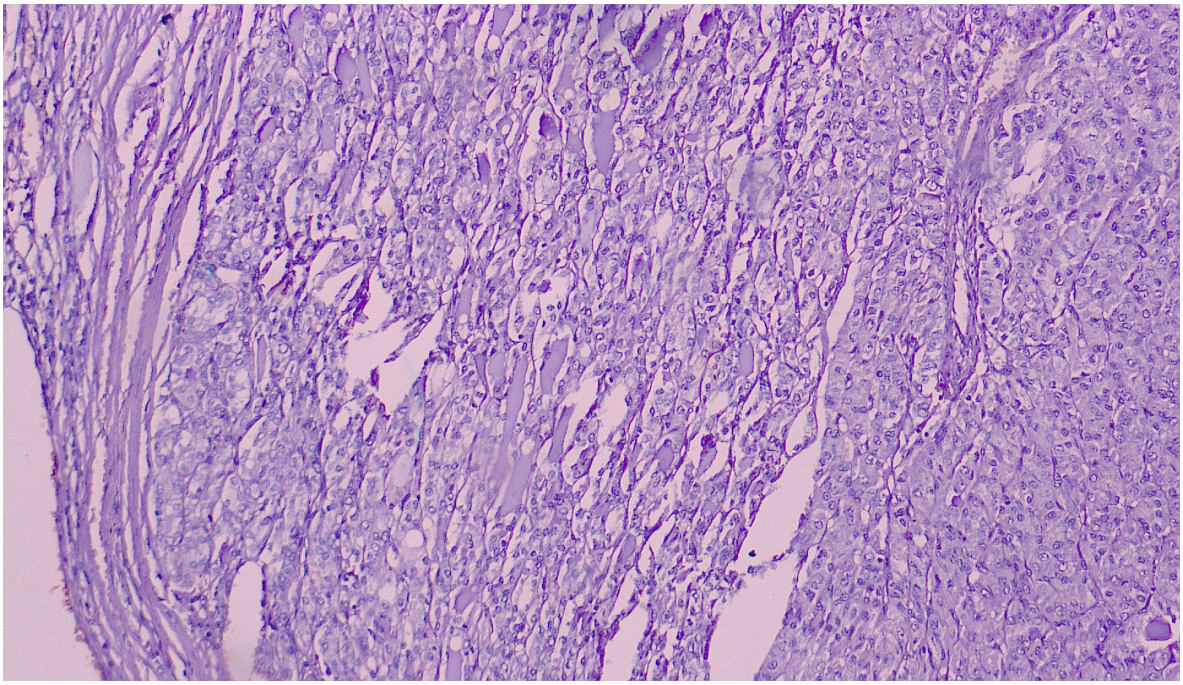
Photograph 1a: Multinodular goiter (H&E 4x)



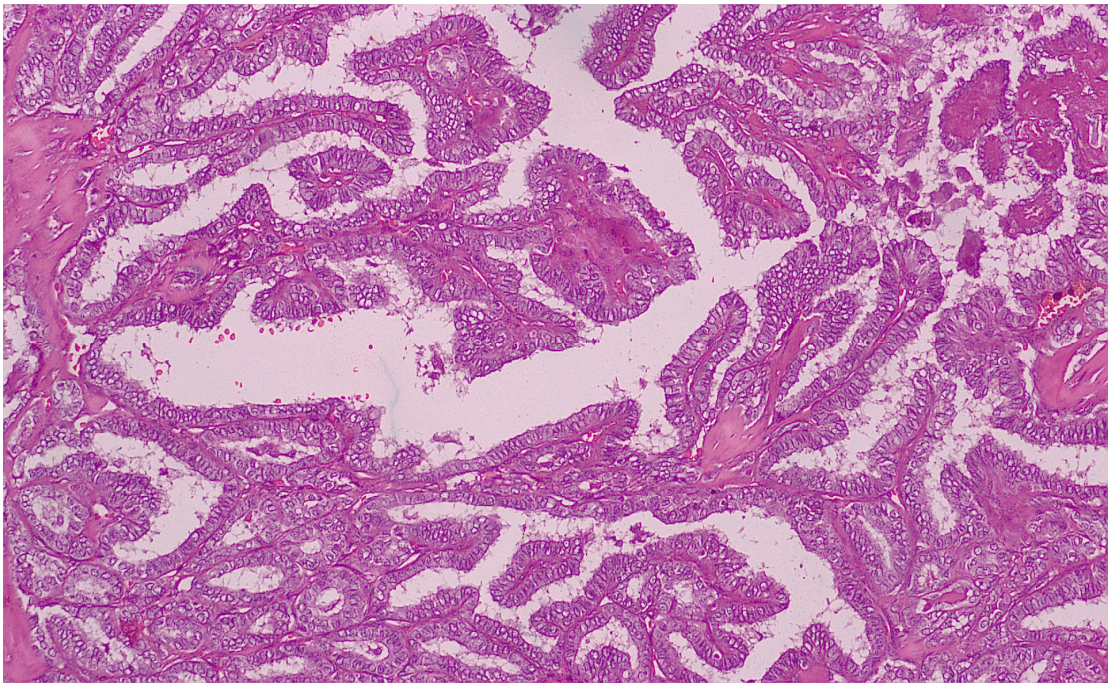
Photograph 1b: Trop 2 negativity in a case of multinodular goiter (IHC stain 10x)



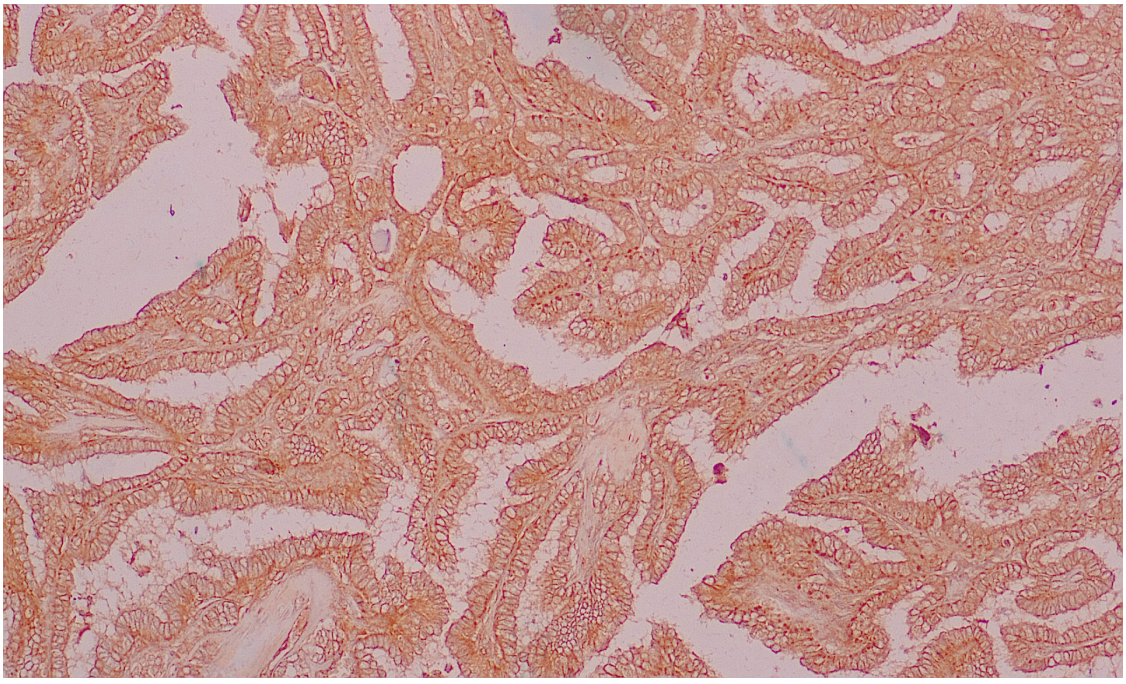
Photograph 2a: Follicular Adenoma (H&E 10x)



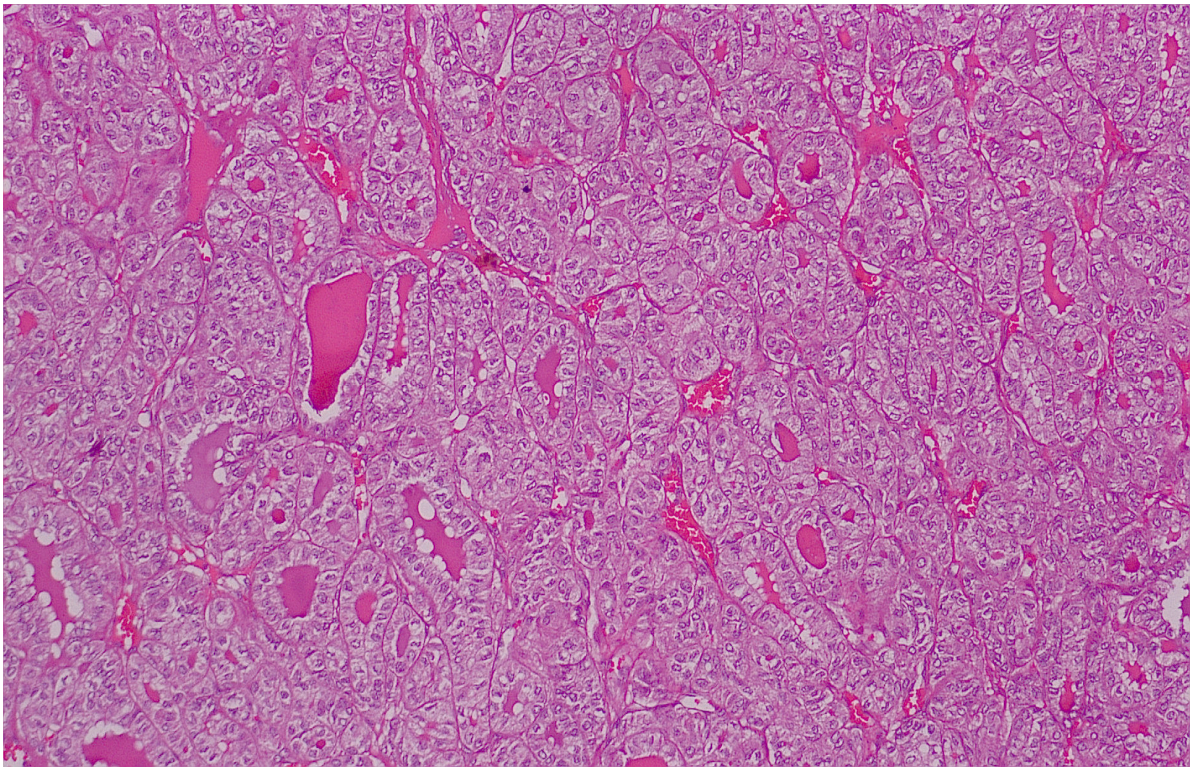
Photograph 2b: Trop 2 negativity in a case of Follicular Adenoma (IHC stain 10x)



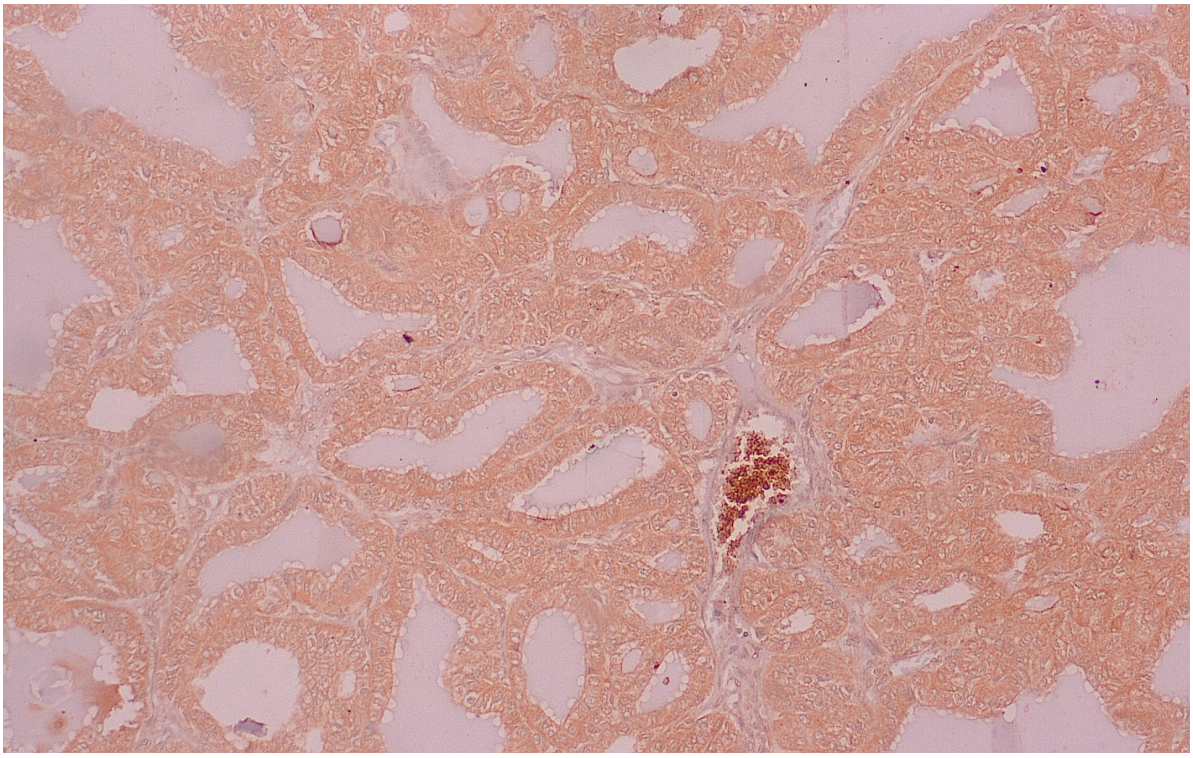
Photograph 3a: Papillary thyroid carcinoma (H&E 10x)



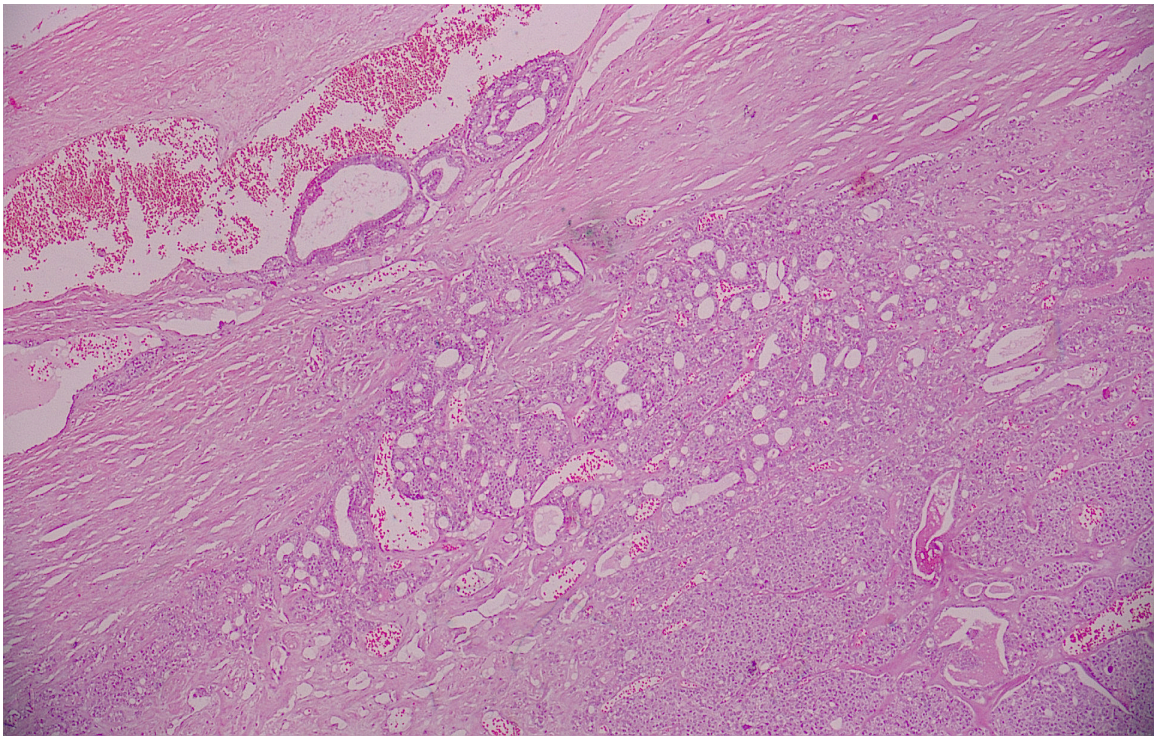
Photograph 3b: Trop 2 positivity in a case of PTC. IHC score- 4 (IHC stain 10x)



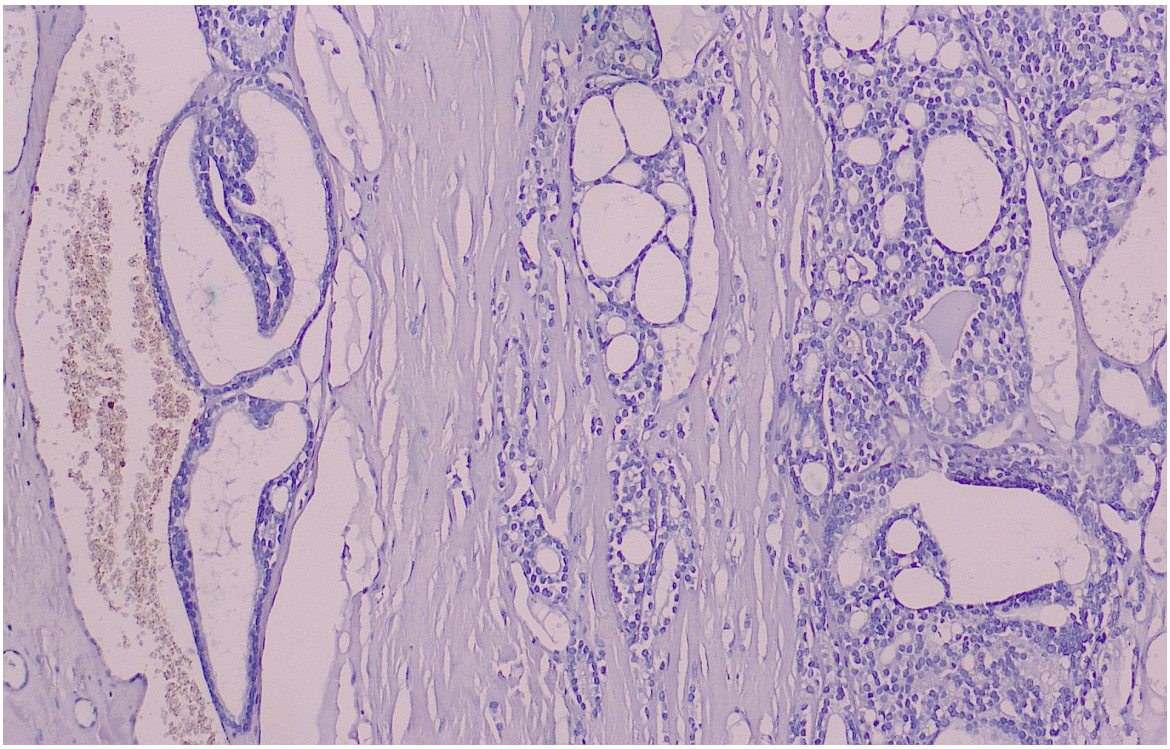
Photomicrograph 4a: Follicular variant of PTC (H&E 10x)



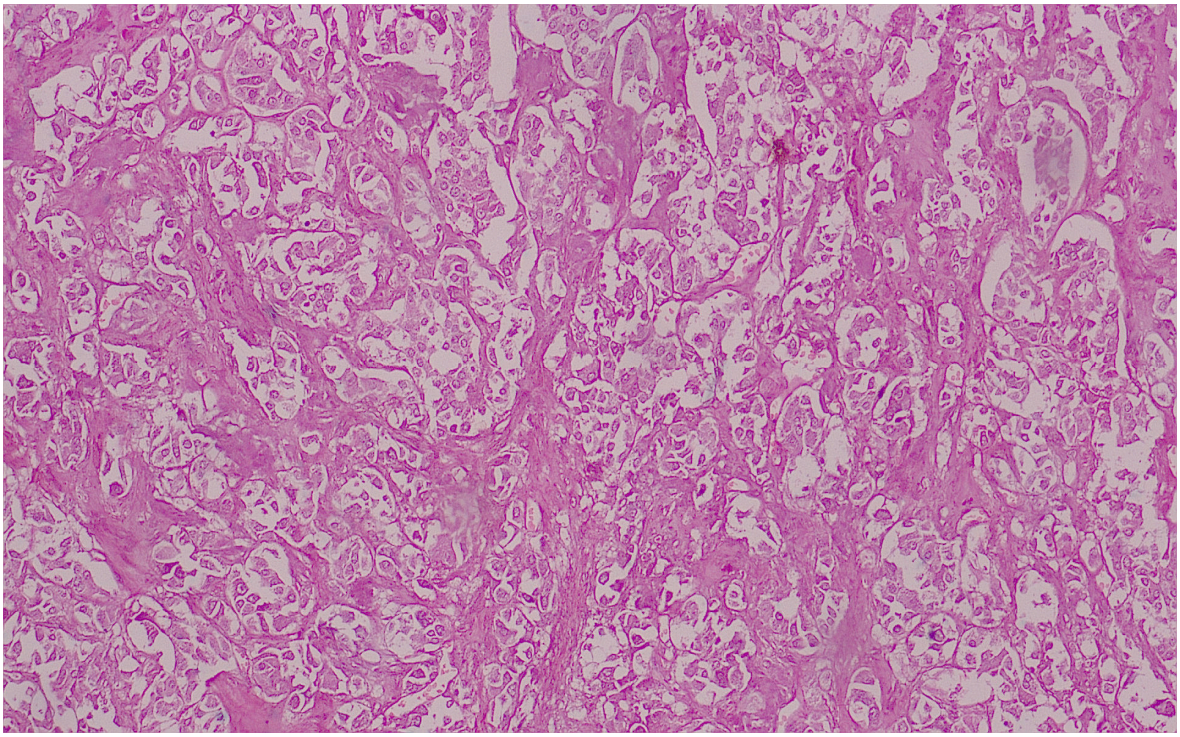
Photograph 4b: Trop 2 positivity in a case of FV PTC. IHC score- 3 (IHC stain 10x)



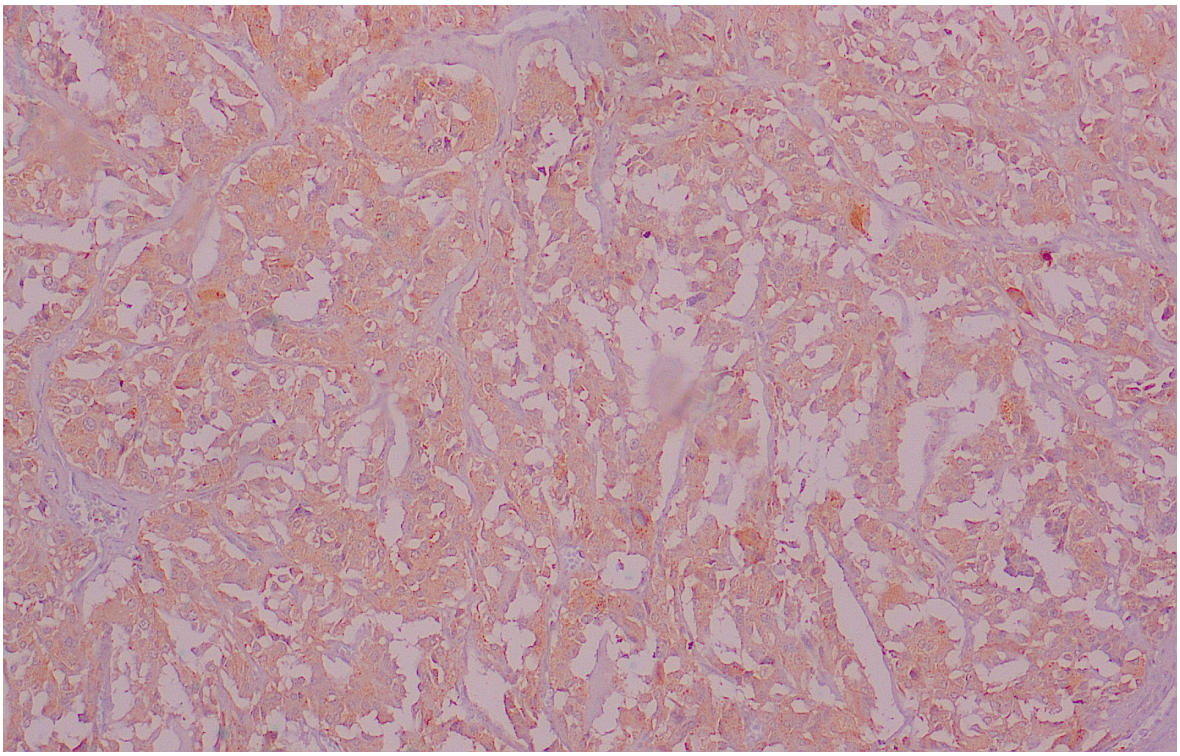
Photograph 5a: Follicular thyroid carcinoma showing LVI (H&E 4x)



Photograph 5b: Trop 2 negativity in a case of FTC (IHC stain 10x)



Photograph 6a: Medullary thyroid carcinoma (H&E 10x)



Photograph 6b: Trop 2 negativity (Non specific staining) in a case of a MTC

(IHC stain10x)

DISCUSSION

Total 45 cases of thyroid lesions were studied in current study in relation to age, sex, site and histopathological characteristics. Cases included benign and malignant thyroid lesions. IHC evaluation with TROP 2 was done to find out the sensitivity and specificity of the marker in diagnosing PTC from other benign lesions.

In the current study, all 45 cases of thyroid lesions were distributed in the range of 22 to 80 years. Peak incidence of thyroid lesion was found in 4^{th} & 5^{th} decade. The youngest patient was 22 years old and oldest 80 years old. Peak incidence of thyroid carcinomas included in the current study was 4^{th} decade. (Table no. 1). In studies done by Parik HK et al[89] & Agate L et al[90] found that both PTC and FTC were seen more common in 5^{th} & 6^{th} decade which is on higher side than the present study.

The mean age of diagnosis in the present study was around 44.73 years for PTC, 54.2 years for FTC, 41 years for MNG, 34 years for FA. Lang BH et al[91] observed mean age at diagniosis was 45 years for both PTC & FTC. Passler C et al[92] observed mean age 52.3 years for differentiated thyroid carcinoma. Sahoo S et al[93] found mean age at presentation to be 47 years for FVPTC & 49 years for follicular adenoma.

Satihal SN et al[94] & Gupta M[95] et al studies comprised of 92% females. Present study comprised of 91.10% females. Hence female outnumbered males in the ratio of 11:1. Also present study found M:F ratio in PTC is 1: 4.75. Bose D et al[96] observed that male: female ratio, overall in case of PTC was 1:6.3 which is higher compared to this study. (Table 2)

In present study, it is found that majority of thyroid lesions were identified in right lobe (43.6%) followed by left lobe (43.6%) and then both lobes (10.3%). These findings

are consistent with other studies[97,98,94]. Choudhury M et al also observed that 68% of thyroid lesions occurred in rt lobe.(Table no. 4)

In current study, majority of patients had undergone Total thyroidectomy (66.70%) followed by hemithyroidectomy (20%). It was in discordant with previous studies where majority of subjects had hemithyroidectomy (55.06%).[97] (Table no. 3)

In present study, majority of patients were euthyroid (88.9%) followed by hypothyroid (13.3 %). These results were similar to the observation of study done by Sengupta A et al[97] in which 90% cases had euthyroid status followed by hypothyroid(6.7%) and hyperthyroid (3.3%) status. Chetan VR et al[98] also noted similar findings stating 90% of the cases had euthyroid status. (Table no 7).

Most of the cases in the present study, including benign and malignant lesions presented with duration of symptoms of < 1 year (53.4%). Similar findings were observed in study done by Satihal SN et al[94] in which patients predominantly presented between 1-6 months (60%). (Table no. 6).

In this study most of the study population presented with chief complaint of localized swelling (82.30%), followed by diffuse swelling (13.30%), change in voice (2.20%) and swelling with dysphagia (2.20%). All cases with diffuse swelling belonged to MNG while most of malignant cases presented with localized swelling without pain. Similar findings were observed in study done by J L wang et al. where 90% of cases presented with localised space occupying lesion in cases of thyroid neoplasms.[99]
(Table no. 5)

In current study most frequent provisional clinical diagnosis was carcinoma(51.10%) followed by colloid goiter (22.20%), MNG (17.80%), follicular neoplasm (6.70%), Graves disease(2.20%). This clinical diagnosis is correlating with

histopathological diagnosis in 57.77% of cases. However, in 42.23% of cases histopathological diagnosis differ from clinical diagnosis.. (Table no 8)

Lymph node metastasis found in current study in PTC cases was 52.17%. It was observed in 41.9% of cases in the study done by Lang BH et al.[91] In study done by Ito Y et al[100] 14.79% PTC patients had LNM. (Table no. 11)

WHO staging system is used for PTC and FTC in this study. This pathological TNM classification is useful & is simplified approach for staging and prognostification of differentiated PTC & FTC.[92]

Most of patients of PTC (65.21%) were in stage I at the time of diagnosis. This finding is consistent with studies done by Lang BH et al[91] & Greene et al[101] in which 61.8% & 64.2% were seen in stage I respctively. Current study also showed that least number of cases of PTC were in stage IV (8%) . This is also in agreement with Greene FL et al study stating 3.1% cases in stage IV. (Table no. 9)

Thyroid cancer is most common endocrine malignancy. It is very important to distinguish between benign & malignant lesions as there are significant differences in prognostic and metastatic characteristics. When morphology is not clear in some cases, IHC has been used as a complementary method for diagnosis in many studies. Various IHC markers used previously are HBME 1, Galectin 3, CK19, CD44, CD56.[12] However none of these markers are enough to produce a reliable diagnosis of malignancy. Also marker panels have their own limitations. So after taking into account all these drawbacks of existing IHC markers, there are continuing efforts to find reliable marker in diagnosing various malignancies.[102]

Several authors have attempted to distinguish papillary from follicular tumors of thyroid & also to differentiate between benign and malignant thyroid neoplasms using TROP 2. Some of the studies in literature have concluded that this marker is beneficial in such cases.

In the present study, most of the PTC (73.91%) showed strong & diffuse membranous staining with or without cytoplasmic (3+, 4+) staining with TROP 2. TROP 2 cytoplasmic positivity might be explained by cross reactivity with epithelial cell adhesion molecule EpCAM/TROP-1 that encoded by another gene of the same family (TACSTD1) and has a high similar sequence with TROP 2.[104] Out of 23 cases of PTC, 2 cases showed staining intensity of 2+ and 6 cases showed TROP 2 negativity. Out of 6 cases of FV PTC, only 2 cases showed membranous staining with or without cytoplasmic staining (2+, 3+). Rest 4 cases of FV PTC showed non specific cytoplasmic staining which was consider as negative. Only 1 case of FTC (20%) showed weak positivity(1+) for TROP 2 marker while MTC was negative for this immunostaining. In follicular adenoma all cases were negative for the immunostain. TROP 2 expression was absent in all cases of MNG. Overall TROP 2 expression was 62.06% in malignant lesions of thyriod.(Table no. 12). These results are consistent with the study done by Canan S et al. in which Trop 2 expression was seen in 74.65% of malignant cases and 1.3% in benign thyroid lesions while MTC was negative for this marker.[12]

Another study conducted by Andrey Bychkov et al[102] in Bankok, Thialand on 226 thyroid lesions including 49 benign lesions and 179 malignant lesions concluded that non neoplastic thyroid lesions, follicular adenomas, follicular carcinomas and medullary carcinomas were negative for TROP-2. While majority of PTC were positive for TROP-2; however pattern of staining significantly differed between the histopathological variants.

Also in present study expression of TROP 2 differed between subtypes of PTC. It was found that TROP 2 is more expressed in cases of CV PTC (88.23%) while it was negative in most of the cases of FV PTC. (Table no 13). This finding was not in concordance with the study done by Canon S et al[12] where 81.8% cases of FV PTC showed immunoreactivity to TROP 2 and helped it in distinguishing PTC FV cases from other follicular neoplasms. Study done by Nooshin Z et al[21] also showed strong TROP 2 expression in cases of FV PTC (95%) followed by CV PTC (93.1%). While study done by Andrey B et al[102] observed all papillary microcarcinomas (mPTC), PTC classic variant (PTC cv), tall cell variant of PTC (PTC tcv) were positive with diffuse staining. In contrast, less than half of PTC fv were positive for TROP-2, with only focal immunostaining. Majority of papillary thyroid carcinoma (94/114, 82.5%) were positive for Trop-2.

It is claimed that lowered TROP-2 expression in FV PTC is related to its genetic profile because RAS mutation is a common event in FVPTC resembling follicular adenoma and follicular carcinoma, which is a rare event in CV PTC. It is reported that tumors driven by RAS mutation usually respond to ERK feedback, resulting in lower MAPK signaling, the pathway involved in TROP-2 signaling.[22]

So overall sensitivity, specificity, PPV and NPV found for TROP 2 in diagnosing CV PTC is 88.23%, 100%, 100%, 88.88% respectively. Study done by Andrey B et al[21] also observed 98.1% sensitivity, 97.5% specificity of TROP 2 in diagnosing CV PTC.

With present study it was found that TROP 2 marker has high sensitivity and specificity in diagnosing classical variant of PTC but not in differentiating follicular variant of PTC from follicular neoplasm.

There is need of studies with larger sample size and with more diverse morphological entities in order to evaluate the role of TROP 2 marker in diagnosing thyroid neoplasms.

CONCLUSION

The present study found that TROP 2 is a very sensitive and specific marker in diagnosis of classical variant of PTC with high overall specificity for PTC. However, it is not much useful in distinguishing follicular variant of PTC from follicular neoplasms.

SUMMARY

This is a retrospective as well as prospective study comprising of 45 cases of histologically diagnosed benign and malignant thyroid lesions. These cases were reviewed. The cases included in the study were MNG, FA, PTC, FTC and MTC.

Findings of the present study are

- Thyroid lesions commonly involved age group of 22-80years. Malignancy was generally seen in 4^{th} decade.
- Females are affected ten times more commonly than males.
- Thyroid lesions were most commonly found in right lobe.
- Localized swelling is the most common presenting complaint in cases of malignancy.
- Most of the cases of malignancy were having euthyroid profile.
- Average duration of symptom in benign and malignant cases was less than a year.
- LNM was seen in 52.17% cases of PTC. Only one case of FTC in the study showed LNM
- Most of the malignant cases were in stage I as per TNM staging system at the time of diagnosis.
- Expression of TROP 2 in CV PTC showed 88.23% sensitivity and 100% specificity.

- Expression of TROP 2 in distinguishing PTC from benign neoplasms showed 73.91% sensitivity and 100% specificity.

- Expression of TROP 2 in distinguishing FV PTC from follicular neoplasms showed 33.33% sensitivity.

So it is observed in the present study that diffuse TROP 2 staining of thyroid lesion favours diagnosis of CV PTC. Negative staining rules out CV PTC but can not exclude FTC and FV PTC.

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ANNEXURE I

INFORMED CONSENT FORM

EVALUATION OF DIAGNOSTIC SIGNIFICANCE OF TROPHOBLAST CELL SURFACE ANTIGEN-2 EXPRESSION IN THYROID NEOPLASMS- A STUDY IN TERTIARY CARE CENTRE OF BELAGAVI.

Purpose of the study: The purpose of this study is to evaluate of diagnostic significance of trophoblast cell surface antigen-2 expression in thyroid neoplasms. This study will help in determining a better diagnostic tool for thyroid cancer.

You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo hemithyroidectomy or thyroidectomy for a thyroid lesion you will be included in this study.

Procedure: During this study , you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge,

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better way to diagnose thyroid cancer which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid/offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

Name of the participant:
(signature/thumbprint)

Name of the witness :
(signature/thumbprint)

Name of the investigator: (signature)

Date:

ANNEXURE II

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 255 **Date: 24/12/2019**

To,
BN0119006
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EVALUATION OF DIAGNOSTIC SIGNIFICANCE OF TROPHOBLAST CELL SURFACE ANTIGEN-2 EXPRESSION IN THYROID NEOPLASMS – A STUDY IN TERTIARY CARE CENTRE OF BELAGAVI ",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Amita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE III

PROFORMA

PATIENT HISTORY

Name : Age :

IP no. : Sex :

Brief clinical history :

INVESTIGATIONS:-

Thyroid function test : 1) T3: 2)T4: 3)TSH:

USG neck :

FNAC(if performed) :

EXAMINATION FINDINGS:-

1) Age:

2) Lobe involved (Rt/Lt/Bilateral):

3) Gross: a) Size :

b) Shape:

c) Colour:

d) Type: Solid/Cystic

e) Consistency: Soft/Hard

CLINICAL DIAGNOSIS :

HISTOPATHOLOGICAL DIAGNOSIS :

1. Hematoxyline and Eosin staining :

2. IHC staining : IHC staining done for TROP-2 which gives positive membranous staining.

ANNEXURE IV

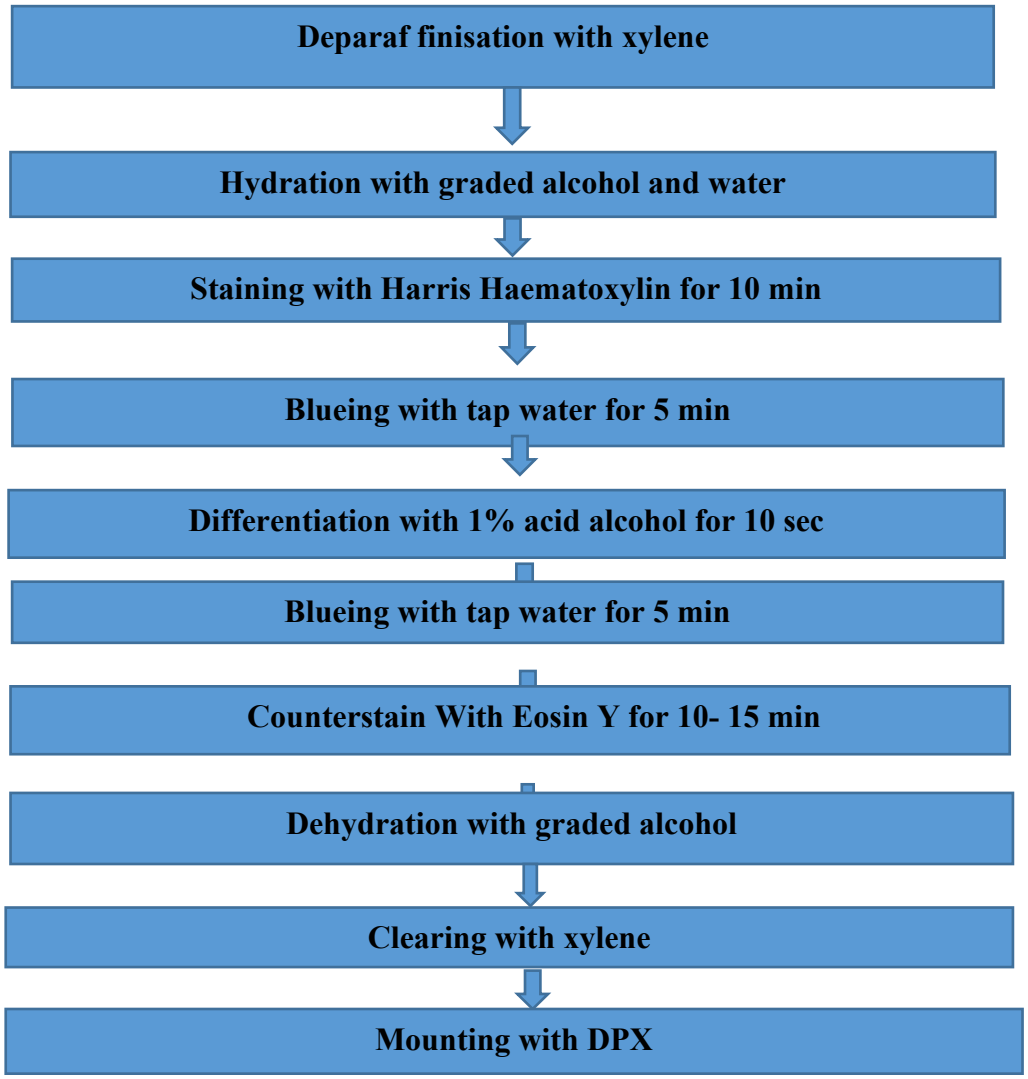
H & E STAINING

HAEMATOXYLIN AND EOSIN STAINING

Reagents required are

• Harris haematoxylin ,Eosin, 1 % Acid alcohol, Graded concentration of iso-propyl alcohol, Xylene, DPX

Procedure:



ANNEXURE V

IMMUNHISTOCHEMICAL STAINING PROTOCOL

PRINCIPLE- In this technique , an enzyme labelled antibody is used to link a cellular antigen specifically to a chromogen that can be more readily visualised under light microscope.

PROCEDURE- For the immunohistochemical technique we used routinely processed paraffin embedded tissue blocks. From each block we obtained histological sections of 3 micrometers of width.

Reagents required :

1.Antigen Retrieval: Tris Buffer-1.21 gms, EDTA-0.37 gms, Distilled water-1000ml, pH-8.5 to 9.0.

2. Wash buffer: Tris buffer- 8.6 gms, Nacl- 9.6 gms, pH- 7.4 to 7.6 (Adjust pH with concentrated HCL addind drop by drop) , Distilled water-1000ml.

3. 3% Hydrogen Peroxide (H_2O_2): H_2O_2-1 ml, Distilled water-100 ml.

4.DAB Solution: Substrate solution- 1 ml, Chromogen- 20 microlitre.

5. Coated slides: 1. Rinse the slides in water- 3 to 4 hours
(Slowly in continuous tap water).

2. Rinse in distilled water.

3. Dry the slides and immerse in Poly L Lysine solution (10 ml distilled water+1 ml Poly L Lysine solution) for 2 to 3 hrs .

4.Dry the slides and a gain immerse in Poly L Lysine solute for 10 m. Dry the slides. Ready to use the slides.

METHODOLOGY

1.Cut the tissue sections on a microtome with thickness of 3 microns and collect them on coated slides.

2.Bake the sections at 37 deg C for overnight.

3. Before test bake it at 60 deg C for 1 hour.

Deparaffinize step

4.Xylene I – 10 minutes.

5.Xylene II-10 minutes.

6.Absolute alcohol I- 10 minutes

7.Absolute alcohol II- 10 minutes

8.Rinse in water- 5 minutes.

9.Rinse in distilled water- 1 minutes.

10. **Antigen retrieval – (Tris Buffer+EDTA)-** Buffer solution

11. Prepare the required amount of buffer and cook the slides in pressure cooker for 3 whistles.

12. Allow it to cool to room temperature for 15 minutes.

13. Wash with wash buffer 2 times with gap of 30 seconds each.

14. Apply 3% Hydrogen Peroxide – 8 to 10 minutes.

15. Wash with wash buffer 3 times with gap of 30 seconds each.

16. Primary Antibody incubate for 45 to 60 minutes . In closed chamber at room temperature. PTEN antibody used was rabbit monoclonal isotype IgG, Clone RM265 obtained from Bio SB.

17. Wash with wash buffer 3 times with gap of 30 seconds each.

18. Apply polymer HRP for 25 to 30 minutes in closed chamber at room temperature .

19. Wash with wash buffer 3 times with gap of 30 seconds each.

20. Apply DAB substrate for 10 minutes.

21. Wash with water- 2 minutes.

22. Wash with distilled water – 1 minute.

23. Counterstain with Haematoxylin – 3 minutes.

24. Blueing in warm water- 1 minute.

25. Clear in xylene and mount with DPX.

ANNEXURE VI

KEY TO MASTER CHART

Sr.No.	Serial number
RHT	Right hemithyroidectmy
LHT	Left hemithyroidectomy
Total	Total thyroidectomy
STT	Subtotal Thyroidectomy
R	Right
L	Left
D swelling	Diffuse swelling
L swelling	Localised swelling
COV	Change of Voice
Dys	Dysphagia
Mts	Months
Yrs	Years
Dx	Diagnosis
F. Neoplasm	Follicular neoplasm
M nod	Multiple Nodules
SCM	Solid Cystic Mass
BS	Bethesda
MNG	Multinodular goiter
Colloid G	Colloid goiter
Complex N	Complex Neoplasm
Papillary N	Papillary Neoplasm

FN	Follicular Neoplasm
PTC	Papillary thyroid carcinoma
FTC	Follicular thyroid carcinoma
MTC	Medullary thyroid carcinoma
FV PTC	Follicular variant of Papillary thyroid carcinoma
FA	Follicular adenoma
MNG	Multinodular goiter
M nodules	Multiple nodules
STN	Solitary thyroid nodule
Neg	Negative

ANNEXURE VII
MASTER CHART

Sr. no.	IP no	Age	Sex	Specimen	Size(cm)	Site	Complaints	Duration	clinical dx	TFT	Imaging	FNAC	Stage	HPE Dx	LVI	ETE	LNM	Grade	Trop 2
1	999234	35	F	Total	3	R & L	D. Swelling	6 mts	MNG	Hypothyroid	M nod	Bs II	Nil	MNG	Nil	Nil	Nil	Nil	Negative
2	13033	45	F	Total	2.5	R	L. Swelling	4 mts	? Carcinoma	Euthyroid	NA	Bs V	Nil	FA	Nil	Nil	Nil	Nil	Negative
3	3032089	47	F	Total	1.5	R	L. Swelling	4 yrs	MNG	Euthyroid	NA	NA	1	FV PTC	Yes	No	No	1	Negative
4	618	30	F	Total	1.8	L	L. Swelling	1 yr	carcinoma	Euthyroid	NA	NA	1	PTC	Yes	No	Yes	1	3+
5	901102	43	F	LHT	2.8	L	L. Swelling	2 yrs	F. Neoplasm	Euthyroid	STN	Bs IV	Nil	FA	Nil	Nil	Nil	Nil	Negative
6	3029918	48	F	Total	3	R & L	D. Swelling	11 mts	MNG	Hypothyroid	NA	NA	Nil	MNG	Nil	Nil	Nil	Nil	Negative
7	903718	40	F	LHT	3.4	L	L. Swelling	4 yrs	?Colloid G	Euthyroid	NA	NA	1	FV PTC	No	No	No	1	3+
8	867481	80	F	Total	3.2	R	L.Swelling& dys	30yrs	Carcinoma	Euthyroid	STN	Bs VI-PDTC	2	FTC	No	No	No	1	Negative
9	5678	46	F	Total	4	R	D. Swelling	10 mts	MNG	Hypothyroid	NA	NA	Nil	MNG	Nil	Nil	Nil	Nil	Negative
10	922053	59	F	Total	3.2	L	L. Swelling	6 yrs	?Carcinoma	Euthyroid	STN+MNG	NA	3	PTC	Yes	No	Yes	1	2+
11	969460	50	F	LHT	3	L	L. Swelling	4 yrs	Carcinoma	Euthyroid	Nil	Bs VI- MTC	2	MTC	No	No	No	1	Negative
12	1040028	68	F	Total	1.5	R	L. Swelling	3 yrs	?MNG	Euthyroid	?MNG	NA	1	FV PTC	No	No	No	1	Negative
13	3020877	44	F	Total	3.4	R	D. Swelling	11 mts	?Carcinoma	Euthyroid	NA	NA	Nil	MNG	Nil	Nil	Nil	Nil	Negative
14	3020496	38	F	LHT	1.3	L	L. Swelling	7 mts	Colloid G	Hypothyroid	NA	NA	Nil	FA	Nil	Nil	Nil	Nil	Negative
15	3019731	80	F	Total	2	R & L	COV	1.5 mts	Carcinoma	Euthyroid	MNG	NA	3	PTC	Yes	No	Yes	1	3+
16	884129	45	F	RHT	4	R	L. Swelling	4 mts	? Carcinoma	Euthyroid	STN	Bs IV	Nil	FA	Nil	Nil	Nil	Nil	Negative
17	3015434	45	F	Total	2	L	L. Swelling	1 yrs	Graves ds	Euthyroid	M nod	NA	1	FTC	No	No	No	1	Negative
18	924365	25	F	RHT	3.8	R	L. Swelling	7 mts	Carcinoma	Euthyroid	NA	Bs VI-MTC	Nil	FA	Nil	Nil	Nil	Nil	Negative
19	907407	28	F	RHT	3.4	R	L. Swelling	6 mts	Colloid G	Euthyroid	STN	NA	Nil	FA	Nil	Nil	Nil	Nil	Negative
20	6230	45	F	STT	5.5	R	L. Swelling	2 yrs	MNG	Euthyroid	NA	NA	3	FV PTC	No	No	No	1	Negative
21	934893	30	F	RHT	1.5	R	L. Swelling	4 mts	Colloid G	Euthyroid	NA	NA	Nil	FA	Nil	Nil	Nil	Nil	Negative
22	879936	22	F	RHT	4.5	R	L. Swelling	6 mts	Colloid G	Euthyroid	NA	NA	1	FV PTC	No	No	No	1	Negative
23	924945	38	F	RHT	4.5	R	L. Swelling	1 mts	F. Neoplasm	Euthyroid	NA	NA	Nil	FA	Nil	Nil	Nil	Nil	Negative
24	723569	36	F	STT	1.6	L	L. Swelling	2 yrs	MNG	Euthyroid	NA	NA	1	PTC	No	No	No	1	negative
25	995989	23	F	LHT	3.7	L	L. Swelling	8 mts	F. Neoplasm	Euthyroid	NA	Bs IV	Nil	FA	Nil	Nil	Nil	Nil	Negative
26	6080158	27	F	Total	3.8	R	L. Swelling	9 mts	Carcinoma	Euthyroid	NA	NA	1	PTC	No	No	No	1	2+
27	3020419	40	F	Total	1	R & L	L. Swelling	4 mts	Carcinoma	Euthyroid	PTC	NA	1	PTC	No	No	No	1	3+
28	1047197	30	M	Total	1.5	L	L. Swelling	4 mts	Carcinoma	Euthyroid	Papillary N	Bs VI-PTC	1	PTC	Yes	Yes	Yes	1	4+
29	908061	25	F	LHT	2.5	L	L. Swelling	5 mts	Colloid G	Euthyroid	NA	NA	Nil	FA	Nil	Nil	Nil	Nil	Negative
30	870166	41	M	Total	4.2	R	L. Swelling	9 mts	? PTC	Euthyroid	NA	Bs V	1	PTC	Yes	No	Yes	1	4+
31	645392	27	F	RHT	1.7	R	L. Swelling	5 mts	Colloid G	Euthyroid	NA	NA	1	PTC	No	No	No	1	Negative
32	3015141	30	F	Total	2	L	L. Swelling	1.5 yrs	Colloid G	Euthyroid	NA	NA	1	PTC	Yes	No	Yes	1	4+
33	918836	57	F	Total	3	R	L. Swelling	6 mts	Carcinoma	Euthyroid	NA	NA	3	FV PTC	No	Yes	Yes	1	2+
34	855281	40	F	Total	3.8	L	L. Swelling	2 yrs	?Carcinoma	Euthyroid	Nil	NA	1	FTC	Yes	Yes	Yes	1	Negative
35	999204	54	F	Total	4.2	R	L. Swelling	5 yrs	?Carcinoma	Euthyroid	NA	NA	4	PTC	Yes	Yes	Yes	1	4+
36	942247	60	F	Total	4.5	L	L. Swelling	15 yrs	?Carcinoma	Euthyroid	STN	NA	4	PTC	No	Yes	Yes	1	3+
37	100794	33	F	Total	3.2	L	D. Swelling	1 yr	MNG	Hypothyroid	NA	NA	Nil	MNG	Nil	Nil	Nil	Nil	Negative
38	958174	78	F	Total	3.5	L	L. Swelling	40 yrs	Colloid G	Euthyroid	SCM	NA	3	PTC	Yes	Yes	Yes	2	4+
39	3011024	45	F	Total	8	R & L	L. Swelling	10 yrs	Carcinoma	Euthyroid	Nil	Bs IV	3	FTC	Yes		No	1	Negative
40	3016563	32	F	Total	2.5	L	L. Swelling	9 mts	?Carcinoma	Euthyroid	STN	Bs VI	1	PTC	No	Yes	Yes	1	3+
41	3026561	40	F	Total	5.5	R	D. Swelling	11 mts	Colloid G	Euthyroid	NA	Bs II	Nil	MNG	Nil	Nil	Nil	Nil	Negative
42	908724	56	M	Total	3.8	R	L. Swelling	3 yrs	?Carcinoma	Euthyroid	STN	Bs V	2	PTC	No	No	No	1	4+
43	879297	47	F	Total	2	L	L. Swelling	1 yr	?Carcinoma	Euthyroid	MNG	NA	1	PTC	No	No	No	1	3+
44	866045	23	M	Total	1.5	R & L	L. Swelling	6 mts	?Carcinoma	Euthyroid	NA	NA	1	PTC	No	No	Yes	1	4+
45	3021252	61	F	Total	4	R	L. Swelling	10 yrs	Carcinoma	Euthyroid	Nil	NA	2	FTC	No	No	No	1	1+