"IMMUNOHISTOCHEMICAL EXPRESSION OF CK-19 IN THYROID NODULES AND ITS CORRELATION WITH HISTOPATHOLOGY"

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

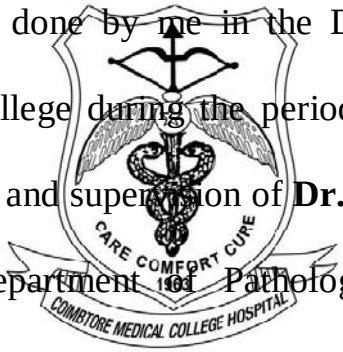
DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

DECLARATION

I hereby declare that the dissertation entitled "**IMMUNOHISTOCHEMICAL EXPRESSION OF CK-19 IN THYROID NODULES AND ITS CORRELATION WITH HISTOPATHOLOGY**" is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from July 2014 to July 2015 under the guidance and supervision of **Dr. A .Dhanalakshmi M.D**., Associate Professor Department of Pathology, Coimbatore Medical College.



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Date: Dr.P. Suriyaprabha

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This is to certify that the dissertation entitled "Immunohistochemical expression of CK-19 in thyroid nodules and its correlation with histopathology" is a record of bonafide work done by **Dr. P. Suriyaprabha** in the Department of Pathology, Coimbatore Medical College, Coimbatore under the guidance and supervision of

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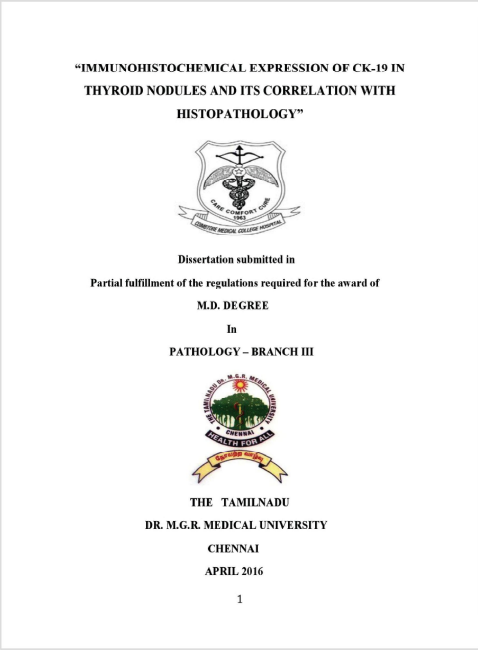

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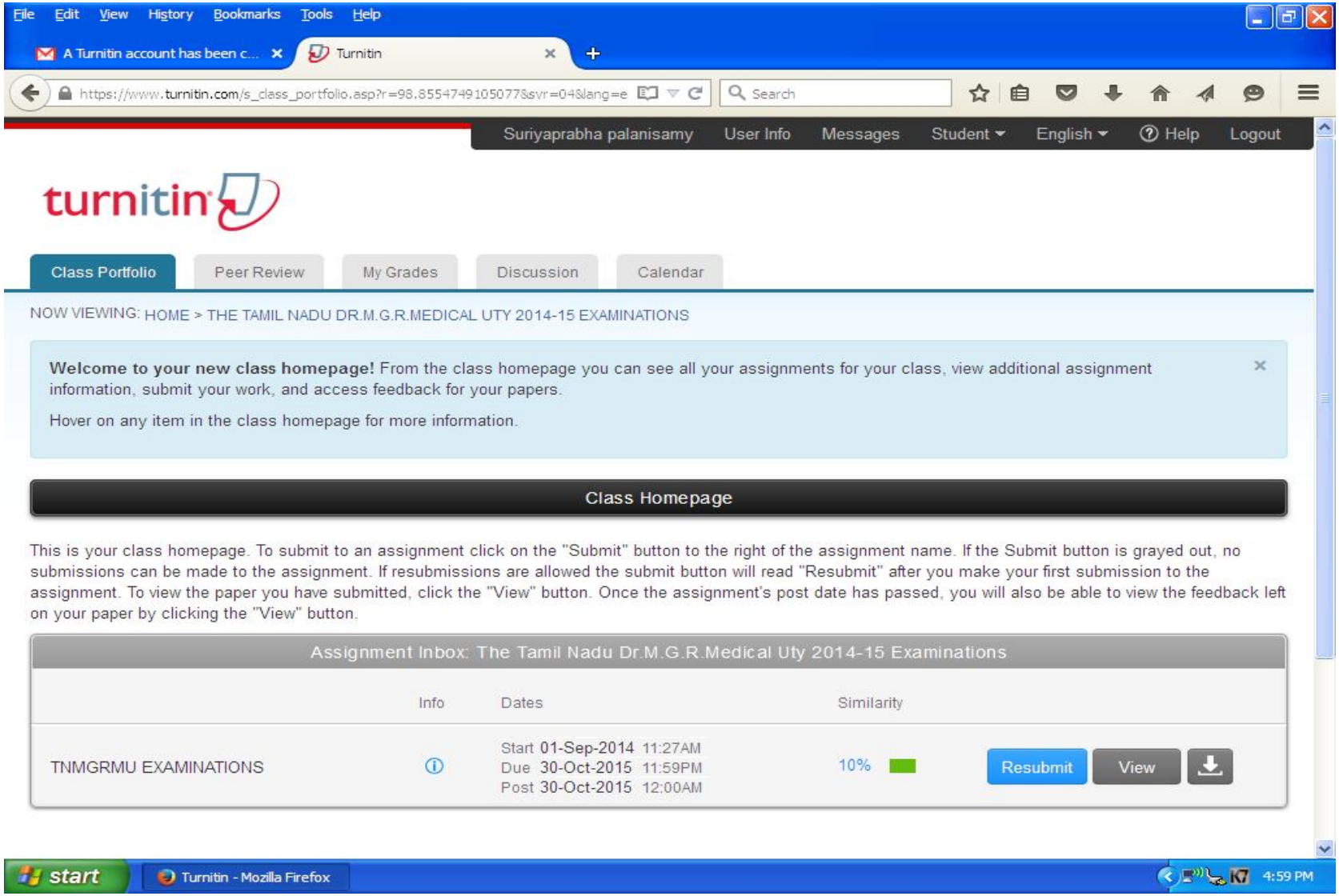
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INTRODUCTION

INTRODUCTION

Thyroid neoplasms constitute the most commonly occurring endocrine tumors worldwide. Thyroid nodules commonly occur between 30-60 years of age. About 4% to 8% of adult women and 1% to 2% of adult men present with thyroid nodules that can be identified by physical examination. With the advent of ultrasonography, the detection rate has increased to 30%. Majority of the thyroid nodules are benign with malignant nodules comprising only 10%. Thyroid tumors can arise either from the epithelial cells lining the follicles or from parafollicular C cells.

Malignant tumors of thyroid are prevalent worldwide. A survey conducted by WHO during 2010 revealed that around 44,670 new cases had appeared of which 1690 deaths occurred due to thyroid malignancy. Papillary carcinoma is the most common malignant tumor constituting 80-85% of all the thyroid carcinomas and that too the classic type, followed by follicular carcinoma comprising 10- 15%.But the mortality rate is only 6.5%. According to the surveillance and epidemiology, the 10 year survival rates for malignant thyroid tumors are

- Papillary carcinoma – 98%
- Follicular carcinoma – 92%
- Medullary carcinoma – 80%
- Undifferentiated carcinoma – 13%

Early diagnosis of thyroid tumors and appropriate management will prolong the survival rate of patients. However distinguishing various thyroid lesions by hematoxylin and eosin sections alone is really challenging to pathologist.

This is well stated by Baloch and Livolsi as, “Thyroid follicular lesions are the bane of the Pathologist” in their article.

As many thyroid tumors have overlapping morphological features, exact diagnosis is very essential for surgical and post- operative management of patients. Especially papillary carcinoma and its follicular variant which mimics follicular carcinoma can be treated by simple thyroidectomy, if diagnosed early. Differentiation of follicular adenoma and follicular carcinoma depends on capsular and vascular invasion. When it is inconclusive, false diagnosis of benignity may lead to extensive vascular dissemination and dismal prognosis. An increasing number of immunohistochemical markers are used in the differential diagnosis of both benign and malignant thyroid lesions. They are cytokeratin19, CD56, HBME-1, Galectin-3, Ret oncoprotein, p17, CITED1, PAX8 and EGFR (epidermal growth factor receptor).

Cytokeratin19, a low molecular weight protein of 40kDa belonging to keratin family is an intermediate filament involved in protein binding and organization of myofibers. Cytokeratin19 is extensively used in the

diagnosis of thyroid tumors. Many studies have reported it as sensitive marker in differentiating benign from malignant thyroid tumors.

The purpose of this study is to analyse the usefulness of Cytokeratin19 in differentiating thyroid nodules by grading the intensity of staining in cytoplasm of cells and to correlate it with the histopathology.

AIM AND OBJECTIVES

AIM OF THE STUDY

To study the immunohistochemical expression of Cytokeratin19 in various types of thyroid nodules and its correlation with histopathology.

OBJECTIVES:

1. To study the expression of Cytokeratin19 in different thyroid lesions.
2. To study the value of cytokeratin19 in differentiating benign from malignant thyroid nodules.
3. To study the correlation of cytokeratin19 expression in thyroid nodules with histopathology.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Thyroid gland is the major endocrine gland that controls the metabolic functions of the human body. THOMAS WHARTON, an English physician and anatomist from London, in 1656 named this endocrine gland as THYROID GLAND, as it resembled the SHIELD, used in Ancient Greece.

The occurrence of palpable thyroid nodules in adults is about 5%. The main goal in clinical medicine is to identify malignant lesions in a cost effective manner. In iodine deficient areas thyroid nodules are more frequent with higher occurrence in women and also with increasing age. The palpability of nodules depends on its location in the thyroid gland and anatomy of patient's neck. These nodules can be detected by thyroid ultrasound, CT scan and pathological studies. Nowadays, image guided biopsies or FNAC is more helpful. But FNAC is not confirmatory test as follicular adenoma and follicular carcinoma cannot be differentiated by this test.

Papillary carcinoma which is identified by its characteristic nuclear features like nuclear grooves , intranuclear cytoplasmic inclusions are also present in atypical adenoma ,Hyalinising trabecular adenoma, medullary carcinoma , parathyroid adenoma and paraganglioma.

Hence, histopathological study of the tissue sections are essential.

But certain tumors of thyroid have overlapping features and often pose a diagnostic difficulty especially in the tumors having follicular pattern such as follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma.

As the prognosis and management are different, differentiating these lesions is essential. As follicular patterned lesions are identified based on cytological criteria like nuclear grooving, nuclear overlapping and intranuclear pseudoinclusions , interobserver variations are common. This leads to inappropriate nomenclature or diagnosis.

Thyroid follicular lesions are either capsulated or unencapsulated but with follicular architecture. The four important lesions that should be differentiated are

- Hyperplastic colloid nodule
- Follicular adenoma
- Follicular carcinoma
- Follicular variant of papillary carcinoma

In an attempt to overcome this diagnostic difficulty many immunohistochemical markers are evaluated in distinguishing papillary thyroid carcinoma from other follicular patterned lesions. They are

CK19, HBME-1, galectin-3, CD 56, Leu-7 (CD 57), CITED-1, fibronectin-1, CD 15, PAX8, CD 44 and platelet derived growth factor. CD 56 expression is lost in papillary carcinoma but is expressed in benign and other malignant lesions of thyroid including normal thyroid.

EMBRYOLOGY:

Thyroid gland starts developing around 2nd or 3rd week of gestation and completes by 11th week and becomes functional at third month.[1] Thyroid gland develops from median endodermal thyroid diverticulum, which arises from foramen caecum, present between tuberculum impar and copula linguae in the base of the tongue. From foramen caecum, thyroglossal duct develops and descends down behind the hyoid bone to the neck. It lies in front of the trachea and bifurcates to form two lobes of thyroid gland.

The thyroglossal duct then obliterates. Sometimes the lower end of thyroglossal duct may persist forming pyramidal lobe or Lalou ette's pyramid. Parafollicular or 'C' cells are derived from caudal pharyngeal complex or ultimobranchial body derived from fourth and fifth pharyngeal pouches.[2] Solid cell nests, having collections of stratified epithelial cells with mucin production focally and cyst formation are the remnants of the ultimobranchial body and seen in 30% of adult thyroid.

On 9th week follicular cells are present as cords and plates. On 10th week follicular lumina appears and is small , by 12thweek colloid secretion begins and at 14th week well formed follicles lined by cuboidal cells, containing colloid within the lumen are present .

HISTOLOGY:

Thyroid gland is covered by a fibrous capsule and the septa arising from it divides the gland into lobules. Each lobule is composed of many follicles of approximately 200µm in diameter. They are lined by follicular cells with central lumen containing colloid. The interstitium contains parafollicular C cells, lymphatics and blood vessels. C cells are called as clear cells or light cells. They are polyhedral with eccentrically placed oval nuclei.

The follicular cells vary in their shape depending upon their function. The normal cells are cuboidal, whereas the inactive or resting cells are flat to squamous and become columnar when hyperactive.[3]

ANATOMY:

The normal thyroid gland weighs about 25 grams in adults and is slightly larger in females than males.[4]It has two lobes, the right and the

left joined by isthmus. Each lobe measures about 5cm x 2.5 cm x 2.5 cm. Isthmus measures 1.2 cm x 1.2 cm. Each lobe extends from the middle of thyroid cartilage to fourth or fifth tracheal ring. The isthmus extends between second to fourth tracheal rings.

Thyroid gland has both true and false capsule. True capsule arises from connective tissue of gland and false capsule from pre-tracheal fascia. Suspensory ligament of Berry connects the thyroid gland to cricoid cartilage, posteriorly.

It has rich blood supply from superior thyroid artery, inferior thyroid artery, thyroidea ima artery in 3%of individuals, branches of tracheal and oesophageal arteries. Thyroid gland is drained by superior, middle and inferior thyroid veins.

Lymphatic drainage is to the upper and lower deep cervical nodes, pre-tracheal and para-tracheal nodes. Nerve supply is from the middle cervical ganglion, mainly and small contributions from the superior and inferior cervical ganglia.

PHYSIOLOGY:

The thyroid gland plays important role in regulation of basal metabolic rate, calcium metabolism, somatic and psychic growth by the production of L- thyroxine -T4 and L – triiodothyronine T3. ***T3 is more***

potent than T4 (prohormone of T3). ***Thyroperoxidase is the primary enzyme of thyroid hormone synthesis.***

These hormones are regulated by negative feedback mechanism of hypothalamic-pituitary-thyroid axis. Thyrotrophin releasing hormone (TRH) from hypothalamus, enters anterior pituitary gland and stimulates secretion of thyroid stimulating hormone (TSH) which in turn acts on the thyroid gland, to produce and release T3 and T4. They bind to thyroid binding globulin (TBG) in plasma. Unbound forms are the active one in tissues.

THYROID TUMORS - AN OVER VIEW:

Thyroid tumors are the most common endocrine tumors. The estimated age standardized annual incidence is 1.0 to 2.9 cases per 1, 00, 000 men and 3.4 to 9.1 cases per 1, 00, 000 women according to GLOBACON 2008. Thyroid tumors are more common in developed countries. The incidence of thyroid tumors has increased in past two decades, predominantly papillary carcinoma of thyroid.[5]The liberal criteria for diagnosis of papillary carcinoma of thyroid and detection of small tumors by imaging techniques and environmental factors led to increase in the incidence of thyroid tumors.[6]

RISK FACTORS FOR THYROID CARCINOMA :

- Irradiation to head and neck
- Age : <20 or >45 years
- Bilaterality
- Female gender
- Iodine deficiency (follicular cancer)
- Positive Family history for thyroid carcinoma or MEN 2 syndrome

FEATURES INDICATING MALIGNANCY:

- Extrathyroidal extension.
- Fixation of nodules to adjacent soft tissues and structures.
- Paralysis of vocal cord
- Involvement of lymph nodes.
- Nodule size > 4 cm.
- Progressively enlarging neck mass.

GENERAL CHARACTERISTICS OF PRIMARY THYROID CANCERS:

1. Most common histologic type is papillary carcinoma.[7]
2. Females are most commonly affected than men. [8]
3. Young patients have well differentiated tumors whereas in older patients less differentiated tumors are common.
4. Young females below 40 years have slightly better prognosis than older individuals.[9]

5. Size of the primary tumor with staging is essential and is an important factor that determines the prognosis.

CHARACTERISTIC FEATURES OF PRIMARY THYROID CARCINOMAS IN CHILDREN:

1. Most common is papillary carcinoma of thyroid. ***National Cancer Institute in 2013*** in their statistical analysis also found that papillary carcinoma is common comprising 70-80%.[10]
2. Radiation exposure plays important role, example : Hiroshima Nagasaki bomb explosion.[11]
3. 60 – 80% of them present with lymph node metastasis and recurrence is more common in these patients.[12]
4. Thyroid carcinomas in children, though aggressive has slightly good prognosis having mortality rate 2.6 % only.

FAMILIAL THYROID TUMORS:

25% of tumors occur in familial form, not only medullary carcinoma, but also familial non - medullary thyroid carcinoma can occur.

Familial non-medullary thyroid carcinoma syndrome is diagnosed if three or more first degree relatives have non - medullary thyroid carcinoma derived from follicular cells[13]

Examples are:

1. Familial papillary thyroid carcinoma with or without oxyphilia chromosome locus 19p13.2 (TCO).
2. Familial papillary thyroid carcinoma with renal papillary neoplasia chromosome 1q21 (FPTC/PRN).
3. Familial non- medullary thyroid carcinoma type –I chromosome 2q21 (NMTC-1).
4. Familial multinodular goiter syndrome chromosome 14q31 (MNG – 1).

SYNDROMES ASSOCIATED WITH THYROID TUMORS:

S. NO	SYNDROME	GENE INVOLVED	INCIDENCE	THYROID TUMORS
1.	Familial adenomatous polyposis	APC (5q21)	2-12%	Papillary carcinoma cribriform - morular variant often
2.	PTEN-hamartoma tumour (Cowden syndrome)	PTEN (10q23.2)	> 10%	Follicular carcinoma, papillary carcinoma occasionally, benign follicular nodules
3.	Carney complex	PRKAR1α (17q22-24)	15%	Follicular carcinoma, papillary carcinoma, benign follicular nodules
4.	Werner syndrome	WRN (8p11-12)	18%	Follicular carcinoma, papillary carcinoma, undifferentiated carcinoma, benign follicular nodules

WHO CLASSIFICATION (2004) OF PRIMARY THYROID TUMORS

Tumors of thyroid follicular or metaplastic epithelium:

1. Follicular adenoma (includes Hürthle cell adenoma)
2. Papillary carcinoma
3. Follicular carcinoma (includes Hürthle cell carcinoma)
4. Mucinous carcinoma
5. Mucoepidermoid carcinoma
6. Sclerosing mucoepidermoid carcinoma with eosinophilia
7. Poorly differentiated thyroid carcinoma
8. Anaplastic / Undifferentiated carcinoma (including squamous cell carcinoma and carcinosarcoma)

Tumors showing C-cell differentiation

1. Medullary carcinoma

Tumors showing both follicular and C-cell differentiation

1. Collision tumor: follicular/papillary and medullary carcinomas
2. Mixed medullary and follicular cell carcinoma

Tumors showing thymic or related branchial pouch differentiation

1. Ectopic thymoma
2. Carcinoma showing thymus-like element (CASTLE)
3. Spindle epithelial tumor with thymus-like differentiation (SETTLE)

Tumors of lymphoid cells

1. Malignant lymphoma
2. Extramedullary plasmacytoma

Mesenchymal and other tumors

1. Benign and malignant mesenchymal tumors such as solitary fibrous tumor, peripheral nerve sheath tumor, smooth muscle tumor, and angiosarcoma
2. Paraganglioma
3. Teratoma
4. Secondary tumor deposits in thyroid gland

Tumor, Node, Metastasis (TNM) Staging of Tumors of the Thyroid:

TUMOR (T):

TX	-	Primary tumor cannot be assessed
T0	-	No evidence of primary tumor
T1	-	Tumor ≤ 2cm in greatest dimension and limited to the thyroid
T2	-	Tumor >2 cm but <4 cm and limited to the thyroid
T3	-	Tumor >4 cm in greatest dimension and limited to the thyroid or tumor with minimal extrathyroid extension (e.g., extension to perithyroid soft tissues or sternothyroid muscle)
T4a	-	Tumor of any size extending beyond thyroid capsule and invades subcutaneous tissue, larynx, trachea, esophagus or recurrent laryngeal nerve
T4b	-	Tumor invades prevertebral fascia / encases carotid artery/ mediastinal vessels.

***All Anaplastic carcinomas are considered T4 tumors*.**

T4a	-	Intrathyroidal anaplastic carcinoma - any size
T4b	-	Extrathyroidal anaplastic carcinoma - any size

REGIONAL LYMPH NODES (N) :

NX	-	Regional lymph nodes cannot be assessed
N0	-	No regional lymph node metastasis
N1a	-	Metastasis to Level VI (pretracheal, paratracheal, and prelaryngeal/ Delphian lymph nodes)
N1b	-	Metastasis to unilateral, bilateral or contralateral cervical or Superior mediastinal nodes.

DISTANT METASTASIS (M) :

MX	-	Distant metastasis cannot be assessed
M0	-	No distant metastasis
M1	-	Distant metastasis present

STAGE GROUPING

Separate stage groupings are recommended for papillary (or) follicular, medullary and anaplastic carcinoma.

Papillary or Follicular (<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	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
Stage III	T1 N1a M0
Stage III	T2 N1a M0
Stage III	T3 N1a M0
Stage IVA	T4a N0 M0
Stage IVA	T4a N1a M0
Stage IV A	T1 N1b M0
Stage IVA	T2 N1b M0
Stage IVA	T3 N1b M0
Stage IVA	T4a N1b M0
Stage IVB	T4b Any N M0
Stage IVC	Any T Any N M1

Medullary Carcinoma :

Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
Stage III	T2 N1a M0
Stage III	T2 N1a M0
Stage III	T3 N1a M0
Stage IVA	T4a N0 M0
Stage IVA	T4a N1a M0
Stage IVA	T1 N1b M0
Stage IVA	T2 N1b M0
Stage IVA	T3 N1b M0
Stage IVA	T4a N1b M0
Stage IVB	T4a N1b M0
Stage IVC	Any T Any N M1

Anaplastic Carcinoma :

All anaplastic carcinomas are considered Stage IV

Stage IVA	T4a Any N M0
Stage IVB	T4b Any N M0
Stage IVC	Any T Any N M1

ROLE OF IMMUNOHISTOCHEMISTRY IN THYROID LESIONS:

As there is morphological overlap between many thyroid tumors with follicular pattern as seen in follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma and the nuclear features characteristic of papillary carcinoma like nuclear grooves and inclusions are also seen in multinodular goiter with papillary hyperplasia and Hyalinising trabecular adenoma , immunohistochemistry is helpful in differentiating the tumors. A panel of immunomarkers that are useful includes ***Cytokeratin 19, HBME-1, galectin -3, CD 56, PAX 8 and Ret – oncoprotein.***

CYTOKERATIN 19:

Cytokeratin 19 belongs to the keratin family. It is a 40 kDa protein that is encoded by KRT 19 gene in human being. It is an intermediate filament involved in protein binding, organization of myofibres and maintains structural integrity of epithelial cells. This acidic protein arranged in pair of heterotypic keratin chains unlike its related family members is not paired with basic cytokeratin in epithelial cells. They are clustered in region of chromosome 17q12 - q21.

Cytokeratin 19 is widely applied as diagnostic marker of papillary thyroid carcinoma.[14]***Hanan Alsaeid Alshenawy*** in his study

found cytokeratin19 as a sensitive marker in diagnosis of papillary carcinoma and its variants.[15]It is also expressed in defined zone of basal keratinocytes, sweat gland, mammary gland ductal and secretory cells , GIT, ectocervix epithelium and urothelium.

HBME-1:

HBME-1 (Hector Battifora mesothelial) is a monoclonal antibody which act against the antigen present on mesothelial cell membrane. It is named after Dr. Hector Battifora, who introduced this marker.The target epitope is located in microvilli. It is expressed in thyroid papillary and follicular carcinoma but is not expressed in nodular goiter or in nodular hyperplasia.[16]

GALECTIN-3:

Galectin-3 is a 31kDa protein belonging to the lectin family and is encoded by the gene LGALS3 located in chromosome 14 in the locus q21-q22. It binds to beta-galactosides and play important role in regulation of cell to cell or cell to matrix interaction, repair of cell damage and cell migration. Galectin-3 also plays an important role in neoplastic transformation and inflammation. It aids in distinguishing papillary carcinoma and follicular variant of papillary carcinoma from other follicular patterned nodules.[17]

CD 56:

CD56, a neural adhesion molecule plays an important role in regulating migrating capabilities of neoplastic cells. Loss of CD 56 expression leads to increase in metastatic potential of tumor cells and leads to poor prognosis. CD 56 is normally expressed by normal thyroid follicular epithelial cells. Low expression of CD 56 is useful in diagnosis of papillary carcinoma of thyroid.[18]

PAX 8:

PAX 8, a transcription factor is essential for the development of thyroid follicular cells and also expresses thyroid specific genes. It is expressed in papillary carcinoma, follicular neoplasms, medullary carcinoma and poorly differentiated carcinoma. It is also expressed in B cell lymphomas, renal cell carcinoma and normal B lymphocytes.

RET ONCOPROTEIN:

Ret gene play an important role in the production of tyrosine kinase, a transmembrane receptor. Ret gene is located in chromosome 10q. It is not expressed in normal thyroid follicular cells, but gene rearrangement commonly occurs in papillary carcinoma and hence useful in the diagnosis of papillary carcinoma, which is proved by the study conducted by Cheung et al.[19]

BENIGN THYROID NODULES:

It is classified as hyperplastic nodules and benign epithelial neoplasm. Hyperplastic nodules includes dyshormonogenetic goiter and nodular hyperplasia. Benign epithelial tumors are follicular adenoma and hyalinising trabecular adenoma.

DYSHORMONOGENETIC GOITER:

It occurs due to defect in hormone synthesis due to peroxidase deficiency, deiodinase deficiency, defective iodide transport, defective coupling, decreased thyroglobulin synthesis and loss of function of pendrin gene (ion channel for transport of iodine).[20] Thyroid gland is grossly enlarged and multinodular. Microscopically it may show microfollicular, solid, papillary and insular pattern. Marked nuclear atypia of cells inbetween hyperplastic nodules and increased mitoses are seen. Follicular carcinoma and papillary microcarcinoma are incidental findings.[21]T4 replacement therapy can produce thyroid tumors in these patients.

NODULAR HYPERPLASIA:

It is also called as multinodular goiter or adenomatous goiter or adenomatous hyperplasia. It exists in two forms, endemic and sporadic goiter. Endemic goiter occurs in geographical areas with low iodine content in soil and water leading to defective thyroid hormone synthesis which stimulates TSH release and causes diffuse or nodular colloid goiter.

Sporadic goiter is due to dietary deficiency of iodine or increased excretion of iodine by kidney or defective hormone synthesis by antibodies. In both, thyroid gland is enlarged and has multiple nodules surrounded by complete or incomplete capsule. Few dilated follicles have conglomerate of small follicles at one pole which are active called as ***Sanderson polsters.***

Some follicles are cystically dilated with papillary hyperplasia and the papillae face towards the center of the cyst which may mimic papillary carcinoma[24]. Nuclear atypia is seen in cells within nodules due to previous irradiation incontrast to dyshormonogenetic goiter where it is seen inbetween hyperplastic follicles. It is also different from adenoma which is solitary and completely encapsulated. Chromosomal abnormalities are rare and may have TSHR mutations, extra copy of chromosome 7 and RAS mutations.[22]

FOLLICULAR ADENOMA:

Follicular adenoma is a solitary benign encapsulated tumor and the patients are in euthyroid state. In radioactive iodine scan, usually follicular adenoma is cold, at times, warm and rarely hot. Hot nodules indicate benign lesion.

PLUMMER ADENOMA :

Follicular adenoma with hyperthyroidism is called as toxic adenoma or ***PLUMMER ADENOMA.***[23]Plummer adenomas have activating mutation of TSHR or GNAS1. Intra luminal calcium oxalate crystals present in thyroid follicles, with hyperfunctioning nodule outside is a sign of hypofunction. Mitosis is rare. Secondary degenerative changes like hemorrhage, cystic degeneration and fibrosis are common.

Some follicular adenoma have papillary structures reported as papillary adenoma in past which gave confusion with papillary carcinoma is now termed as **follicular adenoma with papillary architecture.**[24]

Follicular adenoma has four different patterns:

1) Normofollicular / simple type
2) Microfollicular / fetal type
3) Macrofollicular / colloid type

4) Trabecular /embryonal / solid type

Rarely, papillary pattern can occur.

DIFFERENTIAL DIAGNOSIS OF LARGER FOLLICLES:

1) Hyperplastic nodule

2) Follicular variant of papillary carcinoma

DIFFERENTIAL DIAGNOSIS OF SOLID / TRABECULAR / NESTED PATTERN:

1) Medullary carcinoma

2) Poorly differentiated carcinoma

But they are mostly invasive. Calcification, edema and bone formation are more common.

IMMUNOHISTOCHEMISTRY OF FOLLICULAR ADENOMA:

1) Low molecular weight keratin - cytoplasmic positivity

2) TTF 1- nuclear positivity.

3) Laminin is positive around follicles.[25]

MOLECULAR GENETICS:

No molecular test effectively distinguish follicular adenoma from follicular carcinoma because both has similar chromosomal abnormalities like activating RAS stimulation, PAX 8 / PPARγ rearrangement.[26]

Other Chromosomal abnormalities specific for follicular adenoma - are translocations involving Chromosomes 19q13 having break point at ZNF 331 gene locus and chromosome 2p21 - break point at (THADA) thyroid adenoma associated gene locus.[27]Sporadic follicular adenoma has rarely alteration of PI3K / PTEN/ AKT pathway.

VARIANTS OF FOLLICULAR ADENOMA:

1) Hurthle cell adenoma.

2) Hyalinizing trabecular adenoma.

3) Atypical adenoma – has irregular cytoarchitecture but, lacks capsular and vascular invasion.[28]

4) Adenoma with bizzare nuclei- cells occur in clusters and have huge hyperchromatic nuclei. Other malignant features are absent.

5) Clear cell type

6) Adenolipoma

7) Adenochondroma - adenoma having cartilaginous metaplasia

8) Spindle cell adenoma - resembles meningioma somehow.

9) Black adenoma - minocycline induced.[29]

TREATMENT:

- Lobectomy
- Levothyroxine to suppress the nodule and
- $\mathbf{I^{131}}$ for toxic adenoma.

HYALINIZING TRABECULAR ADENOMA:

It was first identified by ***Langhans*** and the term given by ***Carney.***[30]

MICROSCOPY:

Tumor cells are arranged in trabecular pattern. Cytoplasm shows prominent hyaline material due to intermediate filament accumulation which is also present in extracellular matrix. Hyalinised collagen and basement membrane material are present.

Cytoplasmic yellow body - pale yellow inclusion bodies situated near nucleus with refractile quality is present.[31] Psammoma bodies, nuclear grooves and nuclear pseudo inclusions are seen.

IMMUNOHISTOCHEMISTRY:

Thyroglobulin and TTF -1 is strongly positive, galectin -3 in half of cases and NSE and neurotensin – only focally positive.

Pathogenetic link between hyalinising trabecular adenoma and papillary carcinoma:

- Both Papillary carcinoma and hyalinising trabecular adenoma have nuclear grooves, nuclear pseudoinclusions and psammoma bodies.
- Both express epithelial type keratins.
- Hyalinising trabecular adenoma can have foci of papillary carcinoma.
- Papillary carcinoma with hyalinising trabecular adenoma like pattern with cervical node metastasis is seen.
- Both have RET/PTC rearrangement.
- Because of the overlapping features it is now termed as **Hyalinising trabecular tumor.**[32]

HURTHLE CELL OR ONCOCYTIC TUMORS:

HURTHLE CELL ADENOMA:

It is common in female adults .Tumors are solid, tan, encapsulated and has rich vascularity. The tumor cells have follicular, papillary, trabecular or solid pattern and has inspissated colloid having concentric laminations. Cells have deeply eosinophilic, granular cytoplasm. Nuclei sometimes exhibit pleomorphism and may have prominent nucleoli. Few bizarre forms are also seen. But they do not indicate malignancy. Most of them are benign and show reactivity for thyroglobulin. Benign tumors are called as Hurthle cell adenoma.

ATYPICAL HURTHLE CELL ADENOMA:

It is also called as Hurthle cell tumors of uncertain malignant potential (HCT-UMP) .They have solid or trabecular pattern of growth with increased nuclearcytoplasmic ratio. There is no capsular or vascular invasion and does not metastastize to other sites.

HURTHLE CELL CARCINOMA:

They are aggressive tumors and have solid pattern of growth, increased mitoses and have capsular and/or vascular invasion. They metastatise to bone and lungs. These tumors exhibit aneuploidy and chromosomal gain at 20p and 19q.

Hurthle cell tumors commonly undergo acute infarction following fine needle aspiration. Hurthle cell tumors >4 cm have poor prognosis.

MALIGNANT THYROID TUMORS:

PAPILLARY CARCINOMA:

It is the most common primary thyroid carcinoma and affects any age with mean age of 40 years having female preponderance. In children, 90% of thyroid malignancy is constituted by papillary carcinoma. Irradiation to head and neck causes papillary carcinoma in 5 -10 % of cases and can arise in patients with Hashimoto's thyroiditis.

Papillary carcinoma in thyroid gland alone is 67%, Thyroid and cervical nodes - 13%, Lymph nodes alone -20%.[33]

GROSS APPEARANCE:

Size varies from microscopic to larger nodule and most of the tumor nodules are < 1 cm. Grossly it appears as infiltrating nodule with ill - defined border and is grey white to tan and granular. Encapsulated variant has thick capsule and constitutes < 10%.[34] Calcification and psammoma bodies give gritty feel while cutting.

MICROSCOPIC FEATURES:

Papillary carcinoma consist of numerous branching papillae having central fibrovascular core and lined by stratified cuboidal cells having characteristic nuclear features like Ground glass appearance / orphan annie nuclei / optically clear with nuclear overlapping, nuclear pseudoinclusions (round acidophilic vacuoles due to cytoplasmic invagination into nucleus) and nuclear grooves along the long axis of nucleus due to infolding of redundant nuclear membrane and nuclear microfilament.[59]

Psammoma bodies are seen in papillary stalk or between tumor cells or in fibrous stroma. Psammoma bodies are concentric lamellated basophilic structures occurring as a result of calcification of individual necrotic tumor cells and should be distinguished from inspissated secretions in Hurthle cell tumor.

VARIANTS OF PAPILLARY CARCINOMA:

1) PAPILLARY MICROCARCINOMA:

It is usually ≤1 cm in diameter, formerly called as occult sclerosing carcinoma or non - encapsulated sclerosing tumor. It is most common in males.[36] RET / PTC rearrangements and BRAF mutations are common and has excellent prognosis.

2) <u>ENCAPSULATED VARIANT:</u>

Tumor nodule is completely encapsuled.

D /D: Hyperplastic nodule with central cystic degeneration which appears **hot** on thyroid scan and their papillae face towards the centre of cystic cavity and has pale vacuolated colloid. Immunohistochemistry shows negativity with high molecular weight keratin.

3) <u>FOLLICULAR VARIANT</u>:

Tumor cells are arranged in follicles and is invasive. Psammoma bodies, colloid with scalloped margins, abortive papillae and distinctive nuclear features are present. Follicular variant of papillary carcinoma has many types like solid variant, macro follicular variant, diffuse / multinodular variant and encapsulated variant called as ***LINDSAY TUMOR***.[37]

<u>4) DIFFUSE SCLEROSING VARIANT</u>:

It involves one or both lobes of thyroid gland. Dense sclerosis, solid foci, psammoma body, squamous metaplasia and lymphocytic infiltration are present.[38] Lymph node and brain metastasis are common.

They exhibit both RET/PTC1 and RET/ PTC3 rearrangements. BRAF mutation occur rarely.

5) <u>ONCOCYTIC / OXYPHILIC VARIANT</u>:

Tumor cells have abundant granular eosinophilic cytoplasm with papillary or follicular pattern with nuclear features of papillary carcinoma.[39] It has good prognosis.

6) <u>TALL CELL AND COLUMNAR CELL CARCINOMA</u>:

Tall cell variant has single layer of tall cells whose *height is equal to three times the breadth*.[40] It has papillary structures, nuclear pseudoinclusions and lymphocytic infiltration of stroma. It is more aggressive and affects older age group. Extrathyroidal extension is common.

Columnar cell carcinoma has stratified layer of columnar cells, with subnuclear vacuolation and papillary carcinoma nuclear features are present. It has high proliferative index and has poor prognosis.

7) <u>CRIBRIFORM MORULAR VARIANT</u>:

It has cribriform growth pattern with morular formation. Ultrastructurally - accumulation of microfilaments made of *biotin* leads to nuclear clearing and is different from papillary carcinoma with strong nuclear and cytoplasmic positivity with β-catenin.[41]

8) PAPILLARY CARCINOMA WITH EXUBERANT NODULAR FASCITIS LIKE STROMA:

It has prominent stromal reaction giving, fibroadenoma like appearance. It resembles nodular fasciitis and fibromatosis.[42]

MOLECULAR GENETICS:

Mitogen activated protein kinase pathway which regulates cell proliferation differentiation and survival plays major role in causation of papillary carcinoma. MAPK - pathway activation leads to RET/PTC rearrangements, TRK rearrangements, BRAF mutation and RAS mutation in follicular cells of thyroid. These mutations are mutually exclusive.

REARRANGEMENTS:

Gene rearrangements constitute 20 - 40% of papillary carcinoma. RET oncogene present in chromosome 10q11.2 is a transmembrane tyrosine kinase receptor , the point mutations of which causes medullary carcinoma and rearrangements causes papillary carcinoma. RET rearrangements occurs in intron 11 by intrachromosomal inversions involving long arm of chromosome 10, interchromosomal translocations. RET fuses with 12 different genes leading to 17 different chimeric sequences.

RET/PTC1 (RET fusion with CCDC6 a.k.a H4 or D10S170), RET/PTC3 (RET fusion with ncoa4 a.k.a, RFG, ELE1 or ARA 70), these two occur by intrachromosomal rearrangements and are more common. RET/PTC2 (RET fusion with PRKAR-1A, gene which is inactivated in patients with Carney complex) occurs in one third of the cases. Other mutations are rare.

RET/PTC is common in children and young adults and those exposed to radiation.[43] These rearrangements occur in classical papillary carcinoma or microcarcinoma. They represent low stage with little proliferative capability and less likely to undergo dedifferentiation. These features are more commonly seen in RET/PTC1 rearrangement. RET/PTC3 may behave aggressively. These rearrangements can be detected by reverse transcriptase polymerase chain reaction or by fluorescent in situ hybridization.

BRAF:

BRAF activating mutations are the most common genetic alteration constituting 30-70% of papillary carcinoma. It belongs to RAF family and is a serine threonine kinase of MAPK pathway. Most common molecular alteration is thymidine to adenine transversion in nucleotide 1799 of exon 15 which leads to valine to glutamate substitution in residue 600 of protein activation loop.

BRAF mutations are more specific for papillary carcinoma of thyroid and is present in papillary carcinoma arising in struma ovarii. BRAF mutation is characteristically present in tumors with papillary architecture. It is uncommon in follicular variant of papillary carcinoma. Other rare mutations of BRAF are K601E mutation, paracentric inversion of chromosome 7 and small deletions near codon 600.

BRAF mutations have been attributed to male sex, older age group, extrathyroidal extension, metastasis to lymph nodes and distant sites, recurrence, high tumor stage at initial presentation and reduced survival. BRAF mutation are reduces gene expression required for enzymes production in thyroid hormone synthesis and it makes the tumor refractory to treatment radioactive iodine.

RAS:

RAS mutations are seen in follicular patterned thyroid tumors like follicular adenoma, follicular carcinoma, follicular variant of papillary carcinoma.[44]

NTRK1:

NTRK1 gene encodes a transmembrane tyrosine kinase receptor and it binds with nerve growth factor. NTRK1 rearrangements constitute 5% of papillary carcinoma. It causes inter and intrachromosomal

recombination at NTRK locus in chromosome 1q22 producing chimeric oncogene leading to spontaneous activation of NTRK1 tyrosine kinase.

METASTASIS:

Cervical lymph nodes are commonly involved with cystic degeneration. It is commonly seen in young patients .Blood borne metastasis is less frequent and involves lungs, bone, soft tissue, central nervous system, breast and pancreas. In lung the metastatic foci appear as miliary micronodules and this can be identified with I^{131} scintiscan. Occasionally tumor spreads to nearby parathyroid glands.

PROGNOSIS:

- **Good prognostic factors:**
- Children and adults < 40 years[45]
- Females and
- Encapsulated variant.[46]
- **Bad prognostic factors:**
- Age is > 40 years
- Extra thyroid extension
- Increasing size of tumor

- Multicentric tumor
- Tumors with distant metastasis
- Poorly differentiated and anaplastic carcinoma [47]and
- Tumors with aneuploidy and BRAF mutations.[48]

IMMUNOHISTOCHEMISTRY:

Cytokeratin 19, high molecular weight keratin demonstrated by 34βE12, thyroglobulin and TTF -1 are strongly positive.[49] Other markers are TTF-2, PAX8, S 100, HBME-1, galectin 3, CD 15, CD 57, EMA, CEA, antichymotrypsin, insulin like growth factor, HER2/neu , c-Met/hepatocyte growth factor receptor and vimentin.

FOLLICULAR CARCINOMA:

Follicular carcinoma, a rare neoplasm of elderly females predominantly constitutes 10 – 18 % of all the primary thyroid tumors. It is identified by the capsular or vascular invasion or invasion of adjacent thyroid.[50]

It arises commonly in patients with endemic goiter and iodine deficiency. Rarely, it arises from follicular adenoma. Irradiation and dyshormonogenesis also predispose to follicular carcinoma

GROSS EXAMINATION:

Follicular carcinomas is solid tan to light brown fleshy, sometimes glistening with, areas of hemorrhage and cystic degeneration .Minimally invasive variant are encapsulated. Size varies from 1cm to 10 cm.

MICROSCOPIC FEATURES:

Follicular carcinoma has thick fibrous capsule in which the tumor cells are arranged in closely packed follicles, trabecular pattern or solid sheets. The tumor cells are cuboidal to low columnar and have round nuclei with inconspicuous nucleoli sometimes exhibiting, nuclear pleomorphism. Mitosis is uncommon. Vascular invasion and capsular invasion are the diagnostic features and it differentiates follicular adenoma from follicular carcinoma.

VARIANTS (based on invasion):

1) Minimally invasive follicular carcinoma.[51]

2) Widely invasive follicular carcinoma.[52]

MINIMALLY INVASIVE FOLLICULAR CARCINOMA:

It is encapsulated variant resembling follicular adenoma of embryonal or fetal type. Invasion into vessels of venous caliber within or outside the capsule should be present. Tumor cells are attached to the

wall of vessels lined by endothelium or protrudes into lumen. CD31, Ulex europaeus, Factor –VIII related antigen and Fli –I endothelial cells marker are useful. Capsular invasion should be present and pseudoinvasion has to be ruled out. Pseudoinvasion is due to herniation of tumor tissue due to breech in capsule made by surgeon on fresh specimen.

TERMS USED IN FOLLICULAR NEOPLASM:

- Follicular carcinoma – tumors with definite capsular invasion
- Follicular tumor of uncertain malignant potential – has questionable capsular invasion but does not have nuclear features of papillary carcinoma.
- Well differentiated tumors of uncertain malignant potential – has questionable nuclear changes (? Papillary carcinoma type)

WIDELY INVASIVE FOLLICULAR CARCINOMA:

Tumors that are encapsulated and having four or more blood vessel invasion or those having wide spread infiltration into blood vessels and / or adjacent thyroid tissue are termed as widely invasive follicular carcinoma. Metastasis to sternum, shoulder girdle, skull and iliac bone is common. Those tumors that look alike normal thyroid tissue is called as ***metastasizing adenoma / metastasing goiter/ malignant adenoma*** and

has affinity for radioiodine.[53] < 5% of minimally invasive type of tumors have metastasis.

MOLECULAR GENETICS:

1. Castro and Colleagues postulated that chromosomal gains and aneuploidy leads to microfollicular/ solid / trabecular pattern. Diploidy and near diploidy forms normofollicular pattern.
2. Loss of heterozygosity, (LOH) of 20% per chromosomal arm produce follicular carcinoma and only 5% loss occurs in follicular adenoma and codon 12 and 13 of K-RAS occurs in follicular carcinoma.[54]
3. PAX 8/ PPAR gamma rearrangement due to t (2:3) (q13; p25) is common in females, young age, highly cellular and invasive tumors.[55]
4. PI3K / PTEN /AKT pathway activation is common in *COWDEN SYNDROME, CARNEYS COMPLEX I and WERNER SYNDROME .*[56]
5. TSHR gene mutations are rare and occurs in hyperfunctioning follicular carcinoma
6. VEGFR1 genes are also involved.

POORLY DIFFERENTIATED CARCINOMA:

Tumors falling in between well differentiated and anaplastic type are the poorly differentiated carcinoma .INSULAR CARCINOMA is the one commonly seen, arising from follicular carcinoma.[57]Papillary

carcinoma can also progress to poorly differentiated carcinoma. It is common in old age about 60 years. Recurrence, extrathyroidal extension and metastasis to lymph nodes (14 – 48 %) and distant sites (12-44%) are common. Mortality rate is increased to 50%. TP53 mutation and β catenin mutations are common.[58]Insular carcinoma analogous to "*Langhans' wuchernde struma* " is common in South America and Europe.

Gross and Microscopic Appearance:

The tumor is solid grey white, partly encapsulated or invasive with areas of hemorrhage and necrosis. Microscopically the tumor cells are arranged in insular pattern with retraction artifact or as diffuse sheets. Coagulative necrosis giving ***peritheliomatous appearance*** is common.[59] Tumors cells are small with vesicular or hyperchromatic nuclei and vascular invasion is commonly seen. Tumor cells are positive for TTF-1 and PAX8 and have increased Ki-67 index.

UNDIFFERENTIATED / ANAPLASTIC CARCINOMA:

Anaplastic carcinoma constitutes 2-5% of primary thyroid cancers. Women of >70 years are commonly affected. They are aggressive tumors growing rapidly in a shorter period with increased incidence of recurrence and metastasis. Most of the patients die within a year.

Tumor cells produce granulocyte colony stimulating factor causing marked increase in leucocytes. They are resistant to chemotherapy. Younger patients with tumors < 4 cm can be operated with radical surgery along with adjuvant chemoradiation.

They have mutation in TP53 gene (70%), β-catenin mutation (65%), RAS mutation (30%) mutations in BRAF and RET/ PTC, PTEN, APC, PIK3CA and APC.

GROSS AND MICROSCOPIC FEATURES:

Grossly tumor completely replaces entire thyroid gland and invades surrounding soft tissue. Microscopically cells are either of squamoid type or sarcomatoid type – having spindle cells and giant cells. Epithelial looking cells are arranged in sheets and large polygonal with highly pleomorphic nuclei and many giant cells and bizarre forms are seen. Spindle cell components are alike to undifferentiated pleomorphic sarcoma, with extensive necrosis and hemorrhage.

VARIANTS:

- Angiomatoid variant
- Osteoclastic variant
- Rhabdoid variant
- Lymphoepithelioma like carcinoma

- Paucicellular variant
- Carcinosarcoma
- Adenosquamous carcinoma
- Squamous cell carcinoma

Immunohistochemistry:

Cytokeratin positivity is variable (47-90%) depending on proportion of carcinoma component antigen and PAX8 (76%).[61]

MEDULLARY CARCINOMA:

Medullary carcinoma is a malignancy with parafollicular C cell differentiation. It may be sporadic or part of familial or multiple endocrine neoplasia 2A or 2B.[62] Sporadic form is common in 44- 50 years, and around 10-30 years in MEN syndrome. In sporadic form bilateral tumors are 0-32%, 40-50% of nodal metastasis and 12% of distant metastasis with intermediate prognosis. Hereditary medullary carcinomas have autosomal dominantly acquired RET proto-oncogene mutation and 90% are bilateral tumors.

Familial and MEN-2A associated medullary carcinoma have indolent course with mutation in exon 10, 11, 13, 14 or 15. MEN 2B incur mutation at exon 16 (ATG → ACG; methionine to threonine) and has aggressive course. Other tumors in MEN 2A are Pheochromocytoma,

parathyroid tumors and cutaneous lesions; MEN 2B Pheochromocytoma, neuromas of mucosa and intestine and Marfanoid features. Lymph node metastasis is 10 – 30 % in hereditary form with metastasis to distant sites rarely except MEN 2B (38%).

Grossly the tumor is small firm, greywhite to tan or reddish brown .Tumor is most commonly present in middle third of lateral lobe because of increased C cell number there. They may have capsule. Larger tumors have necrosis and hemorrhage. Microscopically tumor cells are arranged in nest, sheet, trabecular, tubular, pseudopapillary, cribriform or microglandular pattern. Cells are round to polygonal cells having amphophilic cytoplasm with round nuclei having stippled chromatin. Nuclear pleomorphism and mitoses are infrequent. Increased vascularity is a striking feature.80-85% of cases have amorphous eosinophilic material called as ***amyloid.***[63]

VARIANTS:

- Glandular / follicular type
- Oncocytic / oxyphilic type
- Pseudopapillary type
- Clear cell type, small cell type , pigmented variant
- Spindle cell type ,Giant cell variant,
- Paraganglioma like, neuroblastoma like variant

- Pseudoangiosarcomatous like , carcinoid like
- Hyalinising trabecular adenoma like

Immunohistochemistry:

Tumors show strong positivity with cytokeratin, pan-neuroendocrine markers, TTF1, calcitonin, CEA- (80 -100%).

Amyloid can be stained with congo-red and under polarized light gives ***apple green birefringence.***

PROGNOSTIC FACTORS:

- GOOD prognosis:
  - ❖ Female sex
  - ❖ Medullary carcinoma in MEN 2A[64]
  - ❖ Medullary microcarcinoma (<1 cm) and
  - ❖ Small tumors.
- BAD prognosis:
  - ❖ Above 45 years of age
  - ❖ MEN 2B associated medullary carcinoma
  - ❖ Small cell type
  - ❖ Calcitonin poor tumors[65] and
  - ❖ Tumors with somatic RET proto-oncogene mutation.[66]

MUCOEPIDERMOID CARCINOMA:

Primary mucoepidermoid carcinoma is a low grade malignant neoplasm which is rare.[67] Females are commonly affected around 10-83 years of age . 20% of the individuals presented with thyroid mass having extrathyroidal extension. Lymph node metastasis occur commonly but distant metastasis is rare.

Harach stated "Mucoepidermoid carcinoma of thyroid arises from ultimobranchial body".[68] Some other study insisted as tumor of thyroglossal duct origin.

Histologically the tumor is not circumscribed and has cellular islands present in a sclerotic background.some of the cells contain intracytoplasmic mucin. Few of the cells are squamoid. Comedo type of necrosis, nuclear pleomorphism and psammoma bodies are present. Rarely glands that are lined by ciliated columnar epithelium is seen. Mucoepidermoid carcinoma can occur along with papillary carcinoma.two cases of mucoepidermoid carcinoma associated with follicular carcinoma (Hurthle cell variant) are also on record.[69]

IMMUNOHISTOCHEMISTRY:

Thyroglobulin and TTF-1 are positive.

SCLEROSING MUCOEPIDERMOID CARCINOMA WITH EOSINOPHILIA:

It is a rare tumor of low grade malignant behavior and occurs in a background of Hashimoto thyroiditis.[70] It arises from metaplastic squamous epithelium. It is common in adults with mean age of 55 years and with female preponderance. It is an aggressive tumor.[71]

Tumor is composed of nests and anastomising cords of cells in a dense sclerotic stroma which is infiltrated with eosinophils and lymphocytes. Tumor is infiltrative and it extends to perithyroidal tissue. Cells are polygonal with mild to moderate nuclear pleomorphism with prominent nucleoli. Some foci show squamoid nests and mucin pools. Perineural invasion and blood vessel obliteration are common. Lymph node metastasis resembles Hodgkin lymphoma.[72]

IMMUNOHISTOCHEMISTRY:

CYTOKERATIN AND TTF-1 are positive in these tumors.

MUCINOUS CARCINOMA:

Primary mucinous carcinoma is very rare in thyroid .Only seven cases have been reported in literature.[73] these tumors metastatize rapidly with mean survival of about 6 months to 4 years. It is similar to colloid carcinoma occurring in other sites.

Immunohistochemistry show thyroglobulin and TTF-1 positivity.

TUMORS SHOWING DIFFERENTIATION OF BOTH FOLLICULAR AND C-CELL :

COLLISION TUMORS:

Collision tumors are composed of two recognizable types of carcinoma of thyroid. They are

1. Medulllary carcinoma and follicular carcinoma[74,75]

2. Papillary carcinoma and medullary carcinoma[76]

They occur contiguously and are more aggressive.

MIXED MEDULLARY CARCINOMA AND FOLLICULAR CELL CARCINOMA:

It is also called as **Follicular-parafollicular carcinoma** or **differentiated carcinoma of intermediate type.**[77]This rare tumor arises from stem cells hence showing dual component. They are not capsulated. They have features of medullary carcinoma along with follicles. Other patterns like nests, cribriform, trabecular and solid pattern are also seen. Amyloid is present in few cases. These cells show neurosecretory granules, cells having intermediate features, follicular cells and indifferent cells ultrastructurally.

Immunohistochemistry :

Thyroglobulin and calcitonin positivity is present.

TUMORS OF HEMATOLYMPHOID CELLS:

MALIGNANT LYMPHOMA:

Primary thyroid lymphomas, constitutes 2.5 to 3% of extranodal lymphomas and comprises 4 to 5 % of thyroid malignancies. It commonly occurs in elderly females. Lymphomas, commonly arises from lymphocytic thyroiditis or Hashimoto thyroiditis. Thyroid lymphomas form non -circumscribed rubbery or soft mass. Cut surface bulges out and is fleshy homogeneous and light tan coloured. Size of the tumor may vary from 1 to 14 cm.

Non Hodgkin lymphomas are common in thyroid than Hodgkin lymphoma. Diffuse large B-cell lymphoma (constitute 70%) and extranodal marginal zone lymphoma of MALT type occur most commonly.[78] Follicular lymphoma and Burkitt lymphoma are very rare.[79] Some cases of intravascular large B cell lymphoma and T cell lymphoma that expressing γδ receptors of T cells are reported.

METASTATIC MALIGNANT TUMORS IN THYROID:

As thyroid has rich blood supply and lymphatics invasion or metastasis to thyroid is common. Tumors like lung adenocarcinoma, colorectal carcinoma, renal cell carcinoma, malignant melanoma, breast carcinoma and sarcoma metastatize to thyroid gland.[80] Sometimes

psammoma bodies can be seen in metastatic deposits giving misconception with papillary carcinoma.[81]

Metastatic neuroendocrine carcinoma from intra-abdominal site and bronchus present as solitary or multiple nodules within the thyroid gland giving misinterpretation as medullary carcinoma. The metastatic carcinomatous deposits usually present as multiple nodules with increased vascularity and hemorrhage. Immunohistochemistry with appropriate markers is useful in differentiating the tumor.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Design:

Prospective study

Study Period:

From July 2014 – July 2015

Study Place:

Coimbatore Medical College and Hospital, Coimbatore.

Sample Size:

A total number of 30 cases.

From case records brief clinical data were collected, which included age, sex, clinical diagnosis and surgical procedure .

The following inclusion and exclusion criteria were adopted.

Inclusion Criteria :

1. All thyroidectomy specimens (hemithryoidectomy, subtotal and near total thyroidectomy and total thyroidectomy) done for solitary nodule or multiple neoplastic nodules.
2. Patients in all age groups
3. Both male and female patients

Exclusion criteria:

- Multinodular goiter
- Toxic goiter

Methods:

Among the total thyroidectomy specimens that were received in the department of Pathology in our hospital during the study period, 30 cases were taken into study as per inclusion criteria and were evaluated further .

All those 30 thyroidectomy specimens (one with lymph node metastasis) selected were then fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin.

HEMATOXYLIN AND EOSIN STAINING METHOD:

REAGENTS USED:

1. Hematoxylin solution- Erhlich's hematoxylin
2. Eosin Y 1% solution
3. Acid alcohol 1% solution

PROCEDURE:

1. Deparaffinize sections in xylene by immersing for 30 seconds.

2. Place the sections in Isopropyl alcohol for 15 minutes.
3. Wash in running tap water.
4. Stain in Erhlich's hematoxylin for 10 to 15 minutes.
5. Differentiation is done with 1% acid alcohol two to three dips.
6. Blueing is carried out for 10 minutes.
7. Counterstain with eosin 1% solution 3 to 4 dips.
8. Running tap water wash.
9. Air dry
10. Mount with DPX

After hematoxylin and eosin staining, all slides were reviewed by pathologist and categorized as following

1. Follicular adenoma
2. Minimally invasive Follicular carcinoma.
3. Widely invasive follicular carcinoma
4. Papillary carcinoma
5. Follicular variant of papillary carcinoma

The age group varied between 19 to 60 years.

Follicular adenoma:

Follicular tumors that are completely encapsulated having homogeneous architecture and morphology are diagnosed as follicular

adenoma and those showing cystic spaces are called as follicular adenoma with cystic degeneration.

Follicular carcinoma:

MINIMALLY INVASIVE follicular carcinoma are encapsulated tumors and are diagnosed based on following criteria:

1. Tumors showing capsular invasion only.
2. Tumors showing invasion into less than four blood vessels. They have low metastatic potential.
3. Tumors showing invasion into four or more blood vessels. These tumors have higher metastatic potential.

WIDELY INVASIVE follicular carcinomas are diagnosed by widespread invasion into thyroid parenchyma and also the blood vessels. These tumors may lack complete encapsulation. These tumors present with distant metastasis and regional lymph nodes involvement at initial presentation. They invade into surrounding soft tissues and has poor prognosis.

Papillary carcinoma:

Papillary carcinoma is diagnosed based on the major and minor features.

Major features are:

- Ovoid nuclei

- Nuclear crowding
- Nuclei having clear or fine chromatin
- Psammoma bodies

Minor features are:

- Abortive papillae
- Dark colloid
- Irregular follicles
- Nuclear pseudoinclusions
- Multinucleated histiocytes in the lumen of the follicles.

Follicular variant of papillary carcinoma:

Tumor composed of follicles almost entirely (99%) with nuclear features of classic papillary carcinoma.

PROCEDURE OF IMMUNOHISTOCHEMISTRY:

The blocks from control and selected cases were cut and mounted on poly l- lysine coated glass slides. Blocking of endogeneous peroxidase activity was done with 0.3% hydrogen peroxide in methanol, freshly prepared for twenty minutes. Then epitope retrieval was done by heating in microwave oven by using buffer of citrate at pH 6.

Immunohistochemistry was done by utilizing a monoclonal anti – Cytokeratin 19 antibody (clone -RCK 108), a mouse monoclonal antibody obtained from ascitic fluid diluted with phosphate buffered saline containing 1% BSA and 0.09% sodium azide. This is a Ready-to-Use antibody and does not require further dilution.

IMMUNOHISTOCHEMISTRY:

METHOD:

Two – step indirect technique.

PRINCIPLES OF THE PROCEDURE:

Using a two stage process, antigens in cells and tissues were detected.

The first was the binding to specific epitopes of the primary antibody. Second was a colorimetric reaction to detect the binding. Sections of tissues were fixed and attached to slides .The paraffin embedded sections were then dewaxed . Antigen retrieval procedure was done. This consisted of heating the formalin fixed sections in microwave oven in an aqueous solution. It recovered full antigenicity with most of the antibodies. These also included cases that were formerly unreactive with formalin – fixed tissue. Subsequently, the tissue sections were treated with Peroxide –Block and Power-Block for blocking

endogenous peroxidase and non specific protein-protein interactions, respectively.

REAGENTS USED:

1. Peroxide Block: 3% hydrogen peroxide in water.
2. Power Block Reagent: A highly effective universal protein blocking reagent. Contains casein and propriety additives in PBS with 15mM Sodium azide.
3. Chromogen: DAB-3,3-diaminobenzidine.
4. Liquid DAB Substrate: Comprises Tris- buffer containing the peroxide and stabilizers.
5. Super Enhancer Reagent
6. Poly-HRP Reagent
7. Counter stain: Ehrlich's hematoxylin

BUFFER SOLUTIONS:

TRIS BUFFER: (pH- 7.6)

TRIS Buffer salt: 0.605 gm

Sodium chloride: 8gm

Distilled water: 1000 ml

1 N Hydrochloric acid: 3 ml

CITRATE BUFFER: (pH – 6.0)

Trisodium citrate: 2.94 gm

Distilled water: 1000 ml

1 N Hydrochloric acid: 5 ml

TRIS- EDTA: (pH – 9.0)

TRIS Buffer salt: 6.05 gm

Disodium EDTA : 0.744 gm

Distilled water : 1000 m

PROCEDURE:

1. Sections were deparaffinised in two changes of xylene, 15 minutes each.
2. Sections are dehydrated in absolute alcohol for 5 minutes with 2 changes.
3. Sections are then washed in tap water for 10 minutes.
4. Slides are then rinsed in distilled water for 5 minutes.

5. Antigen retrieval was done by placing them with appropriate buffer solution in microwave. Medium temperature – 10 minutes, high temperature – 10 minutes.
6. They were then cooled to room temperature and rinsed in distilled water.
7. Sections are then washed in TBS buffer for 5 minutes with 2 changes.
8. Then treated with Peroxide Block for 10 minutes.
9. Again sections are washed in TBS buffer for 5 minutes with 2 changes.
10. Treated with Power Block for 10 minutes.
11. Slides are then drained and covered with primary antibody (supplied from BioGenex) for 2 hours.
12. Washed in TBS buffer for 5 minutes with 2 changes.
13. Sections were covered with Super Enhancer for 30 minutes.
14. Washed in TBS buffer for 5 minutes with 2 changes.
15. Poly – HRP reagent was applied and left for 30 minutes.
16. Washed in TBS buffer for 5 minutes with 2 changes.
17. Treated with DAB Chromogen with Substrate buffer for 5 to 8 minutes.
18. Washed in TBS buffer for 5 minutes with 2 changes.
19. They were then washed in tap water for 5 minutes.

20. Sections are counterstained with Ehrlich's hematoxylin for 30 seconds.
21. Washed in tap water for 5 minutes.
22. Slides were air dried and mounted with DPX.

Tumor cells were scored positive if there was golden brown cytoplasmic, nuclear or membrane staining in the neoplastic cells. Negative diagnosis was made when no golden brown staining was noted.

Interpretation of immunohistochemistry :

Cytokeratin 19:

Positive staining refers to diffuse staining of cytoplasm. Intensity of positive staining is graded between 0 to 3+ as below:

S.No	Grading	Percentage of cells expressing CK19 positivity
1.	0 (negative)	No positively staining cells
2.	1+ (focally positive)	<25% of positively staining cells
3.	2+ (positive)	25 – 50% of positively staining cells
4.	3+ (diffusely positive)	>50% of positively staining cells

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

The present study is a prospective study conducted in the Department of Pathology, Coimbatore Medical College Hospital. A total number of 30 cases of thyroidectomy specimens with different thyroid neoplasms, one with lymph node metastasis that were received over the period of July 2014 to July 2015, were taken for this study.

Ethical clearance was obtained from Ethics committee of Coimbatore Medical College Hospital, Coimbatore.

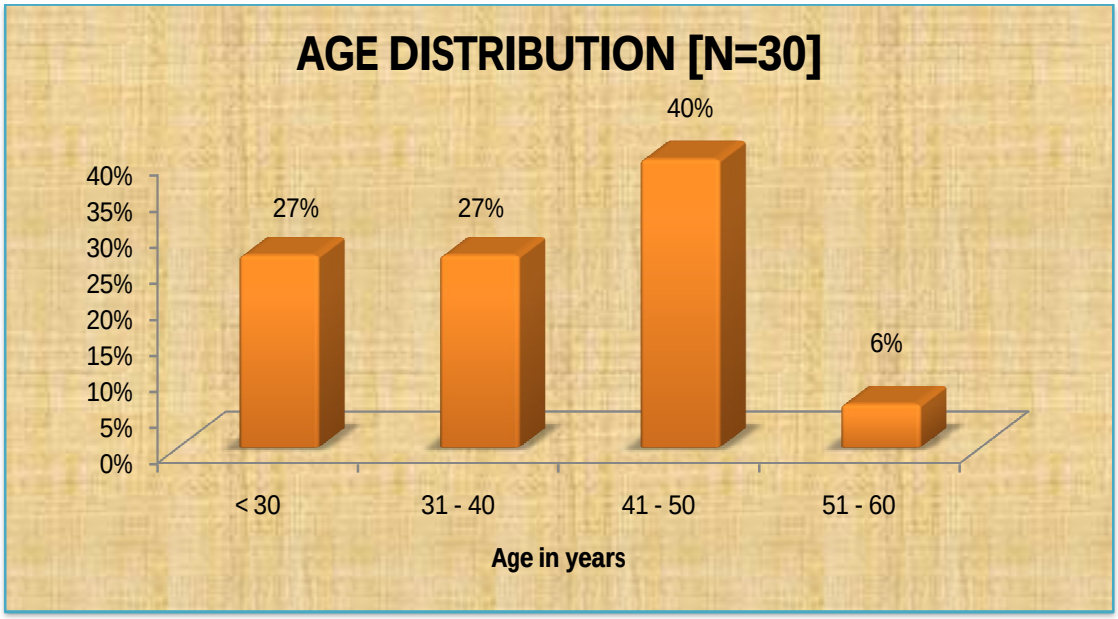
Histomorphological features and immunohistochemical expression pattern of Cytokeratin19 in thyroid nodules were studied, analysed and compared with literature.

TABLE 1: AGE DISTRIBUTION IN THYROID NODULES:

Age in years	No. of cases	Percentage
< 30	8	27%
31 – 40	8	27%
41 – 50	12	40%
51 – 60	2	6%
Total	30	100%

In the present study the incidence of thyroid nodules are common in the age group between 41-50 years comprising about 40% (12 cases) of the total cases followed by 31 to 40 years and less than 30 years each constituting 27% and 6% in the age group of 51- 60 years.

CHART 1: AGE DISTRIBUTION OF THYROID NODULES:

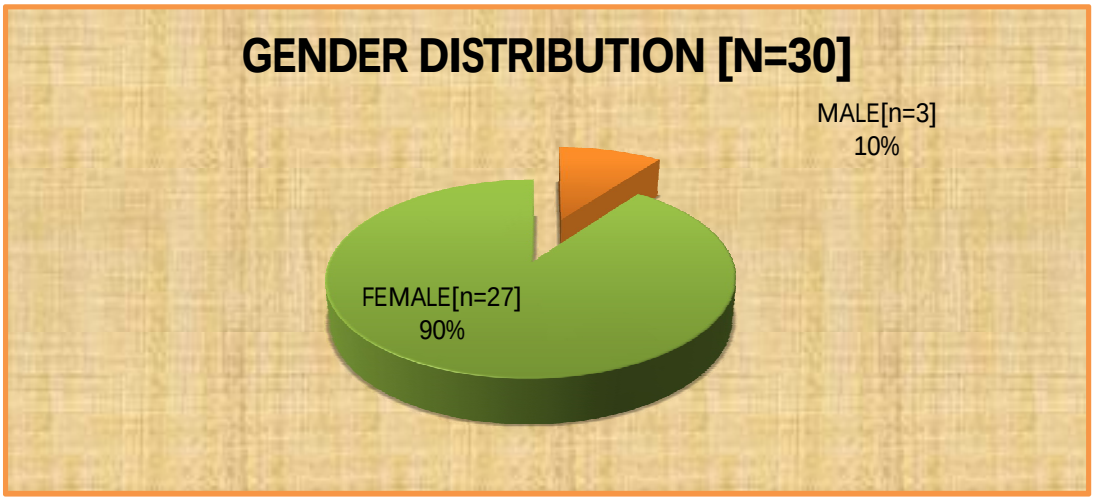


The above bar diagram depicts the age incidence of thyroid nodules. Age group between 41- 50 years is commonly affected followed by 30- 40 years.

TABLE 2: SEX DISTRIBUTION OF THYROID NODULES:

Gender	No. of cases	Percentage
Male	3	10%
Female	27	90%
Total	30	100%

CHART 2: SEX DISTRIBUTION OF THYROID NODULES:



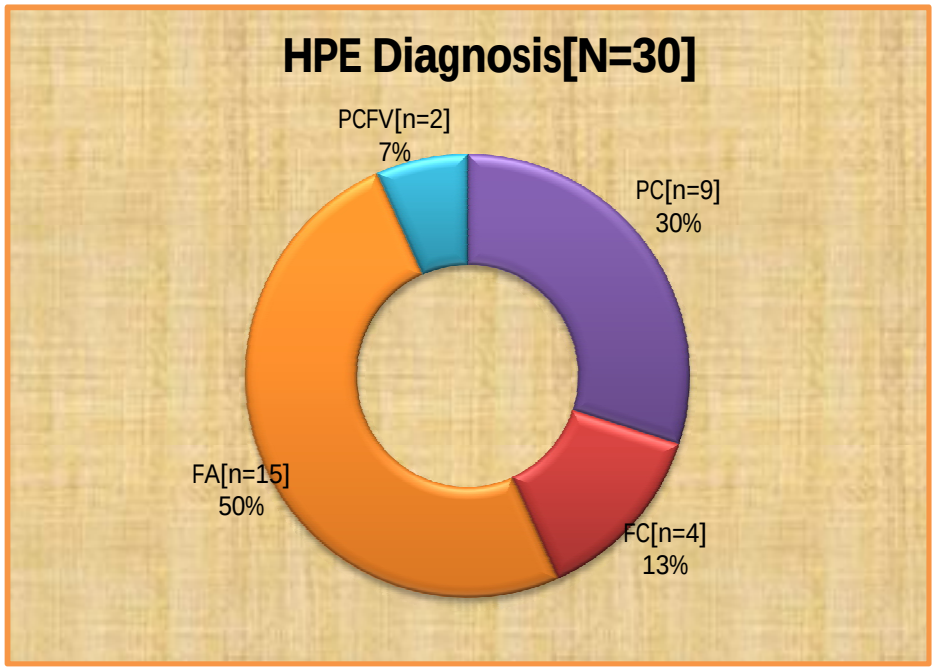
As per the present study ,thyroid nodules are more common among females constituting about 90% (27 cases) in contrast to males with only 3 cases comprising 10%. Female to male ratio is 3:1.

TABLE 3: DISTRIBUTION OF DIFFERENT THYROID NEOPLASMS:

Histopathological Diagnosis	No. of cases	Percentage
Papillary Carcinoma	11	37%
Classic type	9	30%
Follicular variant	2	7%
Follicular Carcinoma	4	13%
Follicular Adenoma	15	50%

In the present study of comparison of different thyroid neoplasms, follicular adenoma constitutes the majority of cases which is 50%, followed by papillary carcinoma- 37% (classic type-30%, follicular variant- 7%) and follicular carcinoma- 13%.

CHART 3: DISTRIBUTION OF VARIOUS THYROID NEOPLASMS:



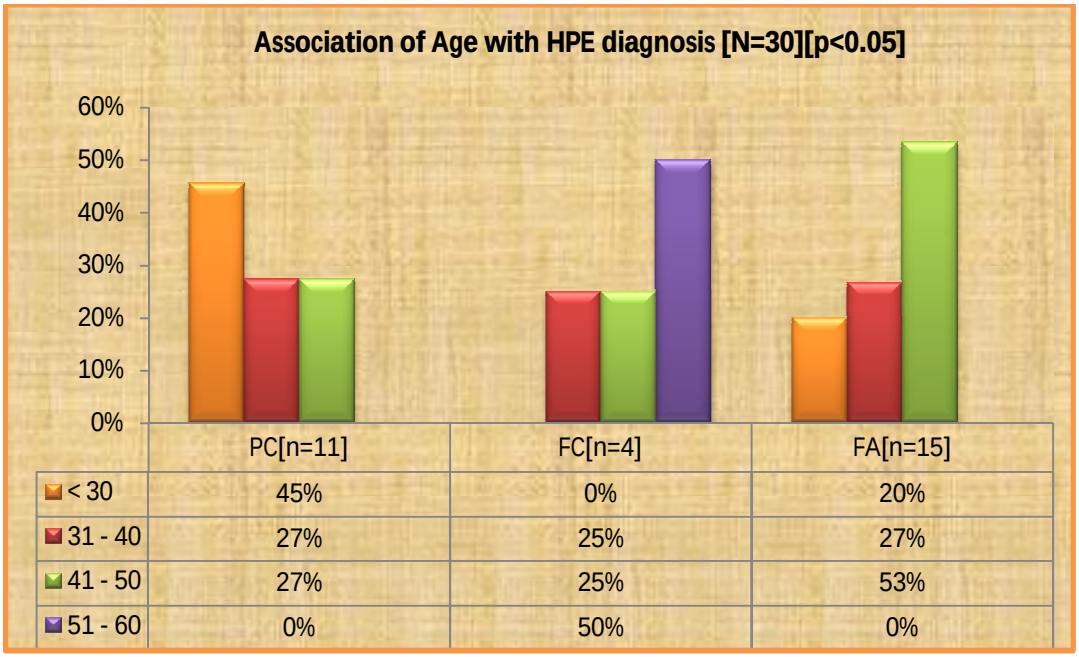
From the above diagram, we observe that follicular adenoma constitutes 50% followed by different thyroid carcinomas constituting totally 50%.

TABLE 4: ASSOCIATION OF AGE WITH HISTOPATHOLOGICAL DIAGNOSIS

HPE DIAGNOSIS				
AGE (years)	**PC**	**FC**	**FA**	**TOTAL**
< 30	5	0	3	8
31 – 40	3	1	4	8
41 – 50	3	1	8	12
51- 60	0	2	0	2
Total	11	4	15	30

From the above table, it is observed that papillary carcinoma is commonly occurring between 30-50 years whereas follicular carcinoma is common in the age group between 51-60 years.

CHART 4: ASSOCIATION OF AGE WITH HPE DIAGNOSIS:



	PC[n=11]	FC[n=4]	FA[n=15]
< 30	45%	0%	20%
31 - 40	27%	25%	27%
41 - 50	27%	25%	53%
51 - 60	0%	50%	0%

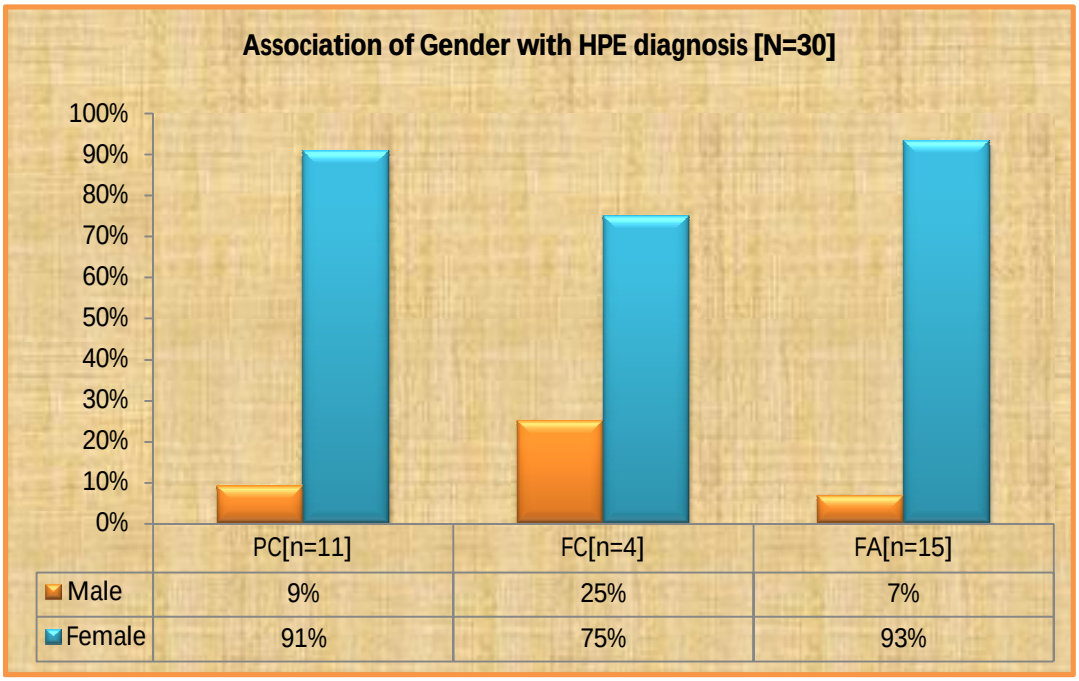
In the present study papillary carcinoma is common in the age group less than 30 years constituting 47%, followed by the age group between 31-40 years and 41-50 years each comprising 27%, whereas follicular carcinoma is common in the age group between 51-60 years constituting 50%. Follicular adenoma occurs in all age groups between 30-60 years with predominance in 41-50 years comprising 53%.

TABLE5: ASSOCIATION OF SEX WITH HPE DIAGNOSIS

HPE DIAGNOSIS				
Gender	**PC**	**FC**	**FA**	**TOTAL**
Male	1	1	1	3
Female	10	3	14	27
Total	11	4	15	30

From the above observations, in the present study, females have higher incidence of thyroid tumors than males. Both benign (14 out of 15 cases) and malignant tumors (13 out of 15 cases) are common in females. In males only 1 case of benign tumor occurred out of total 15 cases and 2 malignant tumors out of total 15 cases.

CHART 5: ASSOCIATION OF SEX WITH HISTOPATHOLOGICAL DIAGNOSIS:



In the present study both benign and malignant thyroid tumors are common in females than males. The percentage of various neoplasms in females are as follows:

Papillary carcinoma – 91%

Follicular carcinoma- 75%

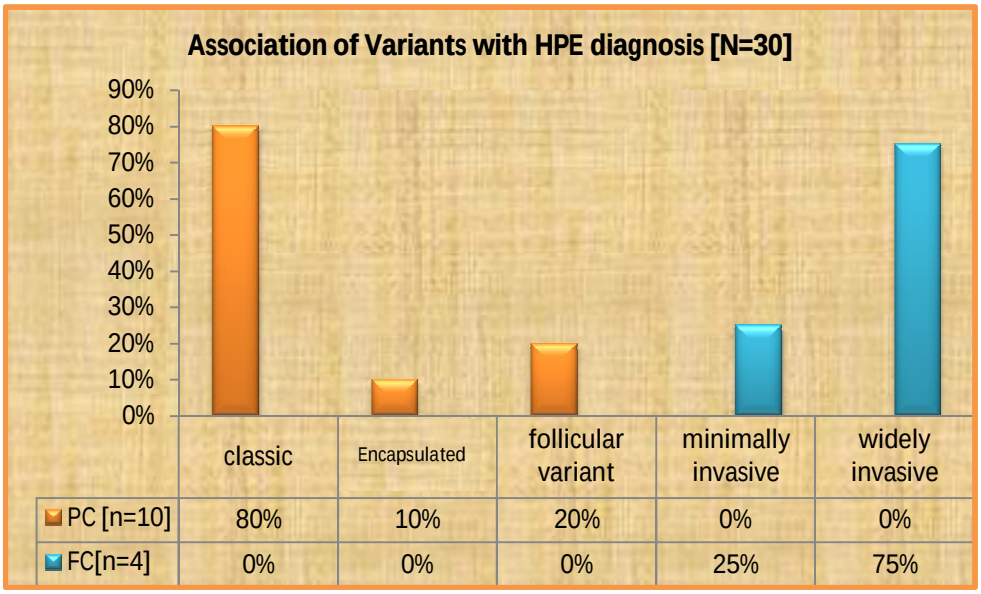
Follicular adenoma- 93%

TABLE 6: VARIANTS OF DIFFERENT THYROID CARCINOMAS [N=30]

Variants of thyroid carcinoma	HPE DIAGNOSIS		
	PC	FC	TOTAL
Classic Papillary Carcinoma	8	0	8
Encapsulated- Papillary Carcinoma	1	0	1
Follicular Variant – Papillary Carcinoma	2	0	2
Minimally Invasive Follicular Carcinoma	0	1	1
Widely Invasive Follicular Carcinoma	0	3	3
Total	11	4	15

In the present study, classic variant of papillary carcinoma constituted majority of cases (9 cases out of 11 cases, one is encapsulated), and follicular variant of papillary carcinoma constituted only 2 cases.

CHART 6: PROPORTION OF VARIANTS OF DIFFERENT THYROID CARCINOMAS:



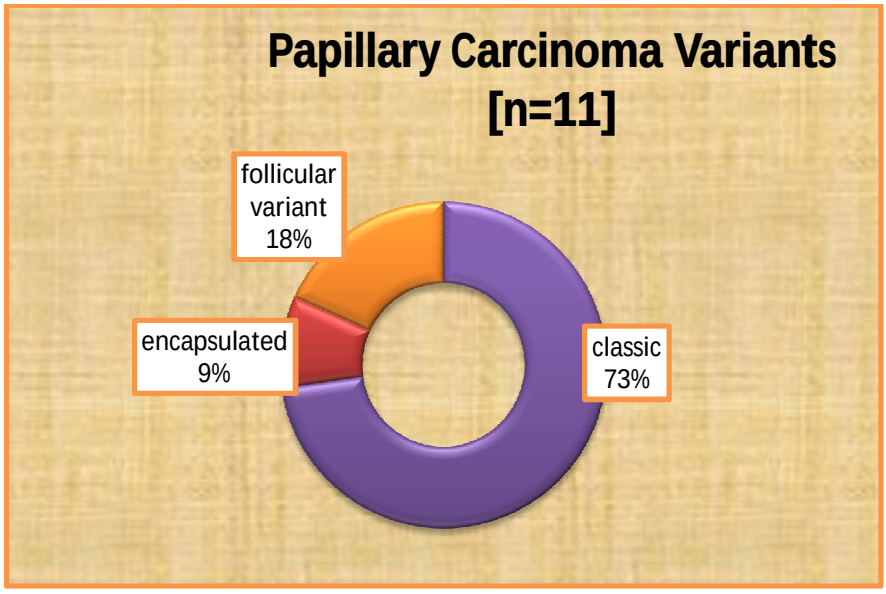
	classic	Encapsulated	follicular variant	minimally invasive	widely invasive
PC [n=10]	80%	10%	20%	0%	0%
FC[n=4]	0%	0%	0%	25%	75%

In the present study the proportion of variants of different thyroid tumors are analysed and in papillary carcinoma classic type constitutes 80% with follicular variant of papillary carcinoma comprising only 20%.

TABLE 7: INCIDENCE OF PAPILLARY CARCINOMA VARIANTS:

Papillary carcinoma variants	No. of cases	Percentage
Classic	8	73%
Encapsulated (classic)	1	9%
Follicular variant	2	18%
Total	11	100%

CHART 7: PERCENTAGE OF VARIANTS OF PAPILLARY CARCINOMA:



The above diagram shows the percentage of papillary carcinoma variants that were received during our study period with classic type 73%, encapsulated variant-9% and follicular variant-18%.

TABLE 8: INTENSITY OF STAINING OF CYTOKERATIN19 IN THYROID NODULES:

Staining of CK 19	HPE DIAGNOSIS				TOTAL
	PC	FC	FA	PCFV	
0	0	3	13	0	16
1+	0	1	2	1	4
2+	1	0	0	1	2
3+	8	0	0	0	8
Total	9	4	15	2	30

(Note: 0: no cells are positive

1+ : <25% of cells are positive

2+: 25%- 50% of cells are positive

3+ : > 50% of cells are positive

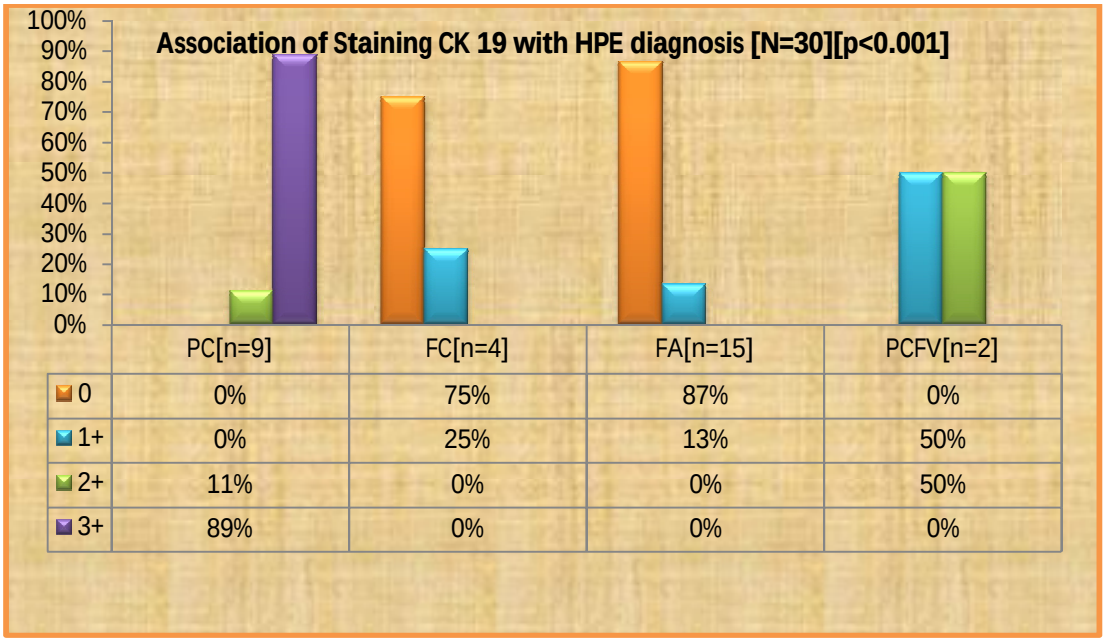
PC : Papillary carcinoma

PCFV: Papillary carcinoma - Follicular variant

FA : Follicular adenoma

FC : Follicular Carcinoma)

CHART 8: INTENSITY OF STAINING OF CYTOKERATIN19 IN THYROID NODULES:



	PC[n=9]	FC[n=4]	FA[n=15]	PCFV[n=2]
0	0%	75%	87%	0%
1+	0%	25%	13%	50%
2+	11%	0%	0%	50%
3+	89%	0%	0%	0%

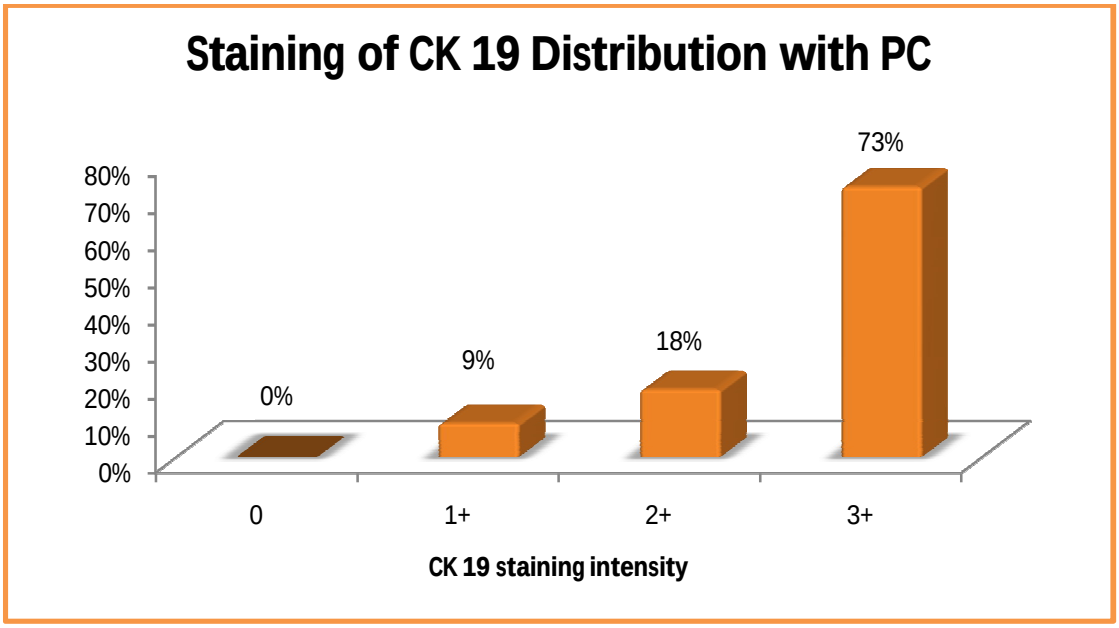
- In the present study, staining intensity of cytokeratin19 in classic papillary carcinoma shows strong and diffuse positivity in cytoplasm of cells i.e. 3+ staining in 8 cases comprising 89% , with only 11% of cases showing moderate or 2 + staining. In follicular variant of papillary carcinoma 50% of cases showed 2 + positivity, 50% of cases had 1 + positivity.
- In follicular carcinoma cytokeratin19 is weakly positive i.e. 1+ in only 25%. 75% of the cases are negative for cytokeratin19
- In follicular adenoma 1+ weak and focal positivity is obtained in 13% of cases only. 87% of cases are negative for cytokeratin19.
- Chi square test showed statistically significant p value of <0.001 from the above variables.

TABLE 9: INTENSITY OF STAINING OF CYTOKERATIN19 IN WELL DIFFERENTIATED THYROID CARCINOMA:

STAINING INTENSITY OF CK19	0	1+	2+	3+	TOTAL
NO. OF FC CASES	3	1	0	0	4
PERCENTAGE OF POSITIVITY IN FC	75%	25%	0%	0%	100%
NO. OF PC CASES	0	1	2	8	11
PERCENTAGE OF POSITIVITY IN PC	0%	9%	18%	73%	100%

The above table shows that Cytokeratin19 positivity is strong and diffuse in papillary carcinoma, whereas focal and weak positivity in follicular carcinoma.

CHART 9: INTENSITY OF STAINING OF CK19 IN PAPILLARY CARCINOMA:



In the present study, the varying intensity of cytoplasmic staining of cytokeratin19 in papillary carcinoma including follicular variant of papillary carcinoma showed diffuse strong positivity of 3+ in 73% of cases , 2+ / moderate positivity in 18% of cases and weak positivity of 1+ in only 9% of cases.

None of the case of papillary carcinoma showed negative staining with cytokeratin19, thus implying its significant role in the diagnosis of papillary carcinoma and its variants.

TABLE 10: STATISTICAL ANALYSIS DATAS OF CYTOKERATIN 19 STAINING IN THYROID NODULES:

Thyroid tumors	Sensitivity	specificity	Positive predictive value	Negative predictive Value
Classic papillary Carcinoma	100%	84%	79%	100%
Papillary carcinoma- Follicular variant	100%	57%	14%	100%
Follicular carcinoma	25%	50%	7%	81%
Follicular adenoma	13%	87%	50%	50%

From the statistical analysis in this present study, it is found that ***cytokeratin19 is a sensitive and specific marker in the diagnosis of papillary carcinoma***.

(*Note: Statistical analysis was done using SPSS software version and the variables are expressed as percentage (%). For statistical comparison Chi square test was used. p value of < 0.05 was considered as statistically significant.)*

REPRESENTATIVE PICTURES

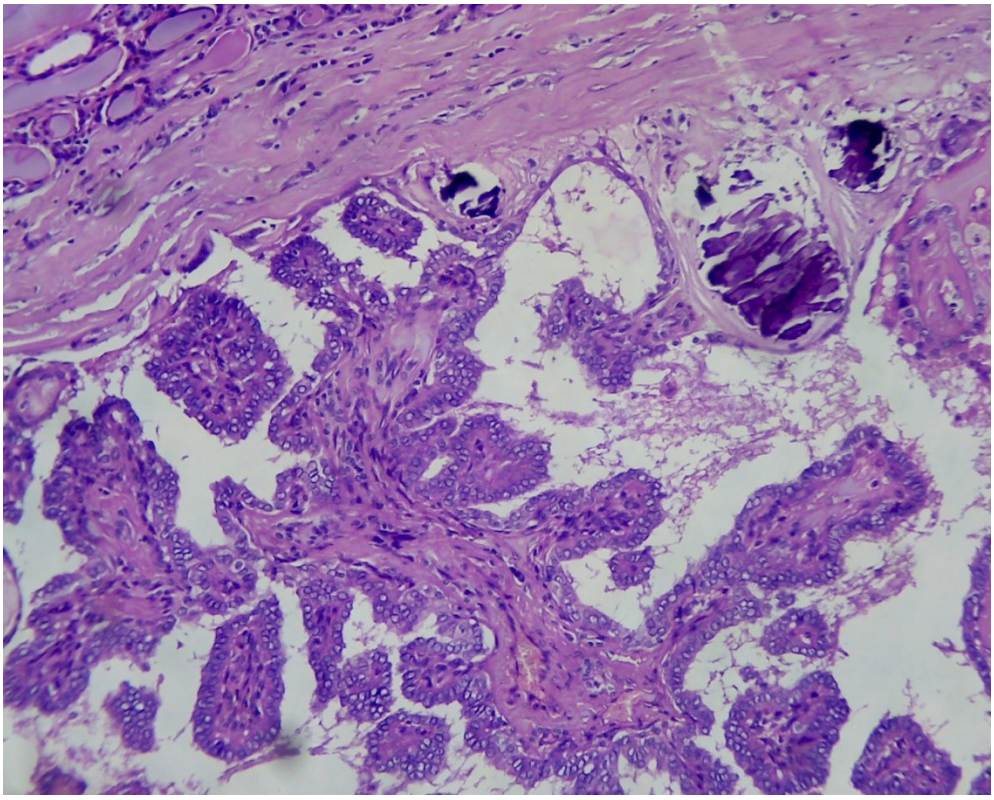


Fig.1. Papillary carcinoma of thyroid- H& E (10X)

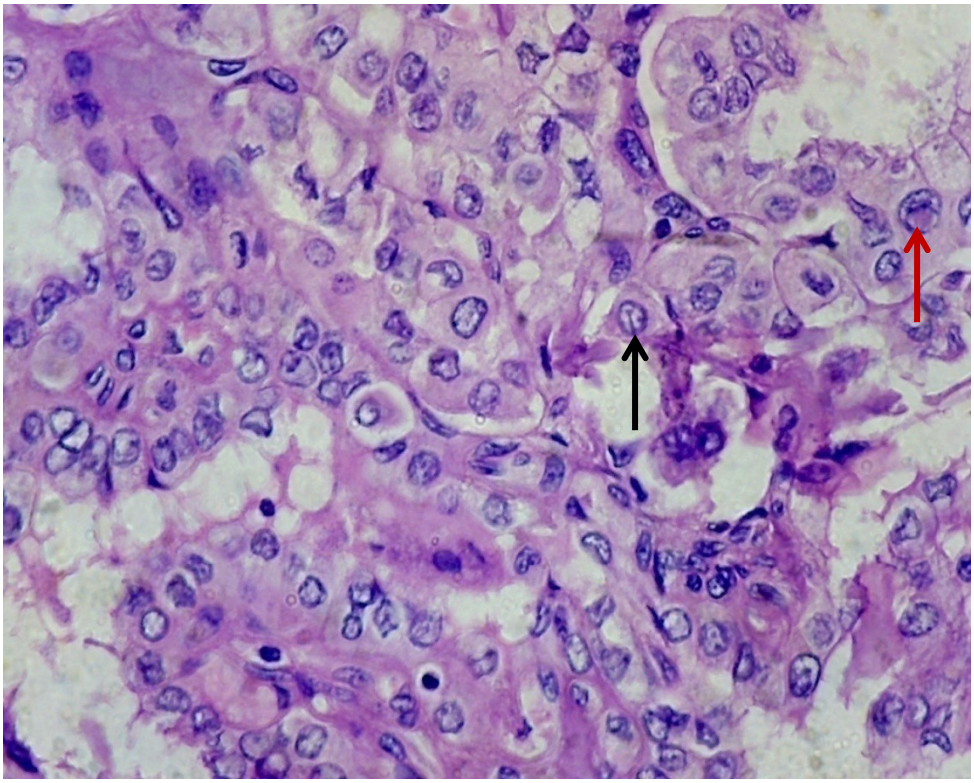


Fig.2. Papillary carcinoma of thyroid showing nuclear grooves (↑) and intranuclear pseudoinclusion (↑) – H & E (40X)

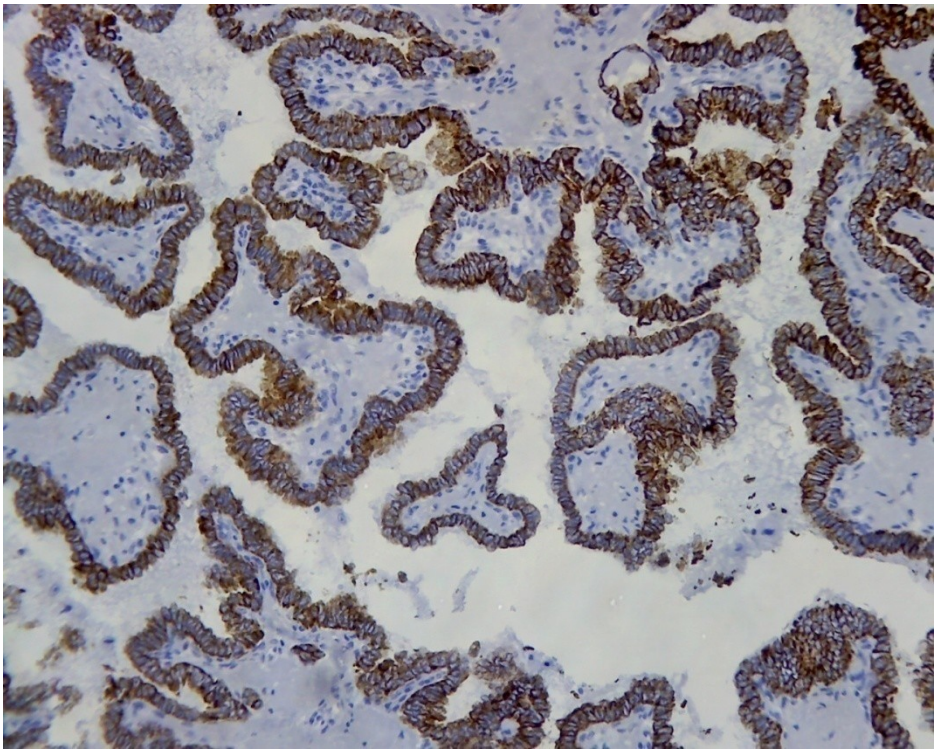


Fig.3. Diffuse 3+ cytoplasmic positivity of cytokeratin19 in papillary carcinoma (10 X)

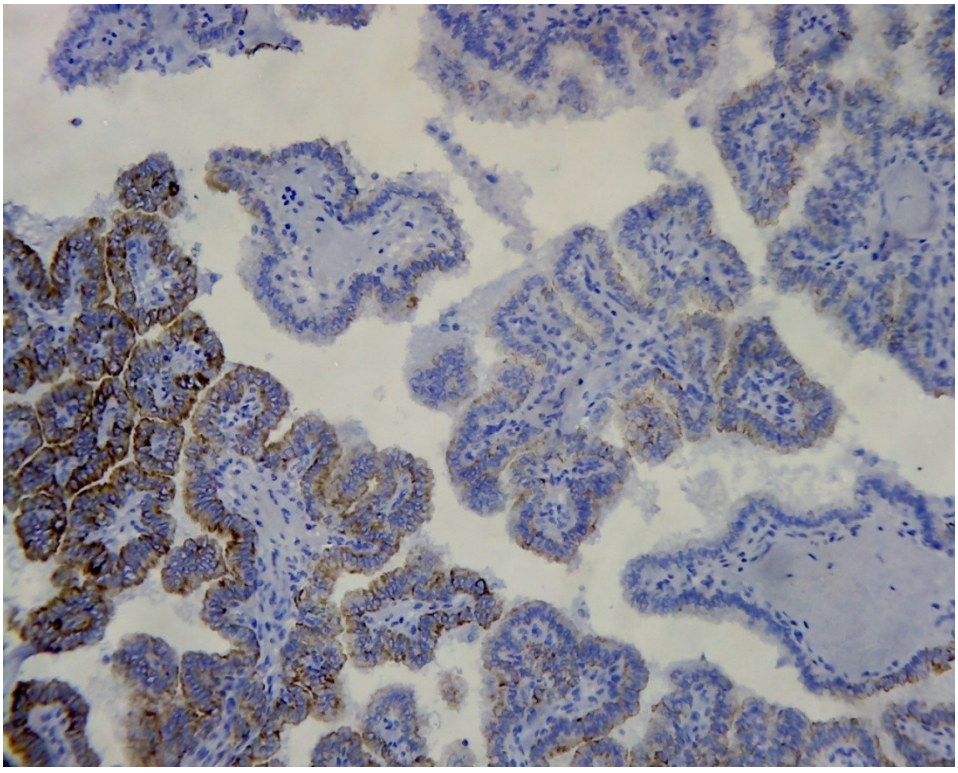


Fig.4. 2+ positivity of Cytokeratin19 in papillary carcinoma (10X)

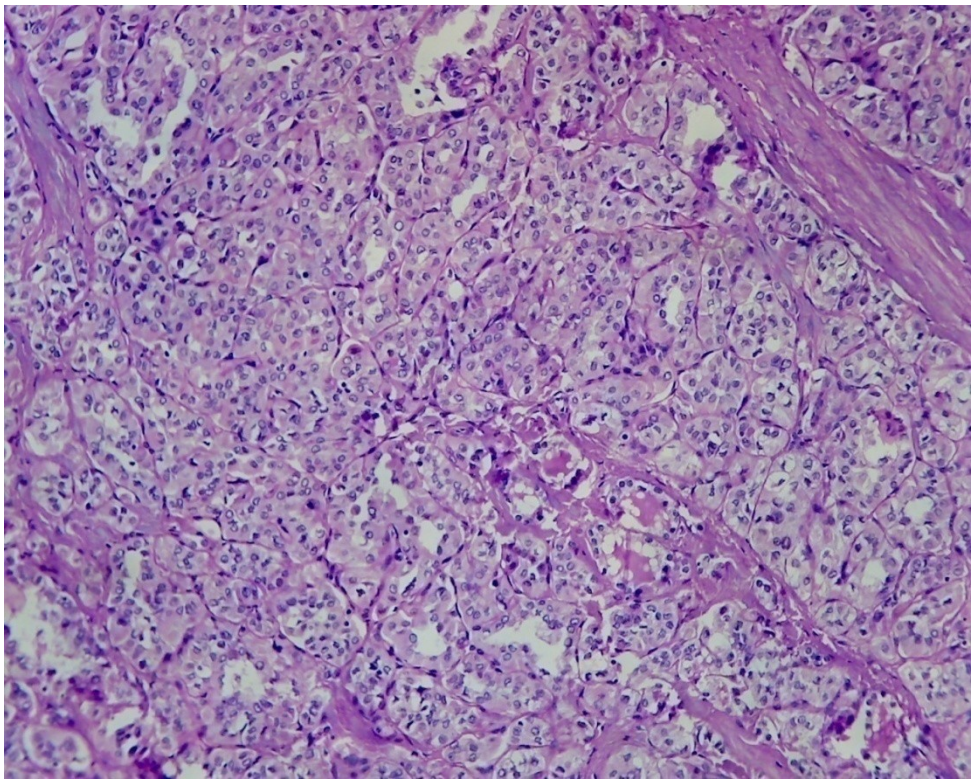


Fig.5. Follicular variant of papillary carcinoma – H & E (10X)

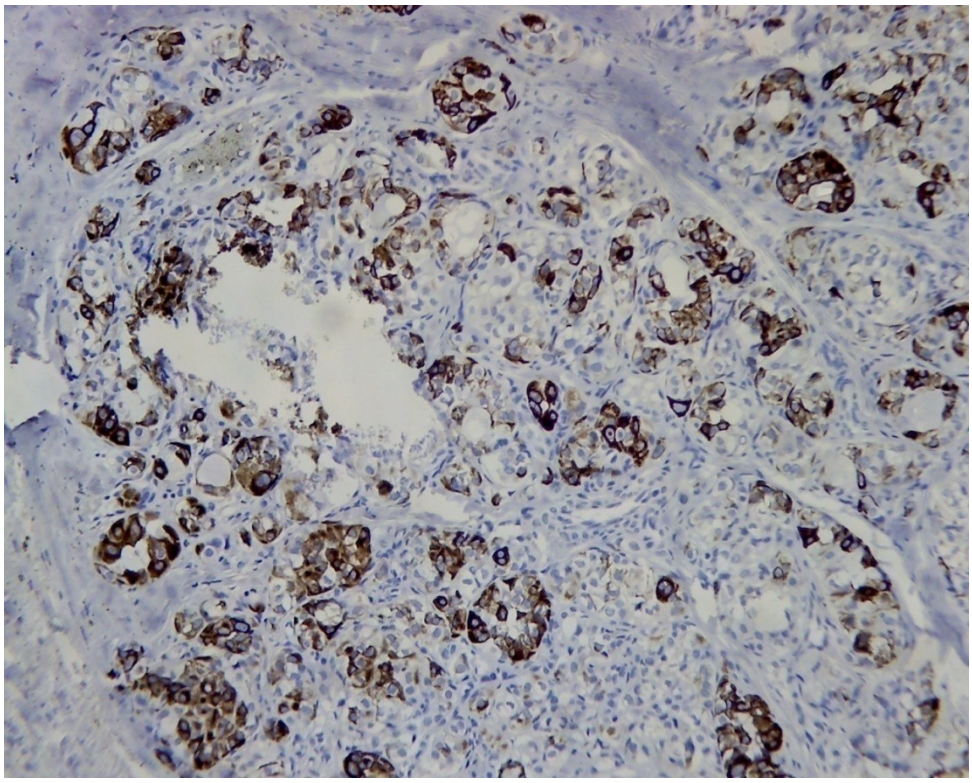


Fig.6 Follicular variant of papillary carcinoma showing 2+ positivity with cytokeratin19 (10X)

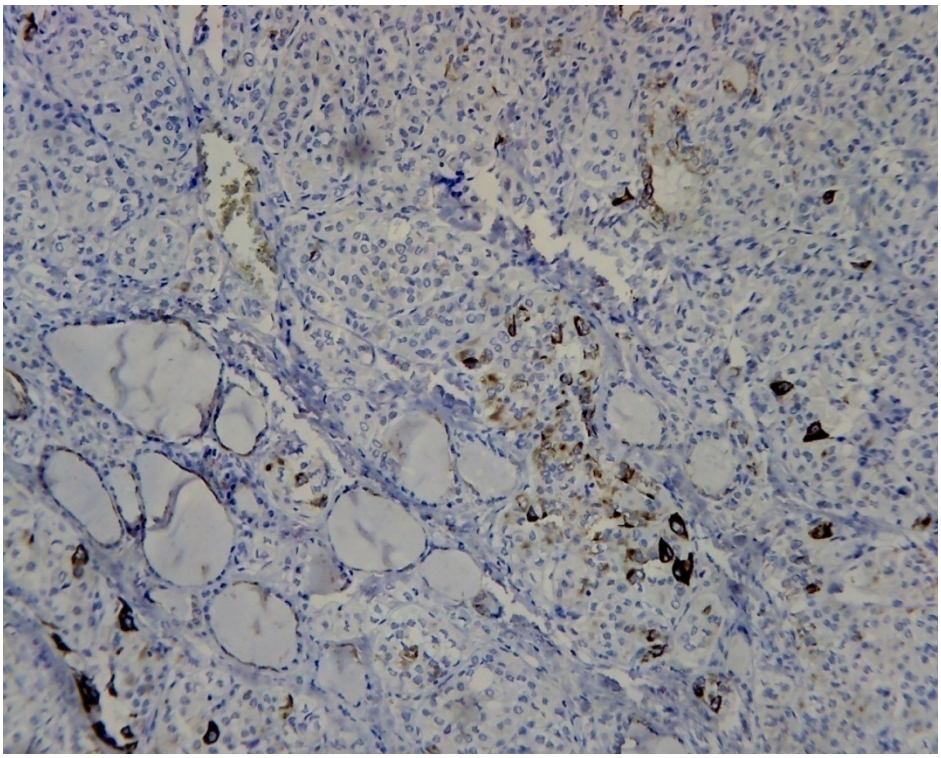


Fig.7 Follicular variant of papillary carcinoma showing 1+ positivity with cytokeratin19 (10X)

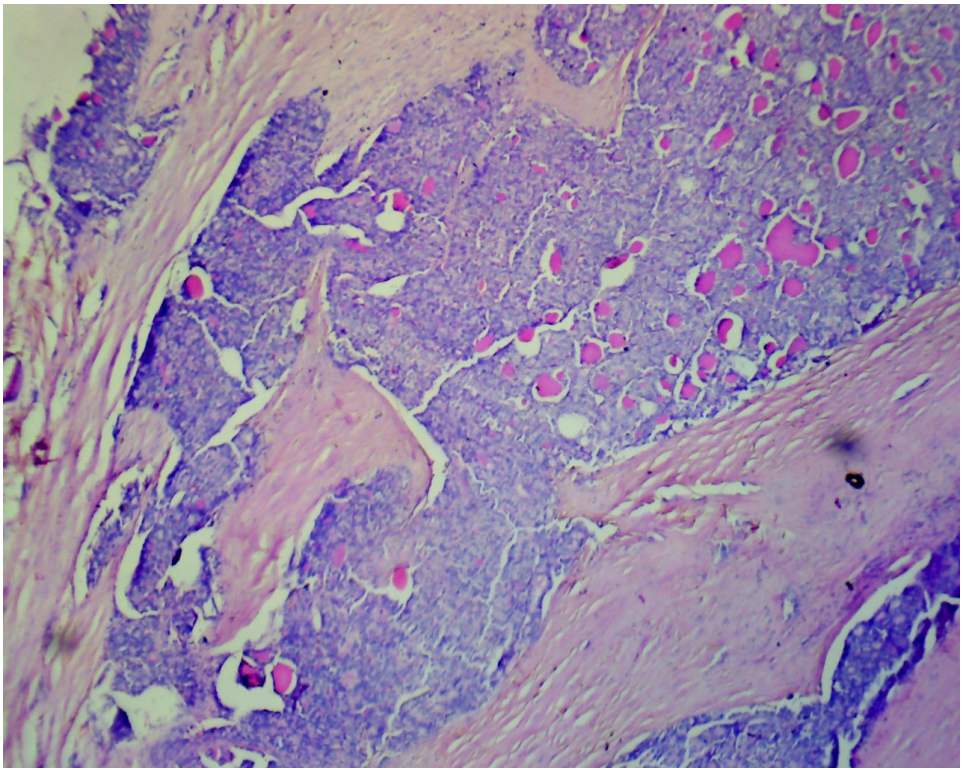


Fig.8. Follicular carcinoma of thyroid with capsular invasion- H & E (10X)

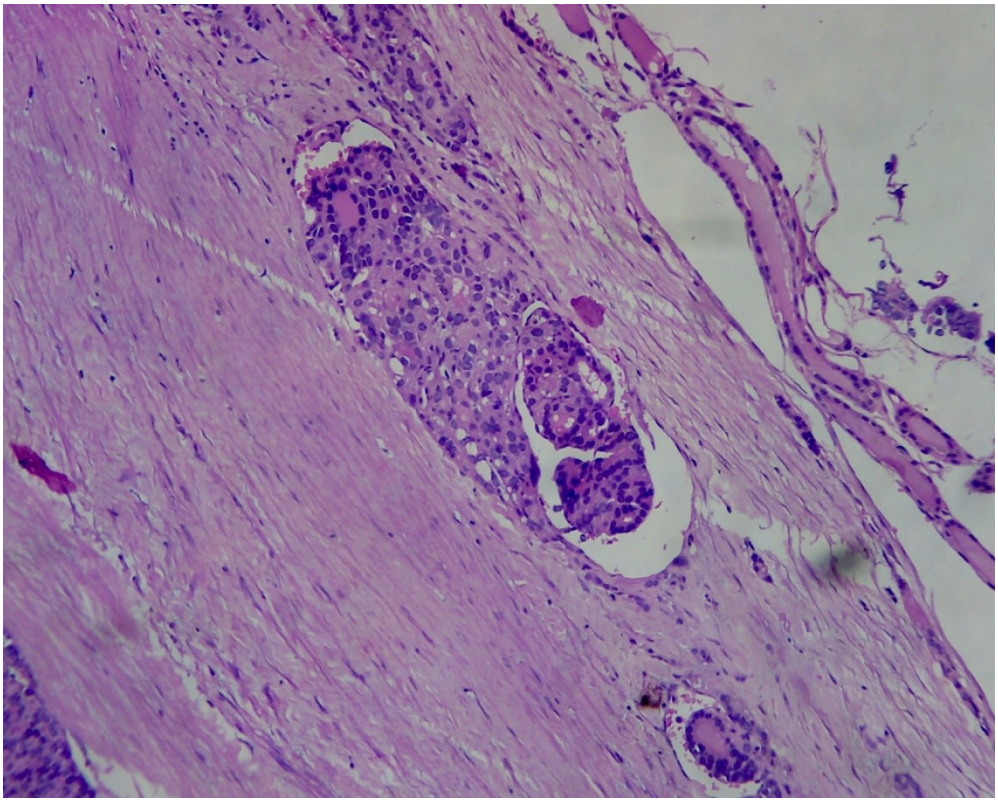


Fig.9. Follicular carcinoma of thyroid showing vascular invasion- H & E (10X)

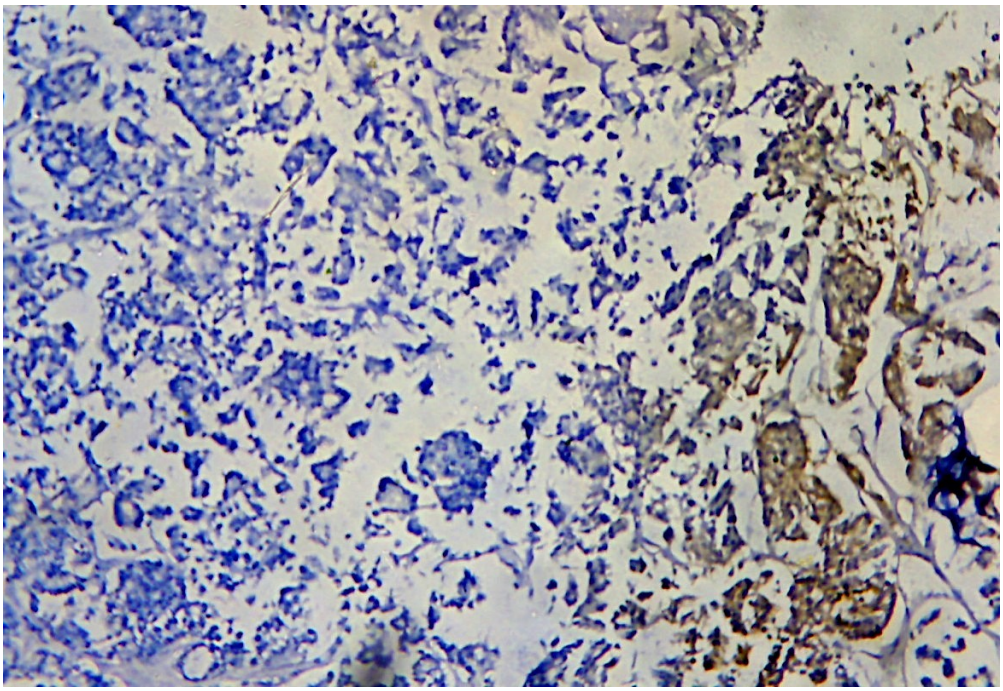


Fig.10. Follicular carcinoma of thyroid showing focal 1+ positivity with cytokeratin19 (10X)

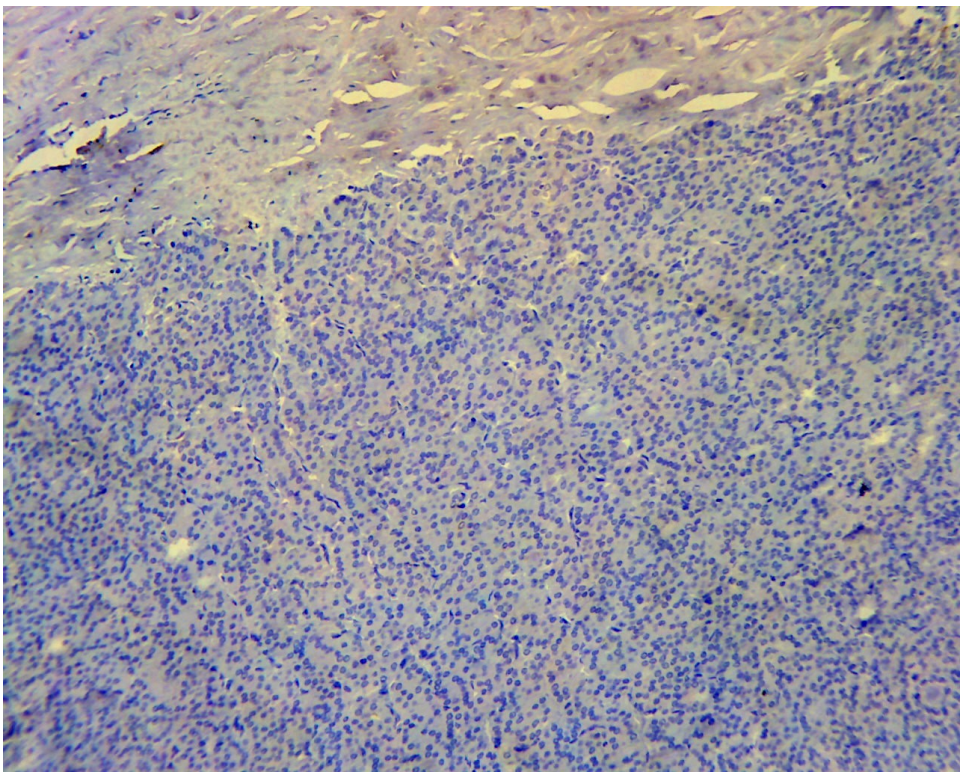


Fig.11. Follicular carcinoma showing negative staining with cytokeratin19 (10X)

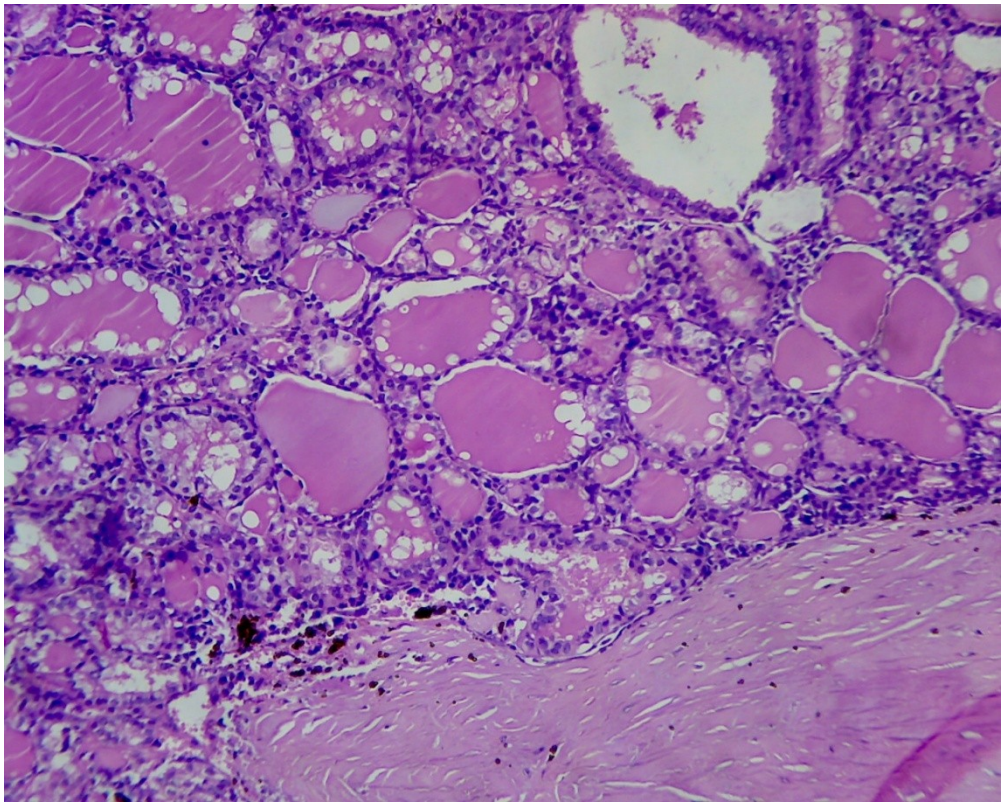


Fig.12. Follicular adenoma – H & E (10X)

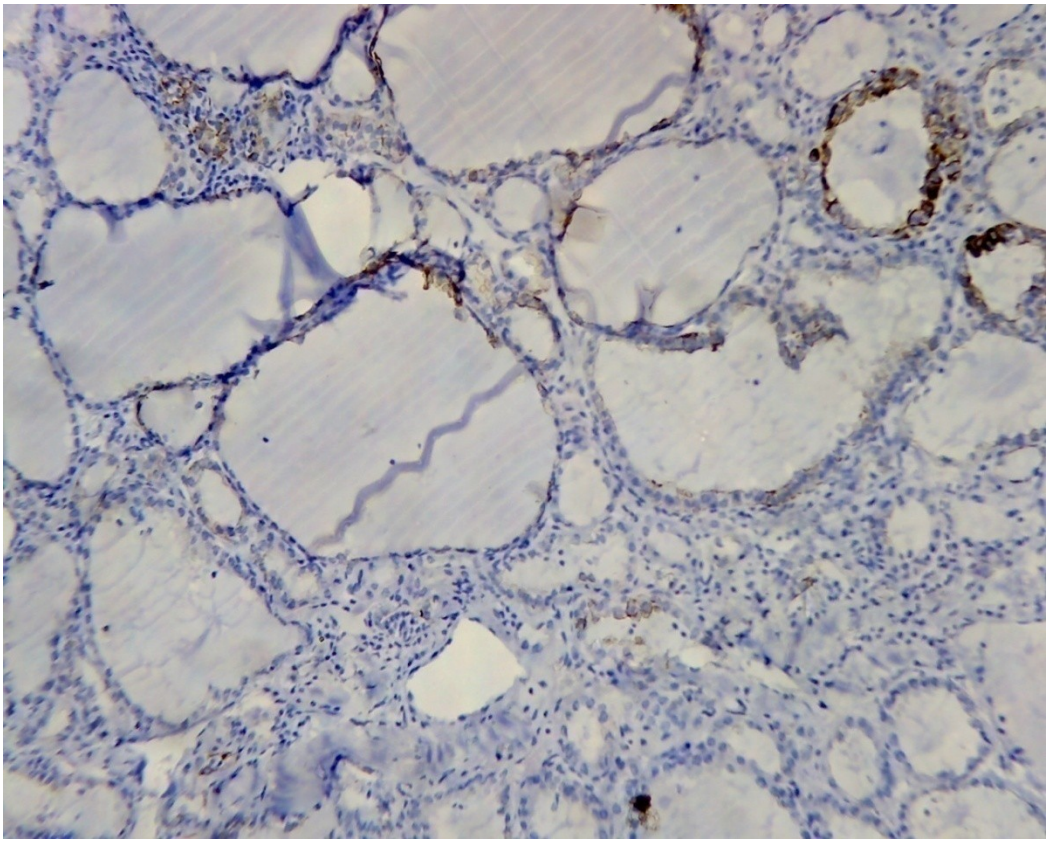


Fig.13. Follicular adenoma showing focal 1+ positivity with cytokeratin19 (10X)

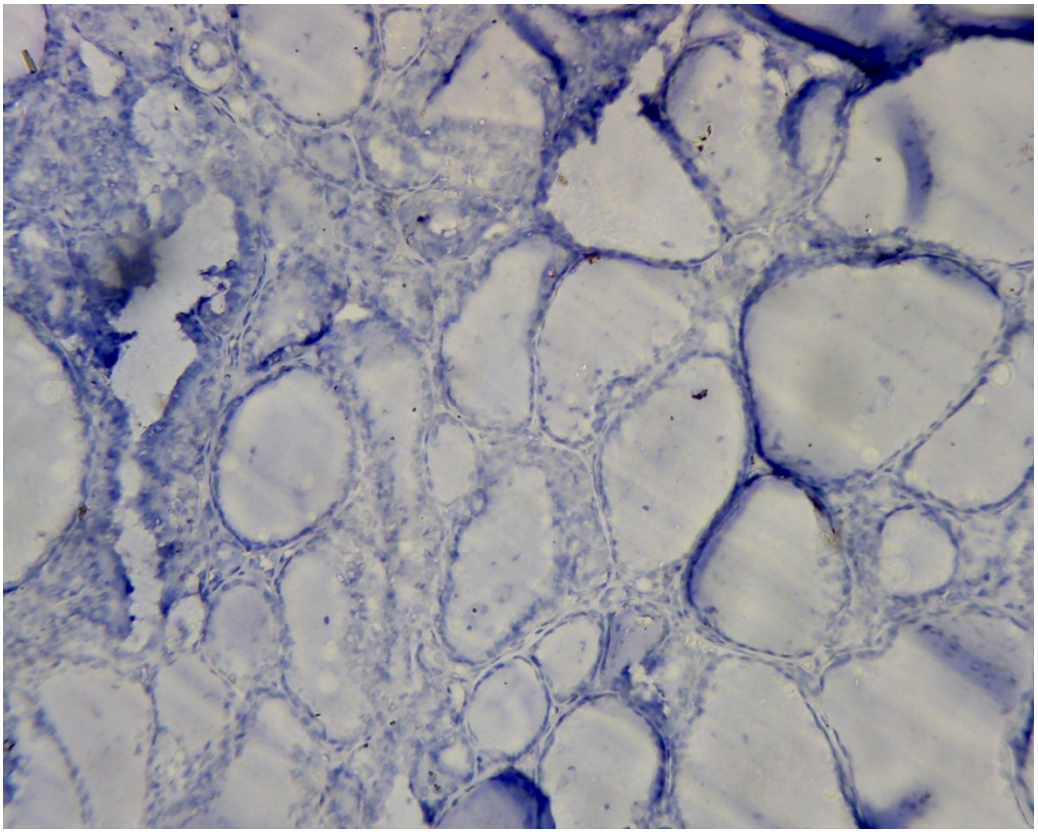


Fig.14. Follicular adenoma showing negative staining with cytokeratin19 (10X)

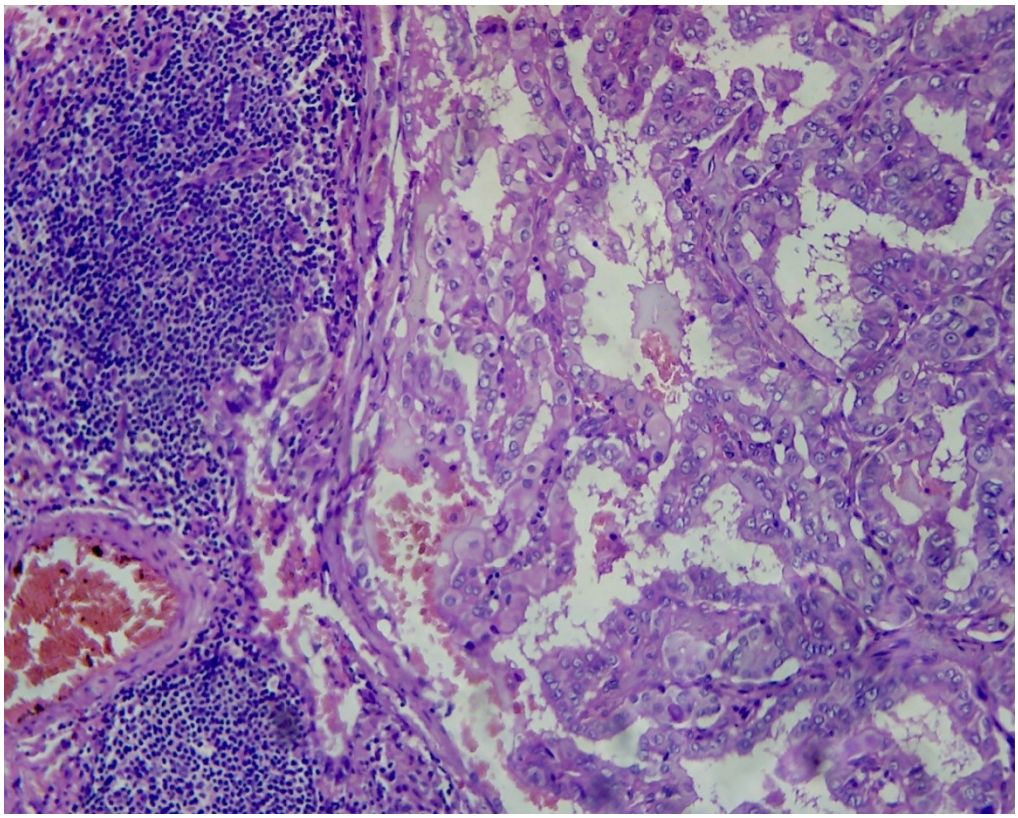


Fig.15. Metastatic papillary carcinoma deposits in lymph node – H & E (10X)

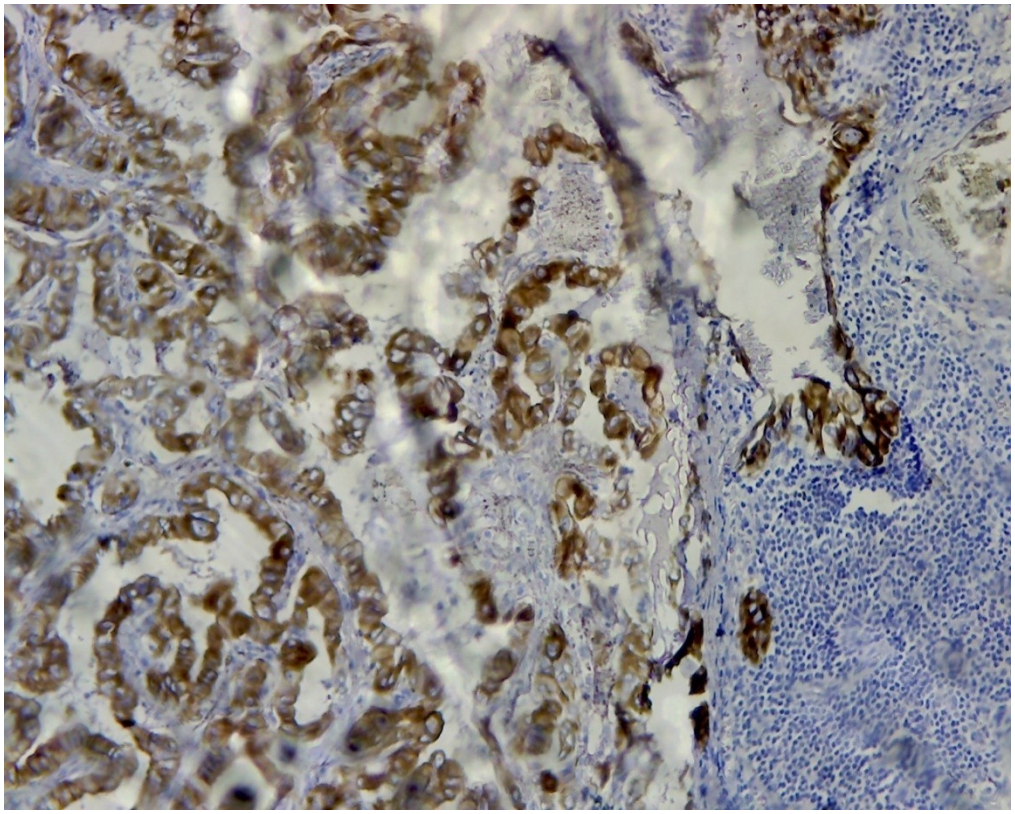


Fig.16. Diffuse 3+ positivity of cytokeratin19 in metastatic papillary carcinoma deposits foci in lymph node

DISCUSSION

DISCUSSION

Diagnosis of thyroid lesions by the routine hematoxylin and eosin stained sections is really challenging because of the overlapping histological features in various thyroid lesions. Interpretation of thyroid lesions with follicular pattern is quite difficult. Also in papillary carcinoma not all the characteristic nuclear features are present in same case.

Additionally, encapsulated tumors with follicular pattern pose dilemma in differentiating benign from malignant thyroid neoplasm, when the neoplastic nodule has incomplete capsular invasion or equivocal vascular invasion. Tumors having some nuclear features of papillary carcinoma but not having papillary structures i.e. follicular variant of papillary carcinoma is difficult to distinguish from other follicular patterned lesions. This issue is clearly pointed out by ***Elsheikh et al,*** in their recent study and was reviewed and confirmed by ***J.Rosai*** in 2008.[82]

Thyroid nodules are common worldwide and affects 40% of the population. They are common in females and in age group between 30-60 years.

But 80% of the thyroid nodules are benign neoplasm or hyperplastic nodules.

Thyroid malignancies are the most common endocrine carcinomas comprising 5- 10%. Each year 3.6% of new cases of thyroid cancer occur. To aid in better clinical management and to obtain maximum benefit it is very essential to identify the benign lesions. Incorrect diagnosis may lead to stress, both psychologically and socially in patients and they may incur unwanted healthcare expenditure. No definite diagnostic modalities are available to differentiate the thyroid lesions. Fine needle aspiration cytology is still routinely used as screening test for thyroid lesions, but it is not reliable in differentiating benign and malignant tumors.

In view of all these shortcomings, nowadays a panel of immunohistochemical markers are used that help in the distinction of benign and malignant thyroid lesions. In this present study, the usefulness of cytokeratin19 in differential diagnosis of various thyroid nodules is analysed and its occurrence is correlated with age and sex of the patients and also with histomorphology.

The observation and results of previously conducted studies that are relevant to this present study are as follows:

As per the study conducted conducted by ***Al-Zaher N et al in 2008***, thyroid nodules most commonly occured in the age group between 25 to 65 years.[83]

In the present study, we have also observed the similar results, and thyroid nodules are common between 30- 50 years with 24 cases out of 30, followed by age group of 51 – 60 years as shown in table 1.

American cancer society in their statistical analysis of thyroid lesions in the year **2010,** reported that thyroid nodules both benign and malignant, are 2 to 3 times, more common in females than males.[84]

In the present study, we also observed that female to male ratio of incidence of thyroid nodules is 3:1as shown in table2 & 5. Thus it correlates with previous study.

National Cancer Institute in 2013, analysed the proportion of various thyroid carcinoma and found that ***papillary thyroid carcinoma*** is the predominant malignancy constituting 70- 80% of all thyroid cancers. They are common in females between 30- 50 years as per their study. The next common malignancy is ***Follicular carcinoma*** constituting 10- 15% of all thyroid malignancies and is common in females in the age group of 40-60 years.[10]

In this present study, we have also obtained similar results with papillary carcinoma constituting 73%, followed by follicular carcinoma 26%. Both papillary and follicular carcinomas are common in females constituting 91% and 75% respectively. Papillary carcinoma is common

in the age group between 30- 50 years (all 11 cases), and follicular carcinoma between 40- 60 years in our study (3 cases out of 4) as shown in table4 & 5. Chi square test was done and these results were found to be statistically significant with p value < 0.05

Fabio Muradas Girardi et al, in 2013 studied the prevalence of thyroid carcinoma variants in a single centre .[85] They have found that

- Classic variant of papillary carcinoma- 31.7%
- Follicular variant – 16%
- Oncocytic variant- 1.9%
- Other variants- 0.1- 0.7%.

In our present study we received 11 papillary thyroid carcinoma of which 9 are **classic type** including one encapsulated variant comprising **82%** followed by follicular variant – 18% as shown in table. We have not received other variants in our study period. Thus it correlates with the previous study of predominance of classic variant.

Hanan Alsaeid Alshenawy studied the usefulness of cytokeratin19 in thyroid nodules during the year 2009-2013 and observed cytokeratin19 positivity in 57% of follicular adenoma i.e. 4 cases out of total 7 cases (1 case focally positive 1+, 3 cases showed 2+ positivity).[15]

***Husain A Saleh** et al* in 2008, in their study reported cytokeratin19 positivity in 30% (23 cases out of 46 total cases) of cases of follicular adenoma and the intensity of staining is weak and focal.[86]

In our present study, we have also obtained the similar results of weak and focal positivity with cytokeratin19 i.e. 1+ (< 25% of cells) in only 2 cases out of total 15 cases, constituting 13% as shown in table and chart 8. Hence cytokeratin19 is not a sensitive marker in the diagnosis of follicular adenoma. But it has higher specificity of 87% in differentiation of benign from malignant tumors.

The sensitivity and specificity of cytokeratin19 in follicular adenoma in this study is 13% and 87% respectively.

***Hanan Alsaeid Alshenawy* ,** in his study found out that cytokeratin19 showed positivity in 53% of cases of follicular carcinoma, i.e. 8cases out of total 15 cases (2 cases with positivity in < 25% of cells - 1+, 2 cases with 2+ positivity about 25-50% of cells and and 4 cases with >50% of cells , 3+ positivity) .[15]

K.Y Lam et al in 2001, reported focal positivity in 68% of follicular carcinoma, 13 cases out of total 19 cases with cytokeratin19.[87]

In this present study we obtained focal and weak 1+ positivity in only 1 case out of total 4 cases of follicular carcinoma constituting 25%.

All other 3 cases are negative as shown in table and chart 8. Thus it correlates with previous studies.

The sensitivity and specificity of cytokeratin19 in follicular carcinoma in this study is 25% and 50% respectively. .

Hanan Alsaeid Alshenawy, in his study found out that cytokeratin19 is always positive in papillary carcinoma incontrast to other thyroid malignancies.[15] He reported that all 14 cases of papillary carcinoma showed positivity with cytokeratin 19 comprising 100%. 13 cases showed strong and diffuse positivity of 3+ and only one case showed 2+ intensity. In follicular variant of papillary carcinoma all 8 cases showed positive staining with 5 cases showing 2+ intensity and remaining 3 cases with 3+ positivity.

He also found that cytokeratin19 has 100% sensitivity and 77% specificity in differentiating papillary thyroid carcinoma from other follicular neoplasm.

Husain A Saleh *et al* reported 85% positivity of classic papillary carcinoma cases with cytokeratin19, (17 cases out of 20) and 83.3% in follicular variant of papillary carcinoma (10 out of 12 cases).[86]

Lei Gong et al in 2012, reported 100% strong and diffuse positivity with cytokeratin19 in papillary carcinoma.[88]

Carol C Chereng et al in 2001, observed strong and diffuse positivity with cytokeratin19 in 80% of classic papillary carcinoma and in 57% of follicular variant of papillary carcinoma and concluded that it is a sensitive marker.[89]

Sahoos et al studied the utility of cytokeratin19 in the diagnosis of papillary carcinoma and reported in 2001 that cytokeratin19 is sensitive marker showing 100% positivity in papillary carcinoma including the follicular variant. [90]They got diffuse 3+ positivity in all 5 classic papillary carcinoma and 3+ in 9 cases out of 10, in follicular variant of papillary carcinoma. Only one case out of 10 showed moderate / 2+ positivity.

Dina El Demellawy et al reported in 2008, cytokeratin19 positivity in 85% of cases of papillary carcinoma which included both classic and follicular variant.[91]

In this present study, the results obtained are so alike with all the aforementioned studies. We studied the cytokeratin19 staining in total of 11 cases of papillary carcinoma including follicular variant. All the 11 cases showed positive staining (100%) with cytokeratin19 but with varying intensity. In classic papillary carcinoma out of 9 cases only one case (11%) showed 2+ intensity of staining. All other 8 cases had diffuse and strong positivity of 3+ (89%).

1 out of the total 9 cases of classic papillary carcinoma had cervical **lymph node metastasis** and cytokertin19 showed diffuse and **strong 3+ positivity in the foci of papillary carcinoma deposits in the node.**

In the total of 2 cases of follicular variant of papillary one showed 1+ intensity and other showed 2+ intensity of staining as in table 8.

The sensitivity and specificity of cytokeratin19 in papillary carcinoma is 100% and 84% respectively. Chi square test of these variables showed statistically significant p value of < 0.001.

The results of this present study are in conformity with the previously conducted studies and thus immunomarker cytokeratin19 plays a pivot role in differentiation of thyroid nodules.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Thyroid lesions are common worldwide with a prevalence of 40 million per year. 80% of the thyroid nodules are benign. As the treatment modalities are improving in recent years, correct diagnosis aids in better patient outcome and survival rate. Immunohistochemistry helps in arriving at exact diagnosis in controversial cases. Cytokeratin19, an intermediate filament protein in epithelial cells shows variable staining in different thyroid lesions and thus helps in differentiating thyroid tumors especially, papillary carcinoma and its variants.

In the present study we included 30 cases of different thyroid lesions which included follicular adenoma (15 cases), follicular carcinoma (4cases) and papillary carcinoma with its follicular variant (11 cases), one case with lymph node metastasis and obtained the following results.

The incidence of thyroid nodules are common between the age group 30-60 years and females are commonly affected than males. Female to male ratio is 3:1.

In the present study we observed that papillary carcinoma is more common comprising 73% followed by follicular carcinoma (26%). These values are studied by chi square test and obtained statistically significant p value of < 0.05.

Both benign and malignant thyroid neoplasm are common in females with an incidence of 90% in contrary to only 10% in males. In females, papillary carcinoma is most common in age group between 30 – 50 years, whereas follicular carcinoma occurred in age group between 40-60 years (75%) .

CYTOKERATIN19 EXPRESSION IN VARIOUS THYROID NEOPLASM ARE OBSERVED AS FOLLOWS:

In classic papillary carcinoma cytokeratin19 showed diffuse and strong 3+ (>50% of cells) cytoplasmic positivity in 89% of cases. Only one case showed 2+ (25%-50%) positivity constituting 11%.

In one of the classic papillary carcinoma with lymph node metastasis, metastatic foci of papillary carcinoma also showed diffuse and strong 3+ positivity with cytokeratin19.

In follicular variant of papillary carcinoma one case showed focal 1+ positivity with cytokeratin19 and the other case showed 2+ positivity. The sensitivity of cytokeratin19 in papillary carcinoma is 100% and specificity is 84%.

The positive predictive value is 79%. Chi square analysis of these variables showed statistically significant p value of < 0.001.

In follicular carcinoma, 75% of the cases showed negativity with cytokeratin19 and 25% of cases showed weak and focal 1+ intensity of

staining (< 25% of cells). The sensitivity and specificity is 25% and 50% respectively.

In follicular adenoma, cytokeratin19 showed negativity in 87% of cases with only 13% of cases showing weak and focal 1+ positivity. The sensitivity is 13% and specificity 87%.

Our findings in the present study indicate that, cytokeratin19 showed varying intensity of staining in different thyroid lesions. Cytokeratin19 showed 100% positivity in papillary carcinoma and is found to be a sensitive and specific marker in the diagnosis of papillary carcinoma and its variants. Cytokeratin19 is also useful in diagnosing papillary carcinoma with tubulopapillary pattern provided the staining is strong and diffuse. Cytokeratin19 in conjunction with TTF1 and PAX8 can also be used to confirm the diagnosis of metastatic deposits of papillary carcinoma of thyroid with occult primary.

Cytokeratin19 does not differentiate follicular adenoma from follicular carcinoma. Though the present study showed more sensitivity in differentiating follicular variant of papillary carcinoma from follicular carcinoma, a definite conclusion cannot be made in this regard, as minimum number of cases of follicular carcinoma and follicular variant of papillary carcinoma have been studied.

In such instances, a panel of markers like *Galectin3, HBME1 and CD56* may aid in exact differentiation of these neoplasms and it needs a further detailed research in future.

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ANNEXURE I

ANNEXURE 1

PROFORMA

Coimbatore medical college

Department of Pathology

Coimbatore

Particulars of the patient:

Name : Age:

Ward : IP. No :

Address: Occupation:

Presenting complaints:

Swelling infront of the neck

Pain

Onset and progression

Difficulty in swallowing

Change of voice

Bowel habits

Menstrual disturbances in female

Hypo/ hyperthyroidism symptoms

Loss of appetite and weight

Other swellings in neck and other regions.

Past history:

History of radiation exposure to head and neck

History of previous surgeries

Family history

Personal history:

Diet

General examination:

Nourishment : Built: Conscious:

Pallor: Jaundice: Clubbing:

Febrile/afebrile Lymphadenopathy : Edema:

Tremors: eye signs:

Vitals:

Pulse rate: BP: RR:

Local examination:

Site : Size: Shape:

Solitary nodule / multinodular

Whether Moving with deglutition or not

Fixity to surrounding structures

Lymphadenopathy: cervical and other region

Clinical diagnosis:

Investigations:

Thyroid hormone profile

USG report

Fine needle aspiration cytology report

Microscopic findings:

Histopathological diagnosis

Immunohistochemistry:

FINAL DIAGNOSIS

CONSENT FORM

Dr. P. Suriyaprabha, postgraduate student in the department of pathology, Coimbatore Medical College is conducting a study on "IMMUNOHISTOCHEMICAL EXPRESSION OF CK-19 IN THYROID NODULES AND ITS CORRELATION WITH HISTOPATHOLOGY". Thyroidectomy done for various thyroid lesions are received in pathology department and are processed and examined under a microscope to obtain diagnostic information or is tested for other studies. I have been informed, to my satisfaction regarding the nature of surgical procedure. The data used herein may be used for research and publication.

Name:

Place:

Signature:

ஒப்புதல் படிவம்

பெயர் :

வயது :

பாலினம் :

முகவரி :

அரசு கோவை மருத்துவக் கல்லூரியில் நோய் குறியியல் மருத்தவ துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி **மரு.ப.சூர்யபிரபா** அவர்கள் மேற்கொள்ளும் **"தைராய்டு கட்டியில் சைட்டோகெராடின் 19னின் வெளிப்பாட்டினை அறிதல்"** பற்றிய ஆய்வில் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெளிவுப்படுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடனும், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த அய்வில் என்னைப் பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம் :

தேதி : கையொப்பம் / ரேகை

ANNEXURE II

ANNEXUREII - MASTER CHART

SERIAL NO.	IP.NO	AGE	SEX	HPE.NO.	HPE DIAGNOSIS	VARIANT	CK19 POSITIVITY	INTENSITY OF CK19 STAINING
1	73070	28	f	3773/14	PC	classic	diffuse positivity	3+
2	565687	19	f	p522/14	PC	encapsulated	diffuse positivity	3+
3	49540	50	f	2737/14	PC	classic	diffuse positivity	3+
4	55804	45	f	3074/14	PC	classic	diffuse positivity	3+
5	81793	32	f	01/15	PC	classic	diffuse positivity	3+
6	80948	40	f	149/15	PC with LN mets.	classic	positive	2+
7	45561	50	f	2601/14	PC	classic	diffuse positivity	3+
8	81924	40	f	297/15	PC	classic	diffuse positivity	3+
9	37888	30	f	1925/15	PC	classic	diffuse positivity	3+
10	80254	27	m	4349/14	PC	follicular variant	focally positive	1+
11	49870	29	f	2528/15	PC	follicular variant	focally positive	2+
12	9499	56	m	638/15	FC	widely invasive	negative	0
13	73517	55	f	4235/14	FC	minimally invasive	negative	0
14	32391	39	f	1620/15	FC	widely invasive	focally positive	1+
15	41535	45	f	2325/15	FC	widely invasive	negative	0
16	35851	35	f	2161/14	FA		focally positive	1+
17	36271	48	f	2169/14	FA		negative	0
18	52681	23	f	3002/14	FA		negative	0
19	54740	28	f	3003/14	FA		focally positive	1+
20	7006	35	f	556/14	FA		negative	0
21	43993	45	f	2544/14	FA		negative	0

22	17294	42	f	1026/14	FA		Negative	0
23	48298	50	f	2777/14	FA		Negative	0
24	53123	50	f	2933/14	FA		Negative	0
25	57595	50	f	3187/14	FA		Negative	0
26	58456	35	f	3747/14	FA		Negative	0
27	28474	32	m	1599/14	FA		Negative	0
28	29424	42	f	1699/14	FA		Negative	0
29	29121	18	f	1716/15	FA		Negative	0
30	33513	44	f	1680/15	FA		Negative	0

KEY WORDS

PC- papillary carcinoma

LN mets - lymph node metastasis

FC- follicular carcinoma

FA- follicular adenoma

CK19- cytokeratin19

ANNEXURE III

ANNEXURE III- LIST OF ABBREVIATIONS

CK19	-	Cytokeratin 19
HBME-1	-	Hector Battifora Mesothelial-1
TTF-1	-	Thyroid transcription factor -1
EMA	-	Epithelial membrane anigen
CEA	-	Carcinoembryonic antigen
NSE	-	Neuron specific enolase
PC	-	Papillary carcinoma
PCFV	-	Papillary carcinoma – follicular variant
FC	-	Follicular carcinoma
FA	-	Follicular adenoma
H&E	-	Hematoxylin and eosin
IHC	-	Immunohistochemistry