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DR. KALYANI R, MD

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DEPARTMENT OF PATHOLOGY

DEPARTMENT OF PATHOLOGY

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LIST OF ABBREVIATIONS

AJCC: American Joint Committee on Cancer

CK 19: Cytokeratin 19

CMVPTC: Cribriform morular variant papillary carcinoma thyroid

CVPTC: Classical variant papillary thyroid carcinoma

FAP: Familial adenomatous polyposis

FVPTC: Follicular variant papillary thyroid carcinoma

FFPB: Formalin fixed paraffin block

Gal 3: Galectin 3

H&E: Haematoxylin and Eosin

HMBE 1: Hector Battifora mesothelial-1

Ig G: Immunoglobulin G

IHC: Immunohistochemistry

m- RNA: Messenger Ribonucleic acid

PTC: Papillary thyroid carcinoma

T3: Triiodothyronine

T4: Tetraiodothyronine

TG: Thyroglobulin

TRH: Thyroid stimulating hormone

TSH: Thyroid stimulating hormone

TTF: Thyroid transcription factor 1

WHO: World Health Organisation

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ABSTRACT

BACKGROUND:

Thyroid neoplasm is one of the most common endocrine neoplasm. Papillary thyroid carcinomas (PTC) are most commonly encountered thyroid malignancies. Diagnosis of PTC is based on the special nuclear features like, anisonucleosis, overlapping of nuclei, intranuclear inclusions, optical clearing and nuclear grooves. However, some of these features may also be seen in benign thyroid lesions secondary to degeneration. As differentiation between benign or malignant lesions has clinical, therapeutic and prognostic significance, it's necessary to make precise diagnosis by using biomarkers.

In recent years many immunohistochemical (IHC) biomarkers have been studied to aid in the differentiation between non-neoplastic and malignant thyroid lesions. CK19, Galectin 3, TG, Ki67, BRAF, Calcitonin, HBME-1, TTF1 and RET are some of the examples of these IHC markers. Inspite of several studies being done related to the utility of these biomarkers in singles as well as various combinations, a uniform consensus is yet to be reached.

AIMS AND OBJECTIVES:

To determine the expression of CK 19 and Galectin-3 in Papillary thyroid carcinoma and non-neoplastic lesions of thyroid. To compare the expression of Galectin-3 and CK19 in papillary thyroid carcinoma and non-neoplastic lesions of thyroid.

MATERIALS AND METHODS:

This is an observational study. 26 cases with papillary thyroid carcinoma and 26 cases of non-neoplastic lesions of thyroid were studied. Relevant clinical data was collected. H&E slides were reviewed and selection of paraffin blocks with lesional area were selected. IHC for CK 19 and Gal 3 were performed. The expression of both the biomarkers were scored, analysed and compared. Specificity and sensitivity was evaluated for each biomarker and their combination. P value of <0.05 was considered statistically significant.

RESULTS:

The specificity and sensitivity of CK 19 to differentiate papillary thyroid carcinoma and non-neoplastic lesions of thyroid was 88.46% and 23.08% respectively. The specificity and sensitivity for Gal 3 was 88.46% and 50% respectively. The specificity and sensitivity for the combination of CK19 and Gal 3 biomarker was 84.62 and 61.54% respectively.

CONCLUSION:

The present study concludes that Gal 3 can be useful to differentiate PTC and non-neoplastic lesions of thyroid. CK 19 and the combination of CK19 and Gal 3 biomarkeris not helpful to differentiate PTC and non-neoplastic lesions of thyroid.

KEYWORDS: Papillary thyroid carcinoma, Cytokeratin 19, Galectin 3

INTRODUCTION

INTRODUCTION

Thyroid neoplasm is one of the most common endocrine neoplasm. Incidence of thyroid neoplasm varies from place to place. Most countries have reported an upward trend in its incidence.[1] A similar trend in the incidence is seen in southern India.[2] According to National Cancer Registry of India, thyroid cancers show an upward trend from 2.3/1,00,000 population in 1990 to 3.8/1,00,000 population in 2014.[1]

Majority of these neoplasms originate from follicular epithelial cells. Of which the most commonly encountered carcinomas are papillary thyroid carcinoma (PTC), follicular carcinoma, poorly differentiated carcinoma and anaplastic carcinoma; the incidence of which are 80%, 15%, <1% and <2% respectively.[3]

The distinction of classical cases with follicular patterned thyroid lesions is considerably easy. However, many a times the diagnosis and the distinction between malignant and benign neoplasm can be difficult, even with histological analysis.[1] General nuclear features of malignancy may not be applicable to endocrine tumours as benign endocrine tumours and some of the non-neoplastic lesions may exhibit them. The only reliable criteria of malignancy in endocrine tumours are invasion and metastasis.

PTC are most commonly encountered thyroid malignancies. PTC diagnosis is based on the special nuclear features like anisonucleosis, overlapping of nuclei, intranuclear inclusions, optical clearing and nuclear grooves. However, some of these features may also be seen in benign thyroid lesions secondary to degeneration. As differentiation between benign or malignant lesions has clinical, therapeutic and prognostic significance, it's necessary to make precise diagnosis by using biomarkers.

In recent years a large number of immunohistochemical (IHC) markers have been studied to assist in differentiating non-neoplastic from malignant thyroid lesions. CK19, Galectin 3, TG, Ki67, BRAF, Calcitonin, HBME-1, TTF1 and RET are some of the examples of these IHC markers.[3] Inspite of several studies being done related to the utility of these markers in singles as well as various combinations, a uniform consensus is yet to be reached. Moreover, minimum number of biomarker s required to make a distinction has not been established. Hence, this study using Galectin 3 and CK19 has been taken up.

OBJECTIVES

OBJECTIVES

- To determine the expression of Galectin 3 in papillary thyroid carcinoma and non-neoplastic lesions of thyroid.

- To determine the expression of CK 19 in papillary thyroid carcinoma and non-neoplastic lesions of thyroid.

- To compare the expression of Galectin 3 and CK 19 in papillary thyroid carcinoma and non-neoplastic lesions of thyroid.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

THE THYROID GLAND:

Thyroid gland is an endocrine gland located in front of the neck. It releases thyroid hormones namely tri iodothyronine (T3) and tetra iodothyronine (T4). These regulate various body functions like growth, development and metabolism.

EMBRYOLOGY:

Thyroid forms 24 days post fertilization hence it is the first endocrine gland to develop. A medial endodermal thickening in the floor of the primordial pharynx gives rise to a small outpouching named as the thyroid primordium. The developing gland goes ventral to the hyoid bone and laryngeal cartilages to reach the neck, anterior to the developing 2nd and 3rd tracheal rings. The thyroglossal duct is formed as the tongue and the gland remain attached for a short time. The hollow primordium solidifies resulting in 2 lobes connected via isthmus. It attains its definite shape, size and location by 7th week. Meanwhile the thyroglossal duct gets physiologically degenerated leaving behind the proximal opening of the duct as a pit in the tongue called foramen cecum.[4]

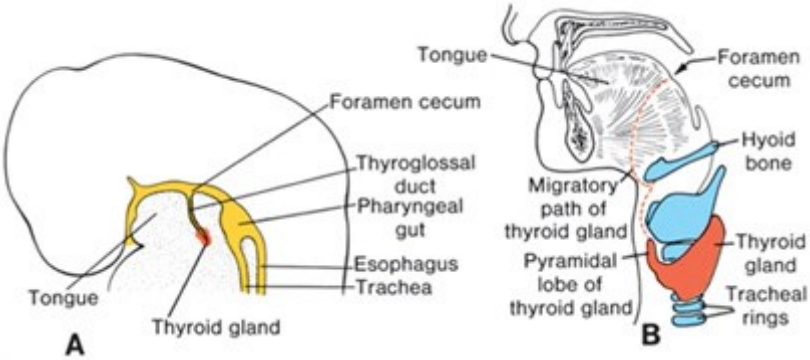

Figure 1: Embryonal development of thyroid gland.[5]

HISTOGENESIS:

The hollow primordium is replaced by endodermal cells. These cells form cords as the vascular mesenchyme intervenes. By the end of 10 weeks, the cords give rise to clusters. These cells get arranged in a single layer forming central lumen. By 11th week colloid is noted in these thyroid follicles.[4]

ANATOMY:

A fully developed thyroid gland is butterfly shaped having pear shaped lobes and central isthmus weighing 25grams. Size of the lobes are 5x2x3cms (length x breath x width) and Isthmus measures1.25x1.25cms (length X width). Blood supply is by superior thyroid artery, a branch of external carotid artery and inferior thyroid artery, a branch of subclavian artery. Venous drainage constitutes of superior and inferior thyroid veins. Nerve supply is by superior and middle cervical sympathetic ganglia.[4,6-8]

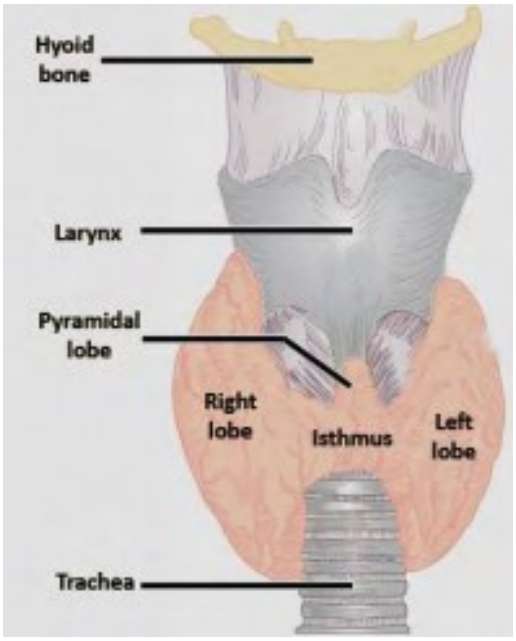

Figure 2: Anatomy of thyroid gland.[9]

HISTOLOGY[4]:

Each lobule contains 20-40 follicles. Each follicular cell has centrally placed round nuclei with homogenous chromatin. Cytoplasm contains liposomes, endoplasmic reticulum and small mitochondria. The intrafollicular spaces are have parafollicular cells or C cells. These are large and pale as compared to follicular cells having polygonal and spindle shape with eccentrically placed nucleus, distinct nucleoli and granular foamy cytoplasm.

PHYSIOLOGY:

Thyroid gland works on the basis of feedback mechanism. Hypothalamus produces thyrotrophin releasing hormone (TRH) which stimulates the pituitary gland to release thyroid stimulating hormone (TSH). TSH acts on thyroid follicular cells to release T3 and T4.

LESIONS OF THYROID:

(1) Non neoplastic lesions:

a) Thyroiditis

i. Infectious:

- Acute
- Chronic

ii. Autoimmune thyroiditis

iii. Grave's Disease

b) Goitre

i. Diffuse nontoxic goitre

ii. Multi nodular goitre

iii. Nodular hyperplasia

(2) Neoplastic lesions:

a) Benign

b) Malignant

NON-NEOPLASTIC LESIONS OF THYROID GLAND

INFECTIOUS THYROIDITIS:

a) ACUTE INFECTIOUS THYROIDITIS:

Acute thyroiditis is more commonly of infective aetiology. It is often associated with acute upper aero digestive tract infections like pharyngitis, sepsis, major neck trauma. Common causative organisms are streptococcus hemolyticus, staphylococcus aureus, pneumococcus, gram negative bacteria, candida, pneumocystis.[10,11] Microscopy shows neutrophilic infiltration in the gland. Severe cases may present with abscess.

b) GRANULOMATOUS THYROIDITIS:

Synonyms: De Quervain's thyroiditis, Giant cell thyroiditis, Painful subacute thyroiditis.

Clinically presents in a 40-50 years old woman with sore throat, odynophagia, fever and malaise. T3 & T4 levels are often elevated.[11]

Gross: Asymmetrical enlargement of thyroid gland. Thyroid gland appears firm in consistency.

Microscopy: Thyroid follicles are surrounded by well-formed granulomas with multinucleated giant cells but absent caseation. Marked inflammation is noted.

Tuberculosis, sarcoidosis, mycoses also presents with granulomatous thyroiditis.[12-16]

AUTOIMMUNE THYROIDITIS:

Autoimmune thyroiditis includes lymphocytic thyroiditis and hashimoto thyroiditis.

a) LYMPHOCYTIC THYROIDITIS:

Clinically, it occours as a painless or silent thyroiditis which may either be sporadic or postpartum predominantly in a 45- 65years old women. It is 10 to 20 times more common in women as compared to men. Often there is a transient hyperthyroid phase followed by hypothyroidism with return to euthyroid state.

Gross: Symmetrical diffuse firm enlargement of thyroid gland with intact capsule. Cut surface is friable, nodular/micronodular, pale, yellowish gray looking like a hyperplastic lymph node.

Microscopy: 2 main abnormalities noted are

1. Thyroid follicular cells with oxyphilic changes.
2. Stroma with dense lymphocytic infiltration/ germinal centers.

   Predomination of T lymphocytes are noted as compared to B lymphocytes.[17]

   Plasma cells, histiocytes and multi-nucleated giant cells can also be found.

   Well-formed germinal centers can be noted.

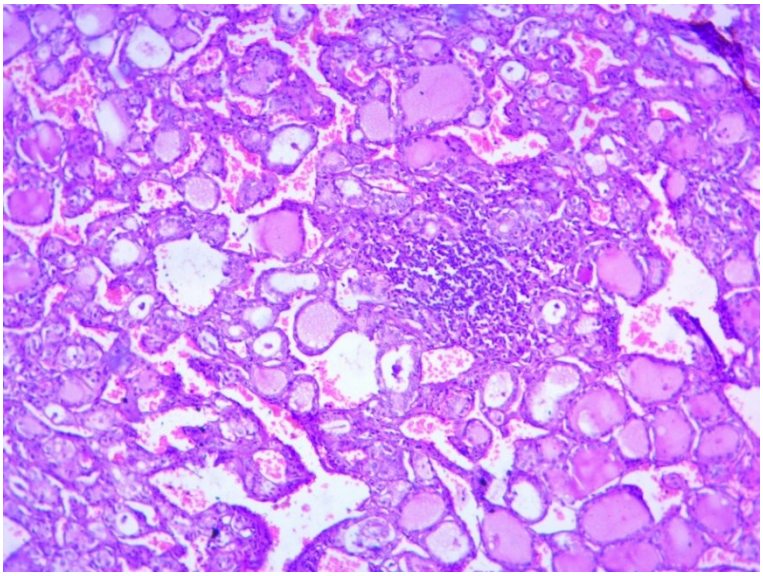

Figure 3: Microphotograph of Lymphocytic thyroiditis. (H&Ex100)

b) HASHIMOTO THYROIDITIS:

Hashimoto thyroiditis is an autoimmune disease characterized by formation of auto antibodies against the thyroid follicular cells. Decreased levels of regulatory T cells and increased T helper cells results in increased production of IL 17 and other pro inflammatory cytokines.[19] Prevalence rate is 46 per 1000 individuals with male to female ratio of 8:1.[20,21]It is histologically lymphocytic thyroiditis with raised anti thyroid peroxidase antibodies level in serum.

GOITER:

a) DISMORPHOGENIC GOITRE:

Etiological factors include lack of responsiveness to TSH resulting in hormone synthesis defect, iodine transport defect, coupling, ossification and defective thyroglobulin synthesis.[22,23]

Gross: thyroid gland is enlarged with single or multiple nodules.

Microscopically, the hypercellular nodules have cells predominantly arranged in solid/ microfollicular pattern. Papillary and insular formation can also be noted in some cases. Fibrosis and nuclear atypia is seen along with scant colloid.[22] It can be commonly confused with Papillary carcinoma and Medullary carcinoma.[23]

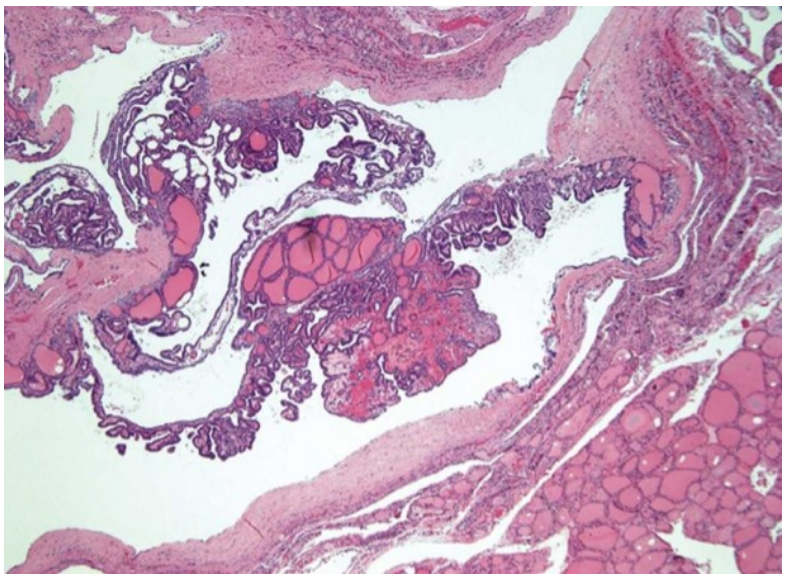

Figure 4: Microphotograph of Hyperplastic thyroid nodule with papillary areas.[18] (H&E x 40)

b) GRAVES DISEASE:

Synonyms are diffuse toxic goitre, thyrotoxicosis, basedow disease. An aautoimmune disease with IgG antibodies against TSH receptors.Commonly occurs in adult females with muscle weakness, weight loss, tachycardia, cold intolerance. Biochemical investigations include raised bound T3, T4 and free T4 levels in blood serum.[24]

Grossly, the gland is symmetrically diffusely enlarged.

Microscopy shows hyperplastic follicles having papillary folding. Lining epithelium is columnar with hyperchromatic nuclei placed basally. Cytoplasm is clear that can show glycogen and fat. Hence, is confused with papillary carcinoma. Scalloping colloid is pale with fine vacuolation. Stroma has lymphoid aggregates.[25] Incidence rate of incidental carcinoma findings in glands removed for hyperthyroidism varies from 1 to 9%.[26]

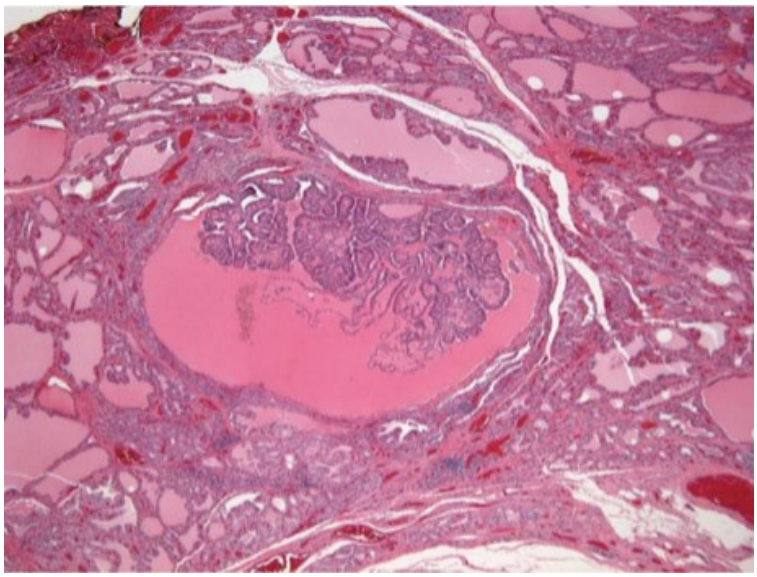

Figure 5: Microphotograph shows thyroid follicles having intrafollicular papillary projections of the hyperplastic epithelium in Grave's disease.[18] (H&E x40)

c) NODULAR HYPERPLASIA:

An endemic goitre due to low iodine intake resulting in increased TSH secretion leading to hyperactive thyroid having tall follicular epithelial cells and scant colloid. It is more commonly seen in adults with incidence of three to five percentage.[27]

Grossly seen are multiple nodules with stretched capsule. Secondary changes like haemorrhage, calcification and cystic degeneration are commonly seen.

Microscopic findings include multiple follicles lined by flattened epithelium. Papillary projections are commonly seen. It can be easily confused with follicular variant of papillary thyroid carcinoma, follicular thyroid adenoma and follicular thyroid carcinoma.[28] Thyroid nodules can lead to thyroid dysfunction. It becomes necessary to rule out thyroid neoplasm.

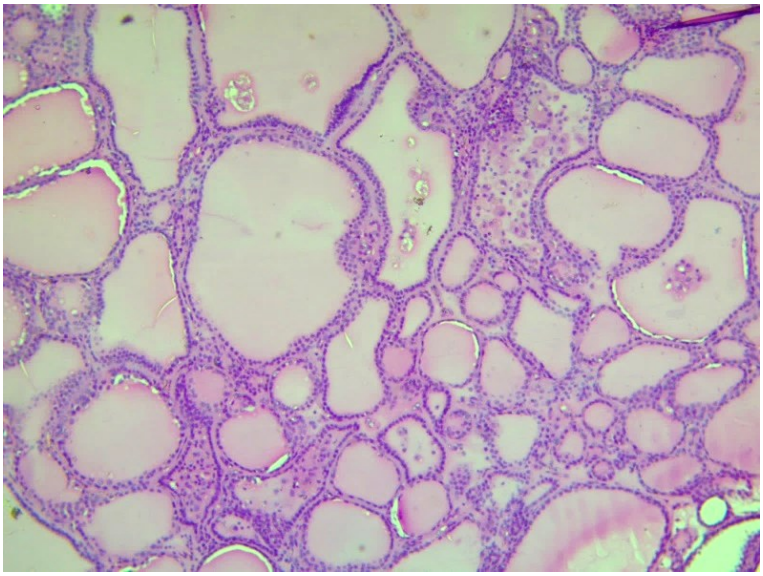

Figure 6: Microphotograph of Nodular hyperplasia. (H&E x100)

WHO (2017) CLASSIFICATION OF THYROID NEOPLASMS.[29]

Tumours of thyroid gland

- Follicular adenoma
- Hyalinizing trabecular tumour
- Other encapsulated follicular patterned thyroid tumours
- Tumours of uncertain malignant potential
- Follicular thyroid neoplasm without invasion and papillary like nuclear features
- Papillary thyroid carcinoma
- Follicular thyroid carcinoma
- Hurthle (oncocytic) cells carcinoma
- Poorly differentiated thyroid carcinoma
- Anaplastic thyroid carcinoma
- Squamous cell carcinoma
- Medullary thyroid carcinoma
- Mixed medullary and thyroid follicular thyroid carcinoma
- Mucoepidermoid carcinoma
- Sclerosing mucoepidermoid carcinoma along with eosinophilia
- Mucinous carcinoma
- Ectopic thymoma
- Spindle epithelial tumour with thymus like differentiation
- Intra-thyroid thymic carcinoma
- Paraganglioma and mesenchymal/stromal tumours
    - Paraganglioma

- Peripheral nerve sheath tumour
- Benign vascular tumours
- Angiosarcoma
- Smooth muscle tumours
- Solitary fibrous tumours
- Hematolymphoid tumours
- Langerhans cell histiocytosis
- Rosai -Dorfman disease
- Follicular dendritic cell sarcoma
- Primary thyroid lymphoma
- Germ cell tumours

AJCC STAGING OF THYROID NEOPLASMS[29]:

TUMOR (T):

Tx - Primary tumour cannot be assessed.

T0 - No evidence of primary tumour

T1 - Tumour < 2cm in largest dimension, limited to the thyroid

T1a - Tumour 1cm or less, limited to the thyroid.

T1b - Tumour >1cm but not >2cm in largest dimension and limited to the thyroid.

T2 - Tumour > 2cm but < 4cm in largest dimension and limited to the thyroid.

T3 - Tumour > 4cm in largest dimension and limited to the thyroid or with minimal extrathyroidal extension.

T4a - Moderately advanced disease. Tumour of any size extending beyond thyroid capsule, invading subcutaneous soft tissues, larynx, trachea, oesophagus or recurrent laryngeal nerve.

T4b - Very advanced disease. Tumour invading prevertebral fascia/ encases carotid artery/ mediastinal vessels. Anaplastic carcinomas are considered under T4 tumours.

T4a - Intrathyroidal anaplastic carcinoma of any size.

T4b - Extrathyroidal anaplastic carcinoma of any size.

REGIONAL LYMPH NODES (N):

Nx - Regional lymph nodes cannot be assessed.

N0 - No regional lymph node metastasis.

N1 - Regional lymph node metastasis.

N1a - Metastasis to Level VI nodes (pre-tracheal, para-tracheal, pre-laryngeal or delphian nodes)

N1b - Metastasis to unilateral, bilateral or contralateral cervical (Level I to V) or retropharyngeal/ superior group of mediastinal lymphnodes (Level VII).

DISTANT METASTASIS (M):

Mx - Distant metastasis cannot be assessed.

M0 - No distant metastasis.

M1 - Distant metastasis present.

STAGE GROUPING:

Based on patient's age and histological type:

Table 1: Stage grouping of Papillary/ follicular carcinoma (< 45 years):

Stage	T	N	M
1	Any T	Any N	M0
2	Any T	Any N	M1

Table 2: Stage grouping of Papillary or follicular carcinoma (45years and above):

Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1	N1a	M0
	T2	N1a	M0
	T3	N1a	M0
IV A	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
IV B	T4b	Any N	M0
IV C	Any T	Any N	M1

PAPILLARY THYROID CARCINOMA:

Thyroid carcinomas are most frequently occurring endocrine malignancy constituting 1% of all the cancers.[30] Different types of thyroid carcinoma are noted. Papillary thyroid carcinoma occurs most commonly, comprising 80% of thyroid malignancies with normal or high iodine intake.[31]

Grossly papillary carcinoma can have several patterns. Typically, on an average the lesion is 2 to 3cms in size, grey white, firm masses with irregular borders or grossly infiltrating the surrounding parenchyma.[32]It can be entirely cystic or solid primary tumour with cystic nodular metastases.

Lesional calcification is a common feature. Necrosis is not noted with typical carcinoma and suggests lesion of higher grade.

The association between PTC and HT were initially described in 1955 by Dailey et al, and became evident because of an increase in new cases of thyroiditis diagnosed by anatomopathological exams over the past decades.[33]The concept of chronic inflammation being a risk factor for development of malignancies has been well established for other tumours. However, with respect to these two entities, the association between their cause and effect remains uncertain.[34]

Microscopic finding certains features of PTC. The neoplastic papillae contain a central fibrovascular core lined by one or occasionally several layers of cells with oval nuclei overcrowding. Thyroid follicles may sometime exaggerate into papillary changes in papillary hyperplasia. Noted are infolded lining epithelium of columnar cells with basal, round and uniform nuclei.

Central core can be absent or composed of oedematous or myxomatous pauci-cellular stroma with small follicles. Psammoma bodies are noted, representing ghost of dead papillae and are differentiated from dystrophic calcification by lamellations. True psammoma bodies are formed by focal areas of infraction at tips of papillae, attracting calcium that is deposited on the dying cells. Psammoma bodies are noted within the core of papillae or in the cores of papillaeor bodies in the stroma and not in the neoplastic follicles. The finding of psammoma bodies in a cervical lymph node strongly point towards papillary carcinoma thyroid.

The nucleus of papillary carcinoma is clear which is described as ground glass empty or as Orphan Annie eye nuclei. These nuclei are larger or more oval than the normal follicular cell's nuclei. Chromatin is hypodense.[35]

Nuclei are often found overlapping one another. Although clear nuclei are characteristic of papillary carcinoma, autoimmune thyroiditis can also show similar nuclear changes. Intra nuclear cytoplasmic inclusions are often found. Another characteristic feature is nuclear grove that are also found in thyroid lesions including hashimoto thyroiditis, adenomatous hyperplasia, diffuse hyperplasia and follicular adenomas.

Foci of squamous differentiation may be found in approximately 15 to 45% PTC. [36]Desmoplastic areasis seen with almost all PTCs, either centrally or at the peripheral zones of lesion. Cyst formation may occur making it difficult to diagnose papillary carcinoma especially if the lesion has metastasized to neck lymph nodes which can be confused for branchial cleft cyst. Papillary carcinoma invades the

glandular lymphatics, accounting for high incidence of metastasis to regional lymph node. Such metastases are most common at initial presentation of usual papillary cancer. This feature does not affect long-term prognosis. Multifocal tumours within the same gland can occur. Papillary carcinoma shows clonal proliferation.[37]

Upto 7% of papillary cancer show venous invasion. Vascular invasion in histopathological examination suggests increased tendency towards haematogenous invasion and consequent increase in the relative percent of metastases. Distant metastasis of papillary carcinoma to lungs and bone occur in less than 10%, despite the presence of multiple metastasis. However, survival may still be prolonged especially if the metastasis can be treated with radio iodine. Death is uncommon.

The electron microscopy of papillary carcinoma shows a nucleus with dispersed chromatin and highly infolded nuclear membrane, cytoplasmic intranuclear inclusions and the cytoplasm that contains many mitochondria and nuclear cytoplasmic filaments.[38] Keratohyalingranules may be found in tumours with squamous foci.

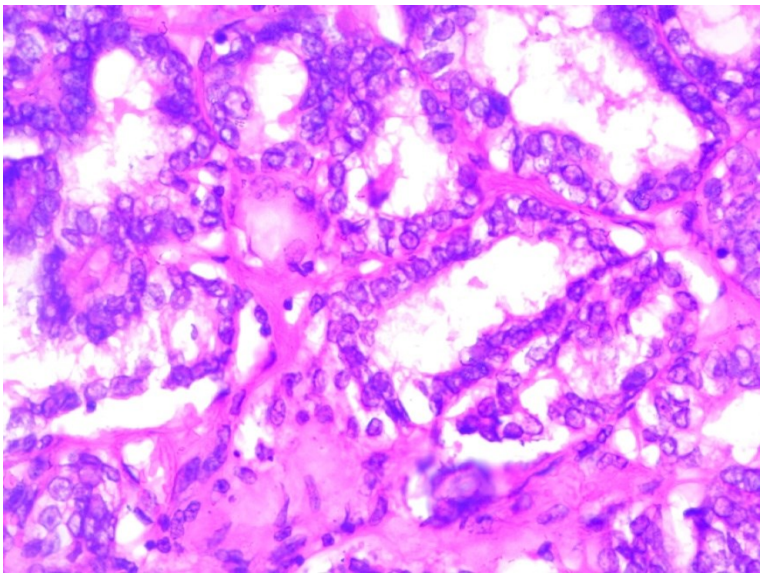

Figure 7: Microphotograph of Papillary thyroid carcinoma. (H&E x400)

DIFFERENT VARIANTS OF PAPILLARY CARCINOMA:

1. Micropapillary variant:

This is a rare variant with an excellent prognosis, also known as occult sclerosing carcinoma. It is most commonly present with lympho-vascular invasion, lymph node metastasis, and distant metastasis. These lesions are small in size hence they can be missed on gross examination. In few lesions, the capsule is thick and fibrous. Focal calcification is seen. Microscopy shows the micropapillary growth pattern without fibrovascular cores in <5% of tumour area.[39] Neoplastic elements of tumour are mostly noted at the periphery and other are seen in centre of the gland.

2. Follicular variant:

Follicular variant of papillary thyroid carcinoma (FVPTC) is the most common subset of PTC also known as Lindsay tumour, seen in 9% to 22.5% of patients with PTC. This variant is composed of only follicles showing an infiltrative growth pattern or is encapsulated. The follicles vary in size and shape. They are elongated or irregular with abortive papillae formation. Colloid is usually deeply stained and scalloped.

Psammoma bodies and sclerosis maybe present. The diagnosis is made by identification of typical nuclear features.

FVPTC has following subtypes:

- Infiltrative
- Encapsulated with invasion
- Macrofollicular
- Diffuse or multinodular follicular

The infiltrative non encapsulated type is the most common form showing obvious infiltration of thyroid parenchyma often accompanied by sclerosis. The growth is similar to conventional papillary carcinoma except the papillae is absent.

The encapsulated variant constitutes 10% of PTCAs the hematogenous spread is rare, it carries an excellent prognosis.[40] Microscopically, it has a typical PTC lesion which is surrounded by a thick fibrous capsule which can either be intact or infiltrated by tumour, focally. The patient tends to be younger and the frequency of lymph node metastasis is lower.[41] Encapsulated follicular variant of

papillary carcinoma behaves more like follicular adenoma and follicular carcinoma.

The encapsulated type is surrounded by fibrous capsule and it may or may not exhibit invasion of capsule or blood vessels. There is low frequency of lymph node metastasis. Presence of RAS mutation/PAX8- PPAR gamma translocation is noted.[42]

Macrofollicular variant is a rare variant resembling the nodular hyperplasia wherein the neoplastic thyroid follicles are cystically dilated. In another rare variant, multinodular follicular type, the whole thyroid gland is diffusely involved by the tumour.

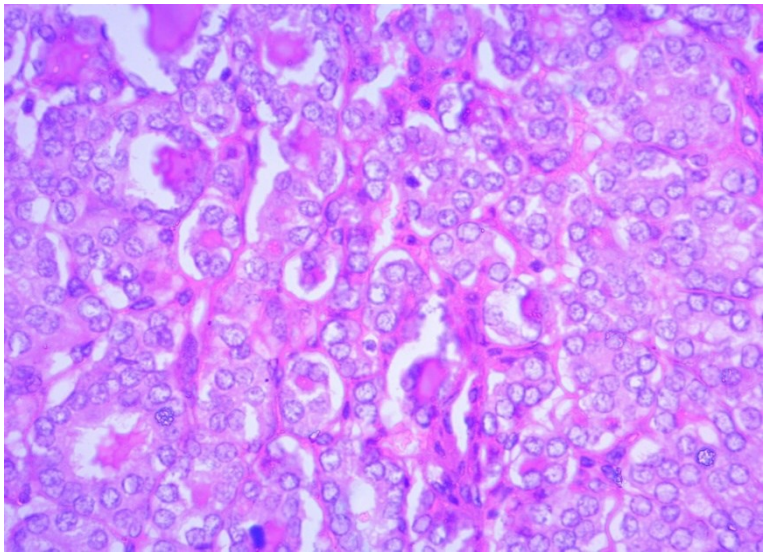

Figure 8: Microphotograph of Follicular variant of papillary carcinoma thyroid. (H&E x400)

3. Diffuse sclerosing variant:

This rare variant mostly affects children and young women.[43] They present with unilateral or bilateral symmetrical diffuse thyroid swelling. Serum antithyroglobulin for anti-microsomal antibody may be positive.[44] This variant is more aggressive as compared to conventional type and is manifested by higher incidence of extra thyroidal extension, lymphnode metastasis and distant metastasis.[44,45] Thyroid shows diffuse replacement of the parenchyma by white firm tissue which is often gritty on cutting.[29]

Microscopy:

- Diffuse sclerosis.
- Dense lymphoplasmacytic infiltrate.
- Psammoma bodies may be abundant.
- Scattered islands of papillary carcinoma having prominent squamoid or squamous differentiation.
- Invasion of intrathyroidal lymphatic spaces.

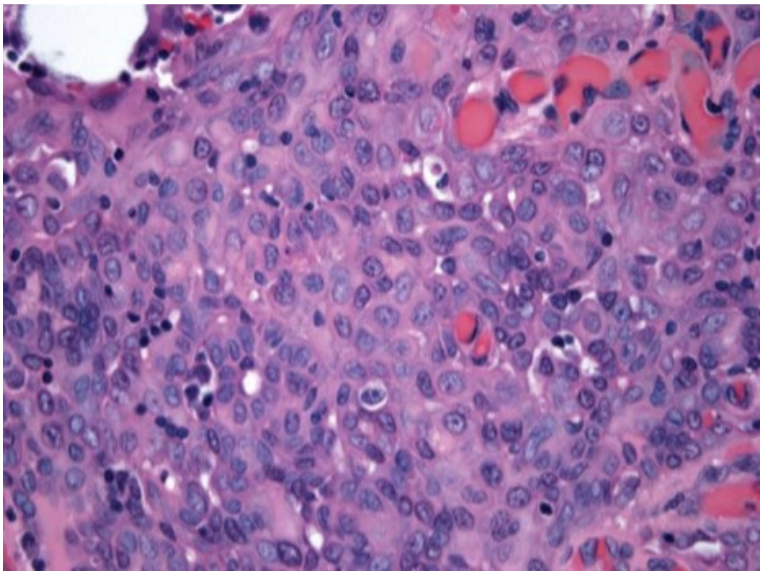

Figure 9: Microphotograph of Diffuse sclerosing variant of papillary thyroid carcinoma.[18] (H&E x400)

4. Tall cell variant:

Tall cell subtype comprises of tall cells. These cells have height at least three times the width, as compared to the conventional papillary carcinoma.[46]This tumour is often bulky and aggressive seen in slightly older age group (52 – 57years).[46] Extrathyroidal extension is common.[47] Higher frequency of BRAF mutation is noted.[46]

One third of cases exhibit RET/PTC rearrangements. Selectively RET/PTC3exhibits more potency than RET/PTC 1.[48] The tall cell variant is highly papilliferous and invasive. Their nuclei are like that of CVPTC, but are mostly basally located. The cytoplasm is abundant and oxyphilic because of accumulation of mitochondria. Focal clearing of the cytoplasm is sometimes present.[29]

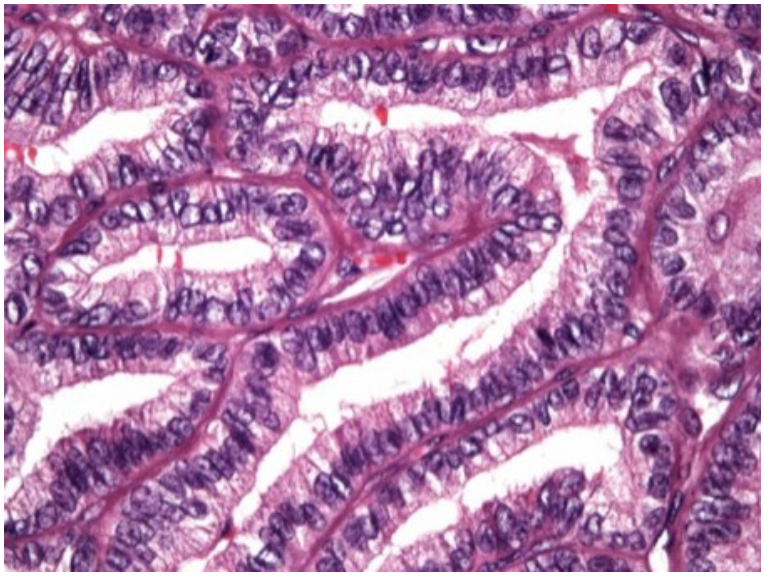

Figure 10: Microphotograph of Tall cell variant of papillary thyroid carcinoma.[18] (H&E x400)

5. Columnar cell variant:

A rare columnar cell subtype was first reported by Evans.[49] This subtype is more aggressive than differentiated thyroid carcinomas.[50] These tumours are invasive with high mortality rate. The Mean age is 57 years.[51] It more frequently metastasizes to lung, vertebra, and regional lymphnodes. Presence of encapsulation or infiltrative architecture determines the prognosis. Microscopy shows hypercellularity with gland like spaces lined by pseudostratified columnar epithelium.[29]

Typical characteristics include:

1. Different patterns are noted: Solid, cribriform, mixed papillary and glandular.
2. Lining epithelium is tall columnar showing pseudo-stratification. Nucleus is hyperchromatic, round to oval in shape.
3. Cytoplasmic clearing and subnuclear vacuolations may be present.
4. Small and polygonal cells are present in solid area.
5. This tumour is thyroglobulin positive.[51]
6. BRAF Mutation is demonstrable in one third of cases.[3]

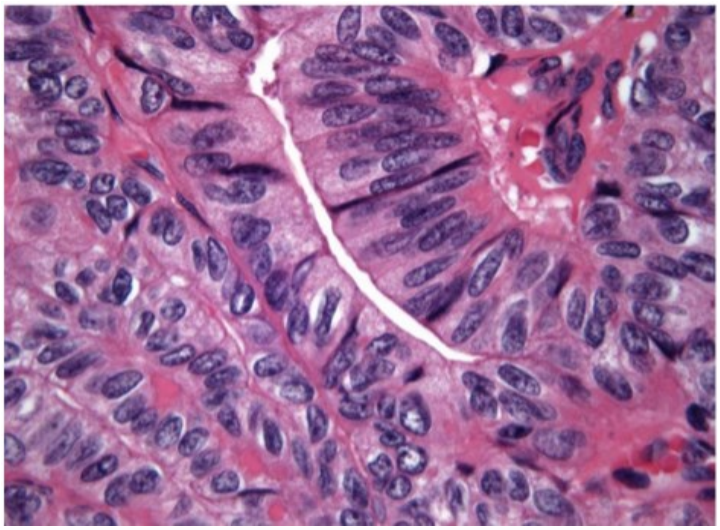

Figure 11: Microphotograph of columnar cell variant of papillary carcinoma thyroid.[18] (H&E x400)

6. Cribriform morular variant:

This is an uncommon variant. This variant of papillary carcinoma can occur as a sporadic tumour or in association with the familial adenomatous polyposis (FAP) coli syndrome. Female predominance is observed with M: F ratio of 1:17. Mean age of diagnosis is 27.7 years. The outcome of this tumour is favourable.[29]

Microscopically, it shows prominent cribriform pattern with interspersed squamoid islands. Cells are arranged in closely packed follicles, papillae or trabeculae. The characteristic feature is that the luminal spaces shows absence of colloid. The tumour cells are columnar or cuboidal. The nuclei are chromatin rich with typical papillary carcinoma like nuclear features seen focally. The nuclei are filled with lightly eosinophilic homogenous, biotin containing inclusions. Some of these cells are spindly in fascicles and whorls. The tumour can be circumscribed, encapsulated, with or without capsular. Vascular invasion can be noted. This type commonly shows RET/PTC rearrangement.[53]

The APC gene shows either germline or somatic mutation. Germline mutation is seen in cases associated with FAP while somatic mutation is observed in some sporadic cases. Somatic mutation in exon 3 of the beta catenin gene, results in nuclear translocation of beta catenin. BRAF mutation is absent. Cytokeratin 19 (CK19) is found to be strongly expressed in PTC. Squamous metaplasia in diffuse sclerosing variant of PTC demonstrates CK19 positivity. Weak expression of CK 19 is noted in the morules of CMV PTC. Morula cells have strong CD10 cytoplasmic and bcl-2 nuclear staining.[54,28]

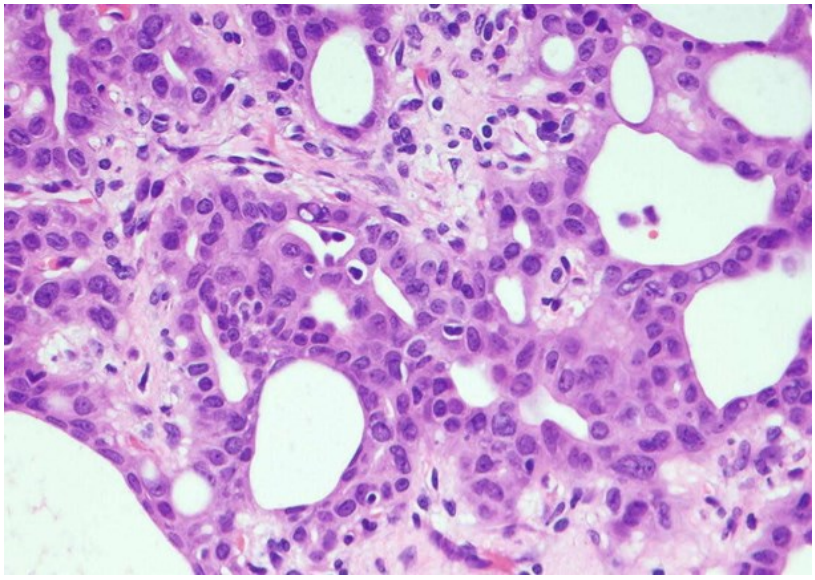

Figure 12: Microphotograph of Cribriform variant of papillary thyroid carcinoma.[18] (H&E x400)

7. Hobnail variant:

It is an aggressive and rare variant of papillary carcinoma. Commonly patient present with cervical lymphadenopathy. Distant metastasis is common. The tumour is multifocal. Microscopic findings include variable sized papillae with cells having atypically placed nucleus, forming surface bulge. Few psammoma bodies are present. Necrosis, mitosis, lymphnode invasion and extrathyroidal spread are common. TTF1 positivity and variable positivity for thyroglobulin is noted. Ki67 proliferation index is 10%. More than 25% of the nuclei are p53 positive. 50% of the cases show BRAF mutation.[55]

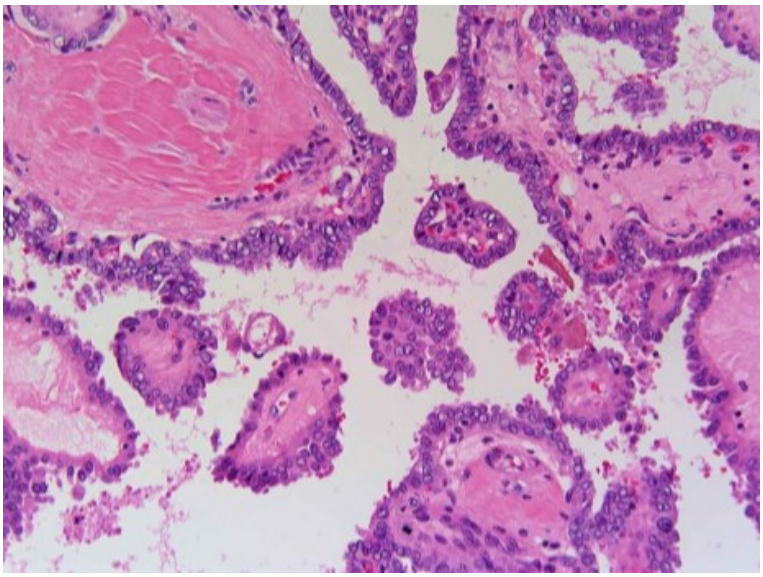

Figure 13: Microphotograph of Hobnail variant of papillary thyroid carcinoma.[18] (H&E x400)

8. Variant with fibromatosis or nodular fasciitis like stroma:

A rare variant associated with an abundant nodular fasciitis or fibromatosis like stroma. Microscopically, the stroma shows myo-fibroblastic spindle cells in vascularised fibro-myxoid matrix and extravasation of red cells. These cells have oval or elongated nucleus with fine chromatin with a distinct small nucleolus. Pleomorphism and mitotic figures are absent. The arrangement of stroma and tumour cells forms an unusual histological pattern similar to fibroadenoma and phyllodes tumour in breast. The tumour cells show positivity for cytokeratin and thyroglobulin but negative for calcitonin. Vimentin and S-100 positivity is also noted in few of the tumour cells. The spindle shaped cells are positive for muscle-specific-actin, vimentin and desmin but negative for S-100 protein.[29]

9. Solid/Trabecular variant:

Solid/trabecular variant is a rare variant commonly occurring paediatric age group. It is associated with a higher frequency of distant metastasis usually carrying poor prognosis. More than 50% of tumour is solid with cells in trabecular pattern. These tumours are large and also invasive. The cells are cuboidal or columnar in long and straight trabeculae. Tumour is transversed by delicate capillaries and nuclear features are of conventional papillary carcinoma.[56,57] This subtype is commonly confused with rare hyalinizing trabecular thyroid tumours due to the presence of trabecular or alveolar pattern.[29]

10. Oncocytic Variant:

Oncocytic variant is an extremely rare variant. These tumours are encapsulated but invasive. Tumour cells have abundant amount of eosinophilic granular cytoplasm due to accumulation of mitochondria. Nuclear features are similar to CVPTC. It can be confused with the tall cell variant of PTC.[29]

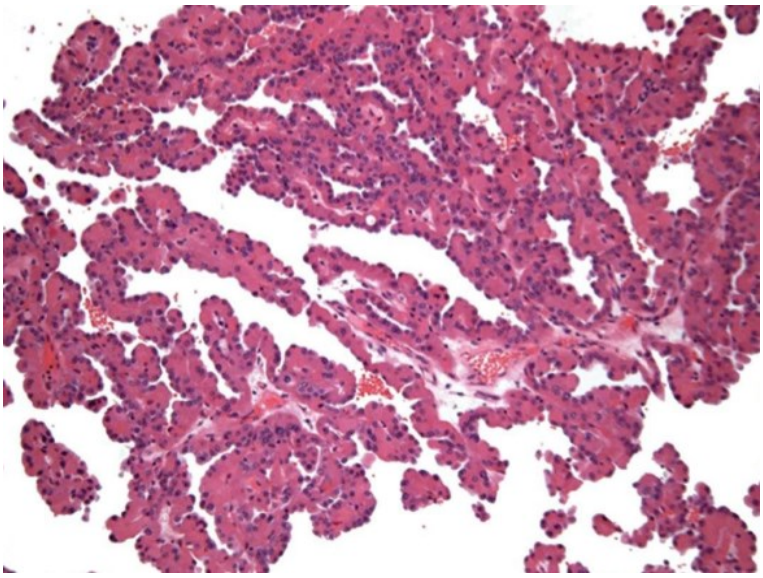

Figure 14: Microphotograph of oncocytic variantof papillary thyroid carcinoma.[18] (H&E x100)

11. Spindle cell variant:

This is a rare subtype having focal spindle cell metaplasia that may constitute <5% to >95% of the tumour. These cells are epithelial in origin. This variant can be distinguished from anaplastic carcinoma with bland spindle cell cytology and absence of necrosis/mitosis.[29]

12. Clear cell variant:

Clear cell variant is a rare subtype of PTC[58] with clear cells arranged in papillary pattern along with cytological features of PTC. Few lesions can occur as combination of oncocytes and clear cells. Cells appear clear due to mucin/ glycogen accumulation or mitochondrial expansion. Differential diagnosis includes clear cell medullary carcinoma, metastatic renal cell carcinoma, intrathyroidal parathyroid gland proliferation.[29]

13. Warthin like variant:

Warthin like variant is a rare variant of papillary carcinoma. Grossly the tumour is circumscribed by rarely encapsulated. Few lesions may be centrally cystic. Microscopic features are similar to warthin tumour of salivary gland in a background of lymphocytic thyroiditis. Cells are large and eosinophilic in papillary pattern. The central core is rich in lymphoplasmacytic infiltrate.[59]

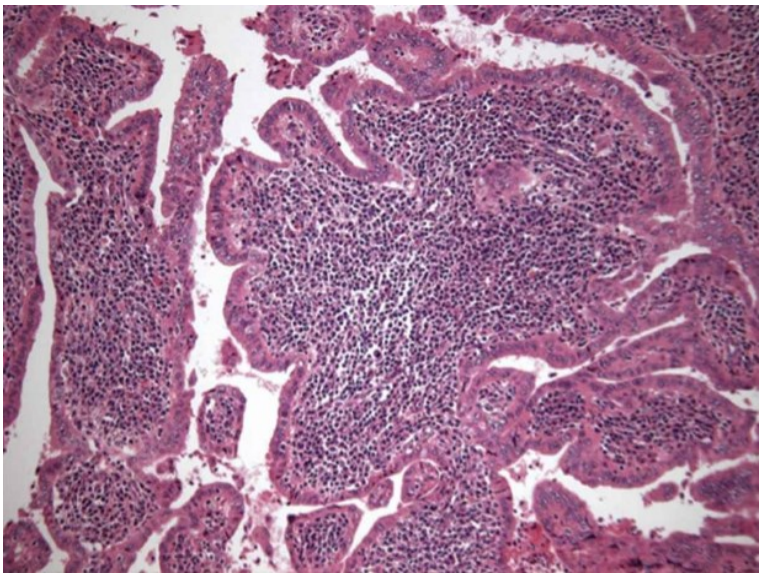

Figure 15: Microphotograph of Warthin like variantof papillary thyroid carcinoma.[18] (H&E x100)

14. Papillary carcinoma with lipomatous stroma: In rare circumstances, adipose cells are interspersed within the papillary carcinoma.[60]

MOLECULAR PATHOLOGY OF PAPILLARY CARCINOMA:

There are 3 molecular alterations are commonly found in papillary carcinoma. These activate the mitogen activated protein kinase pathway.

1. BRAF Mutation

2.RET Mutation

3. RAS Mutation

BRAF mutation

BRAF mutation is the most common genetic mutation in papillary carcinoma thyroid. BRAF gene is located on chromosome 7.[61] This protein kinase is a member of RAF family. They play a major role in signal transduction through mitogen activated protein kinase (MAPK) pathway. A Point mutation of thiamine to adenine transversion occurs on exon 15 at 1799 location (T1799A) that substitutes valine by glutamine in residue 600(V600E). The frequency of BRAF V600E mutation in PTC has risen gradually over past decades. BRAF V600E mutation is infrequent in papillary carcinoma of children and young patients.[62]

BRAF V600E mutation is more commonly found in PTC especially classical and tall cell variant. It is associated with iodide transportation and metabolism which can lead to failure of radio-iodide treatment. This mutation can also supress several tumour-suppressor genes and hence, promote tumorous invasion. Uncommon BRAF includes point mutation of K601E, tiny deletion by codon 600 and chromosome 7 inversion, paracentrically.[29]

BRAF mutation is associated with male gender, old age, extrathyroidal extension, advanced tumour stage, lymph node metastasis, distant metastasis and tumour recurrence.

RAS mutation:

RAS gene is located on chromosome 12.[63] RAS mutation is the second most common genetic alteration identified amidst the thyroid malignancies. 15% of papillary carcinoma cases have RAS mutation. All follicular variant PTC cases are associated with mutation in RAS gene. RAS mutation is identified in neoplasms having follicular arrangement of cells like follicular carcinoma, follicular variant of papillary cancer and follicular adenoma.[29]

RET mutation:

Activation of the ret proto oncogene occurs as a result of chromosomal translocation or inversion intrachromosomally. This mutation is associated with10 to 30 % of PTC cases. This results in nuclear changes including irregular nuclear contour and euchromatic appearance in thyroid follicular cells indicating the presence of a genetic alteration leading to nuclear feature in papillary carcinoma. Non neoplastic thyroid tissues such as hashimoto thyroiditis and benign nodules can also present with RET rearrangements.

The genes expressed on thyroid follicular cells can be fused with tyrosine kinases of RET oncogene. Following are the examples of the same:

PTC 1: inversion of 10^{th} chromosome (q11.2q21)

PTC 2: translocation (10;17), q(11.2q23)

PTC 3: paracentric inversion in 10q11.2

PTC4

PTC 5

RET/PTC I is most common, followed by RET/PTC 3.[64]

RET/PTC gene fusion is high in children and young adults (50- 60%). Chernobyl incident related PTC(60-80%) exhibits RET/PTC 3 rearrangement. RET/PTC 1 fusion is seen in post-radiation therapy cases (60-80%). The tall cell variant and solid variant are asscociated with RET/PTC 3 type fusion whereas RET/PTC 1 is seen commonly in papillary microcarcinoma.[29]

PROGNOSTIC FACTORS:

1. Age: Papillary carcinoma mostly occurring in children and adolescents has good prognosis. Nearly all deaths from papillary carcinoma are noted when it is occurs after the age of 45 years.[65]
2. Sex: Better prognosis in female than male
3. Extra thyroidal extension: papillary carcinoma with extrathyroidal extension has bad prognosis.
4. Previous irradiation history: it does not seem to significantly differ from others.
5. Tumor size: inverse correlation is present between size and prognosis.
6. Capsule and margins: Better prognosis in encapsulated tumour.
7. Multicentricity and distant metastasis: patients with metastasis has bad prognosis.
8. Poorly differentiated squamous or anaplastic foci: bad prognosis. Only 5% of cases presents with this histology.
9. DNA ploidy: Aneuploidy carries a bad prognosis and is associated with the aggressive behaviour in papillary carcinoma.
10. BRAF: BRAF mutation leads to aggressive behaviour of the tumour. These are unresponsive to radioactive iodine.[29]

IMMUNOHISTOCHEMISTRY APPLICATION[29]

Immunostaining shows that most papillary cancer expresses thyroglobulin and thyroid transcription factor -1. A number of biomarkers have been used for differential diagnosis of thyroid lesions such as CD 56, HBME 1, CK 19, Galectin 3 and many more.[29]

RET:

Ret oncogene on Chromosome 10q, encodes for transmembrane tyrosine kinase receptor. It usually is not expressed in follicular epithelial cells of thyroid. PTC is associated with multiple gene rearrangements including Ret oncogene mutation.

Ret oncogene is interpreted as cytoplasmic pattern of staining. It stains the thyroid follicular cells in PTC and its variants. Focal/ moderate immunostaining is noted in benign nodular lesions of thyroid[29].

THYROID TRANSCRIPTION FACTOR 1:

Thyroid Transcription Factor 1 (TTF 1) is also called as NKX2 homeobox I (NKX2.1). It is a nuclear protein from the family of homeodomain transcription factors.It is encoded by a single gene which is located on chromosome 14.[66]It is made up of single polypeptide with 371 aminoacids. TTF 1 is essential for organogenesis and differentiation of thyroid and lung.

TTF 1 immunohistochemical expression was initially identified in thyroid and lung epithelial tissues in neoplastic as well as non-neoplastic lesions. TTF 1 expression in poorly differentiated nonsmall- cell lung carcinomas are helpful to differentiate adenocarcinoma from squamous cell carcinoma. Thyroid follicular cells and the parafollicular cells are diffusely positive for TTF 1.[29]

CD56:

CD56 is a protein. It is associated with neural cell adhesion seen in astrocytes, natural killer cells, myoblasts and lymphocytes. It plays an important role in intercellular and cell matrix adhesion.

CD56 expression is believed to be activating the epithelial-mesenchymal transition (EMT) and modulating genes for metastasis regulation like VEGF. This antigen is expressed in normal thyroid follicular cells.[29]

HBME 1:

Hector Battifora Mesothelial- 1 (HBME-1) is monoclonal type of antibody that reacts with an unknown determinant on the epithelium of trachea and microvilli of the mesothelial cells. They are also expressed in adenocarcinomas of pancreas, breast and lung. HBME-1 is expressed as cytoplasmic and cytoplasmic membrane staining of thyroid follicular cells. Almost all malignant thyroid lesion stains for HBME 1. Oncocytes are rarely stained on HBME 1 immunohistochemistry. FVPTC shows higher expression as compared to follicular carcinoma followed by follicular adenoma.[29]

GALECTIN 3:

Galectin 3 is a protein from the lectin family, encoded by a single LGALS3 gene on locus q21 -q22 of chromosome 14. It is a 31-kDa galactosidase binding lectin having affinity for beta galactosidase present intracellularly, on the cell surface and as extra cellular glycocongugates.[1]Itregulates cell - cell and cell - matrix modulation.

Predominantly expressed in cytoplasm of epithelial and immune cells. Can also be expressed in tissues like breast, thyroid, colon, activated endothelial cells and macrophages. It plays a significant role in the malignant transformation of the thyroid cells, expressed more in carcinomas, especially of the papillary type.[67]

Functions of Galectin 3:

Cytoplasm: Cell survival (growth cycle and apoptosis)

Nucleus: pre-mRNA splicing transcription regulation

Cell surface: Moderate cell-cell, epithelial cell extracellular matrix interaction[68]

CK19:

Cytokeratin 19 (CK19) is the smallest type I filament protein – Intermediate type in keratin family. There are no specific contributors for the filament formation. CK19 is expressed on striated muscle's sarcomere. It can bind with dystrophin glycoprotein molecule via actin binding domain of dystrophin. Dystrophin directly attaches specifically to CK19 protein.[29]

Synthesis of CK 19 protein takes place in simple stratified epithelia. It stains Cytoplasmic membrane and cytoplasm of thyroid follicular cells. Diffuse strong positive IHC expression of CK 19 is noted in PTC cases. Weak or no immunohistochemical staining for Ck 19 is noted in multi-nodular goitre with papillary excrescences, graves' disease, tubular adenoma, follicular adenoma as well as FVPTC. Benign thyroid lesion can sometime present with focal positivity.[69]

MATERIAL AND METHODS

MATERIALS AND METHODS

STUDY DESIGN: An observational study.

SOURCE OF DATA: This observational study was conducted on the thyroidectomy cases received in the department of pathology, R. L. Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical college, Tamaka, Kolar from January 2019 to September 2020.

DURATION OF STUDY:18 months.

DATA COLLECTION:

- Thyroidectomy cases, received in the department of pathology, R. L. Jalappa Hospital and Research Centre, Kolar were assessed and classified according to WHO classification.
- Clinical details of the cases such as the age, gender, clinical diagnosis and other relevant investigations were collected.
- All the histopathological slides of the selected cases were retrieved and reviewed.
- Selection of blocks; 1 FFPB per case, was done on the basis of area of interest in the lesional tissue.
- IHC staining for Galectin 3 and CK19 were performed on all the histopathological slides of the cases using appropriate positive and negative controls as suggested by manufacturers.
- Their expressions were evaluated in the thyroid epithelial cells as per the similar studies.
- The results were statistically analysed.

SAMPLE SIZE:

Sample size was calculated based on the difference in positive rate of Galectin-3 in papillary thyroid carcinoma and nodular goitre as per the studies done.[4]

$$H_0: P_1 = P_2, \qquad H_a: P_1 \neq P_2$$

$$n = \frac{\left\{ Z_{1-\frac{\alpha}{2}} \sqrt{2\bar{P}(1-\bar{P})} + Z_{1-\beta} \sqrt{P_1(1-P_1) + P_2(1-P_2)} \right\}^2}{(P_1 - P_2)^2}$$

Where,

$$\bar{P} = \frac{P_1 + P_2}{2}$$

P_1 : Proportion in the first group

P_2 : Proportion in the second group

α : Significance level

$1-\beta$: Power

p1= 52.58, p2= 97.17

power =90

Sample size = 26 per group= 52

Table 3: Biomarkers used in the present study:

Biomarker	Manufacturer	Dilution	Antigen retrieval buffer	Visualization kit	pH
CK 19	Biogenex	Prediluted	Citrate	Biogenex	6
Gal 3	Master Diagnostics	Prediluted	Tris EDTA	Dako	9

PROTOCOL FOR IHC:

1) Section Cutting:

Approximately 3-4 µm sections were cut from the selected formalin fixed paraffin blocks. They were floated on to the positively charged slides and were incubated at 58° C for 48 to 54 hours.

2) Deparaffinization and De-xylenisation:

Xylene – I - 15 minutes

Xylene – II - 15 minutes

Absolute alcohol - I - 1 minute

Absolute alcohol - II - 1 minute

90%Alcohol - 1 minute

70%Alcohol -1 minute

3) Tap waterwashing – 10 minutes

4) Distilled water washing – 5 minutes

5) Antigen Retrieval:

Method: Microwave method

Buffer: Citrate buffer for CK 19, Tris EDTA buffer for Gal 3

pH: 6.0 for CK 19, 9.0 for Gal 3

Duration: 6 cycles (2 cycles of 6 minutes followed by 4 cycles of 3 minutes each)

Slides were cooled to room temperature.

6) TBS buffer wash – 1 minute

7) Peroxidase block – 30 minutes

8) TBS buffer wash- 1 minute

9) Power block – 30 minutes

10) TBS buffer wash – 1 minute

11) Primary antibody – 120 minutes

12) TBS buffer wash – 1 minute

13) Super enhancer – 30 minutes

14) TBS buffer wash – 1 minute

15) Secondary antibody – 90 minutes

16) TBS buffer wash – 1 minute

17) DAB Colour development – 20 minutes

18) TBS buffer wash – 1 minute

19) Tap water wash – 10 minutes

20) Counterstain by haematoxylin – 30 seconds

21) Tap water wash – 1 minute

22) Xylene dip

23) DPX Mounting

EVALUATION (READING AND SCORING) OF IMMUNOEXPRESSION OF BIOMARKERS:

A semi quantitative analysis was done on the basis of percentage and intensity of staging as per the study done by Kammal WS et al.[70]

Percentage of staining:

- 0-25% staining in the area of the lesion – Score 1
- 26-50% staining in the area of the lesion – Score 2
- 51-75% staining in the area of the lesion – Score 3
- 76-100% staining in the area of the lesion – Score 4

Intensity of staining:

- No staining – Score 0
- Mild staining in the area of the lesion – Score 1
- Moderate staining in the area of the lesion – Score 2
- Strong staining in the area of the lesion – Score 3

Scoring of staining:

Final immunohistochemical scoring was done by multiplying both the scores.

- Negative: Final score 0
- Weakly positive: Final score 1-4
- Moderately positive: Final score 5-8
- Strongly positive: Final score 9-12

STATISTICAL ANALYSIS:

Data was entered into the Microsoft excel data sheet and was analysed using SPSS 22 version software. The categorical data was represented in form of Frequencies and proportions. **Chi-square test** was used for the test of significance for qualitative data. Continuous data was represented as mean and standard deviation.

- Sensitivity = a/(a+c) x 100 = True positive / True positive + False Negative
- Specificity = d/(b+d) x 100 = True Negative / True Negative + False Postive
- Positive predictive value = a/ (a+b) x 100 = True Postive / True positive + False Postive
- Negative predictive value = d/ (c+d) x 100 = True Negative / True Negative + False Negative

Graphical representation of data: Microsoft Excel and Microsoft word was used to obtain various types of graphs such as bar diagram.

P value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all rules of statistical tests.

Statistical software: Microsoft Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

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RESULTS

RESULTS

Age distribution:

Table 4: Age distribution of cases in the present study.

	Non-neoplastic		PTC		Total	
Age	**N**	**%**	**N**	**%**	**N**	**%**
10-19yrs	0	0%	1	1.9%	1	1.9%
20-29yrs	7	13.5%	8	15.4%	15	28.9%
30-39yrs	3	5.8%	4	7.7%	7	13.5%
40-49yrs	11	21.1%	8	15.4%	19	36.5%
50-59yrs	3	5.8%	2	3.8%	5	9.6%
60-69yrs	2	3.8%	3	5.8%	5	9.6%
Total	26	50%	26	50%	52	100%

Overall, maximum number of cases were in their 5th decade at the time of undergoing thyroidectomies followed by 3rd and 4th decade which were 19(36.5%), 15(28.9%) and 7(13.5%) respectively. Amongst the non-neoplastic lesions, maximum cases were in their 5th decade followed by 3rd decade which were 11(21.1%) and 7(13.5%) respectively. Least number of cases were in their 2nd decade, in both the non-neoplastic and PTC lesions which was 0 and 1(1.9%) respectively.

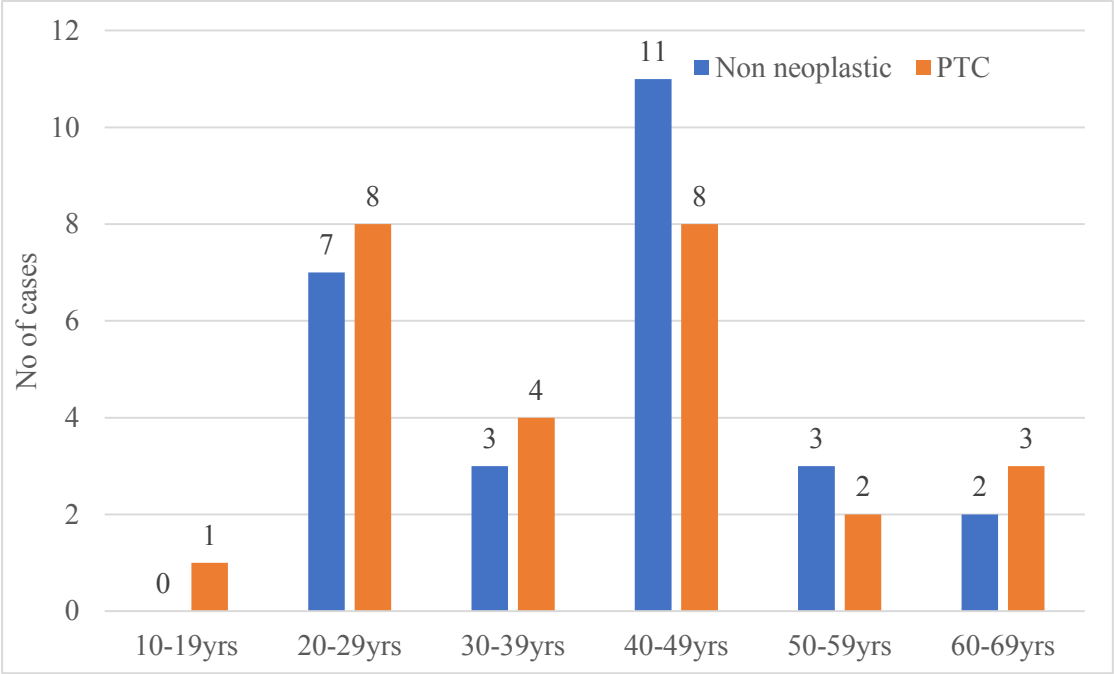

Figure 16: Graphical representation of distribution of cases according to age group in the present study.

Gender distribution:

Table 5: Gender distribution of cases in the present study.

	Non-neoplastic		PTC		Total	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Female	26	50%	24	46.15%	50	96.15%
Male	0	0%	2	3.85%	2	3.85%
Total	26	50%	26	50%	52	100%

Thyroid lesions had higher incidence in females as compared to males with male to female ratio of 1:26. There were 2 male patients diagnosed with papillary carcinoma thyroid.

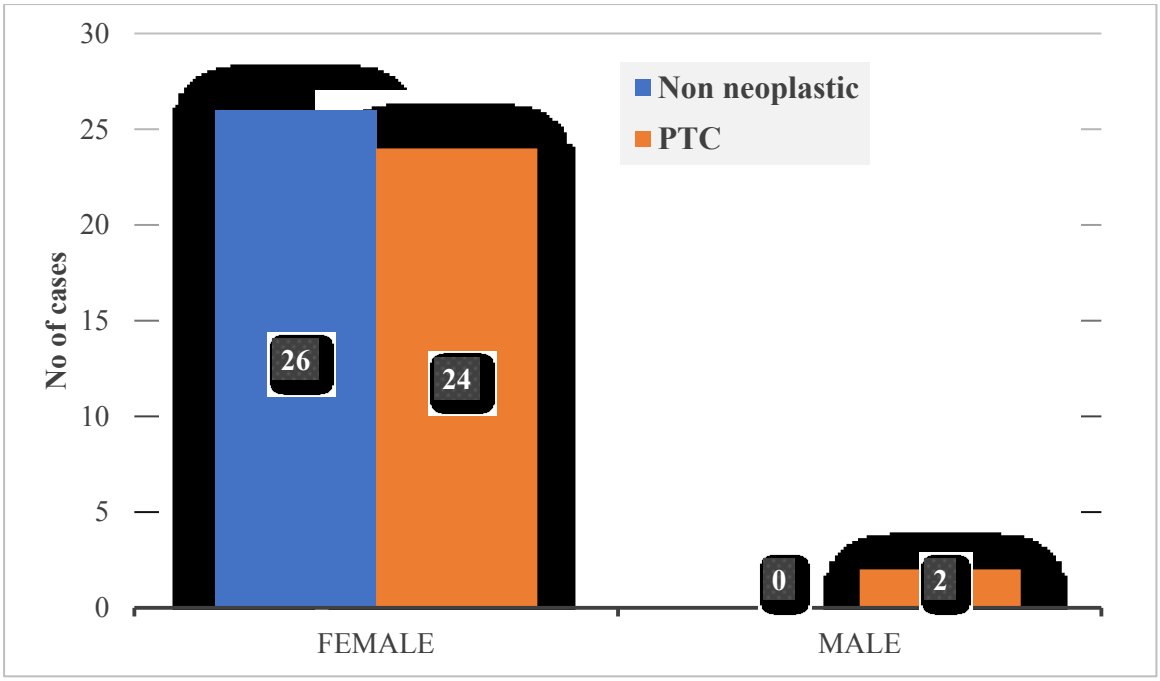

Figure 17: Graphical representation of distribution of cases according to gender in the present study.

Histopathological distribution of cases:

Table 6: Histopathological lesion wise distribution of cases in the present study.

		Frequency	Percentage (%)
Non neoplastic	Lymphocytic thyroiditis	13	25.0
	Nodular hyperplasia	13	25.0
PTC	Classical PTC	6	11.54
	FVPTC	20	38.46
Total		52	100

Our study included a total of 52 cases. 26 were non-neoplastic lesions of thyroid and were 26 PTC cases. Amongst the non-neoplastic cases, 13 nodular hyperplasia cases and 13 lymphocytic thyroiditis were taken up.

Amongst the 26 PTC cases, maximum number of cases were FVPTC followed by CVPTC, which were 6(11.54%) and 20(38.4684%) respectively.

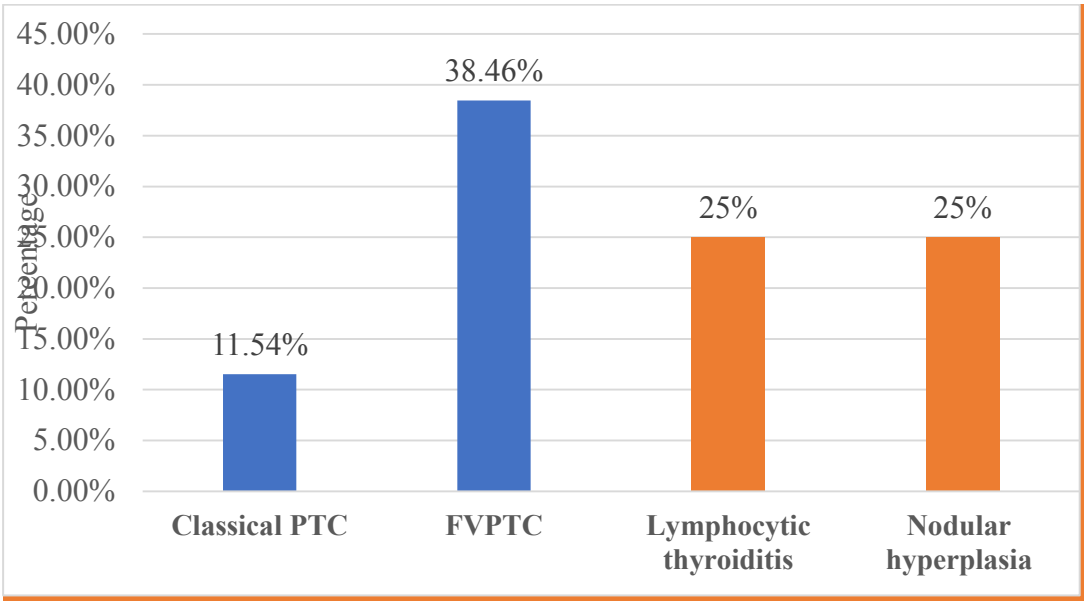

Figure 18: Graphical representation of distribution of cases according to histopathological diagnosis in the present study.

Table 7: Histopathological diagnosis wise thyroid lobe involvement in the present study.

	Both		**Left**		**Right**		**Total**	
	N	%	N	%	N	%	N	%
Classical PTC	1	1.9%	3	5.8%	2	3.9%	6	11.5
FVPTC	4	7.7%	8	15.4%	8	15.3%	20	38.5
Lymphocytic thyroiditis	10	19.2%	0	0%	3	5.8%	13	25
Nodular hyperplasia	4	7.7%	2	3.9%	7	13.5%	13	25
Total	19	36.5	13	25	20	38.4	52	100%

Right lobe was commonly involved followed by bilateral involvement and left lobe involvement; 20(38.4), 19(36.5%) and 13(25%) respectively.

Immunohistochemical expression of biomarkers:

Table 8: CK 19 expression with histopathological diagnosis of non-neoplastic lesions of thyroid and PTC cases in the present study.

CK19	Histopathological Diagnosis		Total
	Non neoplastic	PTC	
Negative	6	3	9
	23.1%	11.5%	17.3%
Positive	20	23	43
	76.9%	88.5%	82.7%
Total	26	26	52
	100.0%	100.0%	100.0%

p value 0.271

There was no statistically significant difference of CK19 expression amongst the non-neoplastic and PTC cases.

Table 9: CK 19 expression in non-neoplastic lesions of thyroid in the present study.

CK 19	Lymphocytic thyroiditis		Nodular hyperplasia	
	Frequency	Percentage	Frequency	Percentage
Negative	2	15.4%	4	30.7%
Weak	9	69.2%	8	61.5%
Moderate	2	15.4%	1	7.7%
Strong	0	0%	0	0%
Total	13	100%	13	100%

p value 0.58

None of the non-neoplastic cases had strong CK 19 expression. Maximum number of non-neoplastic cases had weak CK19 expression; 9(69.2%) and 8(61.5%) cases of lymphocytic thyroiditis and nodular hyperplasia each. There was no statistically significant difference of CK19 expression amongst the lymphocytic thyroiditis and nodular hyperplasia cases.

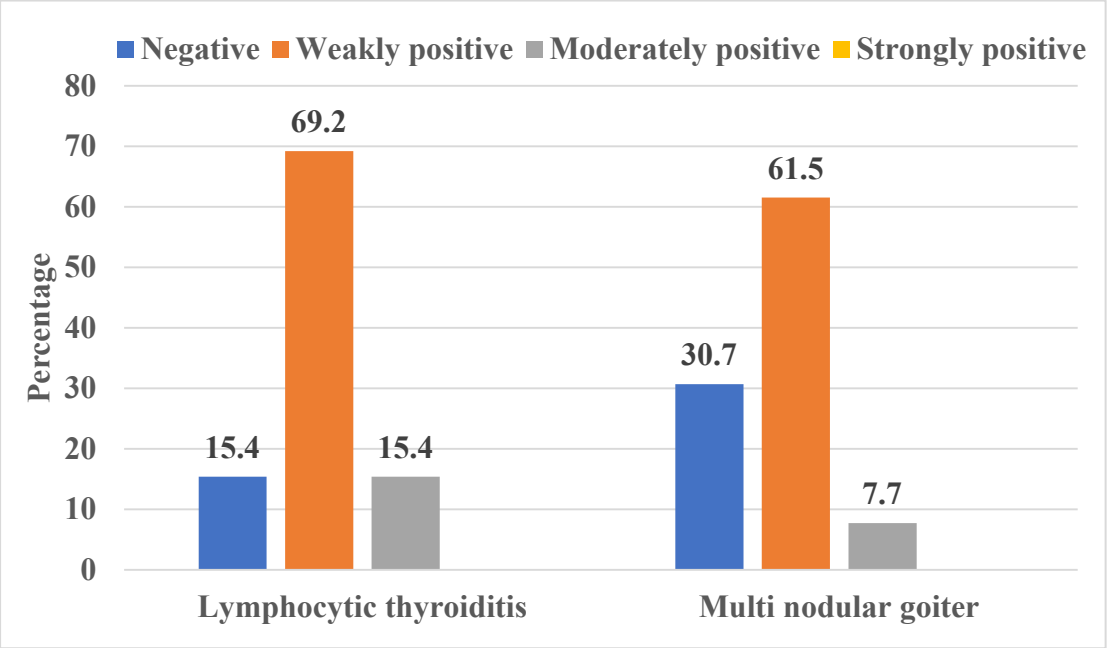

Figure 19: Graphical representation of distribution of CK 19 in non-neoplastic cases in the present study.

Table 10: CK 19 expression in PTC cases in the present study.

CK 19	Classical variant PTC		FVPTC	
	Frequency	Percentage	Frequency	Percentage
Negative	0	0%	3	15%
Weak	0	0%	7	35%
Moderate	0	0%	4	20%
Strong	6	100%	6	30%
Total	6	100%	20	100%

p value <0.01

Maximum number of FVPTC cases; 7(35%) had weak CK19 expression followed 6(30%) and 4(20%) cases with strong and moderate positive CK 19 expression, respectively. All the classical variant PTC had strong positive CK 19 expression. There was a statistically significant difference of CK19 expression amongst the CVPTC and FVPTC cases.

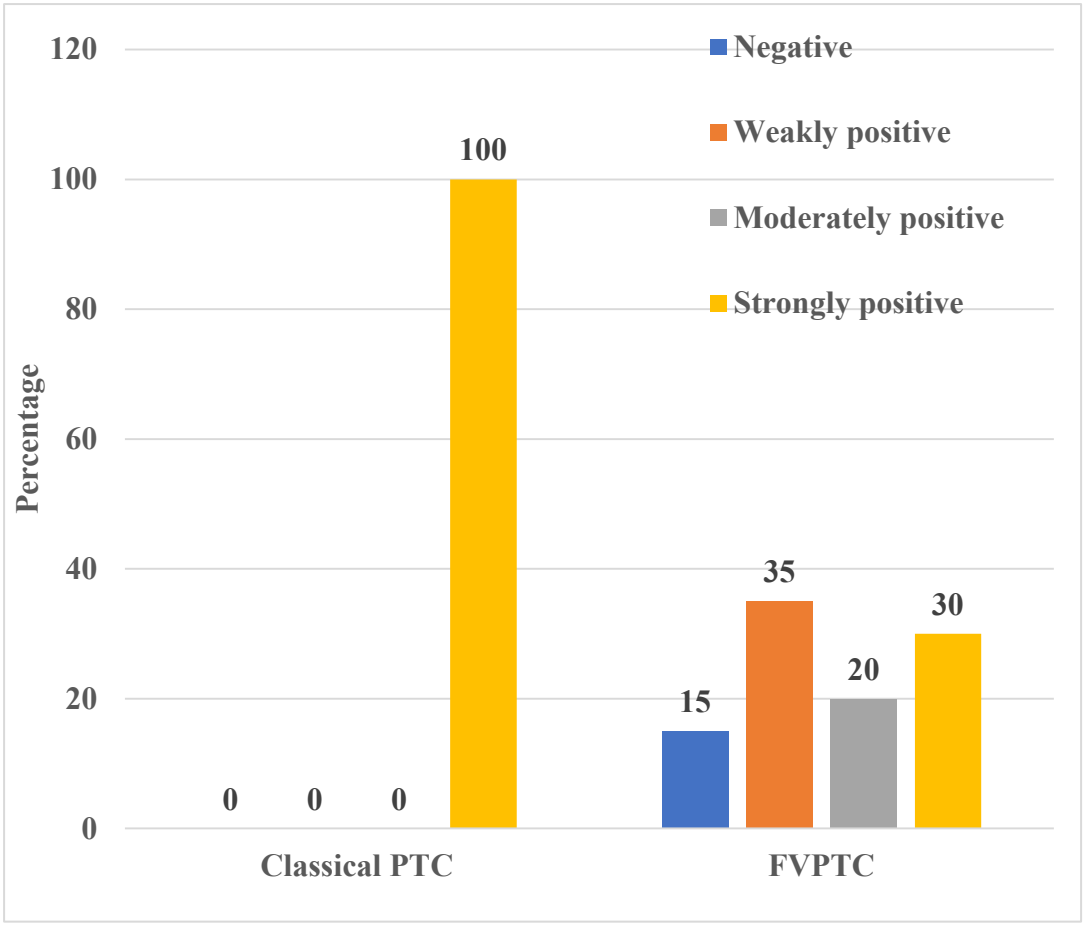

Figure 20: Graphical representation of distribution of CK 19 in PTC cases in the present study.

Table 11: Gal 3 expression with histopathological diagnosis of non-neoplastic lesion of thyroid and PTC cases in the present study.

Gal 3	Histopathological Diagnosis		Total
	Non neoplastic	PTC	
Negative	13	3	16
	50.0%	11.5%	30.8%
Positive	13	23	36
	50.0%	88.5%	69.2%
Total	26	26	52
	100.0%	100.0%	100.0%

p value 0.003

A statistically significant difference was present in Gal 3 expression amongst the non-neoplastic lesions of thyroid and PTC cases.

Table 12: Gal 3 expression in non-neoplastic lesions of thyroid in the present study

Gal 3	Lymphocytic thyroiditis		Nodular hyperplasia	
	Frequency	Percentage	Frequency	Percentage
Negative	3	23.1%	10	77%
Weak	10	76.9%	2	15.3%
Moderate	0	0%	1	7.7%
Strong	0	0%	0	0%
Total	13	100%	13	100%

p value 0.01

None of the non-neoplastic cases had strong Gal 3 expression. Maximum number of lymphocytic thyroiditis cases; 10(76.9%) had weak Gal 3 expression, followed by 3(23.1%) cases with no immunostaining for Gal 3 biomarker. Amongst the nodular hyperplasia cases, maximum; 10(77%) were negative for Gal 3 expression, followed by 2(15.3%) cases with weak Gal 3 expression. A statistically significant difference was present in Gal 3 expression amongst the lymphocytic thyroiditis and nodular hyperplasia cases.

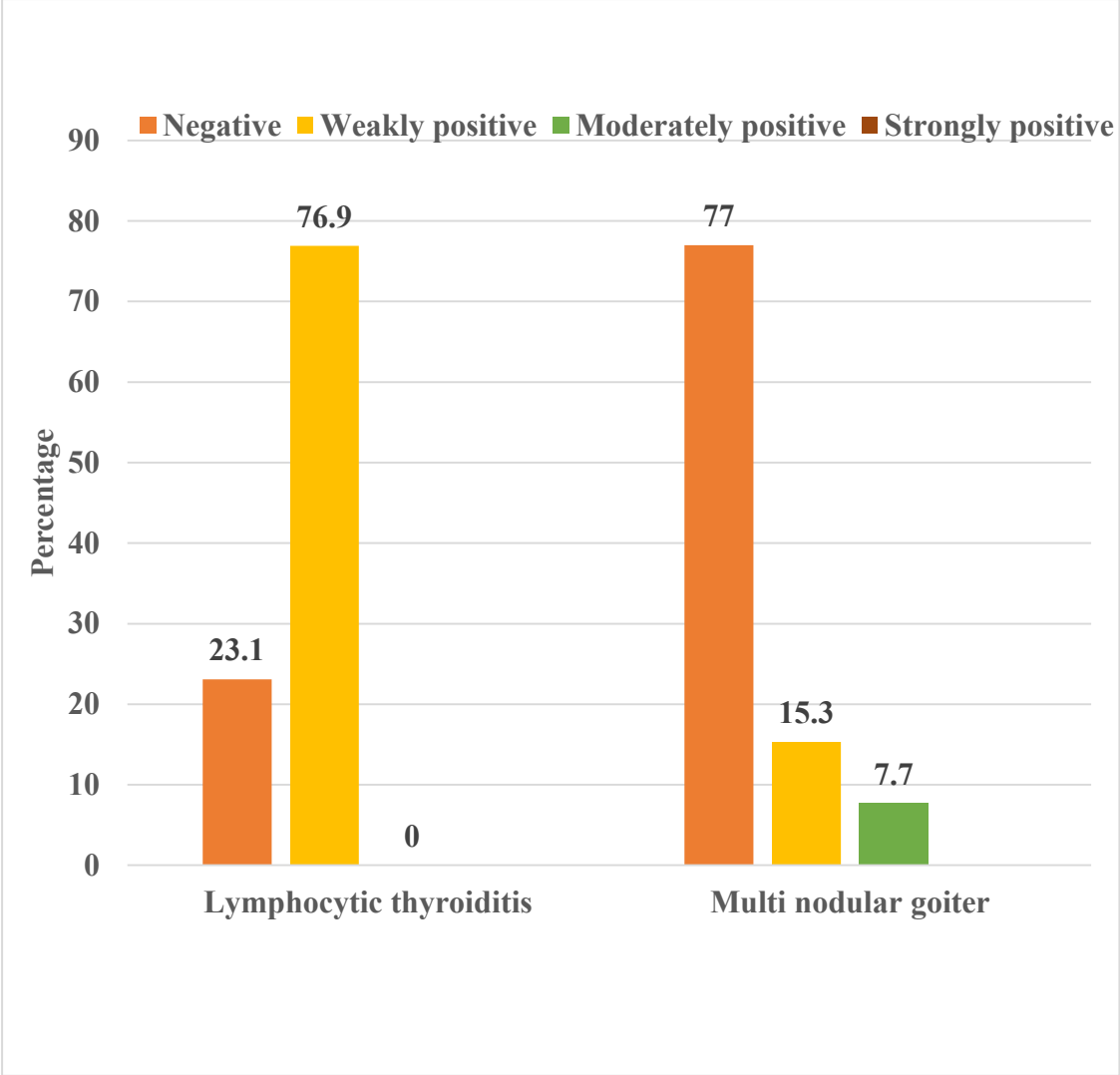

Figure 21: Graphical representation of distribution of Gal 3 in non-neoplastic cases in the present study.

Table 13: Gal 3 expression in PTC cases in the present study.

Gal 3	Classical variant PTC		FVPTC	
	Frequency	Percentage	Frequency	Percentage
Negative	0	0%	3	15%
Weak	0	0%	9	45%
Moderate	0	0%	3	15%
Strong	6	100%	5	25%
Total	6	100%	20	100%

p value <0.01

Maximum number of FVPTC cases; 9(45%) had weak Gal 3 expression followed 5(25%) and 3(15%) cases with strong and moderate positive Gal 3 expression, respectively. All the classical variant PTC had strong positive Gal 3 expression. There was a statistically significant difference of Gal 3 expression amongst the Classical variant and FVPTC cases.

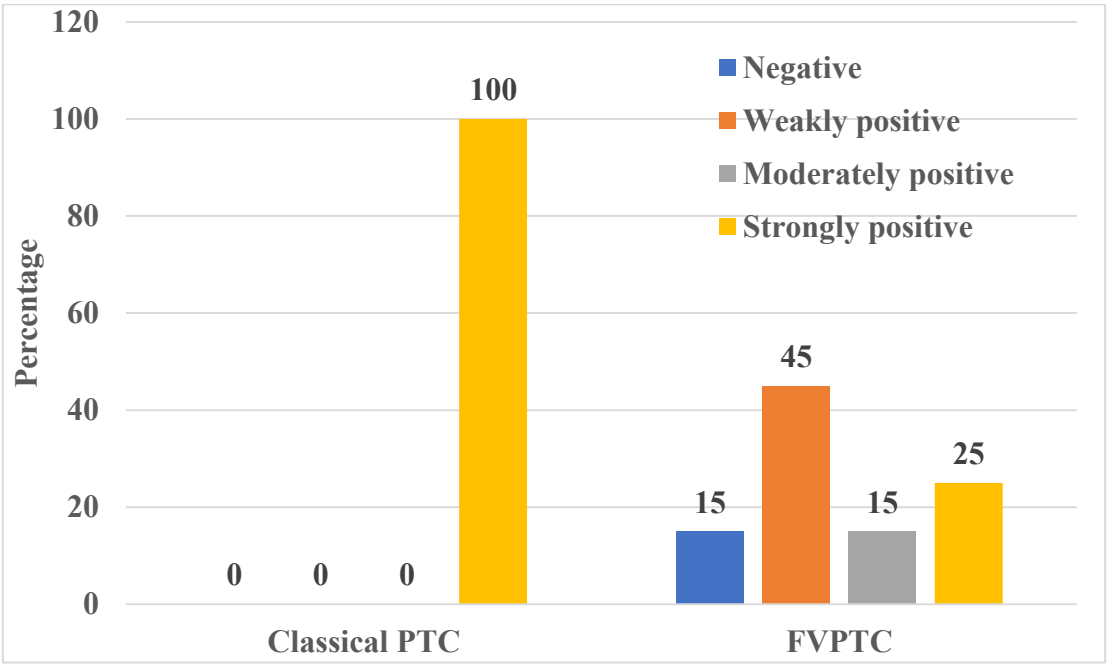

Figure 22: Graphical representation of distribution of Gal 3 in PTC cases in the present study.

Table 14: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy of CK 19 and Gal 3 and their combination in the present study.

	CK 19		Gal 3		CK 19 + Gal 3
Statistic	**Value**	**95% CI**	**Value**	**95% CI**	**Value**
Sensitivity	88.46%	69.85% to 97.55%	88.46%	69.85% to 97.55%	84.62%
Specificity	23.08%	8.97% to 43.65%	50.00%	29.93% to 70.07%	61.54%
Positive Predictive Value	53.49%	47.19% to 59.68%	63.89%	54.04% to 72.70%	68.75%
Negative Predictive Value	66.67%	35.85% to 87.74%	81.25%	58.29% to 93.07%	80.00%
Diagnostic Accuracy	55.77%	41.33% to 69.53%	69.23%	54.90% to 81.28%	73.08%

Comparison of immunohistochemical staining of cases with lymphnode metastasis:

In 10 out of 26 PTC cases in the present study had undergone lymphnode extraction. 2 FVPTC and 2 CVPTC cases showed tumour metastatic deposits in the lymphnodes. All the 4 cases with metastatic deposit showed strong positive expression for CK 19 and Gal 3 biomarkers.

Table 15: CK19 and Gal 3 expression with lymphnode metastasis in Papillary thyroid carcinoma cases in the present study.

	CK 19 (p value 0.788)			**Gal 3 (p value 0.621)**		
	Weak	**Moderate**	**Strong**	**Weak**	**Moderate**	**Strong**
No Metastasis	2	1	3	2	0	4
	33.3%	16.7%	50%	33.3%	0%	66.7%
Metastasis	0	0	4	0	0	4
	0%	0%	100%	0%	0%	100%

There was no statistically significant difference in CK 19 and Gal 3 expression between the PTC cases with/without metastasis to lymph node.

Table 16: CK 19 expression on the basis of size of lesion in the present study:

CK 19	Negative		Weak		Moderate		Strong		Total	
Size	N	%	N	%	N	%	N	%	N	%
Up to 1cm	4	23.5%	10	58.8%	2	11.8%	1	5.9%	17	100
1-2cm	2	28.6%	2	28.6%	1	14.3%	2	28.6%	7	100
2-4cm	3	14.3%	9	42.9%	3	14.3%	6	28.6%	21	100
>4cms	0	.0%	3	42.9%	1	14.3%	3	42.9%	7	100

p value 0.08

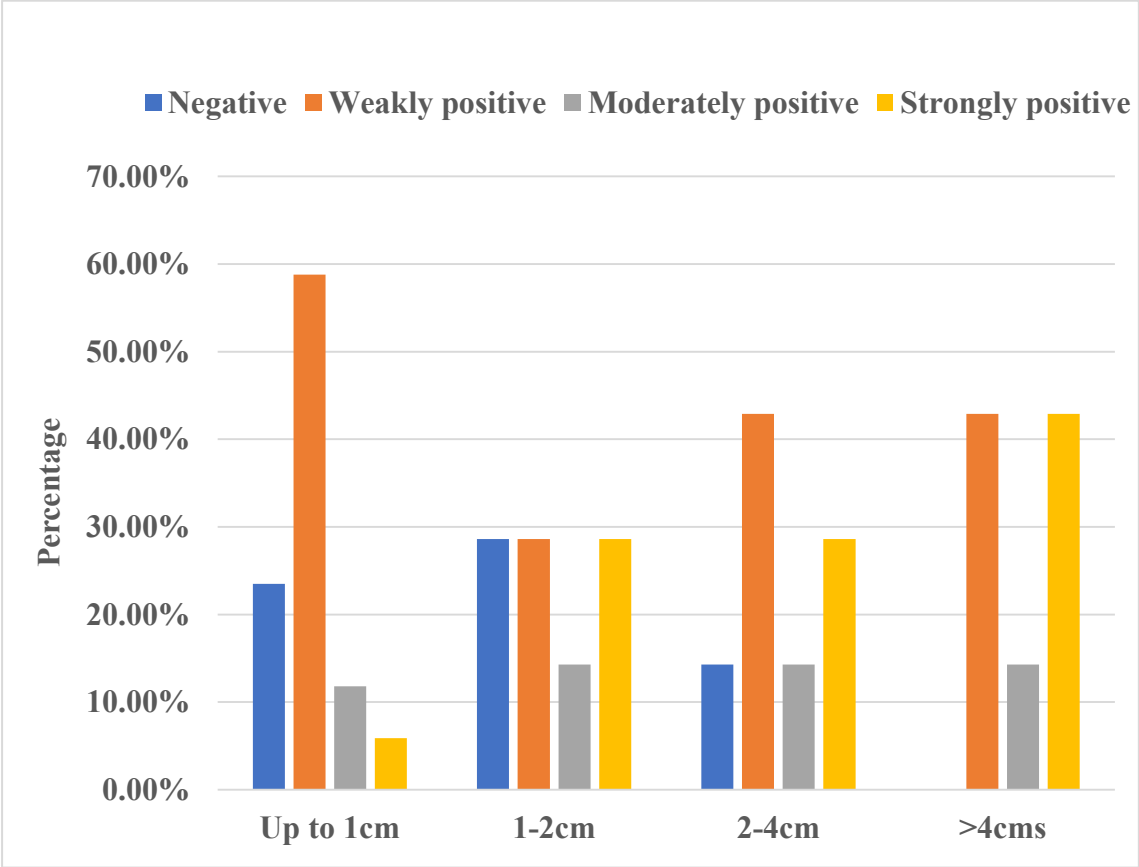

Figure 23: Graphical representation of distribution of CK 19 expression on the basis of size of lesion in the present study.

Table 17: Gal 3 expression on the basis of size of lesion in the present study:

Gal 3 Positivity	Negative		Weak		Moderate		Strong		Total	
Size	N	%	N	%	N	%	N	%	N	%
Up to 1cm	5	29.4%	12	70.6%	0	0%	0	0%	17	100
1-2cm	3	42.9%	0	.0%	1	14.3%	3	42.9%	7	100
2-4cm	7	33.3%	6	28.6%	3	14.3%	5	23.8%	21	100
>4cms	1	14.3%	3	42.9%	0	.0%	3	42.9%	7	100

p value 0.268

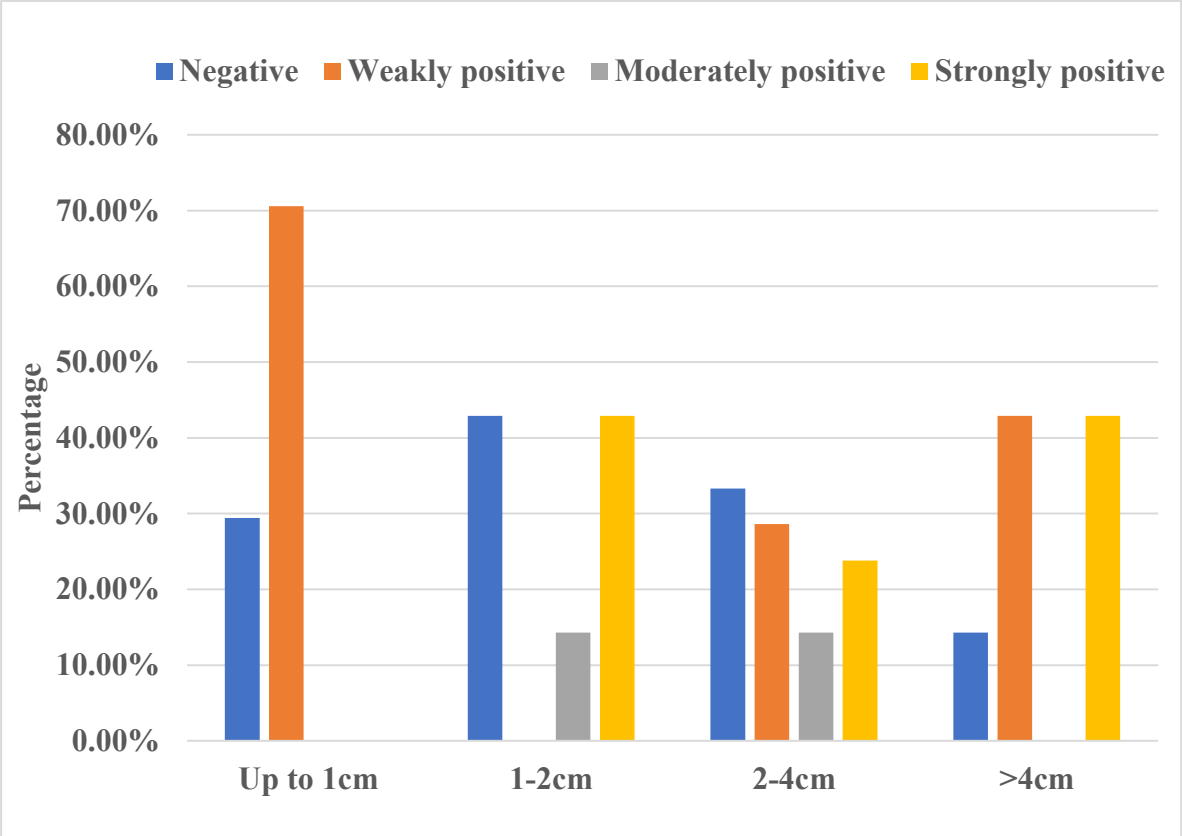

Figure 24: Graphical representation of distribution of Gal 3 expression on the basis of size of lesion in the present study.

There was no statistically significant difference between various lesion size with respect to CK 19 and Gal 3 expression. Maximum number of cases in present study were of <1 cm in size, followed by less than 1cm which were 21 and 17 cases respectively. 7 cases were 1-2cm and more than 4 cm in size, each.

IMAGES

CLASSICAL VARIANT OF PAPILLARY THYROID CARCINOMA:

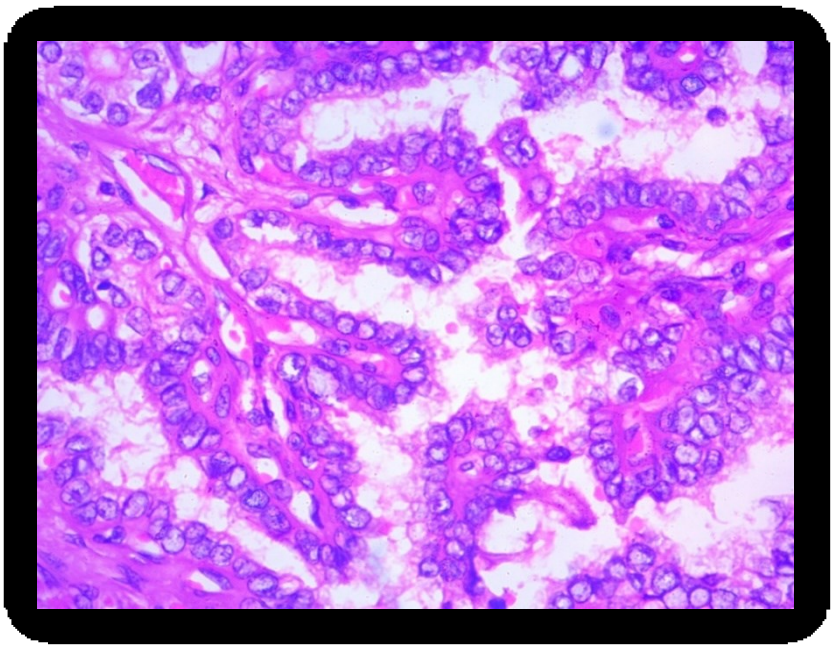

Figure 25: Microphotograph of Classical variant of papillary thyroid carcinoma. (H&E x 400)

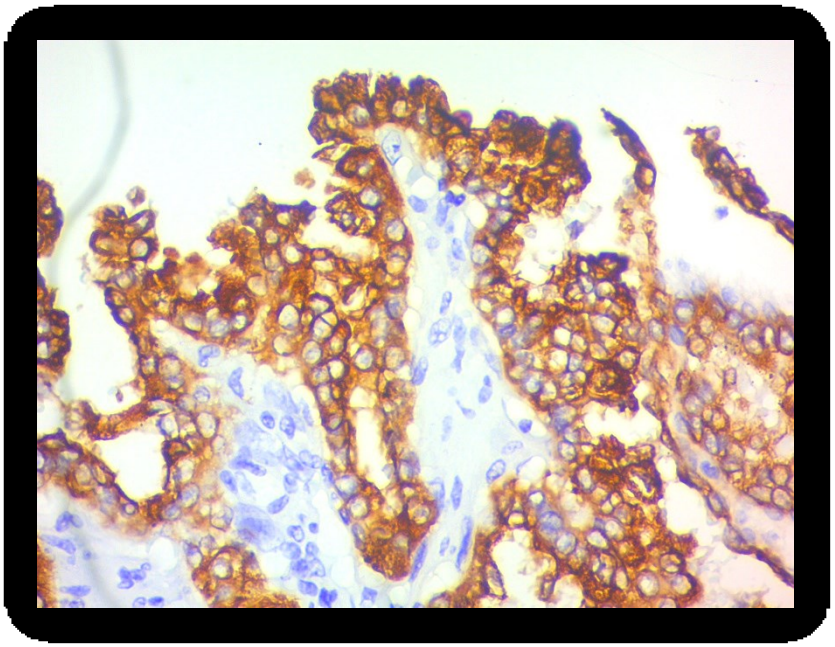

Figure 26: CK 19 expression in Classical variant of papillary thyroid carcinoma – Strongly positive (CK 19 x400)

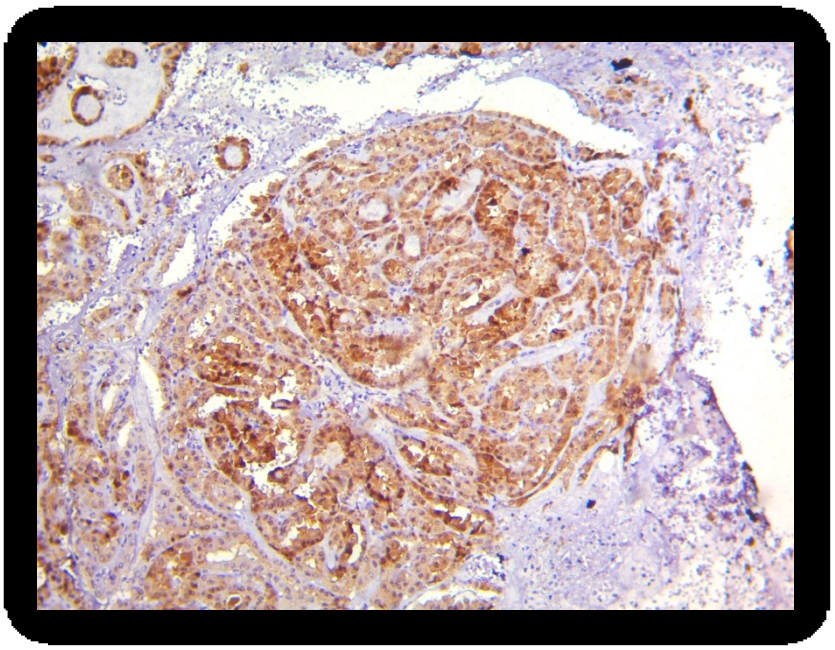

Figure 27: Gal 3 expression in Classical variant of papillary thyroid carcinoma – Strongly positive (Gal 3 x100)

FOLLICULAR VARIANT OF PAPILLARY THROID CARCINOMA:

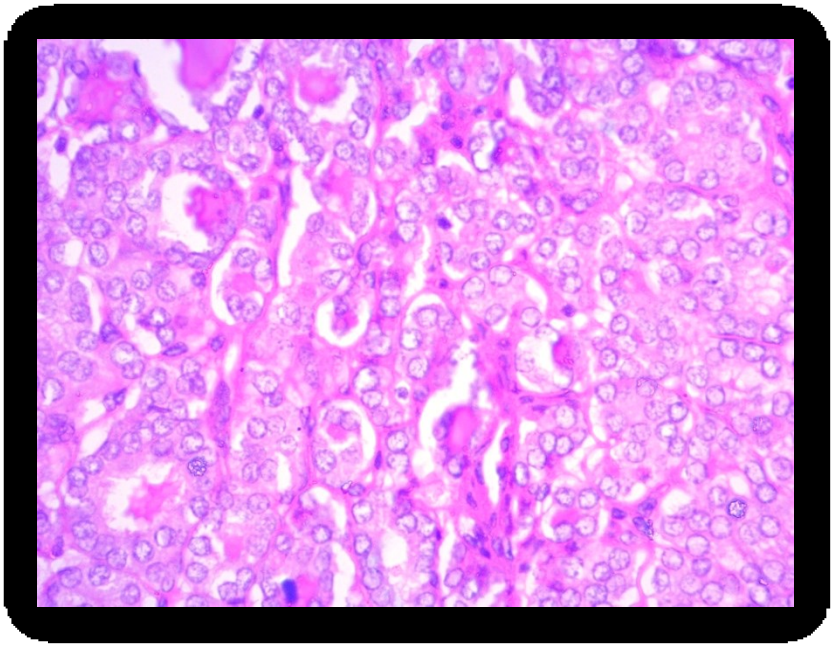

Figure 8: Microphotograph of Follicular variant of papillary carcinoma thyroid. (H&E x400)

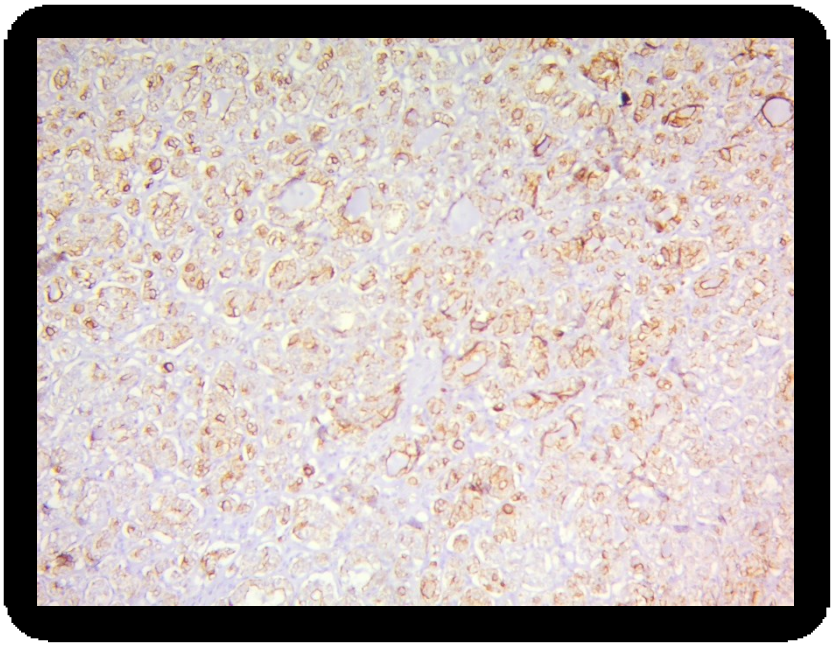

Figure 28: CK 19 expression in Follicular variant of papillary thyroid carcinoma – Moderately positive (CK 19 x100)

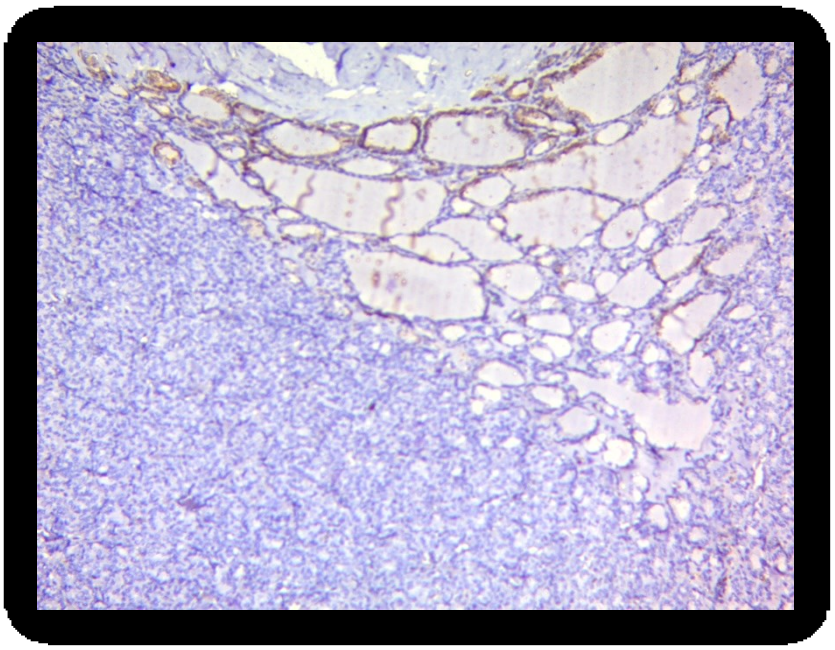

Figure 29: Gal 3 expression in Follicular variant of papillary thyroid carcinoma – Weakly positive (Gal 3 x100)

LYMPHOCYTIC THYROIDITIS:

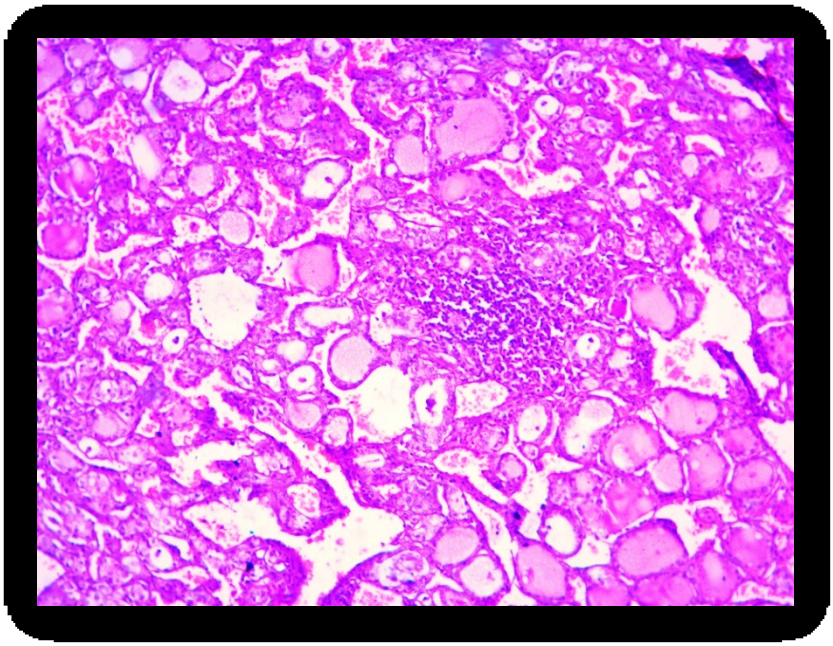

Figure 3: Microphotograph of Lymphocytic thyroiditis. (H&E x100)

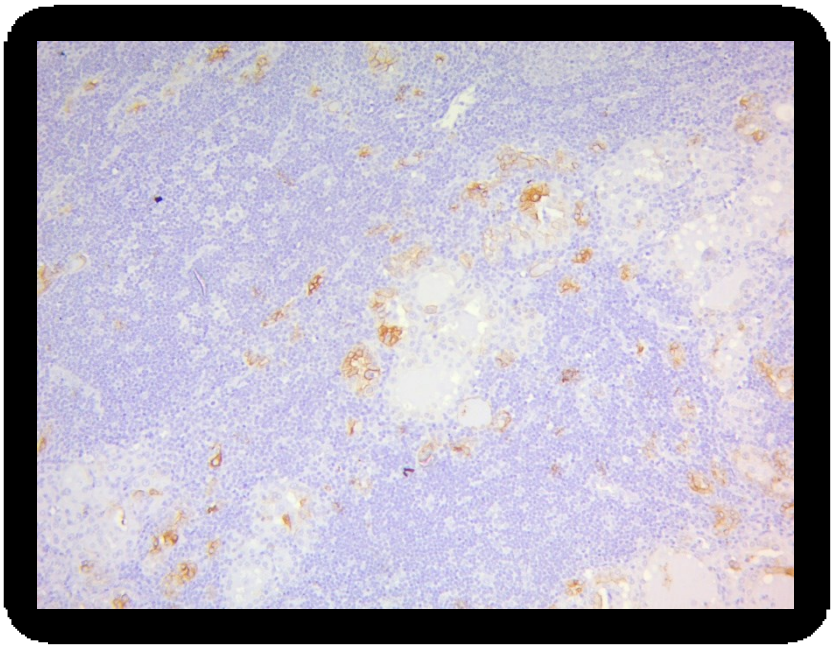

Figure 30: CK 19 expression in Lymphocytic thyroiditis – Weakly positive (CK 19 x100)

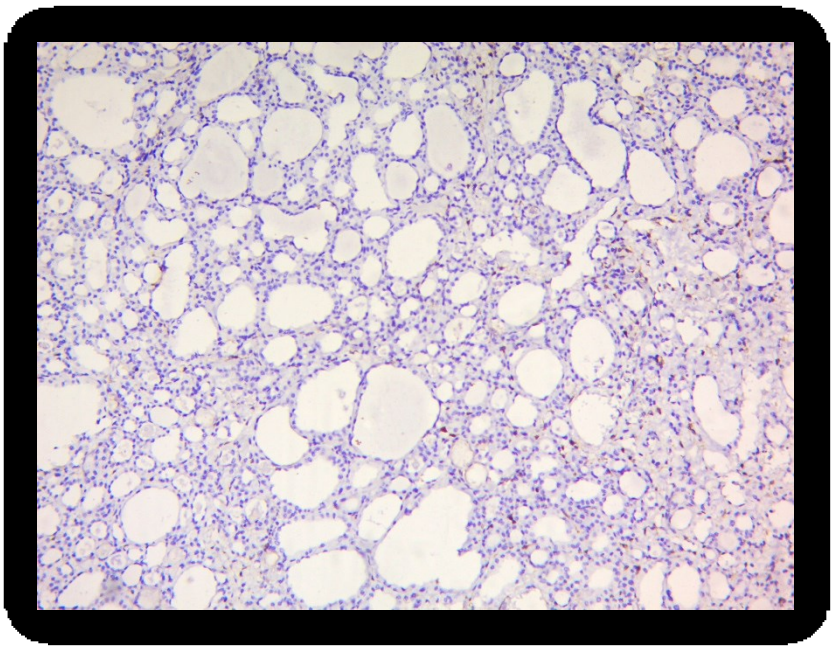

Figure 31: Gal 3 expression in Lymphocytic thyroiditis – Negative (Gal 3 x100)

NODULAR HYPERPLASIA:

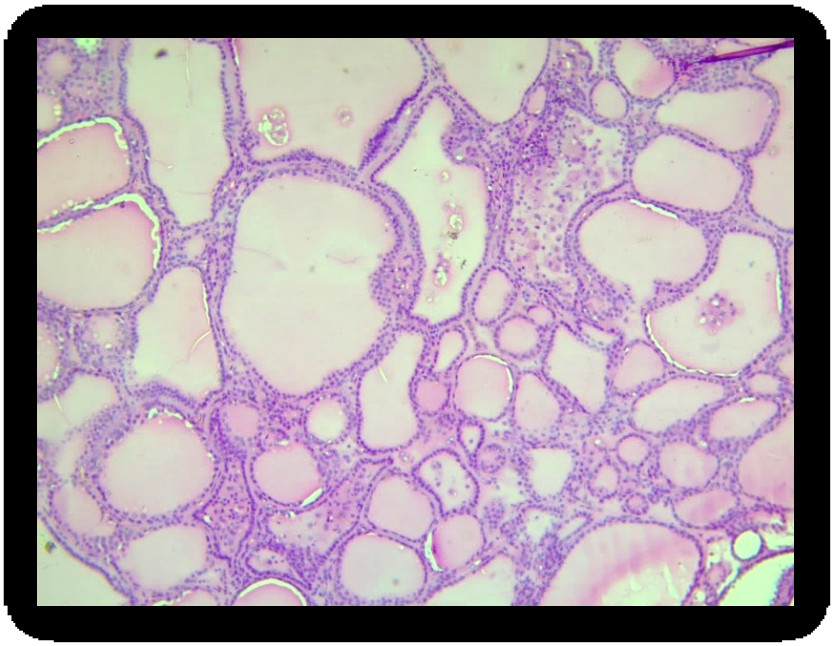

Figure 6: Microphotograph of Nodular hyperplasia. (H&E x100)

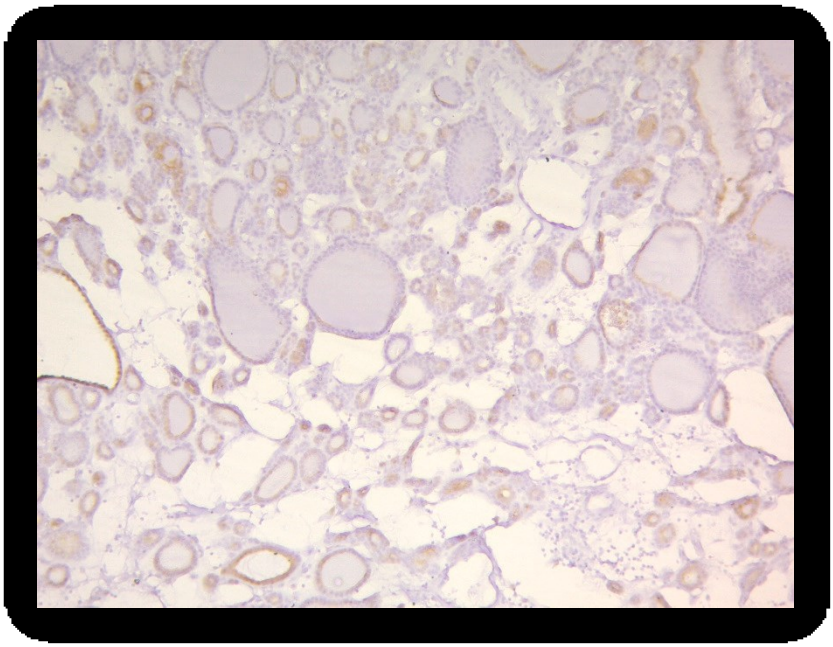

Figure 32: CK 19 expression in Nodular hyperplasia – Weakly positive (CK 19 x100)

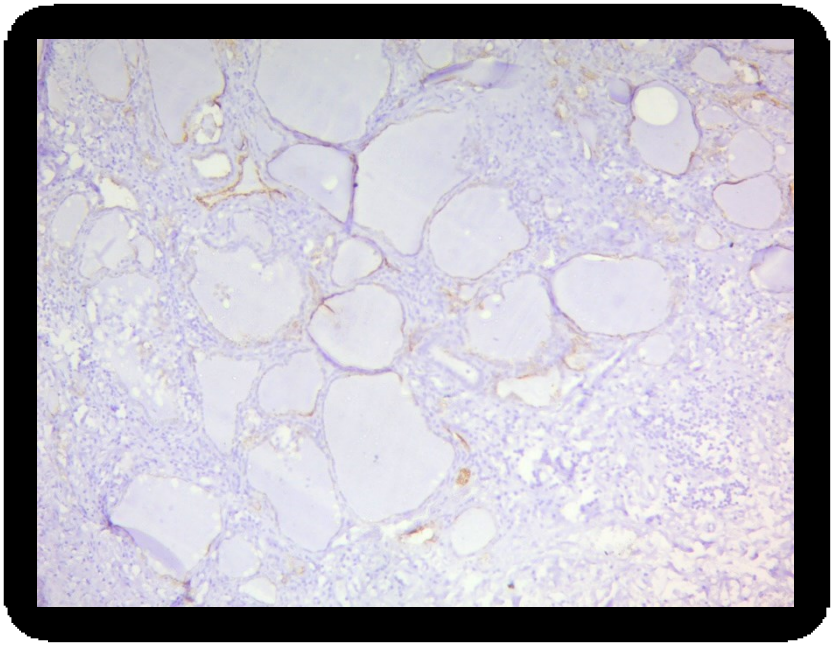

Figure 33: Gal 3 expression in Nodular hyperplasia – Weakly positive (Gal 3 x100)

DISCUSSION

DISCUSSION

Thyroid nodule is a common presentation in common population and yet it is a major concern worldwide. The thyroid lesion can range from non-neoplastic to benign and malignant in aetiology. Even though most of the thyroid nodules are benign, thyroid carcinoma is the commonest cause of malignancy in the endocrine system.[71] Incidence of thyroid malignancies in Kolar region is 3.43%.[72] Many immunohistochemical markers have been useful so far to understand and diagnose the thyroid nodules. Several studies have been conducted to understand the expression of individual markers as well as in combination for differentiating these lesions, when difficult to diagnose. An early detection of these markers in thyroid nodules can help in correct treatment regime and better prognosis.

In our study, we documented CK 19 and Gal 3 expression in both papillary thyroid cancer as well as non-neoplastic lesions. Present study constituted 52 thyroid lesions mostly falling in the age group of 21 to 50 years. Mean age was 40 years.

Table 18: Mean age of cases with thyroid lesions in the present study compared with other similar studies.

	Mean age
Present study (2020)	40 years
Kammal W S et al[70] (2019)	49.4 years
Huang L et al[73] (2017)	52.3 years

Worldwide incidence rate of PTC in women is about 3 times higher than in men (M:F=1:3).[29] A higher rate of prevalence of PTC amongst the women as compared to men is noted in Kolar region.[72] Male to female ratio of the cases taken up in the present study was 1:13.

Table 19: Male to Female ratio in Papillary thyroid carcinoma cases in the present study compared with other similar studies.

	M:F Ratio
Present study (2020)	1:13
Borkar et al[74] (2019)	1:13
Bose D et al[75] (2012)	1:6.3
Sahoo et al[76] (2001)	1:9

Equal distribution of lymphocytic thyroiditis and nodular hyperplasia cases were included in the non-neoplastic group which was 13 each. 26 PTC cases included 6 and 20 cases of classical variant of PTC and FVPTC respectively.

All the cases were stained by 2 biomarkers, namely CK19 and Gal 3. Individual biomarker expression as well as their combination in 2 broad diagnostic categories and the sub categories were studied. CK 19 biomarker showed membranous and diffuse cytoplasmic staining whereas Gal 3 stained the cytoplasm of the thyroid follicular cells.

Predominance of any lobe involvement was not noted in any subgroups of the lesions studied. In the present study, most of the lesions had right thyroid lobe involvement followed by bilateral and left lobe involvement which was 20(38.4), 19(36.5%) and 13(25%) respectively. Comparable studies were not found in the literature.

In present study, most of the nodular hyperplasia cases stained weakly for CK 19 biomarker and negative for Gal 3. Many other studies have demonstrated weak/focal expression of CK19 in nodular hyperplasia [76,77-81] None of the nodular hyperplasia cases had strong positivity for both the biomarkers. Similar expression of both biomarkers was noted in the study done by Mehdi et al.[82]

Table 20: CK 19 and Gal 3 expression in nodular hyperplasia cases in the present study compared with other similar studies.

Nodular hyperplasia	n	Negative		Weak		Moderate		Strong	
		CK19	Gal 3	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3
Present study (2020)	13	30.7%	77%	61.5%	15.3%	7.7%	7.7%	0%	0%
Balcı M et al[83] (2020)	10	0%	70%	10%	20%	-	-	90%	10%
Mehdi et al[82] (2018)	23	39.1%	87%	43.5%	8.7%	8.7%	4.3	8.7%	0%
Bose D et al[75] (2012)	8	50%	-	25%	-	12.50%	-	12.5%	-

Majority of lymphocytic thyroiditis cases stained weakly positive or negative for both the biomarkers. Most of the studies done so far demonstrated moderate or strong positivity. Another study done by Barroeta J E also demonstrated predominantly focal /weak positivity for lymphocytic thyroiditis and grave's disease.[84] This abnormal expression of biomarkers in lymphocytic thyroiditis may be associated with the neoplastic changes similar in PTC and hence identical Ck19 and Gal 3 expressions are observed.[81] Technical errors in the procedure of IHC staining can lead to variability in expression of the biomarkers. This can be a reason for the variation in CK 19 expression in the studies mentioned in table 21.

Table 21: CK 19 and Gal 3 expression in lymphocytic thyroiditis cases in the present study compared with other studies.

Lymphocytic thyroiditis	n	Negative		Weak		Moderate		Strong	
		CK19	Gal 3	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3
Present study (2020)	13	30.7 %	77%	69.2%	76.9 %	15.4 %	0%	0%	0%
Balcı M et al[83] (2020)	10	30%	50%	20 %	50%	-	-	50 %	0%
Mehdi et al[82] (2018)	26	3.7%	7.7%	43.5%	22.2 %	33.3 %	34.6%	40.7%	23.1 %

Present study demonstrates a significant statistical difference in expression of Gal 3 between lymphocytic thyroiditis and nodular hyperplasia cases. There is no such significant statistical difference in the expression of CK 19. Similar findings were noted in the study done by Mehdi A et al.[82]

Table 22: Statistical Significance of CK 19 and Gal 3 expression amongst lymphocytic thyroiditis and nodular hyperplasia cases in the present study compared with other similar studies.

p Value	CK 19	Gal 3
Present study (2020)	0.58	0.01
Mehdi et al [82] (2018)	>0.05	<0.05

Amongst the FVPTC cases studied, 30% and 25% cases showed strong positivity where as 20% and 15% cases showed moderate positivity for CK19 and Gal 3 biomarkers respectively. However, a few cases did stain weakly positive/ negative for both the biomarkers which is 35% and 15% for CK19 and 45% and 15% for Gal 3 respectively. Comparable results from the other studies are as mentioned in table 23.

Table 23: CK 19 and Gal 3 expression in Follicular variant of Papillary thyroid carcinoma cases in the present study compared with other similar studies.

FVPTC	Negative		Weak		Moderate		Strong	
	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3
Present study (2020)	15%	15%	35%	45%	20%	15%	30%	25%
Borkar et al[74] (2019)	-	33%	-	-	-	33%	-	33%
Sumana B S et al[1] (2015)	-	25%	-	50%	-	-	-	25%
Bose D et al[75] (2012)	0%	-	0%	-	37.50%	-	62.50%	-

All CVPTC cases in our study stained strongly positive for both biomarkers. Other studies state similar results as mentioned in table 24.

Table 24: CK 19 and Gal 3 expression in Classical variant Papillary thyroid carcinoma cases in the present study compared with other similar studies.

CVPTC	Negative		Weak		Moderate		Strong	
	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3	CK19	Gal 3
Present study (2020)	0%	0%	0%	0%	0%	0%	100%	100%
Borkar et al[74] (2019)	-	0%	-	0%	-	5%	-	95%
Abdou AG et al[85] (2019)	0%	-	0%	-	0%	-	100%	-
Sumana B S et al[1] (2015)	-	7.1%	-	14.3%	-	-	-	78.6%
Bose D et al[75] (2012)	-	0%	-	0%	-	15.38%	-	84.62%

Present study demonstrates a significant statistical difference in expression of CK 19 and Gal 3 between the FVPTC and CVPTC. Even though CVPTC are easier to detect on histopathology, it is observed that CVPTV had strong expression of both markers studied. FVPTC on the other presents with variable expression of biomarkers. A Study done by Abdou et al demonstrates similar results.

Table 25: Statistical Significance of CK 19 and Gal 3 expression amongst Classical variant Papillary thyroid carcinoma and Follicular variant Papillary thyroid carcinoma cases in the present study compared with other similar study.

p Value	CK 19	Gal 3
Present study (2020)	**<0.01**	**<0.01**
Abdou AG et al[85] (2019)	**<0.01**	**<0.01**

Variable positivity for CK 19 biomarker was noted in non-neoplastic lesions. In the present study, 76.9% non-neoplastic cases showed CK 19 positivity. In contrast to that, PTC shows a relatively higher number of cases staining for CK 19 biomarker. Due to variability of expressions in non-neoplastic lesions, a higher positivity in the cases of thyroid lesion can be incorrectly interpreted as PTC. Hence, CK 19 is not a useful biomarker for differentiating non- neoplastic and PTC cases. Comparative results from other studies are as mentioned in table 26.

There was no statistical difference in CK 19 expression in PTC and non-neoplastic cases in the present study as both the groups demonstrate high expression of CK 19. Similar analysis is noted in the study done by Abdou AG et al.[85]

Present study demonstrated 50% of non-neoplastic lesions with weak to moderate Gal 3 positivity. 88.5% of the PTC cases showed Gal 3 positivity. There is a statistical difference found in Gal 3 expression amongst the PTC and non-neoplastic cases in the present study**.** Similar analysis is noted in the study done by Huang et al.[73]

Table 26: CK 19 and Gal 3 expression in non-neoplastic cases of thyroid and Papillary thyroid carcinoma cases in the present study compared with other similar studies.

	CK 19				**Gal 3**			
	Non-neoplastic		**PTC**		**Non-neoplastic**		**PTC**	
	Negative	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Present study (2020)	23.1%	76.9%	11.5%	88.5%	50%	50%	11.5%	88.5%
Abdou AG et al[85] (2019)	14.3%	85.7%	7.1%	92.9%	-	-	-	-
Huang L et al[73] (2017)	95.17%	4.83%	4.17%	98.83%	83.87%	3.18%	16.12%	96.82%
Song Q et al[3] (2011)	74.17%	25.83%	3.63%	96.37%	-	-	-	-
Barroeta JE et al[84] (2006)	59.5%	40.5%	26.7%	73.3%	66.7%	33.3%	20%	80%
Scognamiglio T et al[86] (2006)	20.84%	79.16%	4%	96%	-	-	-	-

Table 27: Statistical Significance of CK 19 and Gal 3 expression in non-neoplastic cases of thyroid and Papillary thyroid carcinoma cases in the present study compared with other similar studies.

p Value	CK 19	Gal 3
Present study (2020)	**0.21**	**0.003**
Abdou AG et al[85] (2019)	**0.59**	**-**
Huang L et al[73] (2017)	**-**	**<0.001**

Present study shows high sensitivity for both the biomarkers to detect PTC cases; 88.46% for both CK 19 and Gal 3 biomarkers respectively. Specificity for CK19 and Gal 3 was 23.08% and 50.00%. When compared with the result of other studies, specificity of CK19 was quite low. Such discrepancy may be due to unequal distribution of cases in each group of lesions studied. With the given results, this study suggests that CK 19, Gal3 and their combination are not useful to differentiate non-neoplastic lesions from PTC cases.

Table 28: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy of CK 19 and Gal 3 biomarkers in present study compared with other similar studies.

Statistic		Sensitivity	Specificity	PPV	NPV	Accuracy
Present study (2020)	CK 19	88.46%	23.08%	53.49%	66.67%	55.77%
	Gal 3	88.46%	50.00%	63.89%	81.25%	69.23%
Balcı M et al[83] (2020)	CK 19	90%	39.2%	53.7%	53.7%	61.5%
	Gal 3	70.4%	70%	82.6%	82.6%	70.3%
Abdou AG et al[85] (2019)	CK 19	78.65%	66.7%	86%	54%	75%
Dunđerović et al[87] (2015)	CK 19	90%	82%	-	-	-
	Gal 3	93%	86%	-	-	-

Table 29: Sensitivity and Specificity of CK 19 and Gal 3 combination in the present study compared with other similar studies.

	Sensitivity	Specificity
Present study (2020)	84.62%	61.54%
Dunđerović et al87 (2015)	78.2%	92.9%

Lymphnodes were retrieved from 10/26 cases of the PTC cases studied; 5 CVPTC and 5 FVPTC cases. 2 cases from CVPTC group and FVPTC group, each that had metastatic tumour deposits, stained strongly positive for both the biomarkers. Lymphnodes from 3 cases of CVPTC group and FVPTC group each, showed only reactive changes. However, these cases had weak, moderate as well as strong positivity for the biomarkers. No statistically significant difference was found in CK 19 and Gal 3 expression between PTC cases with/without metastasis to lymph node. This suggest that even if a variety of CK 19 and Gal 3 expressions could be found in PTC cases, there is a strong possibility of lymphnode metastasis, if the tumour expresses strong positivity for both the biomarkers. Similar results had been obtained by a study done by Adbou AG et al.[85]

A study done by Dencic TI et al, speculated role of CK 19 in the prognosis of the disease and its association with extra thyroid extension and pTNM staging[88]. This evaluation could assist to avoid unnecessary extensive surgical intervention and clinical surveillance aftersurgery. Various biomarker expressions were seen in all the different tumour sizes. There was no statistically significant difference in expression of the biomarkers in different tumour size. Similar information was found from studies done earlier.[70,89]

LIMITATIONS OF THE PRESENT STUDY:

- Present study comprised of a smaller sample size. Larger group of population has to be studied to validate and confirm the results obtained as above.
- Only two variants of PTC were considered for the study. Lymphocytic thyroiditis and nodular hyperplasia were the only two non-neoplastic lesions considered for the study.
- Scoring system was followed as per the study done by Kammal WS et al.[70]Different criteria of scoring system were used to evaluate positivity and negativity of the biomarker in various published studies. Interpersonal observer bias can make a significant change in expression of the biomarkers. A global standard scoring system is suggested for better comparison and uniformity of the results.

CONCLUSION

CONCLUSION

- CK 19 biomarker was not useful to differentiate non-neoplastic lesions of thyroid and PTC.
- CK 19 biomarker can be used to differentiate CVPTC and FVPTC.
- Gal 3 biomarker was useful to differentiate non-neoplastic lesions of thyroid and PTC.
- Gal 3 biomarker was useful to differentiate CVPTC and FVPTC.
- A combination of CK 19 and gal 3 was not useful to differentiate PTC and non-neoplastic lesions of thyroid, when in doubt.
- No statistically significant difference in the expression of CK 19 and Gal 3 biomarkers was noted in the PTC cases that metastasized to lymphnode.

SUMMARY

SUMMARY

- Thyroid lesions were more commonly noted in 5th decade.
- Male to female ratio was 1:13 with a female preponderance.
- No specific lobe was involved more commonly in thyroid lesions.
- There was no statistically significant difference of CK 19 expression between lymphocytic thyroiditis and nodular hyperplasia.
- There was a statistically significant difference of CK 19 expression between CVPTC and FVPTC.
- There was a statistically significant difference of Gal 3 expression between lymphocytic thyroiditis and nodular hyperplasia.
- There was a statistically significant difference of Gal 3 expression between CVPTC and FVPTC.
- Sensitivity of CK 19 and Gal 3 was high to differentiate non-neoplastic lesions of thyroid and PTC.
- Specificity of CK 19 and Gal 3 was low to differentiate non-neoplastic lesions of thyroid and PTC.
- The combination of CK19 and Gal 3 biomarker had low specificity and low sensitivity to differentiate non-neoplastic lesions of thyroid and PTC.
- No statistically significant difference was found in the expression of CK19 and Gal 3 biomarkers in the thyroid lesions that metastasized to lymph-nodes.
- The expression of CK 19 and Gal 3 biomarker was not associated with the size of the lesion.

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BIBLIOGRAPHY

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ANNEXURES

PROFORMA

CASE NO:

AGE:

SEX: HOSPITAL NO:

PRESENTING COMPLAINT:
CYTOLOGY:

FROZEN SECTION:

CLINICAL DIAGNOSIS: BIOPSY NO:

USG FINDING:

THYROID FUNCTION TEST: T3: T4: TSH:

CYTOLOGICAL DIAGNOSIS:

HISTOPATHOLOGICAL FINDINGS-

MACROSCOPIC- SPECIMEN DIMENSIONS- RIGHT LOBE-

LEFT LOBE-

ISTHMUS-

LESION -

THYROID CAPSULE-

OTHERS-

MICROSCOPIC:

PAPILLARY THYROID TUMOUR:

NON-NEOPLASTIC TUMOUR:

IMMUNOHISOCHEMICAL STAINS-

GALECTIN 3:

CK 19:

INTERPRETATION:

FINAL IMPRESSION:

MASTER CHART