"DIAGNOSTIC UTILITY OF GALECTIN-3 IN PAPILLARY LESIONS OF THYROID"

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LIST OF ABBREVATIONS USED

PTC – Papillary carcinoma of thyroid

FVPTC – Follicular variant of Papillary Carcinoma Thyroid

FA – Follicular adenoma

FTC – Follicular Carcinoma Thyroid

Gal-3 – Galectin – 3

TPO – Thyroid peroxidase

TSH – Thyroid stimulating hormone

CK – cytokeratin

WHO – World Health Organisation

IHC – Immunohistochemistry

ABSTRACT

Introduction: Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm of the neck. Majority cases of PTC are diagnosed on the basis of pathologic criteria. However, few thyroid lesions exist which mimic nuclear features or the architecture of PTC, posing diagnostic problems. Papillary projections may be encountered in benign papillary hyperplasia of multinodular goitre, Hashimoto's thyroiditis and Graves' disease. For this reason, the approach to these challenging lesions should include immunohistochemistry. Galectin-3(Gal-3), is an immunohistochemical marker which shows positivity in PTC. The present study was undertaken to investigate whether strong galectin-3 expression is an important hallmark of PTC or papillary thyroid hyperplasia.

Aim: To determine the efficacy of Gal-3in diagnosis of papillary lesions of thyroid.

Material and Methods: Gal-3expression was sought for by immunohistochemistry in 16 cases of papillary patterns of thyroid specimens received at our institution. The results obtained were statistically analyzed.

Material and Methods: Galectin 3 expression was sought for by immunohistochemistry in 33 cases of papillary patterns (on microscopy) of thyroid specimens received at our institution. The results obtained were statistically analyzed.

Results: Of the 33 cases studied, 17 cases were PTC and 16 were papillary hyperplasia. Immunohistochemical stain with Galectin -3 revealed statistically significant P – value,that proves the tendency for Galectin -3 expression is more in PTC as compared to papillary hyperplasia.

Keywords: Galectin- 3, immunohistochemistry, PTC, papillary thyroid hyperplasia.

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INTRODUCTION

The most common malignancy of the endocrine system is carcinoma of the thyroid gland. [1] The tumors arising from follicular epithelial cells contribute to greater than 95% of the tumors. [2,3] The most common malignant tumour among all the malignancies of thyroid gland is papillary carcinoma of thyroid (PTC) which accounts for 85% of all thyroid cancers. [1]

The increasing incidence of thyroid cancer, especially PTC, is of concern. [4] Although, the burden of Thyroid tumors is 1% worldwide, few tumors pose so much challenge to pathologists. [5] The main diagnostic difficulties are to distinguish follicular variant of PTC (FVPTC) with Follicular adenoma (FA) / Follicular thyroid carcinoma (FTC) and papillary hyperplasia seen in benign conditions of thyroid from PTC. For the diagnosis of PTC, the diagnostic morphological features are papillary fronds, psammoma bodies and characteristic nuclear changes like optically clear nuclei, nuclear pseudoinclusions and nuclear grooves. However, these features may be seen in other thyroid lesions as well. [6,7] Features like optically clear orphan annie nuclei is noted focally in FA, diffuse thyroid hyperplasia and FTC. [8] Nuclear grooves are also present in non-neoplastic lesions of the thyroid and non-papillary neoplasms. [9] In cases of follicular adenoma, intra nuclear cytoplasmic inclusions can also be seen. [10]

The differentiation between papillary thyroid hyperplasia and PTC for many cases is done on histopathology. Lesions like hyper functioning nodule of Multinodular goitre (MNG), Follicular benign neoplasms, Hashimoto thyroiditis, congenital errors of thyroid metabolism and Graves' disease can show benign

papillary hyperplasia. [11] These lesions with papillary thyroid hyperplasia can simulate classical variant of PTC which leads to diagnostic dilemma. The potential diagnostic difficulty is due to the presence of well-developed papillary fronds with fibrovascular core and nuclear pleomorphism in both PTC and papillary hyperplasia. [12] In some cases, diagnostic problems can arise where these features are present focally or multifocally rather than being diffusely distributed throughout the lesion. [13] The pathologist is confronted with lesions where the distinction between benign and malignant lesions are subtle. These group of thyroid lesions which mimic nuclear features or the architecture and growth pattern of papillary thyroid carcinoma, posing diagnostic problems. [14]

The diagnosis of the lesion, either benign or malignant lesion is important as it has clinical significance. Different modalities of treatment are used according to the lesion. Despite the lymphatic dissemination to cervical lymph nodes, patients with these tumors have an excellent long term prognosis if appropriately treated. For this reason, the approach to these tumors must include ancillary techniques like immunohistochemistry which improves standard of morphologic assessment. [2]

Many studies were done in the past to evaluate markers to distinguish benign from malignant thyroid lesions like IHC markers like epithelial membrane antigen, high molecular weight keratins, CEA, vimentin, ceruloplasmin, Leu-7 antigen, thyroid peroxidase, Galectin – 3 (Gal-3), dipeptidyl aminopeptidase IV and CD56. [15] Immunohistochemical marker like Gal-can be used as a diagnostic marker for thyroid cancer. Gal-3 is a carbohydrate-binding protein which binds to galactosidase residues on cell surface glycoproteins. [16] Increased levels of Gal-3 expression are seen in malignant thyroid neoplasms, but not in normal thyroid tissue and adenomas. Gal-3 is

localized predominantly in the cytoplasm, very rarely nucleus and cell surface suggestive of multifunctionality of the marker. It has both physiological and pathological functions like cell growth, adhesion, apoptosis, neoplastic transformation and metastasis. [17]

OBJECTIVE OF THE STUDY

To determine the efficacy of Galectin -3 in diagnosis of papillary lesions of thyroid.

REVIEW OF LITERATURE

EMBRYOLOGY:

The first endocrine gland to develop in the embryo is the thyroid gland. It forms in the floor of the primordial pharynx from the median endodermal thickening. [18] It develops from the thyroglossal duct as a midline structure between 2nd and 3rd weeks of gestation. [19, 20] The gland consists of well-developed follicles lined by follicular cells and colloid in the lumen by 14th week of gestation. [7] The parafollicular cells form from the caudal pharyngeal complex. [20]The development is regulated by the action of multiple transcription factors like TTF-1, TTF-2, PAX8 and HHEX. Discoordination of these factors lead to thyroid dysgenesis. [7]

ANATOMY:

The thyroid gland is present in the lower part of anterior aspect of neck in front of the fifth to seventh cervical vertebrae, first thoracic vertebra and upper most part of the trachea. [21] It is composed of two lobes joined by the isthmus. [7,11] The extent of each lobe is from the midpoint of the thyroid cartilage to the fourth or fifth tracheal ring and measures about 5 x2.5x2.5 cm approximately. [21] The isthmus being smaller extends from the second to the fourth tracheal ring and measures about 1.2x1.2 cm approximately. [21] Weight of the gland ranges from 14 to 18 gram depending on various factors like gender, appropriate iodine intake and nutritional status of the individual. [17,19]

It receives blood supply from the superior and inferior thyroid arteries which are the branches of external carotid artery and thyrocervical trunk respectively. It drains into the superior, middle and inferior thyroid veins. The lymphatic drainage of

the upper and lower parts of the gland is to the upper and lower deep cervical lymph nodes respectively. The major nerve supply is by the middle cervical ganglion and partly by the superior and inferior cervical ganglia. [21]

HISTOLOGY:

The thyroid follicles are lined by a single layer of follicular cells which are flattened to low columnar. [7,16] They vary depending on the state of activity, during the active and resting phases, they are columnar and cuboidal in nature respectively.[21]

On roentgenogram, diffraction, birefringent crystalline material which is confirmed as calcium oxalate is seen in normal or diseased thyroid gland. [11] Parafollicular or 'C' cells are few in number and are present in between the thyroid follicles. They are identified by positive immunostaining for Calcitonin. [11,19]

Ultrastructural studies show that follicular cells comprise of complement of endoplasmic reticulum, liposomes, mitochondria and interstitial tissue shows many fenestrated capillaries.[11]

NON NEOPLASTIC LESIONS OF THYROID

NODULAR HYPERPLASIA [7, 11]

It is the most common disease of thyroid which is one of the thyroid hyperplastic disorders. It is of two types. They are endemic and sporadic (nodular) goitre.

In the endemic type, there is less content of iodine in water and salt of the living habitat and patients have iodine deficiency which stimulates the production of thyroid stimulatory hormone (TSH) leading in production of hyperplastic follicles

which are lined by active tall columnar cells and scant amount of central colloid within the follicles initially which is called as parenchymatous goitre. In the later stages, follicular atrophy with abundant amount of colloid is seen which is called as colloid goitre. In the other type i.e. sporadic goitre, the pathogenesis is unknown.

Gross: The cases of nodular hyperplasia shows enlarged and distorted thyroid gland architecture. On cut section, many nodules are seen with or without secondary changes like haemorrhage, cystic degeneration and calcification.

Microscopy: There is varied presentation of the lesion. Few nodules are hyperplastic and few are atrophic depending on the stage of development. Foci of hurthle cell change, accumulation of small dilated follicles with benign papillary projections of lining follicular epithelium into the lumen is noted called as Sanderson polsters. [22]
Any rupture of follicles within the lesion lead to a granulomatous reaction as a response to colloid resulting in accumulation of macrophages, foreign body giant cells, haemorrhage and calcification.

The treatment to nodular hyperplasia is medical therapy with suppressive exogenous thyroid hormones, larger lesions need bilateral subtotal thyroidectomy whereas mild cases do not need any treatment.

AUTOIMMUNE DISORDERS (LYMPHOCYTIC THYROIDITIS, HASHIMOTO's THYROIDITIS AND GRAVES' DISEASE)

A spectrum of lesions having the pathogenesis of immune mediated inflammation are classified under autoimmune thyroid disorders. [23] It is a polygenetic disease where susceptibility genes and environmental factors act concomitantly and initiate both the cellular and humoral response against the thyroid gland. Hashimoto's thyroiditis is at one end of spectrum which is characterized by

hypothyroidism and on the other end, Graves' disease which is characterized by hyperthyroidism. Many chromosomal foci are linked to the pathogenesis like GD-1,2,3 for Graves' disease and HT-1,2 for Hashimoto disease.[11]

LYMPHOCYTIC THYROIDITIS

It is commonly seen in children. These patients present with asymptomatic goitre. The radioactive iodine uptake is low. On gross, diffuse enlargement of the gland with firm consistency, solid, white, nodular cut surface. On microscopy, majority of the thyroid follicles are unremarkable, few of them are atrophied or few may show oncocytic change, Lymphocytic nodules with germinal centres are present in the interstitial tissue.

GRAVES' DISEASE (DIFFUSE TOXIC GOITRE) [7,11]

It is a hyperplastic disorder of thyroid most commonly seen in young adults and few cases are also noted in children. Females are affected more commonly with a female to male ratio of 9:1 approximately.

Aetiology

It is an autoimmune disorder with a genetic defect in suppressor type of T cells with an increased B cell proliferation leading to the production of antithyroid antibodies like thyroid receptor antibodies.

Gross: The thyroid gland shows diffuse enlargement, on cut surface it is reddish in colour.

Microscopy: There is diffuse involvement of thyroid gland with hyperplastic follicles which are lined by columnar cells, basally placed nucleus with amphophilic or micro

vacuolated cytoplasm. The central colloid is paler with prominent scalloping. Foci of benign papillary infoldings are also noted. Occasionally nuclear clearing of the follicular cells is noted. Stroma shows lymphocytic infiltration frequently.

HASHIMOTO's THYROIDITIS [7,11]

It is an organ specific immune mediated inflammatory disorder most commonly seen in females above forty years of age. Both humoral and cellular mediated immune mechanisms are involved. Clinically patients present initially with features of hyperthyroidism followed by hypothyroidism. Autoantibodies are present against thyroglobulin and thyrotropin receptors. [24]

Gross: The gland is diffusely enlarged, tan yellow in colour due to the presence of abundant lymphoid tissue and firm in consistency due to fibrosis.

Microscopy: The lobular architecture of thyroid gland is prominent due to fibrosis of the interlobular stroma with dense and diffuse lymphoplasmacytic infiltration with well-formed germinal centres. The lymphocytes present in the stroma are polyclonal B and T cell type in 1:1 ratio differing from the T cell predominant lymphocytes in the peripheral blood. Predominantly follicles are atrophic and few of them show oncocytic change in the lining epithelium. Frequently nuclear overlapping, enlargement and clearing is also seen. The intervening stroma is scant with moderate septal thickening whereas in the fibrous variant of Hashimoto thyroiditis, extensive areas of fibrosis are present. Patients with this condition have an increased risk of developing lymphomas- B cell type, leukemia and papillary carcinoma of thyroid.

REIDEL THYROIDITIS

It is a rare disorder of thyroid gland affecting elderly patients presenting with ill-defined enlargement of thyroid and profound dyspnoea. On gross, asymmetric involvement of gland is present. On microscopy, the gland is completely replaced by fibrous tissue and patchy chronic inflammatory cell infiltrate comprised of lymphocytes and plasma cells. The important diagnostic feature is the presence of inflammation in the wall of the veins and encasement by fibrosis. The associated conditions may be sclerosing cholangitis, mediastinal or retroperitoneal fibrosis and inflammatory pseudotumor of orbit. Treatment is by steroid therapy in few cases but majority of them need resection.

DYSHORMONOGENETIC GOITRE

This condition is caused due to the intrinsic defects of thyroid hormone synthesis. It is caused by loss of function mutations of thyroglobulin, thyroid peroxidase, thyroid oxidase 2 and pendrin gene leading to defects of thyroid hormone production like unresponsiveness to thyroid stimulating hormone, transport, organification, coupling, thyroglobulin synthesis and secretion. [25]

Gross: The thyroid gland is enlarged and multi nodular in appearance resembling multinodular goitre. On microscopy, hyperplastic nodules with varied patterns of appearances are present. Predominantly solid, micro, insular and papillary patterns with fibrosis are noted. Nuclear atypia and mitotic figures are present in the internodular tissue with scant amount of colloid. Cases of follicular and papillary microcarcinomas are seen incidentally.

CLASSIFICATION OF THYROID TUMORS FROM THE 2004 WORLD HEALTH ORGANIZATION (WHO) CLASSIFICATION: [26]

I. Tumors of thyroid follicular or metaplastic epithelium

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

II. Tumors showing C – cell differentiation

1. Medullary carcinoma
2. Mixed medullary and follicular cell carcinoma

III. Tumors showing both follicular and C – cell differentiation.

1. Collision tumor: Follicular/papillary and medullary carcinoma

IV. Tumors showing thymic or related branchial pouch differentiation

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

V. Tumors of lymphoid cells

1. Malignant lymphoma

2. Extramedullary plasmacytoma

VI. Intrathyroid parathyroid tumors

1. Parathyroid adenoma

2. Parathyroid carcinoma

VII. Mesenchymal and other tumors

1. Benign and malignant mesenchymal tumors, such as solitary fibrous tumor, smooth muscle tumor, peripheral nerve sheath tumor, angiosarcoma.

2. Paraganglioma

3. Teratoma.

I. TUMORS OF THYROID FOLLICULAR OR METAPLASTIC EPITHELIUM

HURTHLE CELL TUMORS

Hurthle cell tumors are neoplasms composed of large oncocytic cells with large centrally placed nucleus with prominent nucleolus and abundant brightly eosinophilic cytoplasm.

Hurthle cell tumors are usually encapsulated and bright brown in colour due to accumulation of mitochondria. [27,28] Mostly, these tumors present as solitary lesions, but rarely present as bilateral and multifocal lesions. [29] These tumors have different clinical, pathological and molecular profiles from that of other types of epithelial tumors of thyroid. Thus, Hurthle cell tumors (adenoma and carcinoma) are different

from FA and FTC, and are classified as separate entities in the new WHO classification 2017. [30]

The rate of malignancy is higher in hurthle cell neoplasms when compared to that of other non- hurthle cell neoplasms. [31,32]

HURTHLE CELL ADENOMA

Hürthle cell adenoma is a benign tumor without capsular and/or vascular invasion. Tumor tissue is arranged in microfollicular, trabeculae, solid sheets and occasional papillary pattern. Individual tumor cells show hurthle cell change. Occasional tumor cells may show nuclear grooves and pseudoinclusions. [33] These can be treated by lobectomy or nodulectomy. [34]

HURTHLE CELL CARCINOMA

It is a tumor affecting elderly men with features of capsular and/or vascular invasion. These tumors are larger, present at higher pathological stages and relatively radioiodine resistant. The treatment of these tumors is by total thyroidectomy and radiation ablation, as these tumors are capable of recurrence, regional and distant metastasis to lymph nodes, bone and lungs. [34]

Apart from the hurthle cell lesions, another significant change made in the new WHO classification 2017 is addition of a new sub group – The “Borderline” tumor group which includes three lesions as follows.

1. Follicular Tumor of Uncertain Malignant Potential (FT-UMP)
2. Well-differentiated Tumor of Uncertain Malignant Potential (WDT-UMP)
3. Non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP)

1. FOLLICULAR TUMOR OF UNCERTAIN MALIGNANT POTENTIAL (FT-UMP)

Follicular tumor of uncertain malignant potential is a tumor indeterminate between FA and FTC which is an encapsulated or well-circumscribed tumor composed of well-differentiated follicular cells with no PTC type nuclear changes and showing questionable capsular or vascular invasion. [35]

2. WELL-DIFFERENTIATED TUMOR OF UNCERTAIN MALIGNANT POTENTIAL (WDT-UMP)

It is an encapsulated or well-circumscribed tumor composed of well differentiated follicular cells with well or partially developed papillary thyroid carcinoma–type nuclear changes and showing questionable capsular or vascular invasion.

3. NON-INVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES

It is defined as a non-invasive neoplasm of thyroid follicular cells arranged in follicular pattern with PTC nuclear features. It was previously classified as non-invasive type (encapsulated) FVPTC. However in the present 2017 WHO classification, it is a separate entity due to the extremely indolent behaviour of this tumor when compared to other variants of PTC. Also this tumor is associated with RAS mutations rather than BRAF mutations in PTC.

PAPILLARY CARCINOMA (PTC): [33]

PTC is the most common malignant tumor of the gland in patients with iodine sufficient or iodine-excess diets, comprising about 80–85% of thyroid malignancies.

Previous irradiation to the head and neck region, radiation exposure or Hashimoto's thyroiditis increases the risk for papillary carcinoma. [36] It can be seen in any age group. The male to female ratio is 1: 2.5.

Patients generally present with painless neck mass.

Gross: Tumors are white to tan. Surface is granular due to the papillae with irregular and infiltrative border. A gritty surface and hard consistency is common due to calcification. Extension beyond the thyroid gland capsule can be identified along with infiltration into surrounding thyroid parenchyma. Few tumors are circumscribed. [35]

Microscopy: The presence of papillary growth pattern is the characteristic feature for the diagnosis of papillary thyroid carcinoma in the past, however, in the later period, the cytomorphological features are important as well for the diagnosis of PTC. [37] Cytomorphologic features are vital in the diagnosis of papillary carcinoma and are usually constant even between the variants.

The nuclei are enlarged, oval in shape having high nuclear to cytoplasmic ratio and nuclear overlapping. Few of them show variability in size and shape. There is up and down placement of nuclei due to cell overcrowding and loss of polarity. [35] Nucleus stains pale like ground glass in appearance due to clearing which is termed as 'Orphan Annie' nuclei which is due to formalin fixation artefact. The nuclei shows peripheral rim of condensation and margination of chromatin. This is present in more than 80% of PTC cases, however it is not pathognomonic of PTC. It is also seen focally in benign conditions like nodular hyperplasia, Graves' disease, Hashimoto's thyroiditis and FA. The nucleus in these conditions are round, enlarged in the central zone which is due to the 'blowing up' of the nuclei due to delayed fixation while the nuclei in the peripheral zone is hyperchromatic. [33]

Presence of nuclear grooves is also a typical feature of PTC. It is formed due to the deep folding of the nuclear membrane. Nuclear grooves are seen in other lesions like Hashimoto's disease, diffuse hyperplasia, follicular neoplasms, hyalinising trabecular adenoma, poorly differentiated thyroid carcinomas and adenocarcinoma of non-thyroid origin. [38] Nuclear pseudoinclusions are recognized as 'vacuole' formation due to the intra nuclear herniation of pockets of cytoplasm. [35] Mitotic figures are rare to absent in PTC. Cytoplasm is variable and usually not helpful in the diagnosis, although variants are named according to the cytoplasmic features. Squamous differentiation is seen in more than 50% of the cases. [33]

Architectural features:

Most of the cases of PTC are infiltrative, but some of them are circumscribed with cystic change.

Stroma: Abundant sclerotic stroma with calcification, laminated calcified structures known as psammoma bodies are characteristic of PTC. Psammoma bodies are seen in the stalks of the core, stroma or among the tumor cells. Calcified colloid is a mimicker of psammoma bodies which should be differentiated and are seen in Hurthle cell adenoma, Hurthle cell carcinoma and hyalinizing trabecular adenoma.

Many variants of PTC are present based on various types of nuclear, architectural and stroma. [33]

Variants of PTC: [33]

1. Conventional/classic
2. Papillary microcarcinoma
3. Encapsulated
4. Follicular

5. Diffuse sclerosing

6. Tall cell

7. Columnar cell

8. Cribriform-morular

9. Hobnail

10. Papillary thyroid carcinoma with fibromatosis/ fasciitis-like stroma

11. Solid/trabecular variant

12. Oncocytic

13. Spindle cell

14. Clear cell variant

15. Warthin like variant

16. Micropapillary variant

FVPTC:

This group is comprised of macrofollicular and multinodular (diffuse) variants. [30] These tumors are either infiltrative or encapsulated. The encapsulated type is also called as Lindsay tumor. This tumor comprises of exclusively or almost exclusively follicular growth pattern. Tumor tissue is arranged in follicles varying in size and shape lined by the nuclei which show typical features of PTC. Focal abortive papillae can be seen. [39] Many times they can be misdiagnosed as adenomatoid nodule or FA. [40]

Diffuse follicular variant:

This is a tumor in young patients presenting with diffuse involvement of the thyroid lobes. The tumor is arranged in follicular pattern lined by nuclei with typical features of PTC. This variant is rare and aggressive with higher frequency of

metastasis to lymph node, pulmonary and bone compared to conventional PTC. However, the tumor responds excellently to radioactive iodine therapy and has a favourable prognosis.

Solid variant:

The tumor is arranged in solid areas of more than 50% or trabeculae and traversed by multiple delicate capillaries. The typical nuclear features of PTC are retained with absence of necrosis. [41] It is important to see for this variant as it is associated with a less favourable prognosis and slightly higher frequency of metastasis.

Encapsulated variant:

The tumor is totally lined by a fibrous capsule which may or may not show invasion. At foci infiltration can be seen. However, the metastasis to lymph node is not dependent on capsular or vascular invasion. This variant carries an excellent prognosis. This variant constitutes to 10% of total cases of all cases of PTC which have excellent prognosis with almost 100% survival rates.

Diffuse sclerosing variant:

This type is most commonly seen in young adults and children where the thyroid tissue is replaced by the white firm tissue presenting with unilateral or bilateral symmetric thyroid swelling. [42] The typical histological feature is extensive lymphatic permeation. Other features are diffuse involvement, heavy lymphoplasmacytic infiltrate, sclerosis, plenty of psammoma bodies, small islands of PTC and areas of squamous differentiation. This variant is associated with a higher metastatic potential to extra thyroid and lymph node metastasis however with

treatment of this condition, its mortality is comparable to that of conventional PTC probably attributable to the young age.

Tall cell variant:

This variant is seen most commonly in the older age group and these are comprised predominantly of tumor cells where height of each individual tumor cell is at least three times more than their width. They are bulkier with extensive papillae, cytoplasm is abundant and oxyphilic with sub nuclear clearing, hence, this variant is also called as "pink-cell" variant of PTC. Heavy lymphocytic infiltration is present within the papilla and in the stroma. [11,43] Necrosis and mitotic activity is present. These are aggressive and show frequent extrathyroidal extension. Prognosis is less favourable than that of usual PTC.

Columnar cell variant:

This is an aggressive and rare variant of PTC. This variant needs to be identified because it has an extremely poor outcome, with death occurring within 5 years of diagnosis. [44] These present as large tumors measuring more than 6 cm in diameter with a male predilection and mean age of presentation is 57 years. Cases with invasive growth have an aggressive course showing regional lymph node and distant metastases especially to lung and vertebra. Whereas the encapsulated ones have a favourable prognosis. There is prominent papillary growth with markedly elongated, parallel follicles ('railroad tracks') which are separated by scant colloid. [35] Tumors which are invasive often show extrathyroid extension. Encapsulated tumors may show capsular and sometimes vascular invasion. Papillae are lined by tall columnar follicular cells with hyperchromatic oval or elongated nuclei showing pseudostratification. Psammoma bodies are rare. The characteristic and

pathognomonic nuclear features of usual PTC are absent in this variant. Few tumor cells also show clear cell change, in this condition, the cytoplasm clearing is similar to a vacuole confined to the sub nuclear zone differing from the clear cell variant of PTC. [11]

Oncocytic variant:

This variant is comprised predominantly of large tumor cells, large central orphan annie type of nucleus with prominent nucleolus and abundant eosinophilic granular cytoplasm due to the accumulation of mitochondria, however, focal clearing of cytoplasm may be seen due to the mitochondrial ballooning. Pattern of growth may be papillary or follicular, and the tumor may be encapsulated or invasive, this resulting in a number of possible combinations: encapsulated oncocytic, encapsulated oncocytic follicular, oncocytic and oncocytic follicular.[7] The behaviour is similar to conventional PTC and this tumor should be differentiated from Hurthle cell follicular neoplasms as the latter have a poor survival.

Warthin Tumor – like variant:

Previously considered as a subtype of variant of PTC. [30] Now it has been separated as Warthin Tumor – like variant resembling Warthin tumor of the salivary gland by the presence of papillary pattern and rich lymphoplasmacytic infiltrate within the papillary core lined by cells with oncocytic change. [33]Individual tumor cells have abundant eosinophilic cytoplasm. Also, in few tumors at the invasive edge of tumor, transition to tall cell variant is noted. [11] The prognosis of this variant is similar to that of conventional papillary PTC with aggressive clinical behaviour. [30]

Clear cell variant:

This is a rare variant of papillary carcinoma with tumor cells showing clearing of the cytoplasm extensively, due to glycogen accumulation, few cases can also show focal oxyntic change. [33]

Trabecular variant:

This variant presents with large and invasive masses associated with poor prognosis. [45] Tumor tissue shows trabecular arrangement of tumor tissue in more than half of tumor tissue. Individual tumor cells are columnar to cuboidal with cells placed perpendicular to axis of the trabeculae.

Cribriform – morular variant: [11]

This is a rare variant of PTC exclusively seen in women characterized by a prominent cribriform, solid and spindle cell patterns, with interspersed squamoid morules that frequently harbour nuclei filled with lightly eosinophilic, homogenous, biotin containing inclusions. [30,33] This variant show APC Beta catenin gene mutations. Characteristically, the luminal spaces are devoid of colloid. The tumor cells are cuboidal to columnar.

PTC with fibromatosis/ fasciitis –like stroma: [33]

It is characterized by an exaggerated and prominent stromal reaction to the tumor. The stroma is similar to nodular fasciitis or fibromatosis composed of spindle cells which are myofibroblastic in nature present within a vascularized fibromyxoid matrix and extravasated red blood cells. The epithelial tumor cells can be scant and the lesion can be misdiagnosed to a non-epithelial neoplasm, hence a careful search is required to identify the tumor epithelial cells.

Hobnail variant:

This is a rare aggressive variant of papillary carcinoma with high mortality rates. [30,46] Tumor is usually multifocal. Morphologically, this variant is characterized micropapillary growth pattern with absence of true fibrovascular core lying in lacunar spaces. The individual tumor cells are columnar to cuboidal in shape with low nucleus to cytoplasm ratio, eosinophilic cytoplasm and apically placed nucleus with loss of cellular cohesion resulting in a hobnail appearance. These tumor cells must constitute more than 30% of the tumor tissue. Necrosis, mitosis, angiolymphatic invasion and metastasis are common. [30,33]

Micropapillary variant:

This rare variant of papillary carcinoma is associated with a poor prognosis. Morphologically, this variant is characterized by micropapillary growth pattern with absence of true fibrovascular core lying in lacunar spaces and such morphological variation is seen in more than 5% of tumor area. [33]

Microcarcinoma (Papillary Microtumor) [11,33]

Microcarcinoma should be used for a papillary carcinoma, which measures 1 cm or less in diameter. [26] It is found incidentally. Few authors have defined up to 1.5 cm as well. Other terms used for this tumor are occult papillary carcinoma, occult sclerosing carcinoma, nonencapsulated sclerosing tumor and small papillary carcinoma. Occasionally micro carcinomas can present with lymph node metastases. [26] Distant metastasis is very rare. A familial form of papillary microcarcinoma is seen where the tumors are multifocal, increased propensity for vascular and lymphatic invasion, distant metastasis and even result in death. The importance to identify this

tumor is to avoid over treatment as microcarcinoma which is incidentally noticed and confined to thyroid gland is of no clinical importance.

FOLLICULAR NEOPLASM:

The thyroid epithelial tumors showing follicular cell differentiation but lack diagnostic features of PTC are classified as follicular neoplasm.

They include benign and malignant epithelial tumors namely FA and FTC respectively.

Clinical features: follicular neoplasms contribute to less than 5- 10% of thyroid tumors. Incidence being higher in areas of endemic goitre where iodine deficiency is a major contributing factor. [47] Dyshormonogenesis and irradiation are also predisposing factors.

FOLLICULAR ADENOMA: Most common age group is 20 to 50 years with a female preponderance (M:F=1:6). Most patients present with a thyroid nodule.

FOLLICULAR CARCINOMA: It is seen in patients with a higher age group (5^{th}-6^{th} decade) in contrary to PTC. The metastatic spread is via haematogenous route to bone, lungs, brain and liver.

Macroscopy: FA and FTC cannot be distinguished on gross. However the latter has a thickened capsule. The size of the lesion ranges from 1 to 10 cm. cut section shows soild fleshy, tan to light brown in colour with focal areas if secondary changes like haemorrhage and cystic degeneration are present. In invasive FTC, a discrete capsule may not be seen and invasion into the adjacent thyroid gland may be seen.

Microscopy: FA and minimally invasive FTC are enveloped by a fibrous capsule. The arrangement of tumor is in the form of tight follicles, trabeculae, solid sheets and

occasionally papillary pattern. Individual tumor cells are cuboidal to low columnar with dark to pale stained central round nuclei, inconspicuous nucleoli and moderate amount of eosinophilic/oxyphilic/ clear cytoplasm. Scattered enlarged hyperchromatic nuclei may be seen with few tumors showing nuclear pleomorphism.

Criteria to distinguish FA and FTC

The only characteristic distinguishing feature is presence of vascular / capsular invasion in FTC. Therefore careful evaluation of tumor thyroid interface has to be done.

Vascular invasion:

The criteria for definitive diagnosis of vascular invasion applies exclusively to the involved veins which are located within or beyond the fibrous capsule. The polypoid tumor growth within the blood vessel should be covered by endothelium. The tumor tissue in blood vessels present within the tumor tissue is not considered as vascular invasion and is not of prognostic value.

Capsular invasion:

The complete transgression of the fibrous capsule must be seen and the tumor bud has to extend beyond an imaginary line drawn through the external contour of the capsule.

Both vascular and capsular invasion phenomena are interrelated. A tumor bud invades through the capsule to extend directly into a vascular space.

Sub classification of FTC:

(1) Minimally invasive (capsule invasion only),

(2) Encapsulated angioinvasive and

(3) Widely invasive.

Variants of follicular adenoma:

Hyalinising Trabecular Adenoma and Carcinoma:

It is an unusual variant of FA confused for paraganglioma, medullary carcinoma, or papillary carcinoma. It is characterized by long, coiled trabeculae or packets of elongated to polygonal cells with lightly eosinophilic cytoplasm. The cells are arranged perpendicularly in trabeculae. There are frequently interspersed microcysts and larger cystic spaces representing abortive or true follicle formation. The oval nuclei exhibit fine chromatin, and some degree of pleomorphism may be present. Nuclear grooves, pseudoinclusions and perinuclear haloes are frequent findings. Unique cytoplasmic yellow bodies are present, which are often perinuclear, pale yellow, spherical, and refractile, measuring up to 5 μm; they are surrounded by a clear halo and are shown ultrastructurally to represent giant lysosomes. The exceptionally rare examples of hyalinizing trabecular carcinoma are distinguished from hyalinizing trabecular adenoma by the presence of vascular and/or capsular invasion. Neoplasms composed predominantly of large follicles are termed “macro follicular”, those of normalized follicles “normofollicular”, and those of small follicles “micro follicular” or “fetal”. Neoplasms showing a trabecular/solid pattern are termed “trabecular” or “embryonal”. [33]

Clear cell adenoma: This tumor comprises more than 50% of the tumor tissue. [30]

Oncocytic adenoma: These solitary well encapsulated tumors are characterized by a distinct mahogany brown appearance, often with central areas of scarring. Microscopically, the tumor is composed of cells with abundant granular eosinophilic cytoplasm and large open nuclei with prominent nucleoli. Adenomatoid oncocytic

nodules often occur in association with Hashimoto's thyroiditis and are difficult to distinguish from adenomas. [22]

FA with papillary hyperplasia: It is also known as papillary variant of follicular adenoma. It is usually encapsulated and partially cystic. It comprises broad or delicate branching papillae as well as follicles, lined by columnar cells with uniform, round and hyperchromatic nuclei. By definition the nuclear features of papillary carcinoma should be absent. This tumor occurs predominantly in children and adolescents and may be multiple. [22]

Fetal adenoma: This variant is characterized by microfollicular/trabecular structure in an oedematous stroma, particularly in the centre of the tumor. [22]

Signet –ring cell: This variant is characterized by signet ring tumor cells with a discrete cytoplasmic vacuole displacing the nucleus to the periphery. The vacuoles are immunoreactive for thyroglobulin. [22]

Mucinous: A variant characterized by accumulation of abundant extracellular mucin, often accompanied by a microcystic, reticular or multicystic growth pattern. Typical features of follicular neoplasm are often evident in some areas of the tumor, in addition there can be signet ring cell change. [22]

Atypical adenoma: The term atypical adenoma has been variably used to refer to follicular neoplasms exhibiting high cellularity, nuclear atypia or unusual histologic patterns but lacking vascular or capsular invasion on through sampling. [22]

Other variants that can be encountered are lipoadenoma, clear cell follicular adenoma, toxic (hyperfunctioning) adenoma and follicular adenoma with bizarre nuclei.

MUCOEPIDERMOID CARCINOMA:

Primary mucoepidermoid carcinoma is a rare malignant tumor of thyroid. It is a low grade tumor. Females have a slight predominance. On microscopy, tumor tissue is not circumscribed. It is comprised of tumor tissue arranged in cribriform pattern, cellular islands with few nests showing central elongated lumina containing colloid like material in a sclerotic background. The nuclei are hyperchromatic with mild pleomorphism. Two types of tumor cells are seen, squamoid type and other cells show mucinous differentiation. Few larger cell islands also show comedo-type of necrosis. Few psammoma bodies are also noted. [18]

SCLEROSING MUCOEPIDERMOID CARCINOMA WITH EOSINOPHILIA:

The tumor tissue is arranged in small sheets, anastomosing trabeculae and cords. It is seen in cases of lymphocytic thyroiditis. Background shows dense and diffuse inflammatory cell infiltrate comprised of eosinophils and fibrosis. Very frequently they metastasize to lymph nodes and show extracapsular spread, vascular invasion and perineural invasion. [15]

MUCINOUS CARCINOMAS:

It is a very rare tumor of thyroid. On microscopy, the hallmark of the tumor is tumor tissue arranged in strands or clusters present in a background of abundant mucoid lakes. Individual tumor cells show large regular nuclei and prominent nucleoli. Focal squamous differentiation may occur. [24]

POORLY DIFFERENTIATED CARCINOMA: [30]

Poorly differentiated thyroid carcinoma displays biologic, genetic, histologic features indeterminate between well-differentiated and anaplastic thyroid carcinomas.

It is seen in elderly patients with a slight female preponderance. (F:M =1.6: 1 to 2: 1). In general, response to radioiodine treatment is poor. [51]It was previously called as insular carcinoma/ primordial cell carcinoma.

The histologic criteria for poorly differentiated carcinoma are

(1) A diagnosis of carcinoma of follicular cell derivation (by conventional criteria);
(2) Solid, insular, or trabecular growth,
(3) Absence of conventional nuclear features of papillary thyroid carcinoma and
(4) At least 1 of the 3 features: convoluted nuclei ("dedifferentiated" nuclear features of papillary carcinoma), mitotic activity 3 or more per 10 high-power fields, or tumor necrosis. [30]

Extensive necrosis is common and may result in a "peritheliomatous" appearance. The stroma is usually sclerotic and can simulate amyloid. The prognosis is poor compared to that of well differentiated thyroid carcinoma but better than that of anaplastic thyroid carcinoma. [11]

UNDIFFERENTIATED (ANAPLASTIC) CARCINOMA: [33]

Undifferentiated carcinoma of the thyroid is comprised of undifferentiated thyroid follicular cells. This tumor constitutes to 2-5% of the carcinomas of thyroid more commonly seen in elderly women with a mean age of 70 years. Depth of invasion, regional lymphatic and distant metastases involving primarily the lungs and bones are frequent. It is a very aggressive tumor with poor patient survival. [30]

The tumor tissue shows frank invasion and individual tumor cells are highly pleomorphic. It is categorized into 3 patterns: giant cell, sarcomatoid, and epithelial. Focal areas resemble epithelial cells arranged in clusters and sheets of large polygonal

or round cells, occasional squamous cell foci are also noted. Most of the tumors are exclusively sarcomatoid with individual tumor slender or plump spindle cells arranged in intersecting fascicles and show moderate to marked nuclear atypia resembling fibrosarcoma, undifferentiated pleomorphic sarcoma, so-called hemangiopericytoma, angiosarcoma, and rhabdomyosarcoma. Many mitotic figures, coagulative necrosis and neutrophilic infiltrate is seen. Permeation of the blood vessel walls accompanied by obliteration of the vessel lumen by tumor is present which is a characteristic feature of undifferentiated thyroid carcinoma. It may transform into differentiated carcinoma, especially the papillary phenotype being the common one.

Many morphologic variants are present based on the tumor architecture like angiomatoid variant, osteoclastic variant, rhabdoid variant, lymphoepithelioma- like carcinoma, paucicellular variant, carcinosarcoma, adenosquamous carcinoma and squamous cell carcinoma. [11]

Immunohistochemistry: Tumor tissue is positive for cytokeratin (CK). PAX-8 is noted in approximately of 50% of the carcinomas and should be done to confirm the thyroid origin. [30] The genetic profile is complex with multiple genetic alterations most common being p53 mutation.

II. TUMORS SHOWING C – CELL DIFFERENTIATION MEDULLARY CARCINOMA [33]

Medullary carcinoma is a malignancy of thyroid gland with parafollicular C-cell differentiation. [48] Many peptide products are secreted predominantly being calcitonin. The presenting complaints are thyroid mass, pain, dysphagia, hoarseness, cervical lymphadenopathy and diarrhoea rarely, very few patients present with Cushing syndrome which is due to the production of adrenocorticotropic hormone

secretion (ACTH). Most of the cases (70% to 80%) of medullary carcinomas are sporadic, whereas only 20% to 30% of the cases belong to the hereditary form which are inherited by autosomal dominant type. [49]

Medullary thyroid carcinoma comprises less than 10% of all thyroid malignancies. This tumor is of great diagnostic importance because of its aggressiveness, its close association with multiple endocrine neoplasia (MEN) syndromes (MEN2A and MEN2B) and a relationship to a C –cell hyperplasia as the probable precursor lesion. [50, 51]

Gross: Medullary carcinoma is predominantly located in the middle third of the lateral lobe, region where the C-cell density is highest and presents as a small, firm, circumscribed, tumors being more infiltrative in nature. Rarely, it is completely encapsulated. On cut section, tumor is grey-white, tan, or reddish brown, areas of hemorrhage and central necrosis may be seen in larger sized tumors.

Microscopy: The tumor tissue are predominantly arranged in sheets, packets and irregular islands traversed by prominent delicate fibrovascular septa and occasionally pseudo papillary, whorled, micro glandular, trabecular, rosette, tubular or cribriform pattern can be observed. Individual tumor cell cells are polygonal to spindle, spindled, few are plasmacytoid shaped with round or oval nuclei, finely stippled chromatin and indistinct nucleoli. Only mild nuclear pleomorphism is seen with and infrequent mitotic figures. Cytoplasm is moderate in amount and finely granular. Amyloid is seen in 80% to 85% of cases, in the form of globules or massive deposits. Areas of calcification or foreign-body giant cell reaction can be present.

Variants of Medullary Thyroid Carcinoma

Many histologic variants of medullary carcinoma are present. Many of them pose problems in recognition, search for focal areas of conventional medullary carcinoma should be looked for.

Glandular or Follicular:

Tumor tissue predominantly arranged in follicles containing eosinophilic secretion. The luminal side is more deeply eosinophilic granular due to the increased number of neurosecretory granules.

Oxyphilic:

The tumor cells are oxyphilic (oncocytic) appearance due to the accumulation of mitochondria in the cytoplasm of medullary carcinoma.

Giant Cell (Anaplastic):

The tumor tissue may exhibit focal areas of pleomorphic large cells with bizarre nuclei and nuclear pseudoinclusions. However, mitotic rate is low.

Papillary:

The tumor tissue can rarely be arranged in pseudo papillae and focal areas of true papillae. The pseudo papillae are due to the tumor fragments. These tumors have to be distinguished from PTC by their nuclear features. Differentiating it is important because this variant is associated with a more favourable prognosis.

Other variants like pseudoangiomatous, small cell, clear cell, spindle cell, pigmented, squamous, neuroblastoma like, hyalinizing trabecular adenoma like, carcinoid like and paraganglioma like types are also seen.

III. TUMORS SHOWING BOTH FOLLICULAR AND C-CELL DIFFERENTIATION [33]

The epithelial tumor of thyroid gland showing both follicular and C-cell differentiation is called as a collision tumor.

The two types of collision tumors are

1. Follicular carcinoma and medullary carcinoma [52]
2. Papillary carcinoma and medullary carcinoma [53,54]

Both the components are either contiguous or intermingled. The behaviour of these tumors is determined by the more aggressive component present.

FOLLICULAR CARCINOMA AND MEDULLARY CARCINOMA:

Mixed medullary and FTC is a very rare tumor of thyroid showing intermingled follicular and parafollicular components. This tumor is also known as follicular-parafollicular carcinoma or differentiated carcinoma of intermediate type. The hypothesis of origin of two components is predominantly of clonal origin from a common stem cell, but few cases also show a dual origin.

Gross: The tumors are usually unencapsulated.

Microscopy: The tumor shows both features of medullary carcinoma with admixed follicular structures. Few of them also show deposits of amyloid. By definition, all cases show both immunoreactivity for thyroglobulin and calcitonin. Follicular, cribriform areas and focal areas in the solid component show diffuse strong positivity for thyroglobulin whereas strong positivity for calcitonin is seen in solid areas. Rarely, few tumor cells show dual hormone production. If both follicular and parafollicular components can be demonstrated in metastatic sites, the diagnosis is straightforward. Collision tumor must be differentiated from classic medullary

carcinoma with entrapped non neoplastic thyroid follicles. The former should have tumor tissue arranged in follicular pattern located deep within the tumor and should be lined by tumor cells with enlarged hyperchromatic nuclei.

These tumors spread by both lymphatic and haematogenous routes and are more aggressive than the differentiated thyroid carcinomas.

IV. TUMORS SHOWING THYMIC OR RELATED BRANCHIAL POUCH DIFFERENTIATION [33]

These tumors are rare and occurrence of them can be explained by the occasional presence of sequestered thymic tissue or branchial pouch derivatives in the thyroid. These tumors include

1. Ectopic thymoma, [55]
2. Spindle epithelial tumor with thymus-like differentiation (SETTLE)[56] and
3. Carcinoma showing thymus-like element (CASTLE).[57]

ECTOPIC THYMOMA:

Clinical Features: These tumors can occur within or attached to the thyroid. Most patients are middle-aged women presenting with a solitary thyroid nodule.

Gross: The tumor is encapsulated.

Microscopy: The tumor tissue is arranged in jigsaw puzzle–type lobulation. It is comprised of a variable admixture of pale-staining plump or spindled epithelial cells and small lymphocytes.

SPINDLE EPITHELIAL TUMOR WITH THYMUS-LIKE DIFFERENTIATION:

Clinical Features: Spindle Epithelial Tumor with Thymus-like Differentiation **(**SETTLE) is a rare tumor affecting the younger age group (4-59 years, with a mean of

18 years). It is seen most commonly in males. The patients usually present with a painless thyroid mass. The tumor has a tendency for distant delayed metastasis most commonly to the lungs.

Gross: The mean size of the tumor is 3.6 cm.

Microscopy: The tumor is either circumscribed or infiltrative. Stroma is sclerosed and it divides the tumor into incomplete lobules. The tumor tissue is arranged in interlacing fascicles, cords, tubules, or papillae. Individual tumor cells are spindle shaped which merge imperceptibly into epithelial structures. The nucleus is bland with fine chromatin, mitosis is infrequent. Focal areas of squamous differentiation, prominent mitotic activity, or focal necrosis can be seen in few tumors. Rarely, intrathyroidal mucous cysts are noted which are lined by ciliated epithelium present in the vicinity of the tumor, raising the possibility that tumor might have arisen from branchial pouch.

CARCINOMA SHOWING THYMUS-LIKE ELEMENT (CASTLE):

Clinical Features: Carcinoma Showing Thymus-like Element **(**CASTLE) is an intrathyroidal (ectopic) thymic carcinoma affecting the middle and old aged patients (mean age 48.5 years). The presenting complaint is thyroid mass. The tumor usually involves the lower pole of the thyroid and adjacent extra thyroidal tissues with regional lymph node metastasis in one third to one half of the cases. Treatment of choice is surgery followed by radiation therapy. The 5- and 10 year survival rates are 90% and 82%, respectively. The nodal metastasis and tumor extension to surrounding tissues (recurrent laryngeal nerve, trachea, oesophagus, muscle, jugular vein and carotid artery) predict a poor outcome.

Gross: The tumor is well-defined, hard, on cut section it is lobulated and grey tan in colour.

Microscopy: The tumor is infiltrating into the adjacent thyroid gland predominantly in pushing fronts. Tumor tissue is arranged in lobules or cords separated by broad and cellular fibrous septa. Individual tumor cells round to polygonal and few spindle shaped having predominantly indistinct cell borders, with vesicular nuclei and prominent nucleoli or cells show a squamoid appearance with more distinct cell borders. The tumor tissue and fibrous cellular septa show scanty to heavy lymphoplasmacytic infiltrate.

V. TUMORS OF LYMPHOID CELLS [33]

MALIGNANT LYMPHOMA:

Primary lymphoma of the thyroid is extremely rare comprising only 2.5% to 3% of all extranodal lymphomas and 4% to 5% of all thyroid malignancies. It usually occurs in a mean age group of 59-68 years and has a female predilection (M:F = 1: 2.5). The lymphoma commonly arises in a pre-existing case of Hashimoto's thyroiditis or lymphocytic thyroiditis.

Clinical Features: Varied presentation is seen

1. Rapidly enlarging thyroid mass accompanied by dysphagia or hoarseness,
2. A slowly growing thyroid nodule or multinodular goitre.
3. Gradual diffuse enlargement of the thyroid gland mimicking thyroiditis
4. Development of thyroid mass in a patient with long-standing Hashimoto's thyroiditis
5. Asymptomatic, hence as an incidental finding in thyroidectomy specimens.

Regional lymph node involvement can occur. As a group, the overall 5-year survival is 50% to 85%.

Gross: Lymphoma is an ill circumscribed, size ranging from 1cm to 14 cm, rubbery to soft in consistency presenting as a thyroid mass. On cut surface tumor tissue is bulging, fleshy, light tan, homogeneous with or without necrosis.

Histologic Subtypes of Primary Thyroid Lymphoma

Diffuse large B-cell lymphoma (>70% of all cases) and Extranodal marginal zone lymphoma of Mucosa- associated lymphoid tissue Type (MALT) type account for a large number of cases. Rarely, follicular lymphoma, Burkitt lymphoma, Intravascular large B-cell lymphoma and T-cell lymphomas occur in the thyroid.

EXTRANODAL MARGINAL ZONE LYMPHOMA OF MUCOSA ASSOCIATED LYMPHOID TISSUE TYPE (MALT):

These are indolent tumors that tend to remain localized in the thyroid. The prognosis is generally excellent with a good response to radiotherapy but worsened when complicated by large cell transformation. Many reactive lymphoid follicles are often interspersed among the diffuse lymphomatous infiltrate. The lymphoma cells are centrocyte-like cells, with slightly irregularly folded, darkly stained nuclei and scant to broad rim of pale cytoplasm. There is a tendency to invade and expand the thyroid follicles, forming lymphoepithelial lesions. Admixed plasma cells are commonly seen, which exhibit Dutcher bodies or cytoplasmic immunoglobulin inclusions.

DIFFUSE LARGE B-CELL LYMPHOMA:

This tumor may arise either de novo or by the transformation of extranodal marginal zone lymphoma. The diffuse lymphomatous infiltrate obliterates the thyroid tissue. The tumor cells are large and possess round vesicular nuclei with prominent

nucleoli and moderate amount of amphophilic cytoplasm. Many multinucleate or bizarre tumor cells can be seen. Individual tumor cells often infiltrate the thyroid follicles, replacing the follicular epithelium or plugging up the follicular lumens. Vascular invasion can also be seen.

FOLLICULAR LYMPHOMA:[58]

This tumor occurs with a median age of 50 years and predominantly in women. Commonly, it is seen in a background of lymphocytic thyroiditis or Hashimoto thyroiditis. Histologically, tumor tissue is comprised of extensive lymphoid infiltration with formation of neoplastic follicles. A prominent interfollicular component is often present, being more evident than in nodal follicular lymphoma. Lymphoepithelial lesions are also common.

Two distinct groups of thyroid follicular lymphoma are recognized.

1. Group I is similar to classic nodal follicular lymphoma with BCL2 gene rearrangement. The patients usually present with high-stage disease.
2. Group II is similar to the minority BCL-2 negative follicular lymphoma of extranodal sites or lymph nodes with lack of BCL2 gene rearrangement. The patients often have early-stage disease (stage I).

EXTRAMEDULLARY PLASMACYTOMA:[11]

Primary extramedullary plasmacytoma of the thyroid is extremely rare. It is similar to extramedullary plasmacytoma of other head and neck sites, comprising a pure infiltrate of monoclonal plasma cells, which can demonstrate variable degrees of atypia.

LANGERHANS CELL HISTIOCYTOSIS:

Langerhans cell histiocytosis is a clonal proliferation of Langerhans cells involving the thyroid gland as the sole lesion or represent a disseminated disease affecting patients with a wide age range (median 37 years) and no sex predilection. Thyroid gland can show patchy or extensive infiltration by tumor tissue comprised of sheets of Langerhan cells. The individual tumor cells have grooved or convoluted nuclei with a thin nuclear membrane, and moderate amount of eosinophilic cytoplasm. [59] Occasional multinucleated histiocytes are also present scattered throughout the thyroid gland and variable number of eosinophils with areas of eosinophil abscesses are seen. The tumor tissue shows positivity for immunostaining by S-100, CD1a and langerin.

VI. MESENCHYMAL TUMORS AND OTHER TUMORS ANGIOSARCOMA:

Clinical Features: Angiosarcoma is a rare tumor involving the thyroid gland. The aetiology can be iodine deficiency, it affects older adults with a mean age group of 66 years, with a male predominance (M:F = 1.2 : 1). [59] These tumors are highly aggressive, with a median survival of only 3.5 months frequently metastasise to organs like lung, pleura, lymph node, adrenal, gastrointestinal tract, and bone.

Gross: The tumor presents as a single nodule, on cut section the tumor tissue is grey in colour comprised of a central hole filled with coagulated or fluid blood, bordered by a layer of “rubber hyaline” that blends with adjacent rim of tumor tissue. Areas of necrosis and hemorrhage are noted.

Microscopy: The tumor is highly pleomorphic with vasoformative areas in the form of irregular vascular slits or anastomosing channels with pleomorphic tumor cells showing cytoplasmic vacuoles. The epithelioid variant is characterized by polygonal

neoplastic endothelial cells with vesicular nuclei with amphophilic or basophilic nucleoli and abundant eosinophilic cytoplasm.

SOLITARY FIBROUS TUMOR:

Solitary fibrous tumor is a rare tumor occurring as a primary thyroid tumor seen in male adult patients with rare malignant behaviour.

Gross: Tumor has well-circumscribed borders.

Microscopy: The tumor tissue is well circumscribed but focal jagged infiltration or entrapment of thyroid follicles in the peripheral portion of tumor tissue is seen. Hypercellular and hypocellular areas are noted with haphazardly distributed bland-looking spindle to stellate cells and scanty cytoplasm. The stroma comprises of delicate to thick collagen fibres. [11,59]

SMOOTH MUSCLE TUMORS:

Smooth muscle tumors of the thyroid gland are very rare. Spectrum of tumors range from benign lesions like leiomyoma to malignant tumors like leiomyosarcoma. Leiomyoma is an encapsulated lesion ranging from a size of 1.5 to 3.5 cm. On microscopy, the tumor tissue is arranged in interlacing fascicles. Individual tumor cells are spindle cells with blunt ends having cigar shaped nucleus and moderate amount of eosinophilic cytoplasm. On the other end, leiomyosarcoma is comprised of mitotically active atypical spindle cells with eosinophilic cytoplasm. Areas of invasion into adjacent thyroid tissue, necrosis, hemorrhage and extra thyroidal extension are noted. [11]

PERIPHERAL NERVE SHEATH TUMORS: [33]

Nerve sheath tumors arising in the thyroid gland are also very rare. Schwannoma pursues a benign course, whereas malignant peripheral nerve sheath tumor is highly aggressive.

PARAGANGLIOMA: [33,59]

Paragangliomas within the thyroid gland are very rare and probable aetiology is these tumors arise from the inferior laryngeal paraganglia which are located within the capsule of the thyroid gland. They are more commonly seen in females between 40 and 60 years age group.

Gross: These tumors are circumscribed or can extend into the adjacent larynx or trachea of approximately 2 cm in size.

Microscopy: Tumor tissue is arranged in alveolar pattern, individual tumor cells are oval in shape with high N:C ratio, round to oval hyperchromatic nuclei and finely granular cytoplasm these tumor cells are surrounded by an inconspicuous layer of sustentacular cells. The stroma is typically richly vascularized predominantly, areas of oedema, hyalinization, or sclerosis are also noted.

IMMUNOHISTOCHEMISTRY IN THYROID LESIONS

To distinguish benign from malignant lesions and for the definitive diagnosis of follicular lesions of the thyroid many immunohistochemical markers have been used. [60,61]

1. **Cytoskeletal proteins** are the primary valuable markers in tumor diagnosis. In the diagnosis of epithelial cell tumors, a class of intermediate sized filaments,

the CK are utilized as they are expressed in neoplastic epithelium. Cytokeratin 19 (CK19) is a low molecular weight protein expressed in both normal epithelia and tumors of thyroid. Strong and diffuse expression in PTC and heterogeneous expression in FA is seen to CK19 antibodies. Thus, it can be used to distinguish the FVPTC from FA and nodular hyperplasia. [13,61] It is a very sensitive marker for PTC. The staining pattern is diffuse and cytoplasm of tumor cells stain positive. However, its specificity is less as it shows positivity in normal thyroid follicular epithelial lining, Hashimoto thyroiditis and other benign thyroid tumors.[12]

2. **HBME-1** is a monoclonal antibody which is useful in the diagnosis of malignant tumors of follicular or metaplastic epithelium particularly PTC. [61]
3. **ERK** ("extracellular signal-related kinase") is a part of the effector pathway of the RAS oncogene signal transduction pathway. It leads to the activation of RAF kinase which helps in the phosphorylation of the effector genes. One of the isoforms, B-RAF is involved in many tumors including PTC. The RET/PTC oncogenes signal transduction pathway have been found in sporadic as well as radiation-associated PTC. Mutations of RET/PTC, RAS or BRAF are found in about 70% of PTC. [61]
4. **P16INK4a** is a tumor-suppressor gene. It modulates the activity of cyclin dependent kinases (CDK) which are useful for cell cycle progression. In the cell cycle, CDK along with D-cyclins promotes progression through the G1 phase. Loss of function of P16INK4a impairs the control of the cell cycle and its mutation has been associated with the development of various tumors like PTC and medullary thyroid carcinomas. Hypermethylation of the promoter

region of p16 gene, is associated with tumor progression and dedifferentiation in thyroid tumors rather than deletions or mutations. [61]

5. **Gal-3** is a member of b-galactoside binding lectin family. This protein is expressed in many tissues at either nuclear or cytoplasmic levels. It is involved in cell to cell and cell to matrix interactions, cell cycle regulation, cell repair, apoptosis and neoplastic transformation. It is increased in several human malignant tumors like metastasis in breast, gastric, colon carcinoma and well-differentiated follicular-derived thyroid carcinomas. [13,62] Gal-3 is rarely detected in normal thyroid tissue and benign lesions, while its expression is seen in malignant thyroid tumors by immunohistochemistry. Many studies in the literature evaluating Gal-3 reported high sensitivity and specificity in differentiating malignant from benign thyroid lesions. [63] It is expressed predominantly localized at the luminal surface in the cytoplasm of the follicular cells, nuclear localization can also be observed or rarely both nuclear and cytoplasmic positivity. In the thyroid stroma, fibroblasts, endothelial cells, smooth muscle cells, macrophages, lymphocytes and neutrophils also display Gal-3 expression. [64] Gal-3 expression by normal follicular cells in inflammatory areas is due to the permeation of Gal-3 shed by the lymphocytes into the adjacent follicular cells or by the influence of cytokines secreted by the inflammatory cells. [64] In non-neoplastic lesions like lymphocytic and Hashimoto's thyroiditis, follicular cells with oncocytic changes also show strong positivity to Gal-3. It is a positive marker of malignancy (not expressed in benign lesions or normal tissue). In addition, Gal-3 mRNA has been observed in all malignant thyroid lesions, whereas in normal and non-malignant tissues it was not detectable. [65-67] For these reasons Bartolazzi A *et*

al clearly stated that Gal-3 is useful as a marker to differentiate benign from malignant thyroid neoplasms. [68]

6. **Thyroid peroxidase** (TPO) is a negative marker expressed in benign lesions and normal tissue but not in cancer. [69] TPO is a thyroid-specific enzyme involved in the synthesis of thyroid hormone. It reflects normal thyroid function and, therefore, should not be expressed in malignant tissue.
7. **CD56**: It is a neural cell adhesion molecule. It is expressed in thyroid follicular cells and adrenal glands. It shows membranous positivity. However in PTC, the tumor cells are negative for CD56 and occasionally show cytoplasmic positivity. Hence, to differentiate PTC from follicular lesions, CD56 can be used. The sensitivity and specificity is high for differentiating PTC from other follicular lesions.
8. **Claudin 1**: It is a member of claudin family, is one of the components of tight junction. Increased claudin 1 expression is noted in PTC cases. However recent studies show that immunohistochemical expression of Gal-3, HBME-1 and CK-19 demonstrated that a combination of two or a panel of markers may more effectively distinguish benign from malignant thyroid lesions. [62]
9. **p27**: In the literature, the cases of papillary hyperplasia of Graves' disease showed a twofold greater expression than the cases of papillary carcinomas. [70]
10. **Topo II alpha**: It is associated with cell proliferation. During mitoses it is helpful to prevent nondisjunction and chromosome breakage. It has diagnostic and prognostic significance in many tumors. Among thyroid neoplasms, it is commonly expressed in tumors with aggressive clinical behavior like

anaplastic carcinomas, tall cell variant of papillary carcinomas, FTC, Hurthle cell carcinomas, and medullary carcinoma. [70]

11. **PAX 8, Hector Battifora mesothelial-1, Peroxisome proliferator-activated receptor-g and Sodium/iodide symporter.**

METHODOLOGY

A cross-sectional study was carried out on thyroidectomy specimens that fulfill the inclusion criteria and received at Department of Pathology from Department of Surgery & Department of Otorhinolaryngology, BLDEU Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapur from 1st December 2015 to 30th June 2017.

Inclusion criteria:

1) Surgically resected thyroid tissue with histomorphology showing papillary pattern.

Exclusion criteria:

1) On histomorphology thyroid tissue not showing papillary pattern.

Procedure: All thyroidectomy specimens were collected in 10% buffered formalin. Examined for gross characteristics and kept for fixation in 10% NBF (Neutral Buffered Formalin). After overnight fixation, representative areas were selected for paraffin embedding. Sections were cut at 3-4 microns thick and stained with Haematoxylin and eosin. The microscopic features were assessed and diagnosis was rendered accordingly. Sections which followed the inclusion criteria were considered for immunohistochemistry using rabbit monoclonal antibody Gal-3.

Staining procedure of Immunohistochemistry:

- Neutral buffered formalin fixed paraffin embedded tissue sections of 2 to 3µm were taken on poly L lysine coated slides.
- Deparaffinization of slides were done at 60^0 C in oven for one hour.

- Slides were allowed to reach room temperature for 10 minutes
- Rehydration was done by two changes of 10 minutes in xylene, three changes in increasing graded alcohols for 10 minutes.
- It was followed by immersion in distilled water for 5 minutes.
- Antigen retrieval was done with Tri sodium citrate buffer (pH 6.0 to 6.2) in pressure cooker for 15 minutes.
- Slides were brought to room temperature for 15 minutes.
- Slides were then treated with endogenous peroxidase block for 10 minutes.
- Followed by treatment with power block for 10 minutes.
- Further, slides were washed in wash buffer, 2 changes, 10 minutes each.
- Treated with power block for 10 minutes, the solution was allowed to drain.
- Primary antibody with Gal-3 was applied for 45 minutes.
- Washed with TBS wash buffer, 3 times for 3 minutes.
- Super enhancer was added, for 20 minutes.
- Secondary antibody - Polymer HRP was applied for 30 minutes.
- Washed with wash buffer, 2 changes for 10 minutes each.
- Diamine Benzidine chromogen was applied for 5 minutes at room temperature.
- Washed with distilled water to stop chromogen reaction.
- Counter staining was done with Harris Haematoxylin for 30 seconds and then washed with tap water
- Slides were cleared in xylene.
- Slides were finally mounted with DPX.

- The immunohistochemical slides are examined and photographed at 100X. The percentage of positive cells is evaluated by counting number of labelled cells in 10 high power fields for each specimen.

0-3 scoring system [76]

- Negative staining =0
- Weak or rare staining < 5% =1+
- Focal or moderate staining 5-25% =2+
- Strong or diffuse staining >25% =3+

IHC Staining pattern: Gal-3 produces both cytoplasmic and nuclear staining. Staining pattern was compared with control slides. Prostatic tissue was used as positive control and normal thyroid tissue was considered as negative control.

RESULTS

Table 1: Distribution of Papillary patterned thyroid lesions

Diagnosis	N	Percent
Papillary hyperplasia	16	48.5
Papillary carcinoma	17	51.5
Total	33	100.0

Out of 33 cases, PTC were slightly higher compared to that of cases of papillary hyperplasia.

Figure 1: Distribution of Papillary patterned thyroid lesions

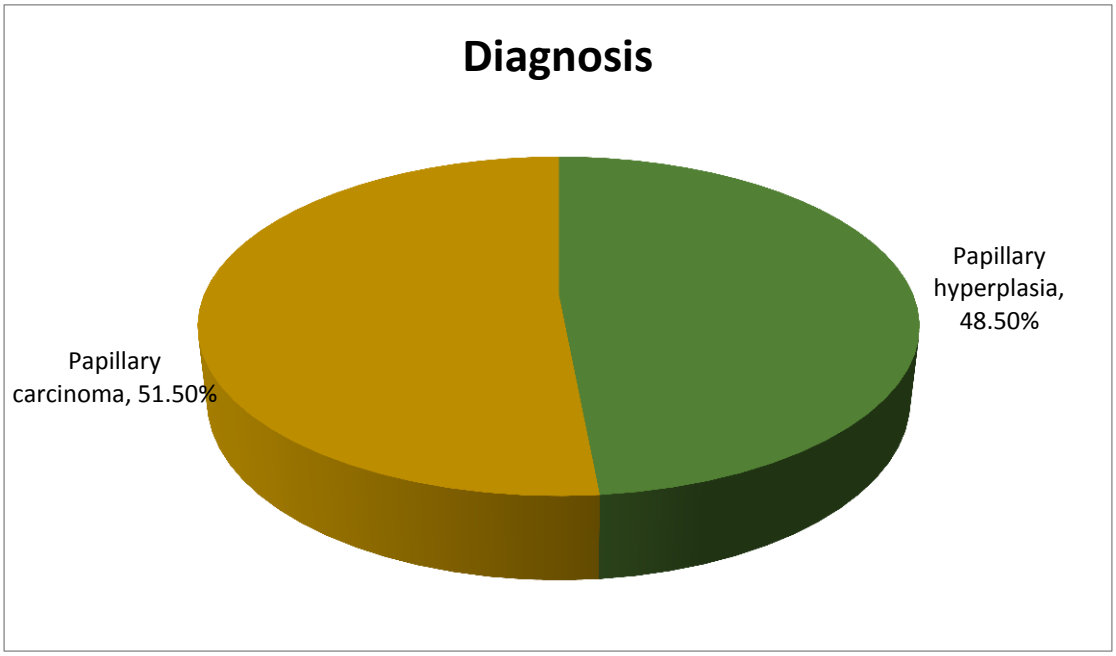
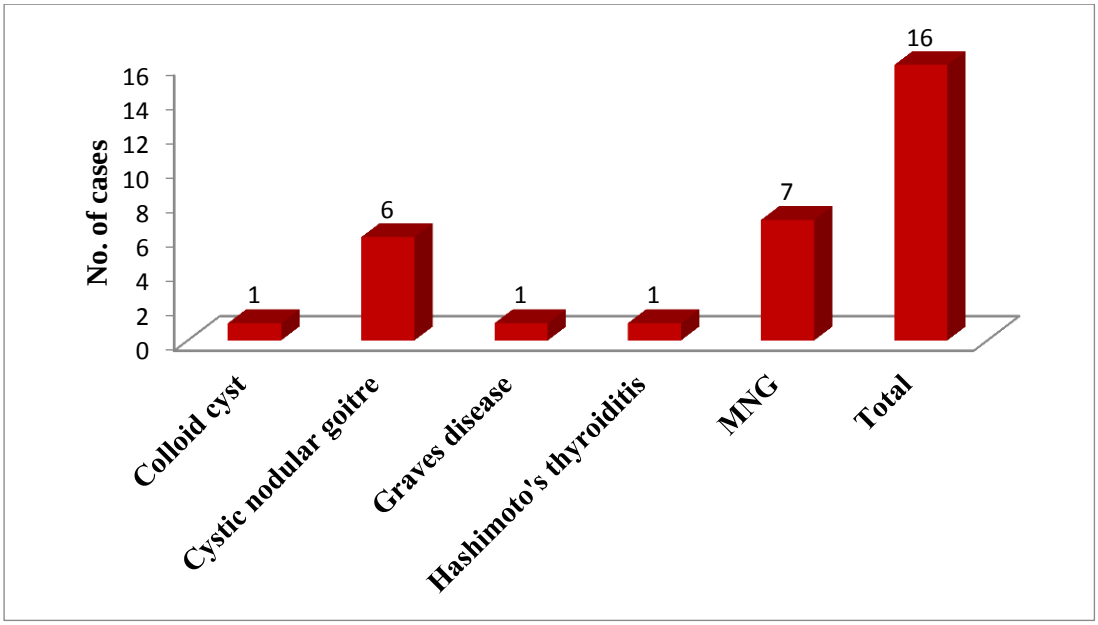


Table 2: Distribution of cases by Papillary hyperplasia and Papillary carcinoma

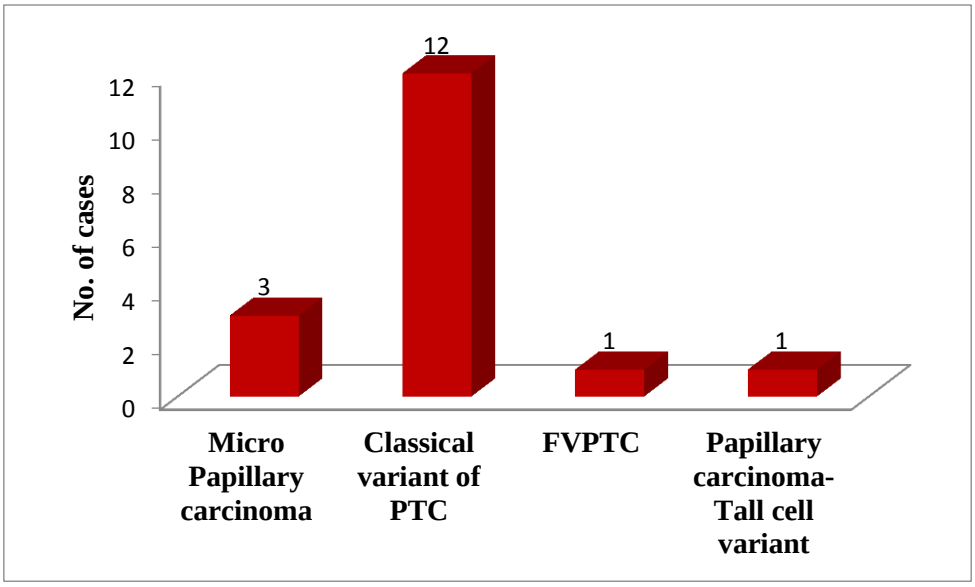
Diagnosis	Diagnosis	N	Percent
Papillary hyperplasia	Colloid cyst	1	3.0
	Cystic nodular goitre	6	18.2
	Graves' disease	1	3.0
	Hashimoto's thyroiditis	1	3.0
	MNG	7	21.2
	Total	16	48.5
Papillary carcinoma		17	51.5

Figure 2: Distribution of cases by Papillary hyperplasia



Out of 16 cases of papillary hyperplasia, majority of the cases are nodular goitre followed by colloid cyst, Graves' disease and Hashimoto's thyroiditis one case each.

Figure 3: Distribution of Variants of Papillary carcinoma cases.



Out of 17 cases of PTC, classical variant were the majority followed by micropapillary carcinoma, FVPTC and tall cell variant.

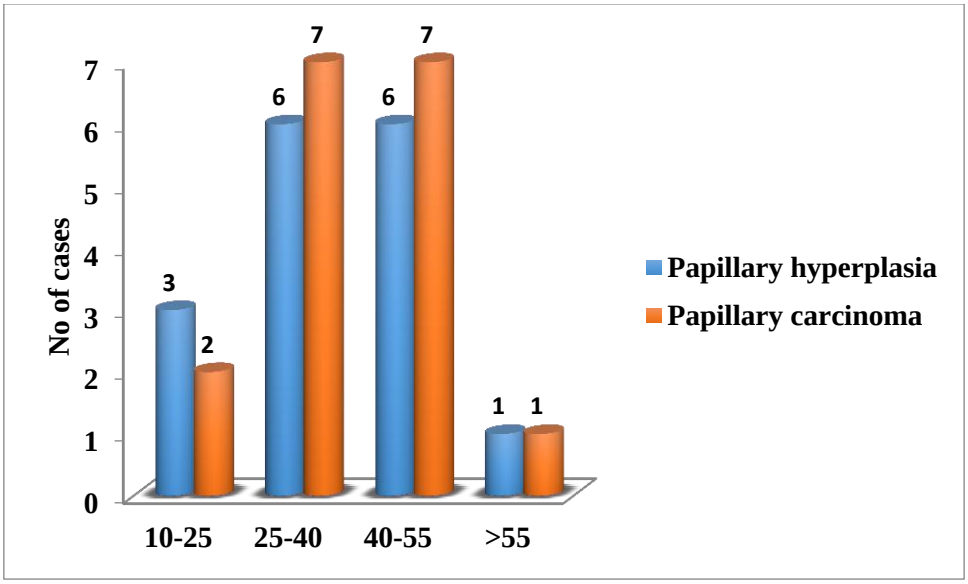
Age distribution:

The age distribution of the cases in our study were in the age group of 10-60 years. Both the non-neoplastic and PTC cases were majority between age group of 25 and 55 years.

Table 3: Age distribution

Diagnosis	Papillary hyperplasia		Papillary carcinoma		p value
Age (yrs)	N	Percent	N	Percent	
10-25	3	18.8	2	11.8	0.955
25-40	6	37.5	7	41.2	
40-55	6	37.5	7	41.2	
>55	1	6.3	1	5.9	
Total	16	100.0	17	100.0	

Figure 4: Age distribution



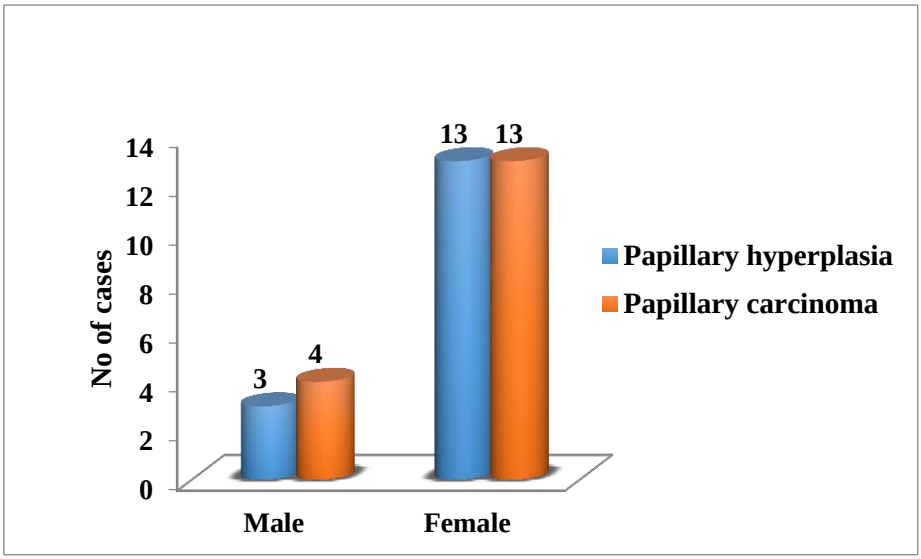
Sex distribution:

Majority of the cases- papillary hyperplasia and carcinoma (78.8%) are common in females.

Table 4: Sex distribution

Diagnosis	Papillary hyperplasia		Papillary carcinoma		p value
Sex	N	Percent	N	Percent	
Male	3	18.8	4	23.5	0.537
Female	13	81.3	13	76.5	
Total	16	100.0	17	100.0	

Figure 5: Sex distribution



Galectin -3 immunohistochemical staining:

Gal-3 immunostaining was sought and the staining pattern was assessed based on intensity and number of cells stained positive. Amongst the benign lesions, 14 cases (87.5%) showed negative staining and 2 cases (11.8%) showed positive staining for Gal-3. Amongst the PTC cases, 15 cases (88.2%) showed positive staining and 2 cases (12.5%) showed positive staining for Gal-3.

Table 5: Distribution of Gal-3 immunostaining in our study

Gal-3	Negative		Positive		p value
Diagnosis	**N**	**Percent**	**N**	**Percent**	
Papillary hyperplasia	14	87.5	2	12.5	<0.001*
Papillary carcinoma	2	11.8	15	88.2	
Total	16	100.0	17	100.0	

Note:*significant difference at 5% level of significance

Figure 6: Distribution of Gal-3 immunostaining in our study

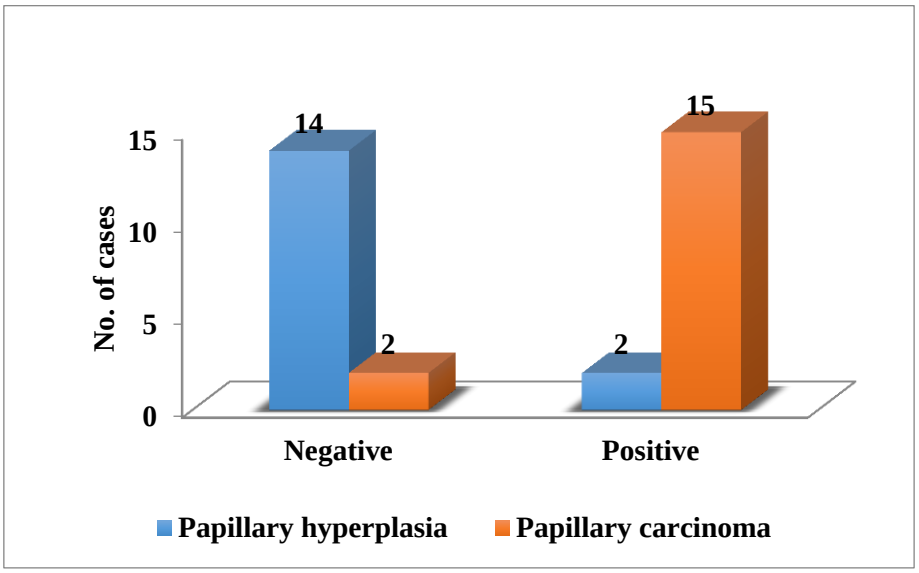


Table 6: Distribution of Gal-3 in Papillary hyperplasia and Papillary carcinoma

Gal-3		Negative		Positive		p value
Diagnosis		**N**	**Percent**	**N**	**Percent**	
Papillary hyperplasia	Colloid cyst	1	6.3	0	0.0	0.086
	Cystic nodular goitre	6	37.5	0	0.0	
	Graves' disease	0	0.0	1	5.9	
	Hashimoto's thyroiditis	1	6.3	0	0.0	
	MNG	6	37.5	1	5.9	
Papillary carcinoma		2	12.5	15	88.2	-

Figure 7: Distribution of Gal-3 in Papillary hyperplasia and Papillary carcinoma

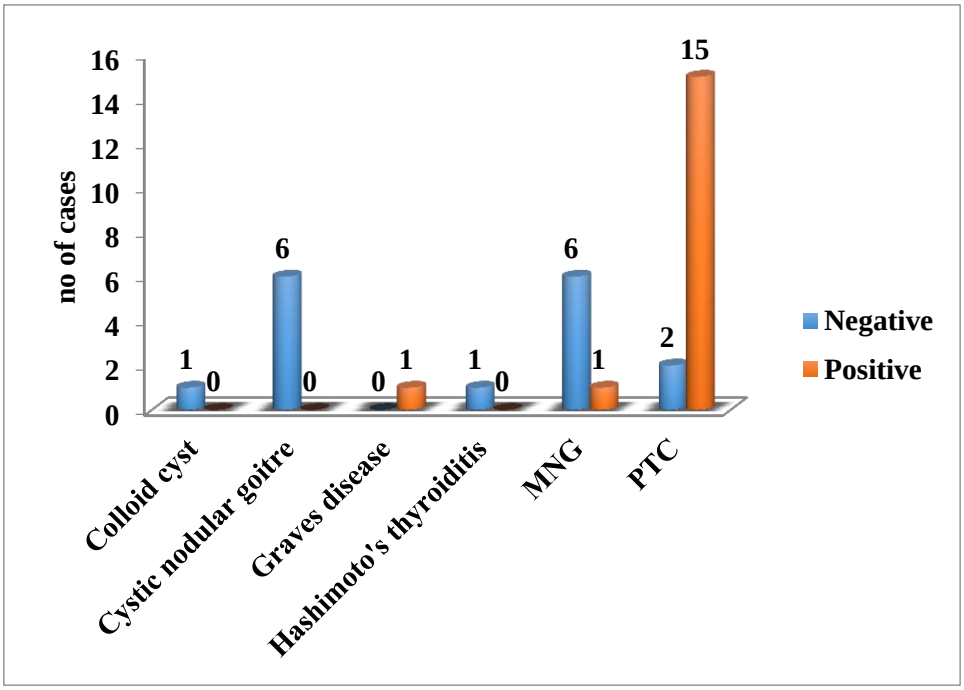


Table 7: Distribution of Gal-3 immunohistochemical Grading

Grade	0		2+		3+		Total		p value
Diagnosis	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	
Papillary hyperplasia	14	87.5	1	6.3	1	6.3	16	100	<0.001*
Papillary carcinoma	2	11.8	1	5.9	14	82.4	17	100	

Note:*significant difference at 5% level of significance

Figure 8: Overall distribution of Gal-3 immunohistochemical Grading

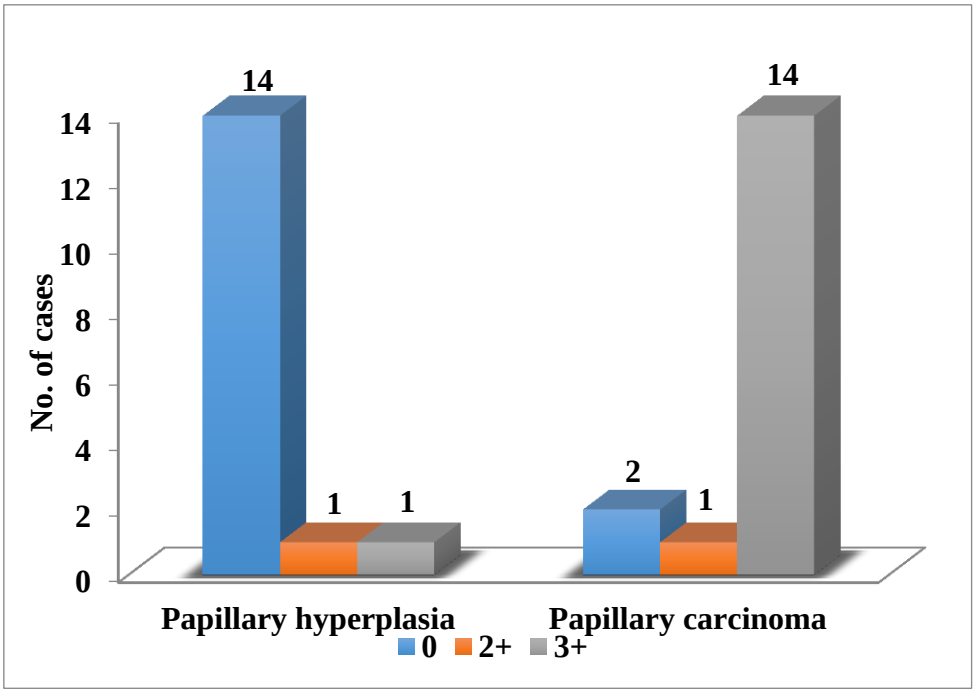


Table 8: Distribution of Gal-3 grading in various lesions

Grade		0		2+		3+		p value
Diagnosis		**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	
Papillary hyperplasia	Colloid cyst	1	6.3	0	0.0	0	0.0	0.027*
	Cystic nodular goitre	6	37.5	0	0.0	0	0.0	
	Graves' disease	0	0.0	0	0.0	1	6.7	
	Hashimoto's thyroiditis	1	6.3	0	0.0	0	0.0	
	MNG	6	37.5	1	50.0	0	0.0	
Papillary carcinoma		2	12.5	1	50.0	14	93.3	-

Note:*significant difference at 5% level of significance

Figure 9: Distribution of Gal-3 grading in various lesions

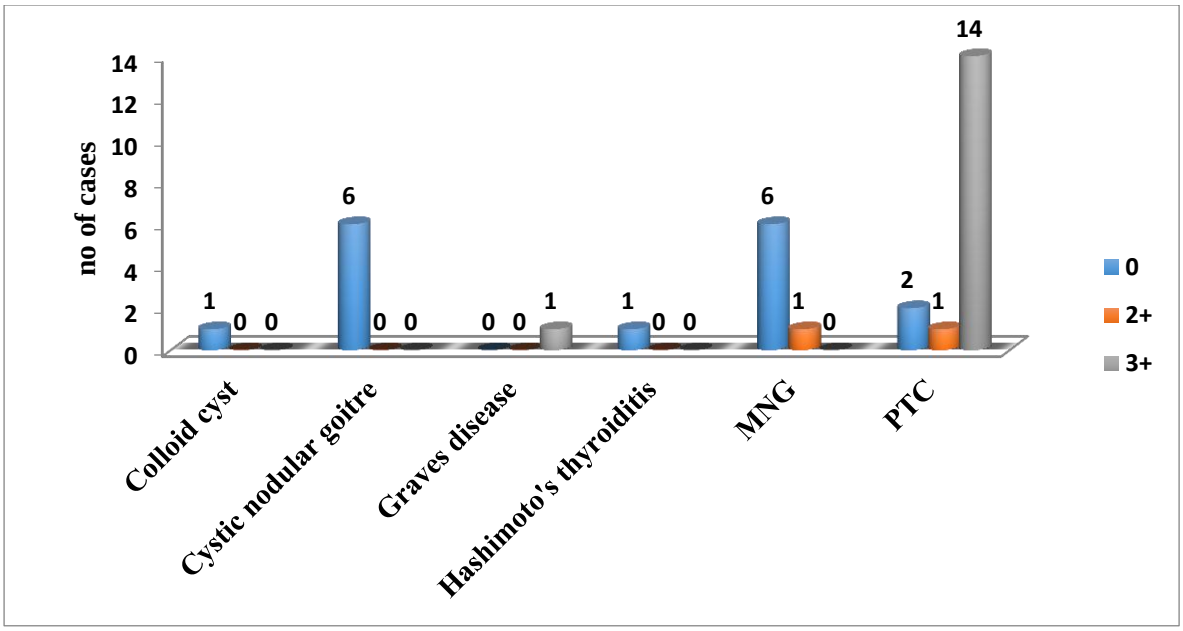


Figure 10: Distribution of Gal-3 grading in PTC & its variants

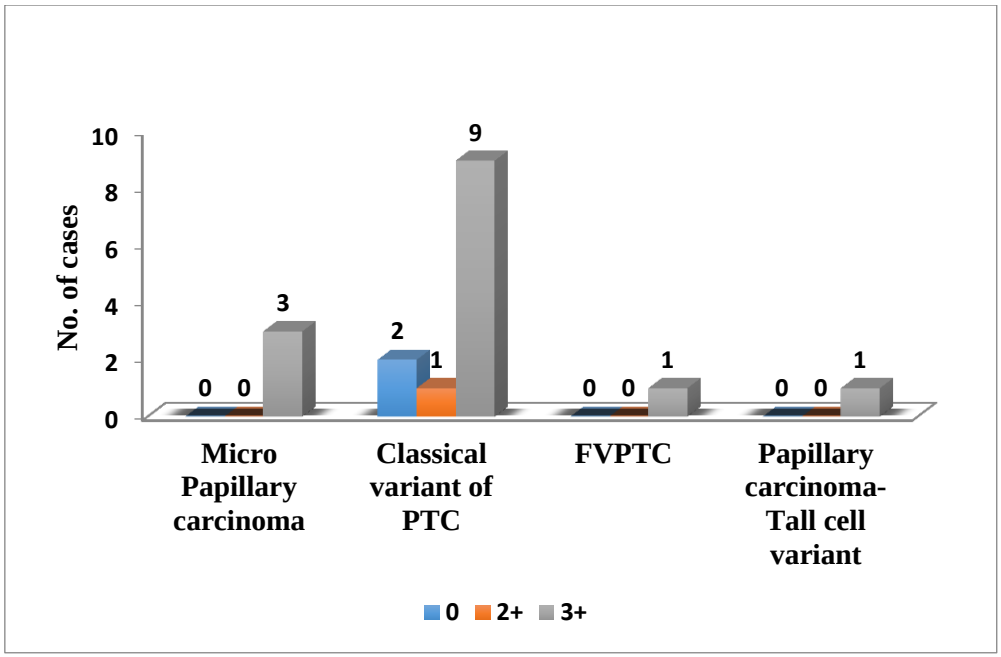


Table 9: Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of the present study.

Sensitivity	**88.24%**
Specificity	**87.50%**
PPV	**88.24%**
NPV	**87.50%**
Accuracy	**87.88%**

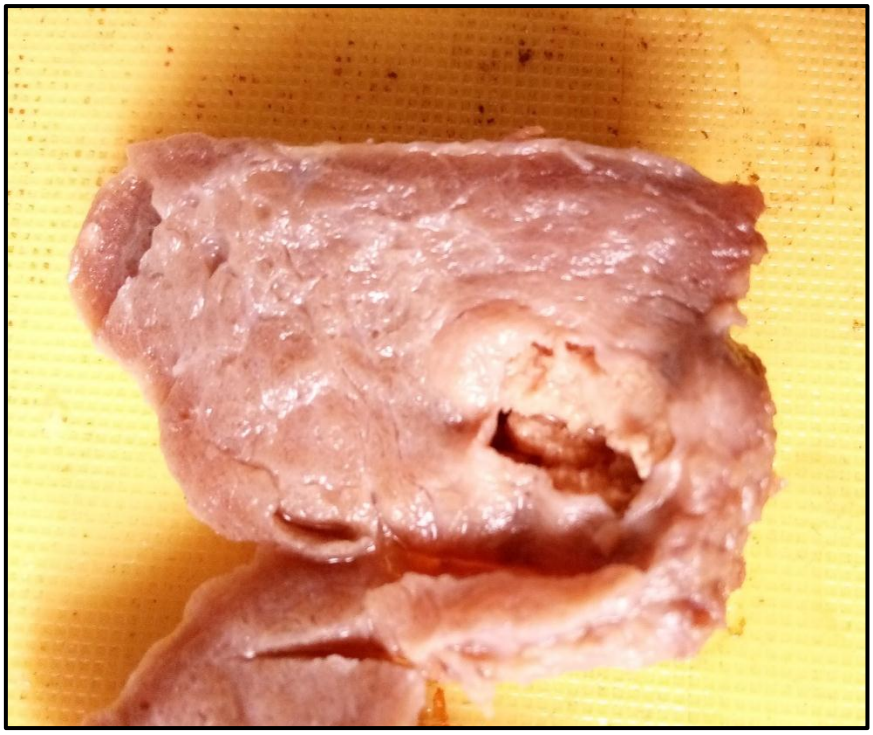


Figure 11: Gross image of PTC

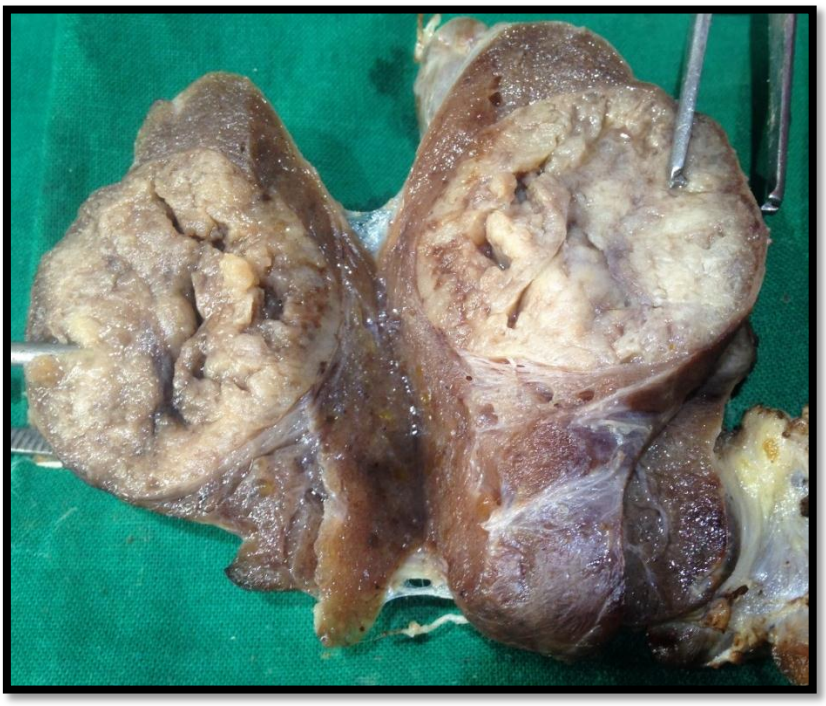


Figure 12: Gross image of Papillary hyperplasia in Nodular goitre

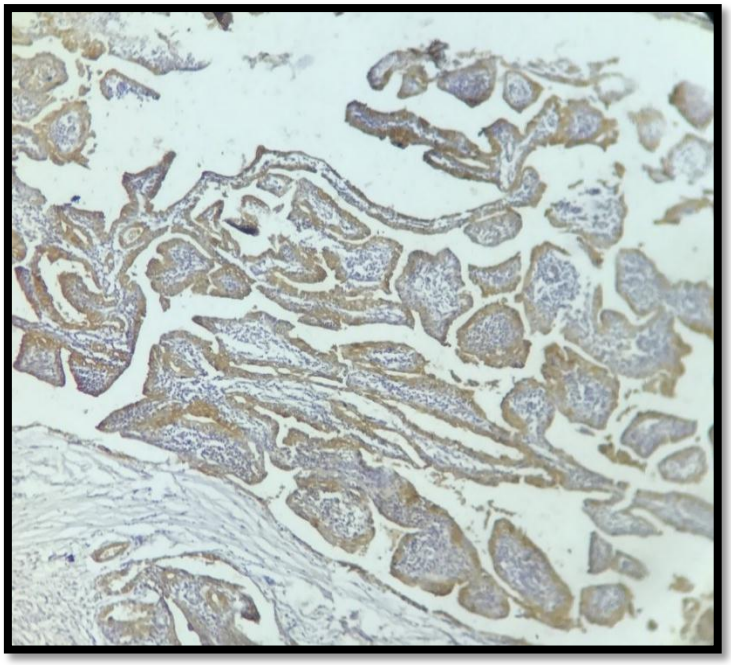


Figure 13: IHC 100X Papillary thyroid carcinoma- classical variant positive for Gal-3, Grade 3

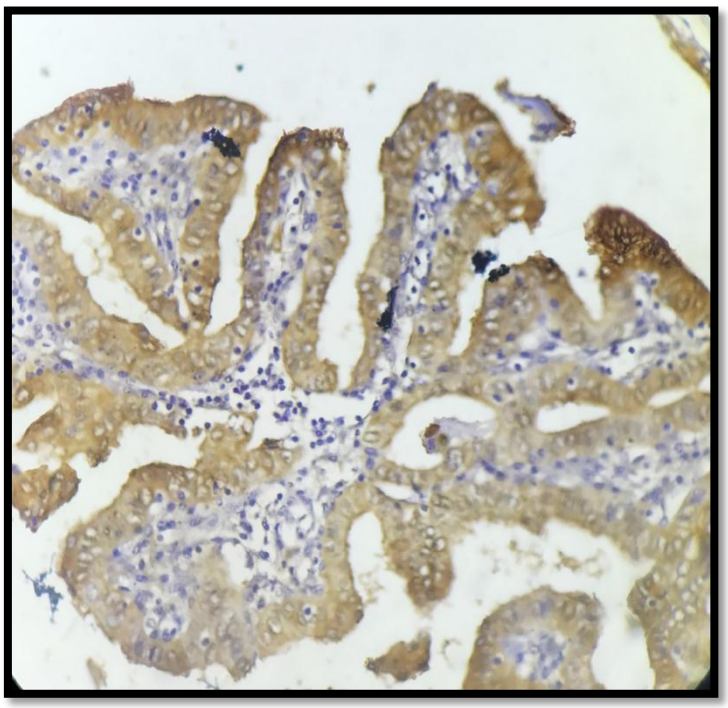


Figure 14: IHC 400X Papillary thyroid carcinoma- classical variant positive for Gal-3 ,Grade 3

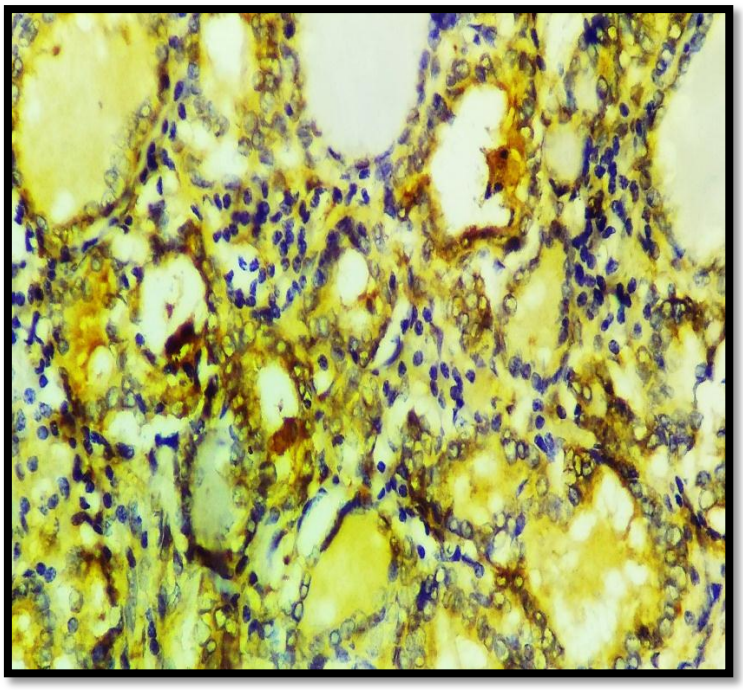


Figure 15: IHC 400X FVPTC positive for Gal-3, Grade 3

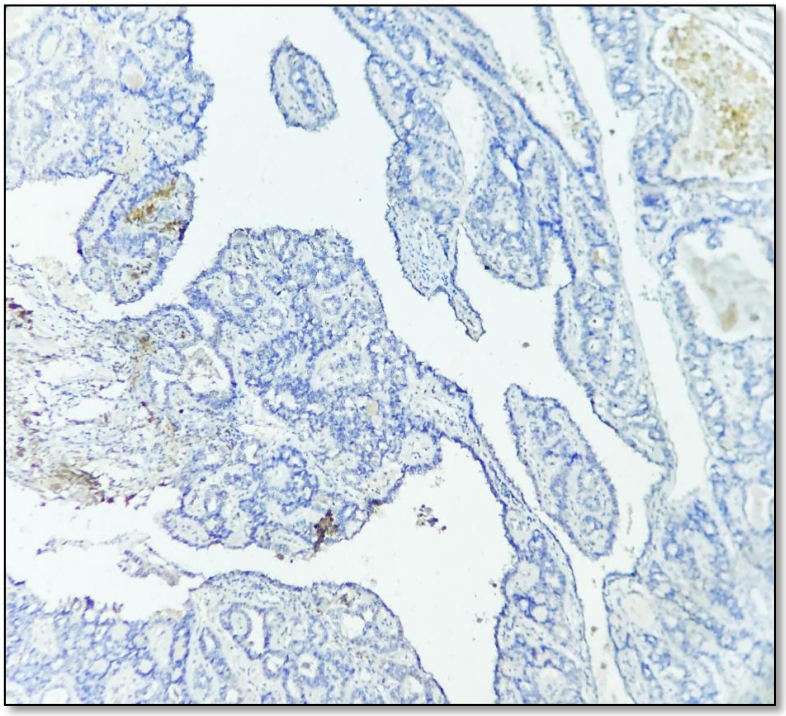


Figure 16: IHC 100X Nodular goiter with papillary hyperplasia- Negative for Gal-3,Grade 0

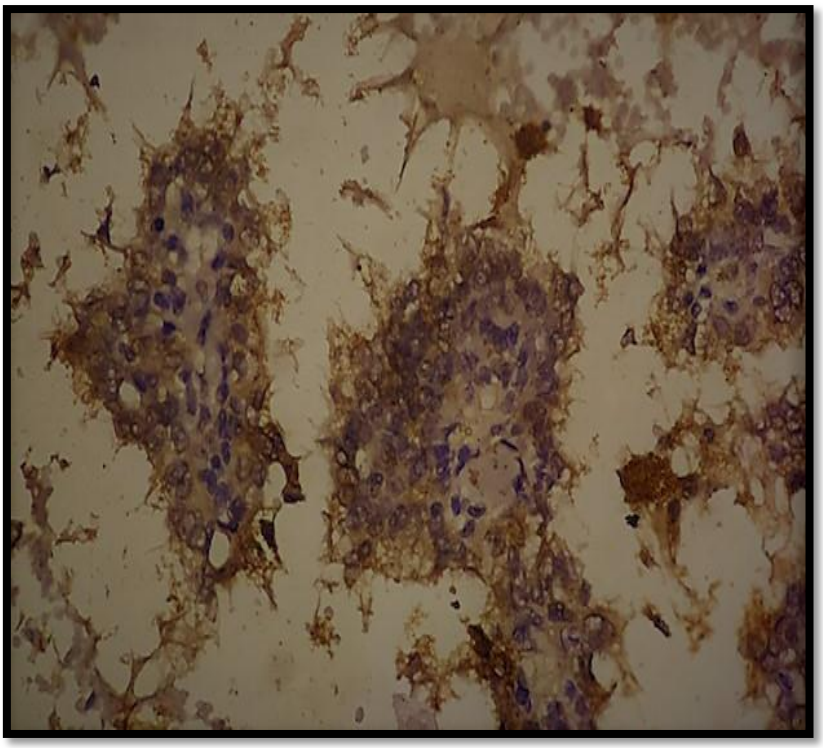


Figure 17: IHC 400X Micropapillary carcinoma - Positive for Gal-3, Grade 3

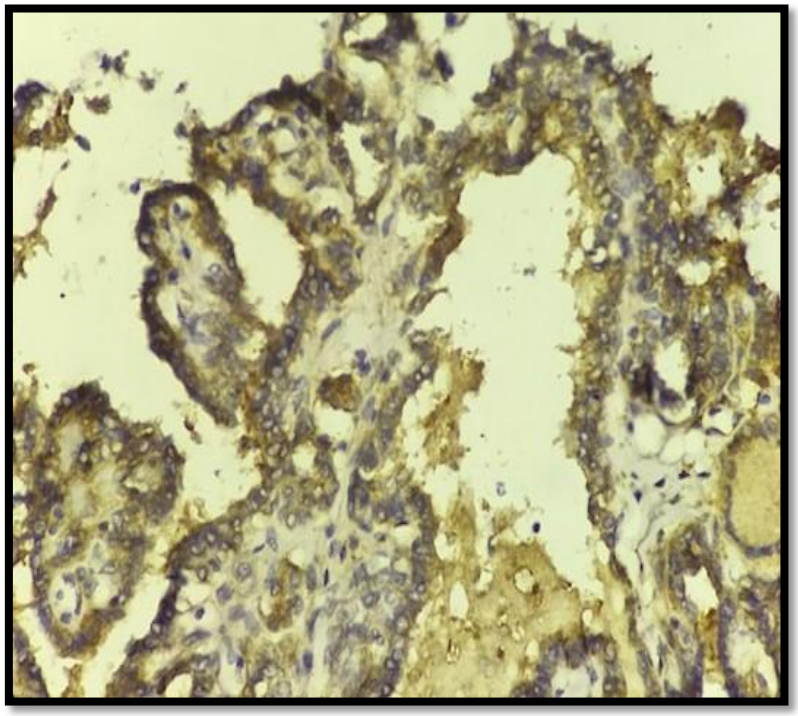


Figure 18: IHC 400X FVPTC - Positive for Gal-3, Grade 3

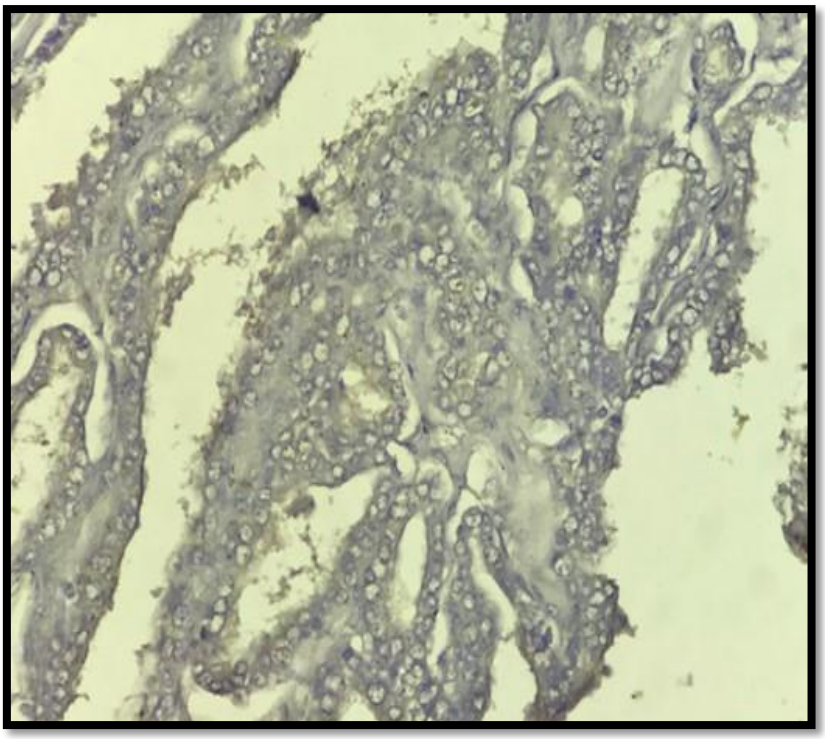


Figure 19: IHC 400X PTC - Negative for Gal-3, Grade 0

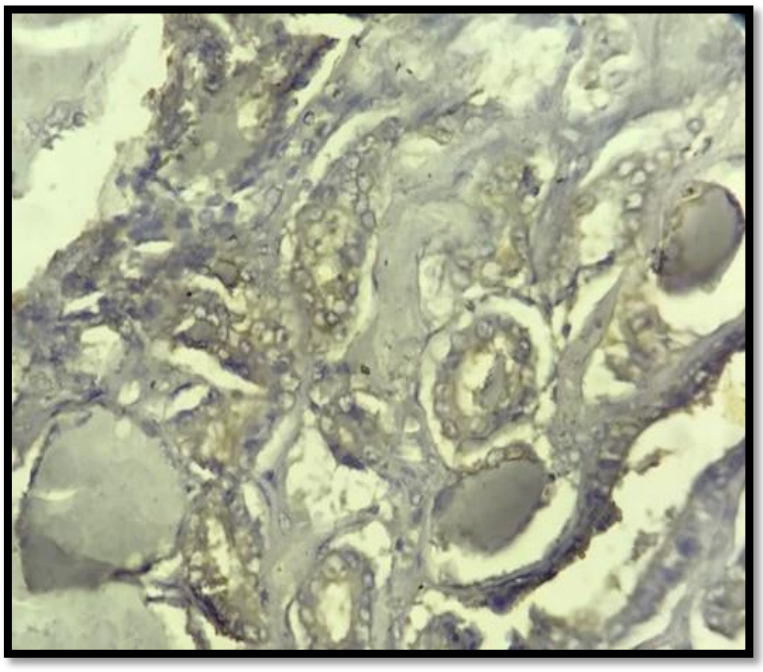


Figure 20: IHC 400X PTC - Positive for Gal-3, Grade 2+

DISCUSSION

Thyroid gland is affected by heterogenous group of lesions that are manifested by varied histomorphological patterns which can either produce diffuse and nodular growth.[11]

Both benign and malignant lesions of thyroid gland are frequently encountered. However, benign lesions are more common than malignancies. [26]

The malignant lesions of thyroid are most common among all the malignancies of the endocrine system. Predominantly, these tumors are of follicular cell origin followed by tumors of C cell origin, primary lymphomas and other non epithelial malignancies. [26]

PTC is the most common histologic type of carcinoma of the thyroid gland showing follicular cell differentiation with a distinct and characteristic nuclear features. However these microscopic features are not pathognomonic for PTC and thus it has to be differentiated from other mimickers as it has an excellent long term prognosis if appropriately diagnosed and treated.[33]

Presence of papillae alone is not a pathognomonic feature of PTC. They are seen in many other non-neoplastic and neoplastic lesions like MNG, thyrotoxicosis, congenital errors of thyroid metabolism, Hashimoto's thyroiditis, FA, Medullary carcinoma, Hurthle cell adenoma and Hurthle cell carcinoma. So, they must not be confused with PTC. [11,33]

The term papillary adenoma was used as a diagnostic term for all the above mentioned lesions with microscopic papillary patterns, however the term should not be used as it encompasses a broad range of lesions, on one end non neoplastic lesions

like nodular goitre, thyrotoxicosis, congenital errors of thyroid metabolism, Hashimoto thyroiditis and on the other end neoplastic lesions like toxic FA, encapsulated PTC, Medullary carcinoma, Hurthle cell adenoma and Hurthle cell carcinoma are present. The proliferation of follicular cells due to hypertrophy and hyperplasia lead to infolding which appear as papillations. [11]

The architecture of papillae is different in various thyroid lesions. The main important feature to distinguish diffuse papillary hyperplasia from PTC is the preservation of gland architecture in the former. [11]

Predominantly papillae in PTC are arborizing with delicate fibrovascular cores, few papillae can also be broad with oedematous, fibro cellular or hyalinised and these are lined by tumor cells which show characteristic nuclear features of PTC. Follicles varying in size and contour are present containing darkly stained colloid. [11]

The papillae in thyrotoxicosis, FA and Hashimoto thyroiditis are short, non-branching, protrude into the follicular lumen without a true fibrovascular core. [33]The diffuse nature of papillae also point towards non neoplastic nature of the lesion. [11]

Whereas papillae in nodular goitre and FA with papillary hyperplasia are predominantly extremely oedematous, haemorrhagic, broad comprised of thyroid follicles in the core and few delicate branching papillae are present which are lined by regular, columnar cells without crowding, basally placed round nuclei without clearing in a ‘beads on a string’ fashion. Absence of Psammoma bodies is also characteristic. [11,33]

Pseudo papillae with ‘rugged surfaces’ are seen in Medullary Carcinoma of thyroid due to cellular dehiscence. A small amount of papillae are also noted in Hurthle cell adenoma and Hurthle cell carcinoma which are non-arborizing and lining cells do not

show crowding. Occasionally, the lining cells show nuclear grooves, inclusions and calcified colloid mimicking psammoma bodies.

Most of the cases of papillary hyperplasia of Graves' disease can be easily distinguishable from papillary carcinoma based on the histologic and cytologic features. But, few cases of Graves' disease may simulate papillary carcinoma. Histologic features which simulate malignancy are well-developed papillary fronds with fibrovascular cores and large vesicular nuclei. Other histologic features, which help to differentiate are preservation of gland architecture, nuclear cytology, lack of stromal desmoplasia and psammoma bodies. However, the distinction between these may be difficult. [70]

Hence, in addition to histopathology, additional ancillary methods are required to distinguish papillary hyperplasia from PTC. Also studies done in the past by Hirokawa *et al,*[71] Franc B *et al*[72] and Fassina *et al*[73] revealed that there exists inter-observer variation in the diagnosis of thyroid neoplastic lesions. Hence immunohistochemistry is useful for diagnosis of thyroid lesions.

Gal-3 is one such marker, its immunohistochemical expression is used to aid in the accurate diagnosis of thyroid neoplasms. Similar to the literature, in the present study, on microscopy, papillary patterns were noted in cases of nodular goitre, colloid cyst, Graves' disease, Hashimoto thyroiditis, PTC and its variants. (Table:1, Fig 1&2) Variants of papillary carcinoma were identified including the classic variant, micropapillary carcinoma, one case of FVPTC and tall cell variant. (Table:2, Fig.3)

Sex distribution of PTC cases

In the current study papillary carcinomas show higher incidence in females than in males. (Table:4, Fig.5) Similarly in the studies done by Heitz *et al* [74] and Shrikhande *et al* [75] noted a higher incidence of papillary carcinoma in females, with female to male ratio of 3.1:1 and 1.9:1 respectively.

Immunohistochemical scoring:

Table 10: Immunohistochemical scoring pattern (0-3 scoring system) [76]

SCORE	STAINING INTENSITY	PROPORTION
0	No staining	-
1+	Slight staining	<5% of cells
2+	Moderate staining	5% -25% of cells
3+	Intense staining	>25% of cells

The percentage of positive cells was evaluated by counting number of positively stained cells in 10 high power fields for each specimen. 2+ and 3+ which indicated diffuse, moderate and intense staining were considered positive. Slides which did not show any staining 'score -0' along with focal and weak staining (1+) were considered negative.

In the study done by Sapio M R *et al*, [62] 27.4% (17/62 cases) including nodular hyperplasia (15/51 cases) and FA (2/11 cases) showed positivity for Gal-3 whereas in cases of PTC, 91.9% (68/74 cases) showed positivity for Gal-3, out of which classical

variant of PTC cases were 78.9% (15/19 cases). The sensitivity, specificity, PPV and NPV of Gal-3 were 70.8, 73.9, 73.9 and 70.8 respectively. (Table: 9)

Similarly, in a study done by Beesley *et al,* [13] predominantly cases of papillary hyperplasia in MNG and FA showed negativity for Gal-3.

In the study done by Mary *et al* [12] where Gal-3 immunohistochemical staining was done to differentiate between papillary hyperplasia and PTC, in cases of papillary hyperplasia, 28 cases (93.3%) showed no expression or rare expression. The other 2 cases (6.6%) showed moderate expression. Whereas in cases of Papillary carcinoma, moderate to strong diffuse staining was seen in 24 cases (80%) out of 30 cases. The remaining six cases (20%) showed rare positivity, resulting in a sensitivity and specificity of 100% and 40% respectively.

Similarly in our study, in cases of papillary hyperplasia, 14 cases (87.5%) showed no staining for Gal-3 whereas 1 case each (6.25%) showed moderate and strong positivity. In cases of papillary carcinoma, 2 cases (11.7%) showed no staining, 1 case (5.88%) showed moderate and 14 cases (82.35%) showed strong positivity for Gal-3. (Table: 5-8, Fig.6-10)

In the various studies reviewed from the literature, the expression of Gal-3 in classical variant of PTC ranged from 82% to 100% of the cases similar to the present study. [16]

Several studies have shown the ability of Gal-3 to discriminate PTC from other thyroid lesions [13,77]

In a study done by Papotti M *et al*[78], although Gal-3 is also expressed by cyst macrophages, which are abundantly present in the aspirated cystic fluid, the positive staining in epithelial cells of thyroid cysts may address towards a diagnosis of cystic neoplasm (i.e. papillary carcinoma) in cytology. [69,78]

Table 11: Positivity of PTC cases by Gal-3

	Study	PTC Classic variant positivity (%)
1	Coli[64]	100%
2	Bartolazzi[68]	97%
3	Weber[69]	92%
4	Nascimento[79]	82%
5	Hermann[80]	86%
6	Giannini[81]	93%
7	Prasad[82]	94%
8	Cejivic[83]	91%
9	Present study	88.2%

Few of the studies in the past done by Martins *et al* and Prasad *et al* [82] showed frequent Gal-3 expression in benign thyroid lesions and in several non-thyroidal cells, including fibroblasts and inflammatory cells, making it a marker with high sensitivity

but lower specificity The presence of Gal-3 positive cells has been associated with the presence of macrophages in benign nodular goitre and Hurthle cells in adenomas.

However in present study, Gal-3 expression showed striking differences between papillary hyperplasia and papillary carcinoma similar to the studies done by other workers.

In our study, the Sensitivity, Specificity, Positive predictive value and Negative predictive value of Gal-3 expression to differentiate PTC from papillary hyperplasia are 88.24, 87.5, 88.24 and 87.5%. (Table 9)

Table 12: Comparison of sensitivity and specificity of Gal-3 in various studies to present study to detect PTC cases.

S. No.	Study	Sensitivity	Specificity
1	Sandra *et al* [(2)]	92	94
2	Mary *et al* [(12)]	100	40
3	Bartolazzi *et al* [(68)]	99	98
4	Weber KB *et al* [(69)]	92	69
5	Papotti M *et al* [(78)]	89.3	100
6	Present study	**88.24**	**87.5**

The results thus obtained in our study indicate that Gal-3 is useful to differentiate benign from malignant lesions of thyroid gland.

Many studies with various combination of immunohistochemical markers were done, in these studies, the values of specificities increased, but values of sensitivity decreased in comparison with single markers values.

Immunopositivity for Gal-3, HBME-1 and CK19 in the diagnosis of differentiated thyroid carcinoma have sensitivity of 85.9 % and specificity of 100 %. The immune panel comprised of p16, ERK, and RET has 51% sensitivity and 89% specificity for carcinoma. However, out of all the markers used, the best immune panel consists of HBME-1, GAL-3, and CK19 with a high degree of sensitivity (54%) and specificity (100%). [61]

CONCLUSION

- Majority of PTC cases can be diagnosed on the basis of histopathologic criteria.
- Although histopathology is a gold standard for the diagnosis of PTC, few cases can mimic the morphology of PTC and can lead to misdiagnosis, hence IHC is an important and valuable adjunctive ancillary technique for these cases.
- In our present study, Gal -3 was strongly expressed in PTC with a high sensitivity and specificity. Thus Gal-3 protein expression can be used to differentiate PTC from other mimickers.
- Hence, the development of Gal-3 as a diagnostic marker for thyroid cancer represents a promising avenue for future study in helping to distinguish PTC from hyperplasia in diagnostically difficult cases.

SUMMARY

- Present study was a cross-sectional study conducted from December 2015 to June 2017 to study the efficacy of Gal-3 in diagnosis of papillary lesions of thyroid.
- Out of all the thyroid samples received at our institute, macroscopic and microscopic examination was done and lesions which had papillary pattern on microscopy were considered.
- A total of 33 samples of papillary lesions of thyroid were found during the duration of the study.
- IHC with Gal-3 primary antibody was sought for the included samples.
- Gal-3 staining immunopositivity was done according to 0-3 grading system.
- Out of all the lesions studied, amongst the benign lesions, 14 cases (87.5%) showed negative staining and 2 cases (11.8%) showed positive staining for Gal-3. Amongst the PTC cases, 15 cases (88.2%) showed positive staining and 2 cases (12.5%) showed positive staining for Gal-3.
- In case of papillary hyperplasia, 14 cases (87.5%) showed no staining, whereas 1 case each (6.25%) showed moderate and strong positivity. In cases of papillary carcinoma, 2 cases (11.7%) showed no staining, 1 case (5.88%) showed moderate and 14 cases (82.35%) showed strong positivity for Gal-3.
- Statistical analysis was done.
- Immunohistochemical staining with Gal-3 revealed statistically significant P – value (<0.0001), with Gal-3 expression being more common in frequency and intensity of staining in PTC as compared to papillary hyperplasia.

LIMITATIONS OF THE STUDY

- Present study investigate was done to correlate whether strong galectin-3 expression is an important hallmark of PTC or papillary projections in other thyroid conditions.
- However, according to the literature, a panel of immunohistochemical markers gives a better sensitivity and specificity in comparison to a single marker. In our study, only single marker is used
- Follow-up was also not done to correlate Gal -3 IHC grading with overall survival and prognosis of PTC patients.

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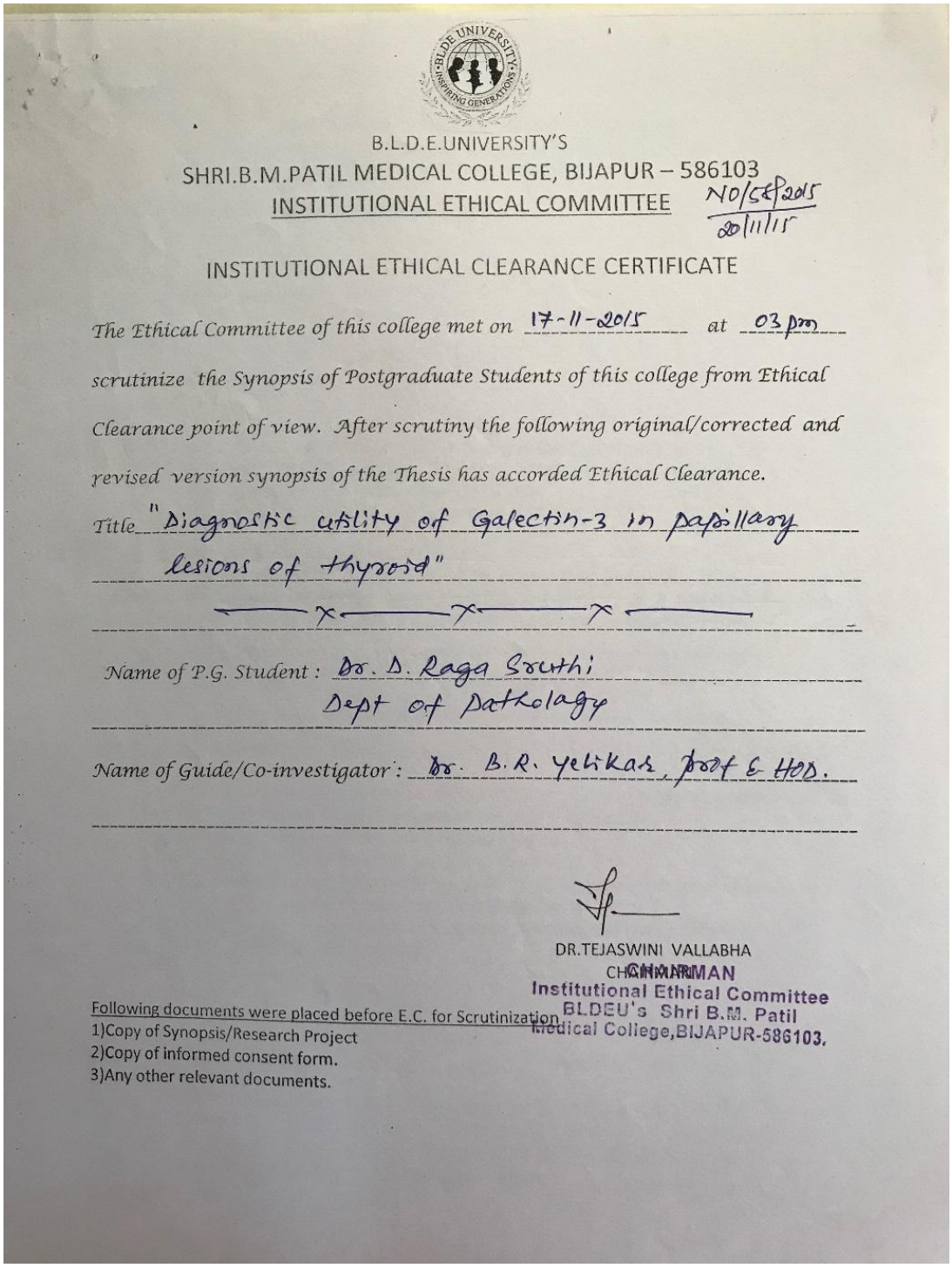
ANNEXURE-I

ETHICAL CLEARANCE



B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE NO/CE/2015 20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "Diagnostic utility of Galectin-3 in papillary lesions of thyroid"

Name of P.G. Student : Dr. D. Raga Sruthi
Dept of Pathology

Name of Guide/Co-investigator : Dr. B.R. Yelikar, Prof & HOD.

DR.TEJASWINI VALLABHA
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

ANNEXURE-II

B.L.D.E.UNIVERSITY , SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER ,VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, _____, S/O D/O W/O _____, aged _____ears, ordinarily resident of do hereby state/declare that Dr _____ of Hospital has examined me thoroughly on _____ at (place) and it has been explained to me in my own language that I am suffering from disease (condition) and this disease/condition mimic following diseases _____. Further Doctor _____ informed me that he/she is conducting dissertation/research titled under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor _____ has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of

other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

PROFORMA

Demographic Details:

- Name:
- Age :
- Sex :
- **OPD / IPD No. :**
- **Lab. No. /Sample No. :**
- **Chief complaints:**
- **Clinical diagnosis:**
- **Histopathological Diagnosis** :
- **IHC grading:**

  0-3 scoring system

  Negative staining 0 :

  Weak or rare staining 1+ :

  Focal or moderate staining 2+ :

  Strong or diffuse staining 3+ **:**

KEY TO MASTER CHART

M – Male

F- Female

PTC –Papillary Thyroid carcinoma,

FVPTC –Follicular variant of papillary carcinoma thyroid

PMC –Papillary micro carcinoma

MNG –Multinodular Goitre

MASTER CHART

Sr No.	Age	Sex	Histopathological Diagnosis	Gal-3 Immunostaining	Gal-3 Grading
1	35	M	PMC	Positive	3
2	35	F	PMC	Positive	3
3	30	M	PTC-classical variant	Positive	3
4	38	F	FVPTC	Positive	3
5	40	F	PMC	Positive	3
6	42	F	PTC-classical variant	Positive	3
7	40	F	PTC-classical variant	Negative	0
8	28	M	PTC-classical variant	Positive	3
9	45	F	PTC-classical variant	Positive	2
10	26	F	PTC-classical variant	Positive	3
11	48	F	PTC-classical variant	Positive	3
12	45	F	PTC-Tall cell variant	Positive	3
13	35	F	PTC-classical variant	Positive	3
14	45	F	PTC-classical variant	Positive	3
15	35	F	PTC-classical variant	Positive	3
16	39	F	PTC-classical variant	Negative	0
17	40	F	PTC-classical variant	Positive	3
18	35	F	MNG	Negative	0
19	50	F	MNG	Negative	0
20	28	F	Graves' disease	Positive	3
21	25	F	Nodular Goitre	Negative	0
22	40	F	Hashimoto's thyroiditis	Negative	0
23	50	M	Nodular Goitre	Negative	0
24	30	F	Colloid cyst	Negative	0
25	20	F	MNG	Negative	0
26	32	F	Nodular Goitre	Negative	0
27	22	M	MNG	Negative	0

28	50	F	MNG	Positive	2
29	55	M	Nodular Goitre	Negative	0
30	44	F	Nodular Goitre	Negative	0
31	44	M	Nodular Goitre	Negative	0
32	55	F	MNG	Negative	0
33	22	F	Nodular Goitre	Negative	0