HISTOPATHOLOGICAL ANALYSIS AND STUDY OF EXPRESSION OF PSMA IN THYROID FOLLICULAR NEOPLASMS

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I, **Dr.SYED ABDULLAH MOHAMED AMEEN,** solemnly declare that the dissertation titled **"HISTOPATHOLOGICAL ANALYSIS AND STUDY OF EXPRESSION OF PSMA IN THYROID FOLLICULAR NEOPLASMS"** is a bonafide work done by me at Department of Pathology, Madurai Medical College & Government Rajaji Hospital, Madurai during the period from May 2016 to August 2018. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any reward, degree and diploma to any university, board either in India or abroad. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, towards partial fulfilment of requirement for the reward of **M.D Degree in PATHOLOGY.**

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CONTENTS

INTRODUCTION

INTRODUCTION

Thyroid cancers are one of the common endocrine cancers. There is a notable increase in the incidence of thyroid cancers worldwide over the past two decades, probably due to the use of more sensitive detection techniques and environmental changes. Most thyroid neoplasms are primary tumours. Traditionally, they have been classified as adenomas and carcinomas.

Lesions exhibiting follicular cell differentiation comprise more than 95% of the total cases. [1]

Thyroid follicular neoplasms are composed of wide range of lesions which includes benign adenomatous nodule, follicular adenoma (FA), follicular carcinoma (FC) and follicular variant of papillary carcinoma (FVPC).

The Diagnosis of follicular carcinoma is based on the presence of capsular invasion or vascular invasion or nodal or distant metastasis, which is sometimes problematic for pathologists [2]

For the purpose of easing the diagnosis, different tools such as Immunohistochemistry and molecular profiling have been used to differentiate between benign and malignant follicular neoplasms.

Prostate Specific Membrane Antigen (PSMA) is a type II integral membrane glycoprotein expressed in prostate cancer cells. Later it was discovered that it is also expressed in the neovasculature of the various other solid tumours. But neither normal endothelium nor endothelial cells of benign tissue are positive for PSMA [3].

This study is conducted to evaluate the expression of PSMA by immunohistochemistry in neovasculature of thyroid follicular neoplasms and to determine its usefulness in distinguishing between adenoma and carcinoma.

AIMS
AND
OBJECTIVES

AIMS AND OBJECTIVES

1. To study the frequency of occurrence of Thyroid Follicular neoplasm in specimens received at the Department of pathology, Madurai Medical College, Madurai.
2. To study the age and sex related incidence of thyroid follicular neoplasms.
3. To assess the expression of prostrate specific membrane antigen (PSMA) in selected cases of thyroid follicular neoplasms and it's utility in differentiating between benign and malignant lesions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Historical Aspects

THOMAS WHARTON, an English physician and anatomist from London in 1656 named this endocrine gland as thyroid gland as it resembled the shield used in ancient Greece.

Embryology

The thyroid gland develops as an epithelial proliferation in the floor of pharynx between the tuberculum impar and copula at a point which is later represented as foramen cecum. After development, the thyroid descends in front of the pharyngeal gut as a bilobed diverticulum.

During the course of this migration the thyroid gland remains connected to the tongue by a narrow canal, called the thyroglossal duct which disappears later.

With further development, the thyroid gland descends in front of hyoid bone and laryngeal cartilages. By seventh week it reaches its final position.

By this time it acquires a small median isthmus and two lateral lobes. Approximately, by the end of third month, thyroid begins to function, at which time the first follicles containing colloid becomes visible. This development of the thyroid gland is controlled by the

coordinated action of specific transcription factors such as TTF-1, TTF-2, PAX8, and HHEX, and the altered expression of which likely plays an important role in thyroid dysgenesis. [4]

Anatomy

The thyroid is the largest of the endocrine glands, weighing between 20 and 25 gm. The thyroid gland is located in the neck, just below the larynx. It consists of two lobes; each measures about 5 cm, connected anteriorly by a bridge of tissue called the isthmus. It receives an abundant blood supply of about 80–120 ml/min, through the paired superior thyroid artery and the paired inferior thyroid artery which are branches of external carotid arteries and subclavian arteries respectively. The venous drainage is through the paired superior and middle thyroid veins which passes to the internal jugular veins and through the inferior thyroid veins that empty into brachiocephalic veins. [5]

Histology

Thyroid follicles are the functional units of thyroid glands**.**They are composed of a single layer of cuboidal epithelial cells, with a basement membrane, the follicles are of variable sizes and they contain a homogeneous colloid. The thyroid gland is enveloped by a fibrous capsule, which forms fine collagenous septa extending into the gland and dividing it into lobules and also conveys rich blood supply, along with

lymphatics and nerves. Tiny capillaries pierce the thyroid tissue and surround the follicles. They are usually difficult to see in a Haematoxylin & Eosin preparation, but they can be highlighted using an immunohistochemical method for an endothelial marker.

The function of thyroid follicles is mainly to store thyroglobulin (an iodinated glycoprotein) the storage form of thyroxine (T4) and tri-iodothyronine (T3). The follicles epithelial cells are responsible for the synthesis of the glycoprotein component of thyroglobulin and for the conversion of iodide to iodine. The inactive thyroid epithelial cells are flat or cuboidal cells, whereas cells actively synthesising or secreting thyroid hormones are tall and columnar.

C cells

A second type of cell is the C cell or parafollicular cell that has ultrastructural characteristics of neuroendocrine cells. The major secretion by the C cell is calcitonin. C cells are relatively difficult to identify in haematoxylin-eosin–stained sections, even though they sometimes exhibit cytoplasmic clearing. They can be recognized in Diff-Quick–stained smear because their cytoplasm contains metachromatic granules. C cells are most reliably identified by immunehistochemical methods by using antibodies to calcitonin or chromogranin A. C cells occupy an exclusively intrafollicular position and are separated from the

interstitium by the follicular basal lamina and from the colloid by the cytoplasm of adjacent follicular cells. C cells are seen in highest concentrations in a zone of the junction of the upper and middle thirds of the lateral lobes. They are generally present as single cells or as small cell clusters. As many as 50 C cells are present per single low-power field (×100) in adults.

A detailed morphometric study, demonstrated a significant inter-individual variation in the maximum C-cell surface area, which ranged from 28×10^3 to 470×10^3 μm^2in adults [6]. They also demonstrated that C-cell density in males was more than twice that of the females, paralleling the known higher levels of calcitonin in males.

The primary transcript of the calcitonin gene gives rise to two different mRNAs by tissue-specific alternative splicing events, calcitonin and the calcitonin gene–related peptide mRNAs. The calcitonin gene–related peptide is expressed in thyroid and nervous tissue, but calcitonin is being produced in large quantities only in the thyroid gland. In addition to calcitonin production, the C cells also produce a variety of other peptides that includes somatostatin, gastrin-releasing peptide, and thyrotropin - releasing hormone. They also have biologically active amines, including serotonin. Ultra structurally, C cells contain

membrane-bound secretary granules, which are representative of the sites of storage of calcitonin and other peptides.

Physiology

Synthesis of thyroid hormone begins by active transport of iodide which is the ionized form of iodine, into the follicular cells, which is mediated by the sodium-iodide symporter. The intracellular iodide is then oxidized by an enzyme called thyroid peroxidase and is bound to tyrosine to form the hormonally inactive iodotyrosines, monoiodotyrosine and diiodotyrosine. The formation of T4 occurs as results from coupling of two diiodotyrosine molecules, as the formation of T3 results from the coupling of one molecule of monoiodotyrosine and one molecule of diiodotyrosine.

The iodothyronine molecules are further incorporated into thyroglobulin. The stimulation of the follicular cells by thyroid-stimulating hormone (TSH) results in the resorption of colloid through the apical aspect of the follicular cell and the subsequent release of T4 and T3 at the basal aspect of the cell from where they subsequently enter the systemic circulation.

The iodotyrosines undergoes subsequent intra thyroid iodination, with recycling of the resulting free iodide. Thyroid hormone synthesis and secretion are controlled by the actions of TSH and thyroid hormone

releasing in a classic negative feedback pathway. Thyroid-releasing hormone stimulates the synthesis and release of TSH, whereas T3 and T4 inhibit these activities. The activity of the genes coding for thyroglobulin, thyroid peroxidase, the TSH receptor, and sodium-iodide transporter are mainly controlled by three thyroid transcription factors, TTF-1, TTF-2, and Pax-8.

Thyroid Nodules and Thyroid Cancers -General Considerations

The prevalence of thyroid nodules in population depends on many factors that include age, geographic location, and the sensitivity of the detection system. The prevalence of thyroid nodules is five to ten times higher in glands studied through ultrasonography.Of those nodules which have been surgically removed, 42% to 77% proved to be hyperplastic nodules, 15% to 40% were adenomas, and 8% to 17% were carcinomas.[7]

The estimated age standardized annual incidence is 1.0 to 2.9 cases per 1000,000 men and 3.4 to 9.1 cases per 100000 women, with higher incidence in developed and lower incidence in developing countries [8].In the Framingham study conducted, thyroid nodules were identified clinically in 6.4% of female population and 1.5% of male population, with a nodule with an accurate rate of 0.09% per annum. [9]

Approximately 95% of all thyroid cancers are primary carcinomas. With less than 5 % of thyroid cancer contributed by Primary lymphomas

of the thyroid, majority of them possibly evolved from autoimmune (lymphocytic) thyroiditis.

Sarcomas of the thyroid are very rare. About 20% of patients, who died of metastasizing malignancy, had metastatic deposits in the thyroid gland, most commonly from renal cell carcinomas, malignant melanoma, and bronchogenic carcinoma.

Epidemiology

The true incidence of follicular adenomas is generally difficult to establish with high accuracy since they cannot be discriminated from solitary the hyperplasic nodules based on clinical parameters or FNA.

The incidence of adenomas in adults can be estimated to about 3% to 5% based up on autopsy series [(10)].Palpable thyroid nodules are found in between 3% to 7% of adults who are living in iodine-sufficient areas, and three fourths of those are solitary nodules on palpation and might represent adenomas [(11)]. Follicular adenomas are frequently seen more in female population, with the female-to-male ratio of 4 to 5:1. They are generally seen in all age groups, although most of the patients present during the fifth and sixth decades of life.

Follicular carcinoma is the second most common type of thyroid cancer following papillary carcinoma. The absolute incidence of follicular carcinoma has not changed significantly during the last several

decades. According to Survival, Epidemiology, and End Results (SEER) registry, the annual incidence in the United States from 1973 to 2003 was about 0.8 per 100,000 persons per annum [12].Females are more commonly affected than males, with a slightly higher female predominance among conventional type carcinomas (2.5:1) than among oncocytic carcinomas (1.7:1) [13].These tumours are rare in paediatric age group. Their incidence increases with age which reaches a peak in the 5th decade of life.

The data collected over the past 20 to 30 years indicate that follicular carcinoma, when including both the conventional type and oncocytic variant, makes up to approximately 15% of all thyroid cancer cases [66].Recently this trend is decreasing to approximately 10%. There are several likely reasons that are responsible for this trend. First, the incidence of occurrence of thyroid papillary cancer has been increasing in many countries around the world; it has almost tripled in the United States over the last 30 years, making the incidence of follicular carcinoma proportionally lesser [15].Secondly an increased incidence of follicular carcinoma was associated with dietary iodine deficiency. Severe iodine deficiency has been largely eliminated or diminished by widespread iodization of salt and other food supplies.

Etiopathogenesis

The vast majority of follicular adenomas are sporadic but some may even arise as manifestations of known inherited syndromes. The risk factors commonly implicated in the development of sporadic adenomas are exposure to radiations and iodine deficiency.

External Radiation is considered as the single most important environmental factor associated with increased risk of developing thyroid carcinoma. Japanese atomic bomb survivors and in individuals who were living in the vicinity of nuclear accident sites had greater incidence of thyroid neoplasm. X-ray or γ-radiation exposure during childhood and adolescence is found to increase the risk of follicular adenomas as much as, up to 15 fold [16]. The risk is mostly dose-dependent and exists even after a dose as small as 0.25 Gy. Most follicular adenomas develop about 10 to 15 years of duration following exposure, but elevated risk prevails for at least 50 years after exposure. Follicular neoplasms that develop following a radiation exposure are generally solitary nodules and they exhibit conventional histology, although oncocytic follicular adenomas may also be found [17].

Iodine deficiency is one of the important risk factor that contributes to the higher incidence of benign thyroid nodules. Palpable thyroid nodules are about 2 to 3 times more common in areas of low

iodine consumption when compared with areas with sufficient iodine and a significant proportion of those are adenomas [18]. To support this findings, follicular adenomas can be induced in experimental animals in labs, by the administration of low iodine diet or drugs which interfere with iodine uptake and metabolism in thyroid cells [19].

The exact process of how iodine deficiency induces adenoma formation is not clear as of now, but it may probably involve an increase in thyroid stimulating hormone (TSH) levels which stimulates the proliferation of thyroid cells [20].

A cholesterol lowering agent, HMG-CoA reductase inhibitor simvastatin is also known to increase incidence of thyroid adenomas in female rats [21]. This is most likely due to enhanced liver clearance of thyroid hormone, which might be the reason to cause elevation in TSH levels.

Follicular neoplasms have been reported to develop in patients affected with inherited diseases like Cowden disease and Carney complex. Cowden disease also known as multiple hamartoma syndrome, is caused due to germ line mutation of the PTEN tumour suppressor gene which is located on chromosome 10q23.PTEN which codes for a dual specificity phosphatase, that functions as a negative regulator of the PI3K/AKT signalling pathway which is responsible for cell survival and

proliferation .The inherited mutations result in the loss of PTEN function and the chronic stimulation of AKT and its downstream targets. Follicular adenomas in the affected patients develop at a young age which are almost always multiple and bilateral. They are found to have conventional type of follicular adenoma appearance, occasionally clear cell adenomas, adenolipoma and oncocytic adenomas have also been reported [22].

Molecular Genetics

Clonality

Follicular adenomas are true neoplasms and hence are seen to have a monoclonal origin i.e., arise from a single cell. This is in contrast to non neoplastic lesions, which have a polyclonal origin. The clonality of a lesion can be determined in female tissue based on the randomness of inactivation of the two X chromosomes. This can be achieved by polymerase chain reaction-based HUMARA assay. Most of the studies have confirmed that follicular adenomas, which are diagnosed based on common morphologic criteria are generally monoclonal, that are consistent with their neoplastic nature [23].

Cytogenetic alterations are generally found in about less than half of follicular adenomas and they are most frequently as trisomy 7 or

translocations involving the long arm of chromosome 19 (19q13)[24]. Other molecular alterations involved are point mutations of the *RAS* genes, most frequently involving *N-RAS* codon 61, that are found in approximately 30% of adenomas[25]. Besides studies have found that they are not very specific for adenomas and they can also occur in follicular carcinomas and papillary thyroid carcinomas of thyroid.*PAX8-PPAR*γ rearrangement have been found in about as many as 8% of adenomas, although it has been reported much more common in thyroid follicular carcinomas, and hence the identification of *PAX8-PPAR*γ rearrangement in a follicular tumour should prompt search for vascular or capsular invasion. Hyperfunctioning adenomas usually show point mutations in the TSH receptor gene and sometimes mutations in the *Gs*α (*GSP*) gene [26].

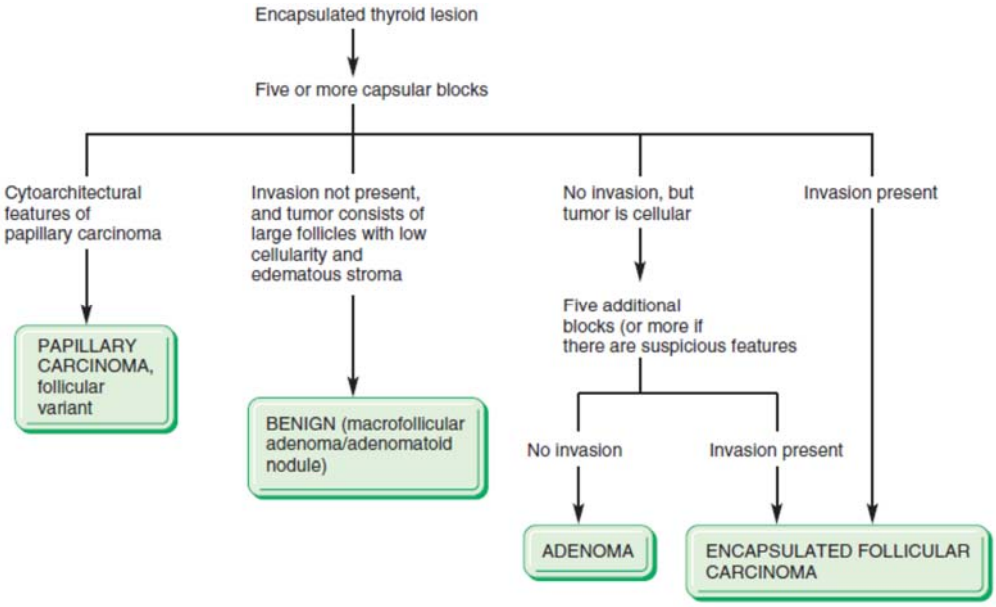
Hurthle cell neoplasms usually shows commonly large deletions of mitochondrial DNA (mtDNA), mutations of *mtDNA* genes that code for oxidative phosphorylation (OXPHOS) proteins, and mutations of nuclear genes codes also for mitochondrial OXPHOS proteins. These alterations are generally thought to lead to energy production defects and they lead to an increased proliferation of mitochondria as a compensatory mechanism [27]. Some of the cases show mutation in the *GRIM-19* gene

[28].*PAX-8/PPARγ* gene rearrangement is also found in 27% of cases of Hurthle cell carcinoma [29]. Surprisingly *RET/PTC* rearrangements which is a hallmark of papillary thyroid carcinoma, is also found in about one third of Hurthle cell neoplasms.

Follicular Neoplasm

Follicular adenoma and carcinoma are respectively benign and malignant epithelial tumours of the thyroid showing follicular cell differentiation and lacking the diagnostic features of papillary carcinoma [30]. Even though the neoplastic nature of follicular carcinoma is indisputable, the nature of follicular adenoma is more controversial [31].

Histochemical and molecular studies provide strong evidence regarding follicular adenoma diagnosed using strict morphologic criteria, in particular the presence of a well-defined fibrous capsule. They are indeed clonal lesions which supports that the lesions represent neoplasm rather than hyperplastic nodules [32].



Follicular Adenoma

Follicular adenomas are benign, encapsulated tumours which show evidence of follicular cell differentiation. Molecular studies have proven that most adenomas have a clonal origin [33].

Follicular adenomas are seen mostly in adults of age between 20 to 50 years, but no age is exempt. It is more common in female (M/F ratio 1: 6). Most of the patients presents with a solitary thyroid nodule noticed for variable periods. The adenomas usually lack uptake on iodine scans ("cold" nodules), but rare ones may be "hot" and can cause hyperthyroidism, which are so-called toxic adenoma. They are totally benign and can be adequately treated by lobectomy.

Clinical Features and Imaging

Thyroid adenomas typically presents as a painless thyroid nodule that are incidentally discovered by palpation or during thyroid ultrasound carried out for any other reasons.

Most of the adenomas are generally asymptomatic, even though tumours of large size cause difficulty in swallowing and other local symptoms. They generally grow very slowly, and some patients seek medical attention many years after discovering the nodule. Sometimes, bleeding into the tumour may occur and cause sudden pain, tenderness, and increase in nodule size, which may occur spontaneously or after procedures like vigorous neck palpation or FNAC.

On palpation, adenomas are generally felt as a discrete mass which are not fixed to the neck and shows movement with the thyroid. Most of the patients are euthyroid. On ultrasound examination, follicular adenoma are seen typically as a solid, homogeneous mass that may be hyperechoic, isoechoic, or hypoechoic when compared to the surrounding thyroid tissue .The margins of the nodule appear well-defined and smooth. A peripheral hypoechoic halo which is a fibrous capsule may be seen. Blood flow can be seen by use of a colour Doppler ultrasound, low blood flow is more suggestive of a benign nodule, even though no ultrasound features allow for a reliable diagnosis of adenoma. By radionuclide scan,

adenomas typically appear as “cold” nodules because they concentrate radioiodine or other tracers less avidly than adjacent thyroid parenchyma .Small subset of adenomas are hyper functional and presents with variably prominent symptoms of hyperthyroidism. Overt thyrotoxicosis, are generally very rare, and most of the patients reveal subclinical hyperthyroidism (i.e., normal thyroid hormone levels and decreased serum TSH level). TSH suppression in these patients is as a result of the negative feedback regulation of TSH secretion due to excess thyroid hormone production by the nodule. Hyper functioning adenomas appear as “hot nodule” on imaging when compared with the adjacent thyroid tissue that is hypofunctional due to deprivation of TSH stimulation.

Macroscopic and Microscopic Appearances

Follicular adenoma and minimally invasive follicular carcinoma are encapsulated which are usually in distinguishable macroscopically, except for the capsule tends to be thicker in follicular carcinoma. Their size ranges from less than 1 cm to over 10 cm. they are solid, fleshy, and tan to light brown, sometimes having glistening quality, degeneration might be present. The widely invasive follicular carcinomas might lack a discrete capsule and show, in addition, obvious invasion beyond the main tumour bulk, and tumour thrombi may also be seen distending the blood vessels.

Secondary changes like haemorrhage, hemosiderin deposition, sclerosis, calcification, necrosis, and cystic change are usually very common. Rarely, amyloid may also be present [34]. Usually, the pattern of follicular growth is uniform within the adenoma, which is in contrast to hyperplastic nodules, which commonly exhibit variations in the sizes and shapes of individual follicles.

Adenomas are sub classified according to the predominant pattern of follicular growth. The embryonal (trabecular or solid) adenoma is made of nests of follicular cells with few or no follicles. Micro follicular (fetal) adenomas are composed of small follicles which contains scanty luminal colloid. Simple (normofollicular) adenomas are composed of follicles more or less of the same size as those in the adjacent normal thyroid, whereas macrofollicular adenomas are composed of follicles which are substantially larger than those seen in adjacent normal follicles.

Foci of clear cell change may be seen throughout the tumour or it can be restricted to small groups of follicles. The factors responsible for clear cell change in follicular adenomas are the presence of cytoplasmic vesicles that may originate from degenerated and cystically dilated mitochondria, or from dilatation of the endoplasmic reticulum or golgi vesicles. In certain cases clear cell change were traced to the intracellular accumulation of glycogen, lipid, or thyroglobulin. Some of the adenomas

may occasionally have cells with a signet-ring appearance. The cytoplasmic vacuoles are typically positive for thyroglobulin and also contain mucin. Hence such tumours have been referred to as signet-ring adenomas [35].The differential diagnosis of adenomas includes adenomatous (hyperplastic) nodules, follicular carcinoma, the follicular variant of papillary carcinoma and the follicular (tubular) variant of medullary carcinoma.

Most of the adenomatous nodules are multiple and exhibit considerable variation in the size of the component follicles, whereas the follicular structure of adenomas are seen to be more uniform in morphology. The surrounding capsule of adenomas is complete, whereas it is often incomplete around adenomatous nodules. The distinction of follicular adenoma from carcinoma depends on the demonstration of capsular or vascular invasion, or both.

Differentiating between true capsular invasion and the changes associated with fine-needle aspiration biopsy may be exceedingly difficult. These changes are referred to as WHAFFT (worrisome histologic alterations following fine needle aspiration of the thyroid) [36]. Acute changes (the changes that occur less than 3 weeks after aspiration) include haemorrhage and granulation tissue with hemosiderin-laden macrophages, capsular distortion, and sometimes, infarction may also be

seen .Typically the foci of haemorrhage and granulation tissue are linear and are perpendicular to the centre of the nodule. Chronic changes , that occur about1 to 6 months after aspiration includes linear fibrosis adjacent to hemosiderin-laden macrophages; oncocytic cells, squamous metaplasias and spindle cells ; pseudoinvasion of the capsule; and calcification. Foci of pseudoinvasion tend to be linear, and the presence of haemorrhage, hemosiderin deposition, granulation tissue, and the focal and geographic pattern of the lesions indicates a fine-needle aspiration biopsy–induced change rather than true capsular invasion.

The follicular variant of papillary carcinoma is characterized by a series of nuclear changes, which will be discussed later. The follicular (tubular) variant of medullary carcinoma is characterized by presence of chromogranin or calcitonin within the tumour cells, whereas staining for thyroglobulin is negative.

Adenoma variants

Oncocytic (Hurthle Cell) adenomas

Oncocytic cells seen in the thyroid have been known by different names including Hurthle, Askanazy, and oxyphilic cells. At present the term “oncocytic” is recommended to describe these cells and tumours [(80)].Oncocytic adenomas are benign tumours composed predominantly of oncocytic cells more than 75%. Oncocytic adenoma is a common microscopic type of thyroid follicular adenomas. Approximately 10% to

15% of all adenomas belong to this variant. The tumours are usually round to ovoid shaped which are separated from the adjacent thyroid parenchyma by a fibrous capsule. In larger tumours with foci of degenerative changes, groups of oncocytes might become entrapped within the capsule. In such cases the foci should be differentiated from the areas of true capsular invasion to differentiate it from oncocytic carcinoma. On cross section, the tumours appear dark brown in colour and are solid in consistency. Oncocytic tumours might also undergo FNAC –induced or spontaneous infarct-like necrosis [37].The pattern of growth is usually follicular but occasionally may be also solid or trabecular in rare circumstances .The intra follicular colloid may be weakly basophilic and may also contain psammoma body–like calcifications .The nuclei are generally large and vesicular with coarsely clumped chromatin and prominent nucleoli, where in the cytoplasm is deeply eosinophilic and granular. The granular appearance of the cytoplasm is because of the presence of numerous mitochondria. Marked swelling of the mitochondria may lead to a clear appearance of the tumours cells.

Hyperfunctioning adenoma

Hyperfunctioning adenomas contribute to about 1% of all follicular adenomas approximately [38]. They are generally associated with variably prominent symptoms of hyperthyroidism caused due to excessive thyroid

hormone production by the tumour cells. The nodules appear “hot” on radionuclide scans. Most of the tumours carry an activation mutation of the TSHR or Gsα genes, leading to chronic upregulation of the cAMP signalling pathway that mimics constant TSH stimulation.

Microscopically they mostly show normofollicular or microfollicular architecture .The follicles are shaped irregularly and frequently show significant variation in size and shape. They are lined by tall columnar or cuboidal epithelial cells. Delicate papillary projections into the lumen are also seen. The cells contain abundant pale eosinophillic and frequently vacuolated cytoplasm and uniform, basally located nuclei. The colloid appears usually bubbly, pale, and watery, often showing peripheral scalloping. Examination of thyroid parenchyma surrounding the adenoma typically shows evidence of suppressed TSH stimulation (i.e., large follicles with dense colloid lined by flattened epithelium).

Adenolipoma and adenochondroma

Adenolipoma are follicular adenomas which are composed of follicular elements are separated by mature fat cells. The stromal fat most probably arises as a sequel of metaplasia of connective tissue elements [(39)].Similar changes may be also seen in normal and hyperplastic thyroid nodule and in papillary carcinoma thyroid [(40)]. The stromal components of

adenomas may undergo cartilaginous metaplasia (adenochondroma) very rarely [41].

Follicular adenomas with papillary hyperplasia

Follicular adenomas may sometimes also exhibit foci of papillary hyperplasia. These changes in the tumours are more common during the second and third decade of life. Some patients yield a history of exposure to ionizing radiation [42].The papillary processes are typically short and they point toward the lumina of cystically dilated follicles.

The follicular cells seen are tall with basally situated, round nuclei. The central regions generally appear cystic. Alternatively, these lesions were referred to as hyperplastic papillary adenomas [43]. Even though this term has resemblance to papillary carcinoma and its use should be avoided. Some of the hyperfunctioning (toxic) adenomas may show papillary hyperplasia. The follicles and papillary projections in these tumours are lined with tall epithelial cells. Clinical symptoms of hyperthyroidism are evident.

Adenomas with bizarre nuclei

Follicular adenomas may contain groups of cells with markedly enlarged and hyperchromatic nuclei, like other benign endocrine tumours. Thyroid tumours with these types of features have been termed adenomas with bizarre nuclei [44]. Although the presence of such nuclei suggests a

possibility mostly of malignancy, they occur, in fact, more often in benign lesions than in malignant follicular lesions of the thyroid. This type of nuclear atypia is more commonly seen in hyperfunctioning adenomas, after radiation exposure, and in tumours that are composed of oncocytic cells.

Atypical adenoma

The term atypical adenoma has been generally used for those type of follicular adenomas which display atypical features but they do not reveal any definitive capsular or vascular invasion [45].This term was introduced by Hazard and Kenyon to describe adenomas which contained follicles that lack a central lumen with solid columns of cells, sheet like masses of cells, or areas of spindle cell growth (spindle cell metaplasia). As a result of their broad histopathologic definition of atypical adenoma, many pathologists have used this term indiscriminately for any adenoma with features that deviate from usual or typical adenomas [46].This approach are generally discouraged, and, in fact, Rosai and colleagues suggest that the term atypical adenoma be replaced with the designation hypercellular adenoma [47].In any event, lesions with any atypical features should be carefully examined to rule out evidence of capsular or vascular invasion.

Follicular adenoma with signet-ring cells

Follicular adenoma with signet-ring Cells is one of a rare variant of adenomas that is characterized by cells with large intra cytoplasmic vacuoles which displace and compress the nucleus to the side. In the H&E-stained sections, these vacuoles appear pale eosinophillic or clear with homogeneous or finely granular texture .The vacuoles generally reveal strong immune reactivity for thyroglobulin and positive staining for PAS after diastase digestion, whereas negative for other mucin stains, such as mucicarmine and alcian blue at pH 2.5 [48]. This pattern of staining of vacuoles is consistent with the presence of protein-polysaccharide complexes that are derived from partial degradation of thyroglobulin [49]. These types of adenomas are generally mistaken for metastatic carcinoma of signet-ring type from breast or stomach. Immunostaining for thyroglobulin and TTF-1 is useful in differentiating them.

Follicular adenoma with clear cells

This type of adenoma is also an uncommon variant which is predominantly composed of cells with clear cytoplasm due to the accumulation of glycogen, thyroglobulin, lipid or distended mitochondria.

Their cytoplasm appears abundant watery clear or has a pale eosinophilic hue or fine granularity .The nuclei are placed centrally and

have smooth contours. The tumour is composed entirely of clear cells or may contain small clusters of cells showing typical follicular cell cytoplasm.

The cells of this tumour retain thyroglobulin immunoreactivity, but it is typically focal and weak. In certain cases, they are PAS positive and diastase sensitive, which is due to their glycogen content.

On the ultrastructural level, the cells show multiple cytoplasmic vacuoles. In certain cases, the vacuoles reveal the presence of residual cristae, suggestive of massively dilated mitochondria [(50)]. This suggests that at least some of the clear-cell tumours might evolve from oncocytic adenomas as a result of progressive enlargement and conversion of mitochondria into vesicles. Follicular adenomas with this unusual appearance should be differentiated from follicular, papillary, and medullary carcinomas with clear cells, metastatic renal cell carcinoma, and parathyroid tissue.

Differentiation of these lesions from follicular carcinoma follows the usual invasive criteria. Thyroglobulin and TTF-1 immunoreactivity is also useful in distinguishing this tumour from parathyroid tissue or renal cell carcinoma.

Other variants of follicular adenoma

Black adenomas of thyroid gland have been reported in patients treated with minocycline [(51)].These tumours have black discoloration on

gross examination with the cells showing abundant black pigment with appearance and staining qualities.

Follicular carcinoma

Definition

Follicular carcinoma is a malignant well-differentiated tumour of the thyroid follicular cells which lacks the diagnostic nuclear features of papillary carcinoma.

Incidence and Epidemiology

Follicular carcinoma is regarded as the second most common type of thyroid cancer following papillary carcinoma. The data collected over the past 20 to 30 years show that follicular carcinoma, when combining both the conventional type and oncocytic variant, accounts for about 15% of all thyroid cancer cases [52].Follicular carcinoma affects patients with a higher mean age compared with follicular adenoma. Most of these patients present with a thyroid mass and up to 11% of patients present initially with distant metastasis, such as bone fracture, pain, or a pulsatile mass in soft tissue [53].This is in contrast to papillary carcinoma, which spreads by hematogenous to mostly bone and lung rather than by lymphatic spread [54]. The likelihood of metastasis and prognosis depends mostly on the extent of local disease, that is, minimally invasive versus widely invasive type. In some cases, the follicular neoplasm shows no

vascular invasion and only equivocal capsular invasion. Initially follicular carcinoma used to comprise up to 10% to 20% of all primary thyroid cancers but its frequency has considerably reduced to less than 5% to 10% in recent years [55]. This drop in incidence is attributable to increased detection of early papillary carcinoma and adoption of a liberal approach in diagnosis of follicular variant of papillary carcinoma [56].Incidence of follicular carcinoma is greater in areas of endemic goiter,for which the iodine deficiency appears to be the main contributing factor,due to addition of iodine supplement to the diet ,the incidence of follicular carcinoma has significantly reduced in these geographic areas [57].In rare circumstances, follicular carcinoma may also arise in a pre-existing follicular adenoma[58].Irradiation and Dyshormonogenesis predispose to the development of follicular carcinoma [59], Few cases of follicular neoplasm also occur as a part of hereditary non medullary thyroid cancer syndrome.

Gross Features

Follicular carcinomas are typically seen as an encapsulated nodule of round or oval shape .Most of them are of 2 to 4 cm in size. The cut surface is solid and fleshy, and in some cases of non fixed samples the tumour is generally seen bulging from the confines of the capsule. The colour of the nodule appears as gray-white in conventional type

carcinomas and as brown-tan in oncocytic type of carcinomas. A thick fibrotic capsule frequently surrounds these tumours. A very thick capsule is suggestive more of malignancy. Invasion of the capsule is found rarely on gross examination, although it can be identified in some cases. Widely invasive tumours are seen to have widespread invasion through the capsule, and in those cases no residual capsule are found. Foci of infarction, hemorrage and other secondary changes, either spontaneous or FNA induced may be seen. Very careful inspection of the capsule is generally required to identify areas of invasion. The entire pericapsular zone are essentially required to be submitted for the microscopic examination unless the nodule is very large. If this is not feasible at least 10 sections from the capsule should be processed as the chance of detecting invasion progressively increases when 1 to 10 blocks are examined [(60)].

Microscopic Features

Follicular carcinomas have a well-defined and complete capsule typically; its thickness ranges from 0.1 to 0.3 cm [(61)]. This capsule consists of parallel layers of collagen fibers and frequently may contain medium calibre blood vessels. In some rare instances, carcinomas have a thin or incomplete capsule. Architectural patterns of follicular carcinomas

are mostly similar to those seen in follicular adenomas, even though the proportion of more cellular growth patterns is greater in carcinomas.

A microfollicular or trabecular/solid growth pattern is seen in about 80% of cases and normofollicular or macrofollicular patterns with increased colloid content in approximately 20%.

A insular or nested architecture may also be seen. Even though the growth pattern does not have any diagnostic value per se, but it should alert a pathologist to search more extensively for invasion, especially if the lesion is found to be very cellular. And also the architectural patterns does not correlate with the frequency of metastases or cancer-related death [62].Tumours that having solid, trabecular, and insular growth patterns should be distinguished from the poorly differentiated thyroid carcinomas, which tend to have significantly more aggressive behaviour.

Typically the cells are cuboidal with moderately abundant cytoplasm, which is light eosinophillic to amphophilic. Their nuclei are generally small to medium, round, with smooth contours and dark or with more vesicular chromatin. Small nucleoli are often seen in conventional type carcinomas but they are much more common in oncocytic follicular carcinoma. Some of the tumours have more irregular nuclei and coarse chromatin. Random cells having very large, hyperchromatic, and highly irregular nuclei may also be rarely found .This exuberant nuclear atypia

of single cells does not increase the chance of malignancy in thyroid tumours and they are also seen in follicular adenomas and hyperplastic nodules.

Mitotic figures might be found, typically may range from 1 to 2 per 10 high-power fields .Higher mitotic activity might mark the areas of recent FNAC or of any other secondary changes. Atypical mitoses are uncommon. Well-differentiated follicular carcinoma does not show any tumours necrosis unless seen in association with spontaneous or FNA induced secondary changes. If present, they suggests the emergence of poorly differentiated thyroid carcinoma [(63)].The stroma is generally scant and they might show some hyalinization and edema..The growth pattern, thickness of the capsule, or cytologic features does not differentiate between follicular adenoma and follicular carcinoma.

Distinction between follicular carcinoma and follicular adenoma

In a follicular-patterned neoplasm which lacks the cytoarchitectural features of papillary carcinoma, the only distinguishable feature of a carcinoma from an adenoma is that of the presence of unequivocal vascular and/or capsular invasion. The features heightening the suspicion of follicular carcinoma are

1. Thick fibrous capsule

2. High cellularity with trabecular or solid growth pattern

3. Diffuse nuclear atypia

4. Readily identified mitotic figures

Due to the excellent prognosis of minimally invasive follicular carcinoma the criteria for invasion has to be strictly applied to avoid an over diagnosis of cancer [64].In cases where the invasion remains doubtful even after assessment of multiple deeper levels and multiple sampled blocks, the case should be best labelled as follicular adenoma because the risk of metastasis is nearly close to zero. An alternative suggestion has also been given to label these cases as follicular neoplasm of indeterminate malignant behaviour, with suggestion to treat them as follicular adenoma [65].

The literature appears somewhat confusing in that some studies have showed development of distant metastasis in certain patients with equivocal or definite capsular invasion alone; this raises the issue that follicular carcinomas with capsular invasion only may not be that innocuous [66]. However, these patients actually already had metastases at the time of diagnosis, a feature well-known to be associated with a poor prognosis. [67]

Vascular invasion

In order to qualify for the criteria of vascular invasion, the involved blood vessels must be located within or outside of the fibrous capsule and

the intravascular polypoid tumours growth should be covered with endothelium [68].The only situation where the requirement for endothelialization of the tumours island may be relaxed is when the intravascular tumours cluster is attached to the wall and is associated with thrombus formation.

Mete and Asa have recently redefined vascular invasion as "tumours cells that invade through a vessel wall and endothelium, and those which are associated with thrombus adherent to intravascular tumour,"[69].But such criteria as such generally too strict to be used in diagnosis of follicular carcinoma. The collections of follicles bulging slightly into the thin walled capsular vessels may be disregarded if deeper cuts and further sampling fail to reveal more convincing vascular invasion. Retraction artifacts around tumours islands when present can also simulate the appearance of a vascular invasion, but no endothelial lining is found in the space. Sometimes irregular clusters of nonendothelialized tumours with ragged borders which are not conforming to the contour of blood vessels may be seen inside the capsular vessels; they result from artifactual dislodgment of the tumours during sectioning of the specimen and they should not be counted as vascular invasion.

A rare occurrence which might simulate vascular invasion is when intravascular endothelial hyperplasia that occurs in capsular blood

vessels. In such cases careful scrutiny will reveal that the intravascular polypoid plug is formed by plump pericytes and spindle endothelial cells, which are quite different from neoplastic follicular epithelial cells, even though intravascular endothelial hyperplasia per se does not constitute vascular invasion, this finding should warrants more careful sampling and examination to look for genuine vascular invasion if any.

Capsular invasion

To fulfil the criteria for capsular invasion, complete transgression of the fibrous capsule should be seen. The tumour bud should extend beyond an imaginary line which is drawn passing through the external contour of the capsule [70].Tumours showing the features short of complete capsular transgression even after extensive sampling and after careful assessment should not be given a label of carcinoma, Even though some authors accept incomplete capsular invasion as being sufficient for the diagnosis of follicular carcinoma [71, 72].

One of the major differential diagnosis is capsular rupture caused because of prior fine-needle aspiration. Capsular invasion and vascular invasion are in fact closely related phenomena. Tumours showing vascular invasion also frequently show capsular invasion. A tumour bud generally invades into or through the capsule to extend directly into a vascular space.

They are further divided into three groups based on their risk of metastasis

1. Tumors “having capsular invasion only” these tumors *have* practically zero risk of metastasis [73].
2. Tumors “with invasion of lesser than four blood vessels “they have a low rate of metastasis of approximately 5 %[74].
3. Tumors “having invasion of four or more blood vessels “they generally have a higher risk of metastasis.

   The collective event rate is approximately 18 %[75]. In view of the excellent prognosis of low risk group, total lobectomy with or without suppressive thyroxine therapy is adequate treatment for low-risk patients [76]. Total thyroidectomy and a radioactive iodine therapy should be considered for high-risk patients.

Categories of follicular carcinoma

After the diagnosis of follicular carcinoma has been made, it is also important to categorize it further into the following categorise.

Minimally invasive follicular carcinomas.

They are grossly encapsulated tumourswhich shows focal capsular or vascular invasion which is usually apparently visible only on histological examination.

Widely invasive follicular carcinomas

These are tumours that show widespread infiltration of the thyroid parenchyma and blood vessels and they generally lack complete encapsulation.

At least some proportion of cases with “widely invasive follicular carcinomas” reported in the literature actually represents poorly differentiated thyroid carcinomas. At presentation, regional lymph node metastasis and distant metastasis are already found in 7% and 29% to 66% of these cases, respectively [(77)].

On follow-up, most of the patients are found to have further lymph node and distant metastases, especially to the bones and lungs [(78)].

According to one long-term follow-up study conducted, 29% of patients died of disease, 41% remained alive with disease, and only 22% were alive without disease. Extension of the tumour into the soft tissues of the neck generally has an unfavourable influence on survival of the patient. This has an event rate 53% at 6 years, compared with 28% for carcinomas confined to the thyroid gland [(79)]. Widely invasive carcinomas are very aggressive neoplasms and should to be treated by total thyroidectomy, radioactive iodine, and suppressive thyroxine.

Papillary carcinomas –follicular variant

This is a common variant of papillary carcinoma which comprises 15% to 20% [80] .Their diagnostic criteria includes:

(i) Complete absence of a well formed papillae

(ii) Exclusively a follicular growth pattern, and

(iii) Presence of characteristic nuclear features of papillary carcinoma.

A solid, nested, or trabecular architecture can be present as well as scattered, poorly formed papillae, but a follicular pattern should be the predominant pattern (>50%) in a tumour that lack a well-developed papillae.

Historically, all thyroid tumours showing follicular growth were diagnosed as follicular carcinomas. The term follicular variant of papillary carcinoma was originally described by Lindsay[81].In 1960 and they are further characterized as a distinct variant by Chen and Rosai,who realized that, despite the follicular architecture, tumour showing nuclear features of papillary carcinoma have biologic properties of papillary carcinoma rather than that of follicular carcinoma [82].Even though they are designated as papillary carcinomas, they represent a distinct variant of papillary carcinoma with a number of characteristic molecular and biological features, some of which are closer to follicular tumours.

Follicular variant papillary carcinomas generally harbour RAS mutations, which are common with follicular tumours and are seen rarely in classic papillary carcinoma[83]. They generally reveal a higher frequency of aneuploidy and they exhibit patterns of chromosome gains and losses resembling those of the follicular tumours and show gene expression profiles that are distinct from the classic papillary carcinomas[84,85].

Investigation and Diagnosis

Follicular carcinomas present as a solitary thyroid nodule which is a slowly growing, and painless. Patients might be asymptomatic or have dysphagia, stridor, and hoarseness. In rare cases, the presenting symptoms are bone pain or pathologic bone fracture due to distant metastases.

On physical examination, the nodule moves along with swallowing. Nodules fixed to surrounding tissues give a strong suspicion for invasive malignancy. Thyroid function tests are usually normal. Radionuclide scans typically reveal a “cold” nodule as follicular carcinomas concentrate radioiodine and other tracers less likely than adjacent thyroid parenchyma. On ultrasound examination, tumours show a solid hypoechoic nodule with a peripheral halo, the halo representing the fibrous capsule. The outlines of the nodule are smooth and well-

defined, unless the tumour is widely invasive. Ultrasound features generally cannot differentiate follicular carcinomas from adenomas. But the picture of irregular, poorly defined margins, a irregular thick capsule, and chaotic intra nodular blood flow on colour Doppler imaging are features that are more of carcinoma. A routine chest radiograph shall reveal tracheal deviation and pulmonary metastases. Computed tomography (CT) and magnetic resonance imaging (MRI) can be also used when invasion is suspected to better evaluate the extension of tumour into the adjacent neck structures. Fine-needle aspiration cytology (FNAC) is routinely performed for solitary thyroid nodules. Because FNAC cytology typically reveals a cellular aspirate suspicious for a follicular neoplasm, then patients are referred for surgery.

Immunohistochemistry in Diagnosis of Follicular Neoplasms

Immunohistochemistry is generally not required except in case of rare tumours showing unusual morphologic features such as prominent fibrovascular septa, signet ring cells, clear cells, or follicular nature of the neoplasm. Various antibodies have been studied for their potential value for the distinction between follicular carcinoma and adenoma in practice, but none so far has-been shown to be foolproof[(86)].

1. Immunostaining for endothelial markers has been attempted for finding the vascular invasion, but the results were of low success [87].
2. Various other tumour markers like carcinoembryonicantigen (CEA), oncogene products (RAS p21,CMYC), epidermal growth factor cyclin-dependent Kinase inhibitor ,proliferation marker like Ki-67,P-glycoprotein, and high mobility group IHMGI(Y) protein have not been much helpful.[88]
3. Although some antibodies such as tissue polypeptide antigen, Leu-7, dipeptidyl aminopeptidase IV, cyclooxygenase-2, thyroid peroxidase (MoAb 47), HBME1, galectin-3,CITED-1,matrix metalloproteinase-2 and matrixmetalloproteinase-7 are reported to show differential staining of follicular adenomas and follicular carcinomas[89]. Their low discriminatory power precludes their application for routine diagnostic purposes [90].They showed marked differences in the reported positivity rates in the different studies suggesting technical factors and interpretation criteria may significantly affect the results. More recent studies have also promoted the application of a panel of antibodies instead, but no consensus exists on the most optimal panel.[91]

PSMA

Prostate Specific Membrane Antigen (PSMA) is an integral membrane protein, anchored to the epithelial cells which were used to detect prostatic carcinomas. Wide use of [68Ga] PSMA-HBED-ccPET/CT for prostate imaging yielded a plethora of reports with unexpected detection of non prostatic tumours, including primary and metastatic breast, renal, neuroendocrine and other malignancies. Several consecutive case reports described [68Ga] PSMA-positive thyroid tumours, including adenomas and carcinoma. A growing number of such reports reflect increased use of [68Ga] PSMA-HBED-ccPET/CT and also high incidence of thyroid neoplasm in the population [(91)].

It is been widely acclaimed that the thyroid cancer today is the fastest growing malignancy in the developed world. Prognosis in most of patients is good due to high efficacy of treatment based on thyroidectomy followed by radioiodine (RAI) ablation of thyroid remnants. But up to 15–20% of all thyroid cancers might lose their ability to trap RAI, therefore they are being hidden for RAI imaging and therapy.RAI-refractory thyroid cancer, whether local or disseminated, needs an alternative imaging strategies. It is compelling that 68Ga-PSMA PET/CT imaging can potentially identify RAI-refractory metastases in patients with negative RAI scan, and serve as a potential therapeutic opening.

PSMA expression has not been studied systematically in thyroid cancers, and only limited evidence is available about absence of PSMA expression in benign thyroid. Tissue microarray study has found occasional and weak PSMA expression in less than 5% of follicular and papillary thyroid cancers.

Treatment and prognosis

Since follicular adenomas cannot be reliably distinguished from carcinomas by imaging or FNAC, and therefore most patients are referred for surgery [92].A lobectomy is mostly performed in these cases. Occasionally, patients are treated with levothyroxine to suppress TSH and followed without surgery, if the nodule decreases in size. Hyper functioning nodules are generally associated with a very low risk of malignancy and are typically managed conservatively.

The treatment of follicular carcinomas is surgery. Because the diagnosis is rarely established preoperatively, the initial surgical approach is lobectomy. If invasion is identified from intraoperative frozen sections from a lobectomy specimen, the surgery may be expanded to total or near-total thyroidectomy. However, mostly minimally invasive follicular carcinomas are diagnosed only during microscopic evaluation of routine sections. The need for second surgery to remove the remaining lobe is mostly debatable. Many studies have found that total thyroidectomy has

an overall positive effect on outcome in patients with tumours larger than 1 to 1.5 cm and in high-risk groups; whereas other studies report that there is no effect of the extended surgery on survival [93, 94]. Most of the current guidelines suggest total thyroidectomy for patients with follicular carcinomas. The main reason for completion thyroidectomy is to allow subsequent radioiodine therapy and monitoring of tumour recurrence by measuring serum thyroglobulin in these patients. Most patients with follicular carcinoma undergo postoperative treatment with 131I, which improves the outcome of the patient significantly [95].External beam radiation therapy can be reserved only for tumours that cannot be completely excised. On follow-up study, it has been found that tumour recurrence at local site or distant metastases occurs in 15% to 30% of cases [96].The 10-year survival rates in U.S. patients was 83% to 85% for conventional follicular carcinoma and 73% to 76% for the oncocytic follicular carcinoma [97].The Factors consistently found to negatively affect survival are older age of the patients larger tumour size more than 4 cm, distant metastases at presentation, and extra thyroidal extension [98].Additional factors that might correlate with mortality and/or recurrence include degree of invasiveness, (i.e., minimally invasive vs. widely invasive carcinomas marked vascular invasion (≥4 vessels) and oncocytic appearance[99,100].

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN

The present study was a prospective study conducted in the Department of Pathology; Madurai Medical College during the period May 2016 to August 2018.Ethical clearance for the study was obtained from the Ethical Committee of Madurai Medical College, Madurai. A total sample of 113 cases of Thyroid lesions was analyzed during this period.

INCLUSION CRITERIA

1. Follicular thyroid Adenoma (FTA)
2. Follicular thyroid carcinoma (FTC)
3. Follicular variant of Papillary Thyroid carcinoma (FVPTC)

EXCLUSION CRITERIA

1. Other neoplasms of thyroid
2. Other non neoplastic lesions of thyroid

METHODOLOGY AND TECHNIQUES

The study material included 113 Thyroid lesions. (Annexure VI) Clinical and morphological details of the cases were recorded according to the Proforma (Annexure III). Operated resection specimens were collected and fixed in 10% neutral buffered formalin for 12 hours.

After adequate fixation, the specimens were photographed and sliced in 5–10 mm intervals. The margins were best evaluated by sections taken perpendicular to the specimen surface closest to the deep margin of the tumour. Representative bits were taken from the tumour, adjacent soft tissue, surgical margins and lymph nodes. They were processed routinely and multiple 4 to 6 micron thin paraffin sections were obtained. Staining was done by Haematoxylin and Eosin staining technique (Annexure IV)

HISTOMORPHOLOGICAL EVALUATION

Stained slides were evaluated under light microscopic examination. Tumours were classified as benign or malignant based on cellularity, nuclear features, nuclear atypia and presence or absence of atypical mitotic figures. Tumours were categorized broadly according to their pattern of differentiation (Annexure VI).

IMMUNOHISTOCHEMICAL EVALUATION

Selected cases of follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma were subjected to immunohistochemical evaluation with PSMA. Blocks with minimal necrosis or haemorrhage and representative amount of tumour tissue were selected for IHC study. IHC was performed using monoclonal rabbit PSMA, according to the manufacturer’s protocol. Prostate tissue was

considered as positive control. Also we performed CD31 staining to confirm the localization of neovasculature.

The stained sections were assessed for the extent and intensity of endothelial cell staining in tumour micro-vessels and scored semi-quantitatively.

Percentage of stained endothelial cells(%)	Interpretation	Intensity	Interpretation
0-9	Negative	No reaction	0
10-39	Minimal	Faint reaction visible only at high power	1+
40-69	Moderate	Moderate intensity at low power	2+
≥70	Diffuse	Strong reaction easily visible at low power	3+

STATISTICAL ANALYSIS

Data obtained was entered into Microsoft excel spread sheet. The data was analyzed using ratios and percentage. All quantitative and qualitative data were analyzed using Fischer's exact test. P-value less than 0.05 were considered significant. Observations and results were compared with other studies and inferences drawn.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Table 1: Average incidence of thyroid specimens in our institution (N=9823)

Total specimens	Thyroid specimens
9823	1521 (15.48%)

9823 specimens received in our institution during the study period were studied. Thyroid specimens were about 15.4% of the total specimens received.

Chart 1: Average incidence of thyroid specimens in our institution (N=9823)

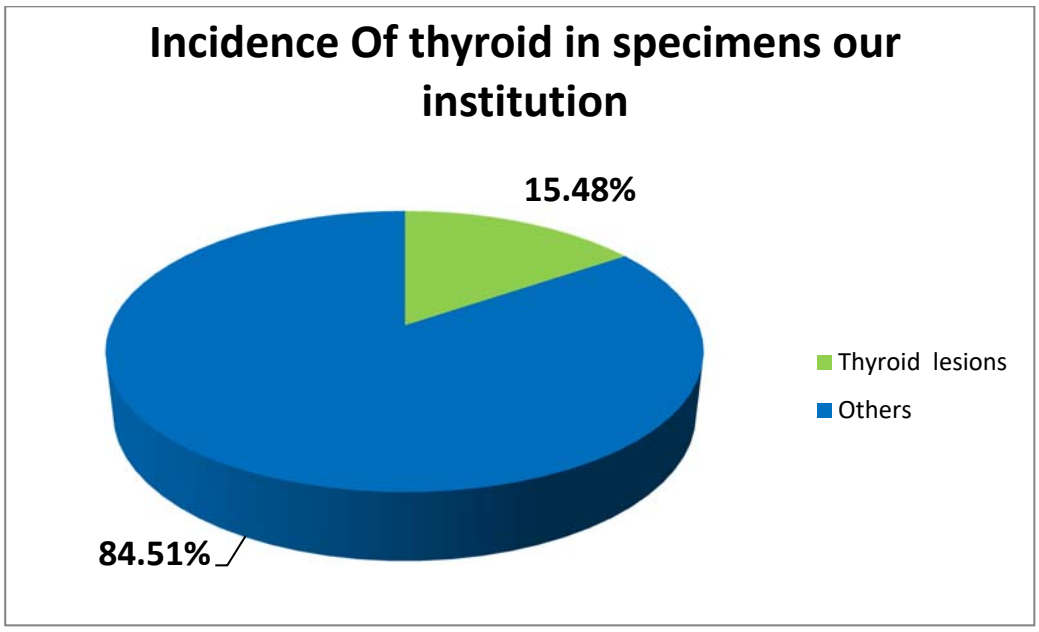


Table 2: Incidence of Benign and Malignant Thyroid lesion (N=1521)

Variable	Frequency (Percentage)
Malignant	42(2.76%)
Benign	1479(97.24%)
Total	1521

The most common were benign thyroid lesion which contributed around 97% and malignancy about 2.67% among total 1521 specimens studied.

Chart 2: Incidence of benign and malignant thyroid lesion (N=1521)

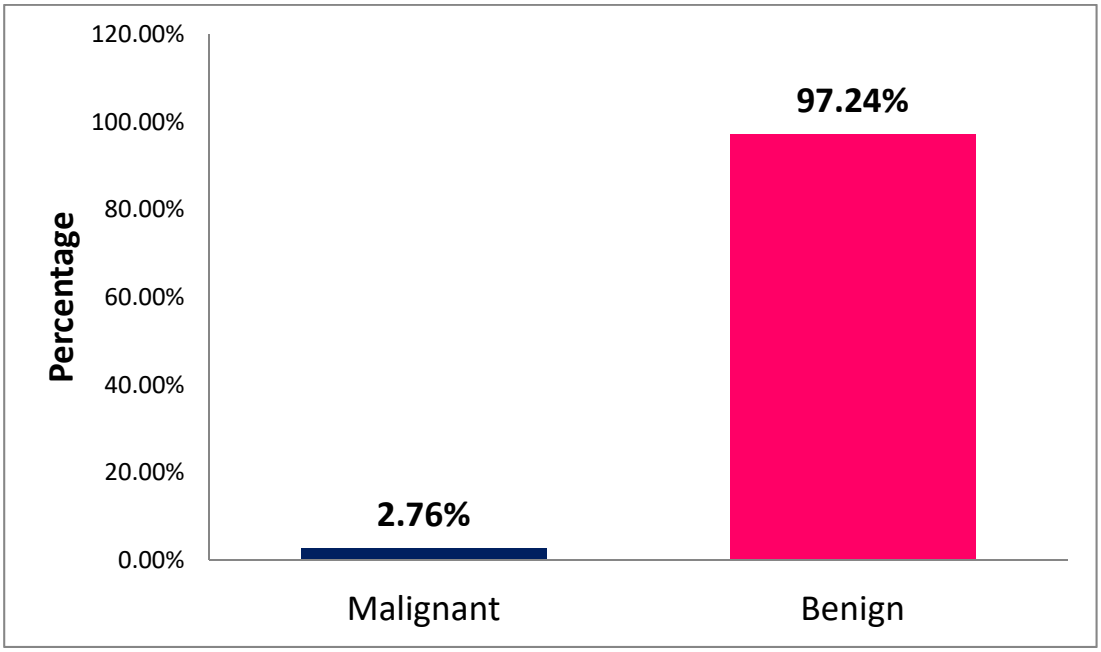


Table 3: Incidence of thyroid follicular neoplasm (N=1521)

Variable	Frequency (Percentage)
Thyroid follicular neoplasm	113(7.43%)
Others	1408(92.57%)
Total	1521

Among the 1521 specimens studied, among that 7.43% were thyroid follicular neoplasm.

Chart 3: Incidence of thyroid follicular neoplasm (N=113)

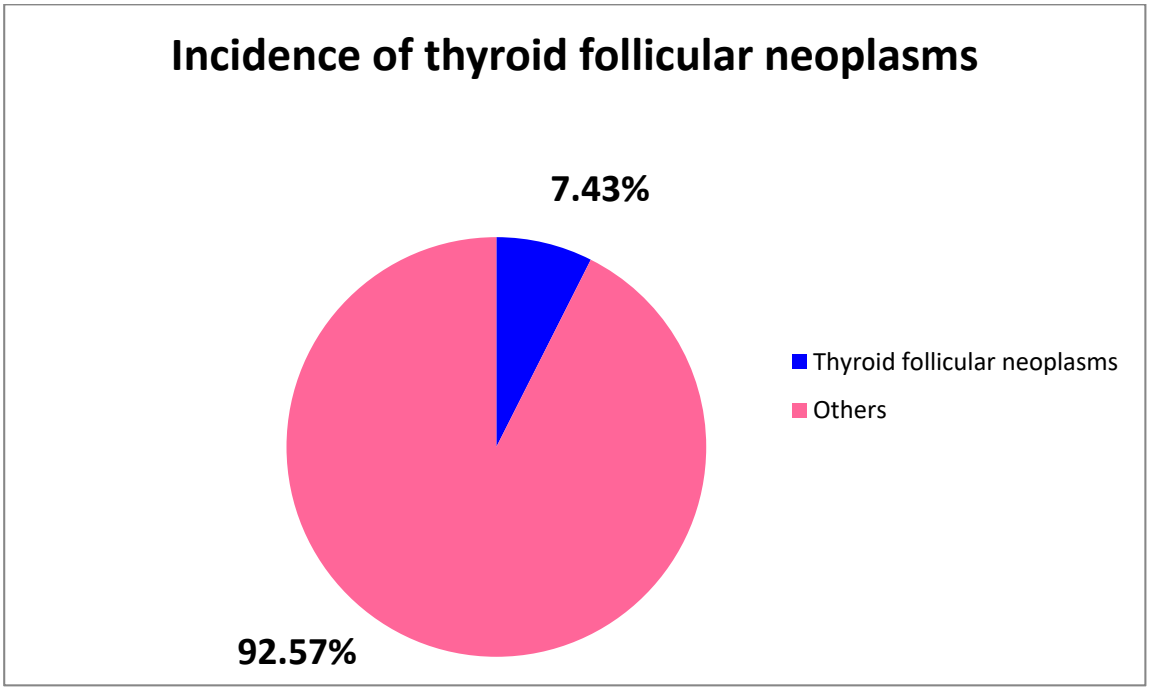


Table 4: Incidence of malignant lesions in total follicular lesions (N=113)

Variable	Frequency (Percentage)
Benign	95(84.07%)
Malignant	18(15.92%)
Total	113

Among 113 specimens of thyroid lesions, 84% of them were benign and 16% were malignant.

Chart 4: Incidence of malignant lesions in total follicular lesions (N=113)

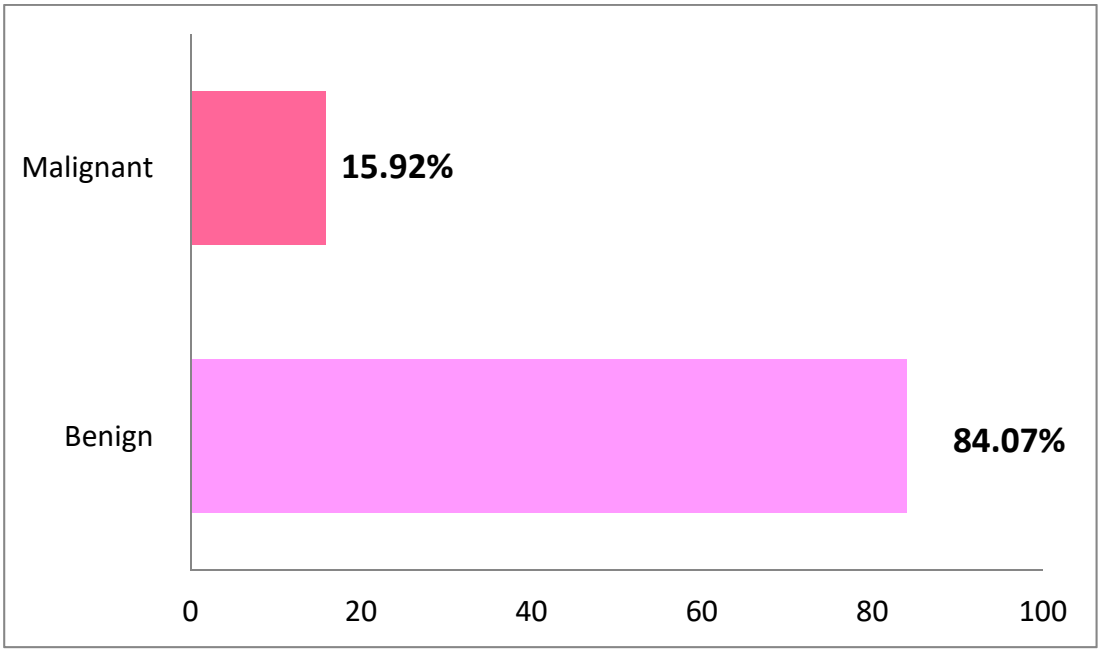


Table 5: Categorisation of thyroid follicular neoplasm (N=113)

Variable	Frequency (Percentage)
Follicular adenoma	95(84.07%)
Follicular carcinoma	1(0.88%)
Follicular variant of papillary carcinoma	17(15.04%)
Total	113

Among 113 thyroid lesion studied, 84% of were follicular adenoma, 15% were of follicular variant of papillary carcinoma and only 0.88% were follicular carcinoma.

Chart 5: Categorisation of thyroid follicular neoplasm (N=113)

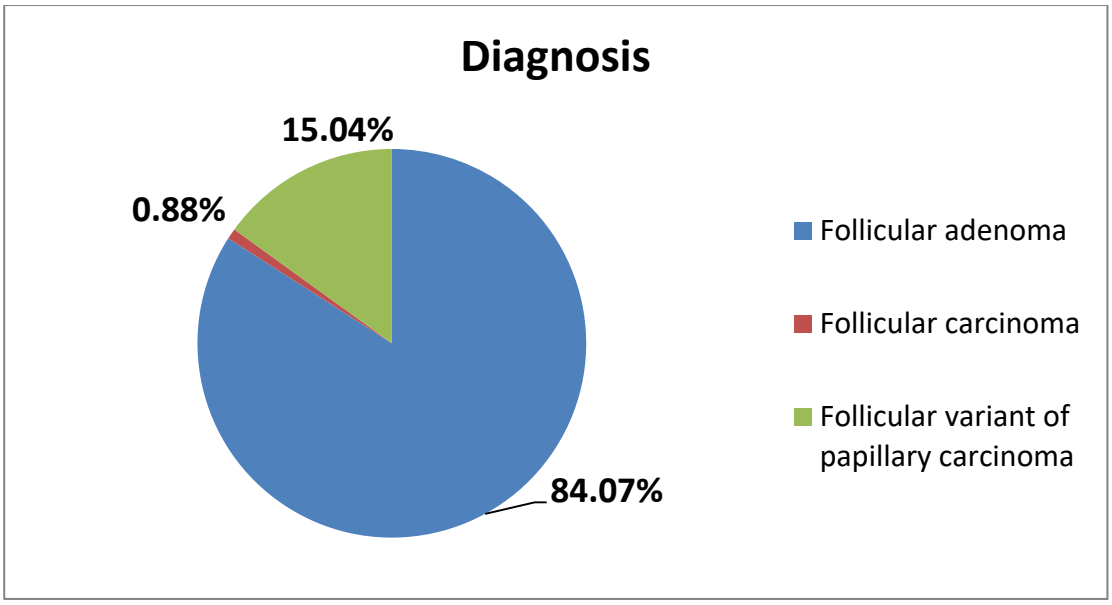


Table 6: Gender wise distribution of follicular neoplasm (N=113)

Variable	Frequency (Percentage)
Male	14 (12.39%)
Female	99 (87.61%)
Total	113

Females contributed around 88% of total follicular neoplasm and males about 12%. The female male ratio was 7:1.

Chart 6: Gender wise distribution of follicular neoplasm (N=113)

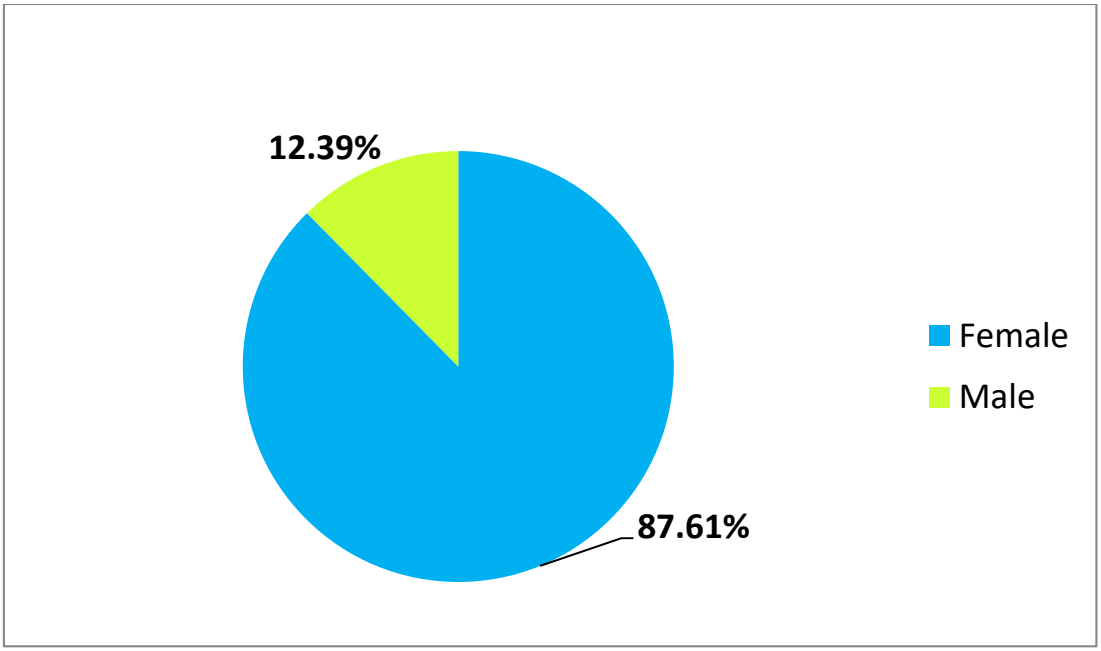


Table 7: Gender wise distribution of malignant follicular neoplasm (n=18)

Variable	Frequency (Percentage)
Female	15(83.33%)
Male	3(16.66%)
Total	18

Among 18 cases of malignant follicular neoplasm, 84% were females and 16% were males.

Chart 7: Gender wise distribution of malignant follicular neoplasm (n=18)

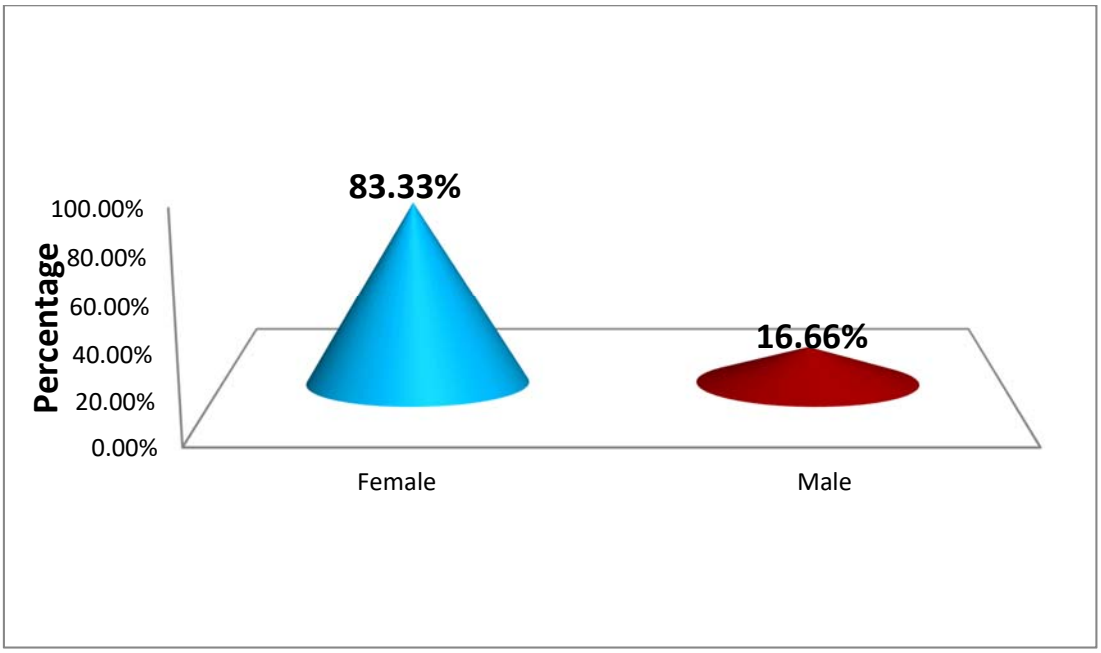


Table 8: Age wise distribution of thyroid follicular neoplasm (N=113)

Variable	Frequency (Percentage)
Below 19	5(4.43%)
20 to 29	20(17.70%)
30 to 39	40(35.40%)
40 t0 49	28(24.77%)
50 to 59	14(12.39%)
60 to 69	6(5.31%)
Total	113

Among 113 cases of thyroid follicular neoplasm, 35% belonged to age group 30-39yrs, 25% of them were between 40-45 yrs, and 17% between 20-29 yrs, 12% were between 50-59 yrs. around 4.43% below 19 yrs and 5.31% between 60-69 yrs

Chart 8: Age wise distribution of thyroid follicular neoplasm (N=113)

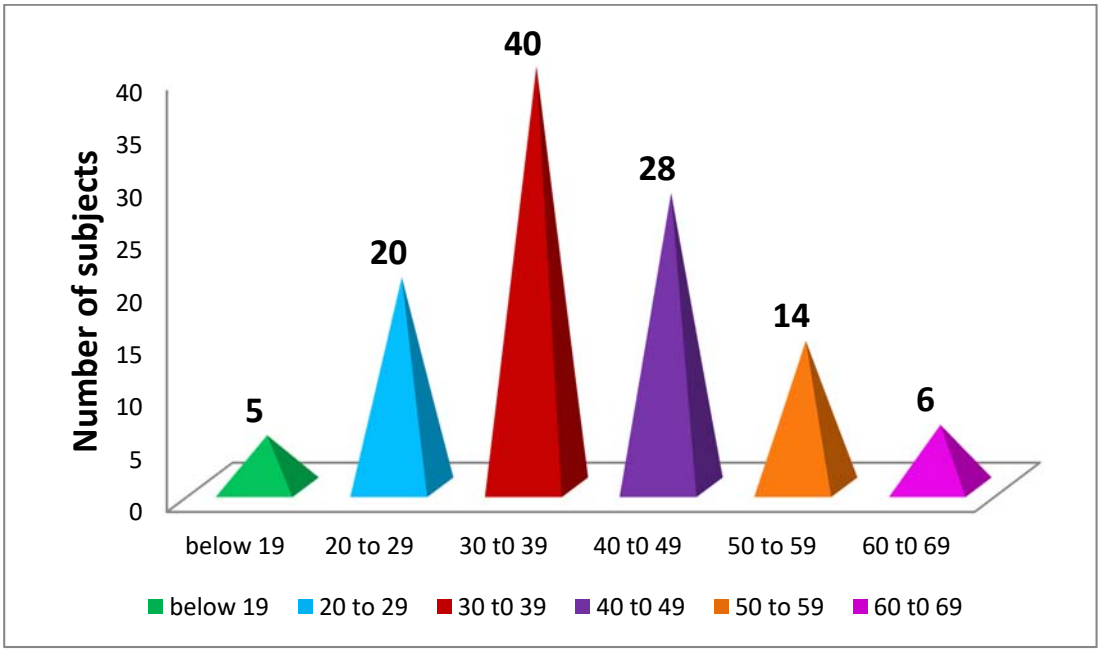


Table 9: Age wise distribution of malignant follicular lesions (n=18)

Variable	Frequency (Percentage)
Less than 19	1(5.56%)
20 to 29	2(11.12%)
30 to 39	8(44.44%)
40 to 49	3(16.66%)
50 to 59	2(11.11%)
More than 60	2(11.11%)

Among 18 cases of malignant follicular neoplasm, 44% belonged to age group 30-39yrs, 17% of them were between 40-49 yrs, and 11% between 20-29 yrs, 50-59 yrs and more than 60 yrs respectively. Around 5.56% were below 19 yrs.

Chart 9: Age wise distribution of malignant follicular lesions (n=18)

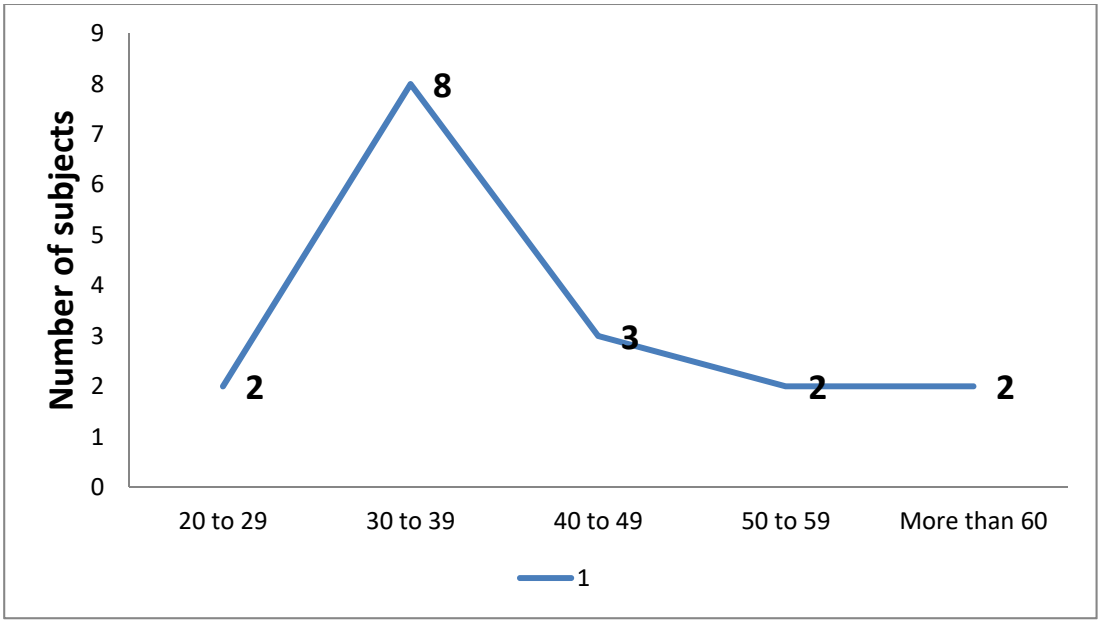


Table 10: Clinical presentation of Thyroid follicular neoplasm (N=113)

Variable	Frequency (Percentage)
Solitary nodule	81(71.68%)
Multiple nodule	32 (28.32%)

About 71% of thyroid follicular neoplasm presented with solitary nodule and 28.32 % with multiple nodule.

Chart 10: Clinical presentation of Thyroid follicular neoplasm (N=113)

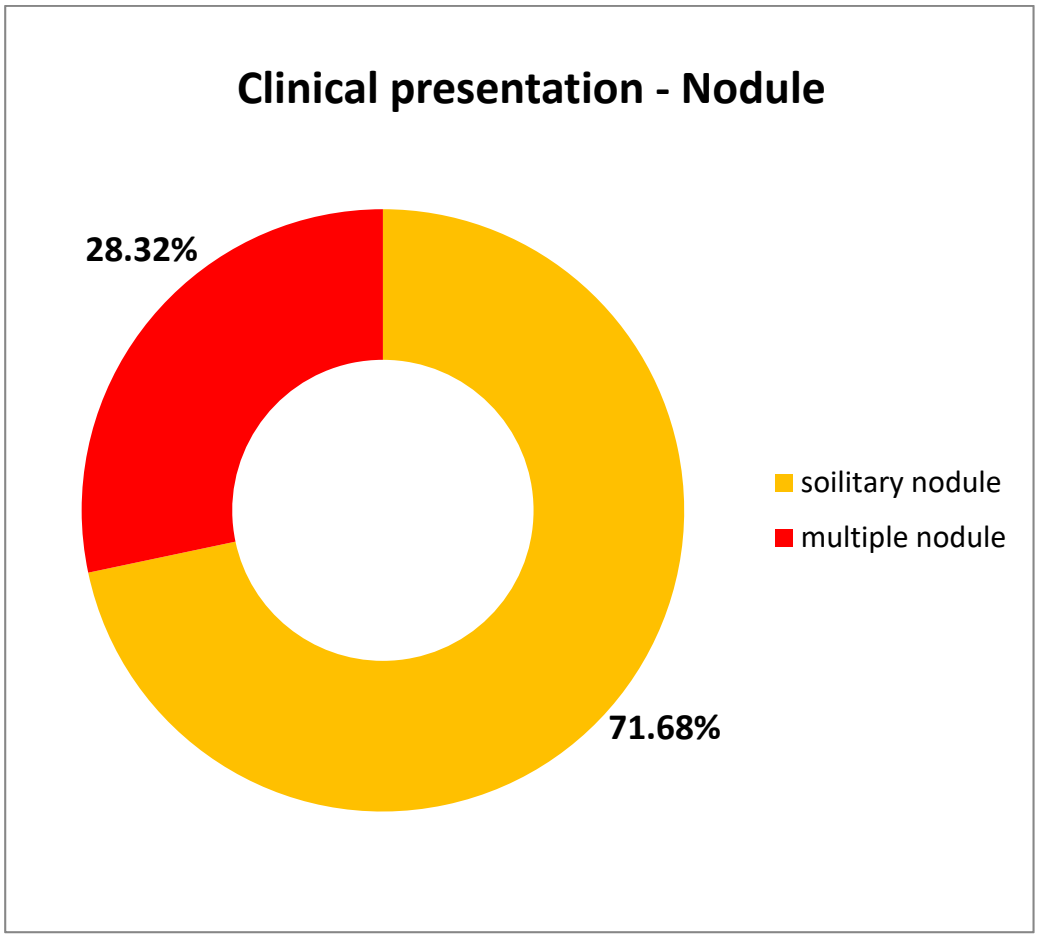


Table 11: Surgical procedure done (N=113)

Variable	Frequency (Percentage)
Total thyroidectomy	38(33.63%)
Hemi total thyroidectomy	74 (65.49%)
Near total thyroidectomy	1(0.88%)

Among 113 cases of thyroid lesion, 65% of them underwent hemi-total thyroidectomy, 33.63% of them underwent total thyroidectomy and 0.88% near total thyroidectomy.

Chart 11: Surgical procedure done (N=113)

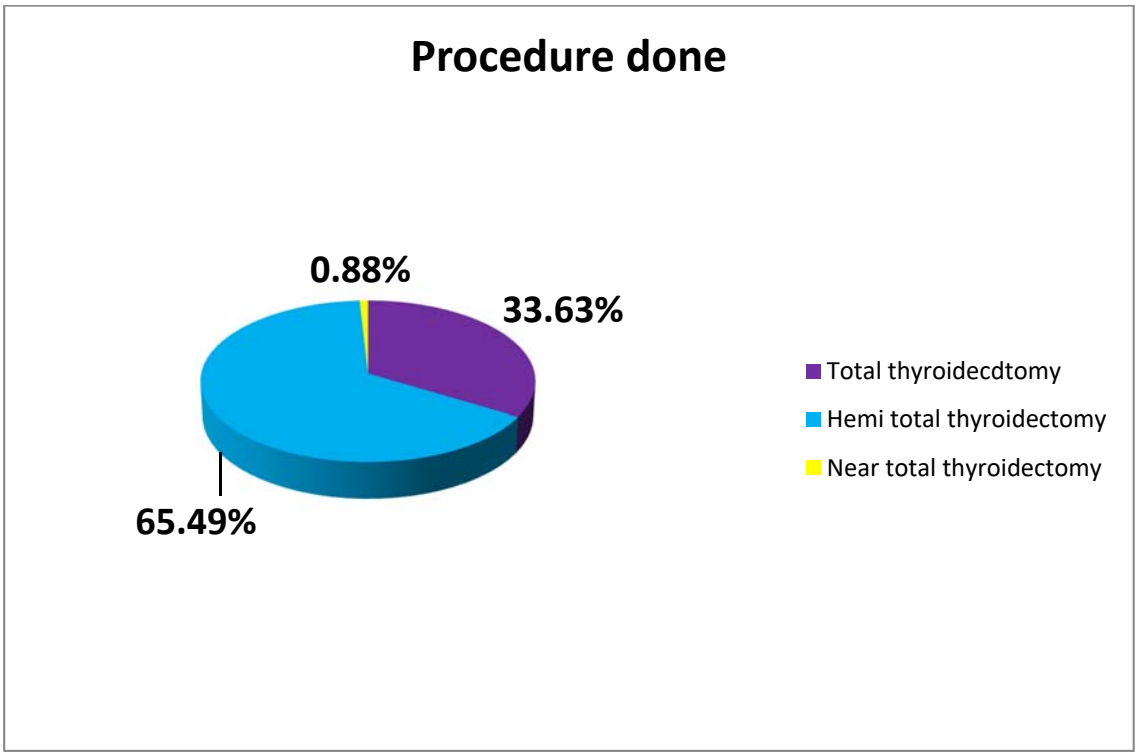


Table 12: Variants of follicular Adenoma (n=95)

Variable	Frequency (Percentage)
Simple /Normofollicular	81(85.27%)
FA with papillary hyperplasia	5(5.27%)
FA Fetal variant	3(3.15%)
FA Hurthle cell variant	4(4.21%)
FA colloid variant	2(2.10%)
Total	95

Among 95 cases variants of follicular adenoma, 85% of them was simple/normo follicular, 5.27% were FA with papillary hyperplasia, 4.21% FA hurthle cell variant, 3.15% FA fetal variant and 2.10% FA colloid variant.

Chart 12: Variants of Follicular Adenoma (n=95)

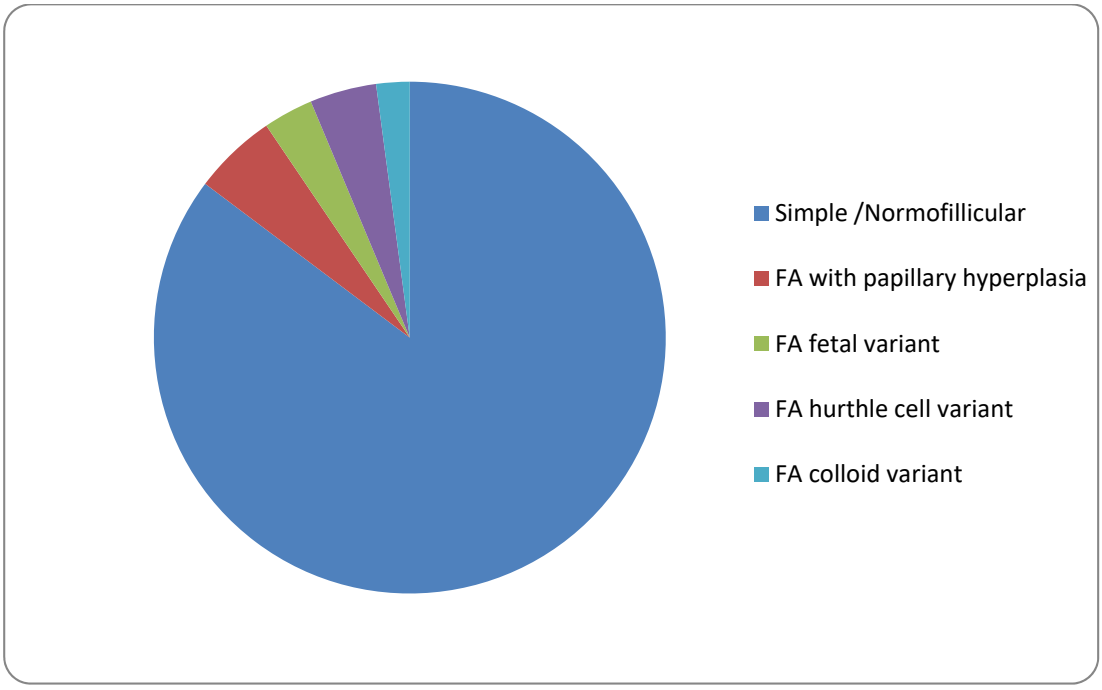


Table 13 :The intensity of PSMA staining by immunohistochemistry in FTA, FTC and FVPC

Intensity score	0	1+	2+	3+	Total	Fischer's exact test (df)	p value
Follicular Adenoma	30	4	1	1	36	10.384 (3)	0.013
Follicular carcinoma and Follicular variant of papillary carcinoma	6	4	4	0	14		

In the present study 83% of follicular adenoma had negative staining.11% of follicular adenoma had 1+ (faint reaction at high power).2.7 % of follicular adenoma had 2+ (moderate intesity at low power) and 2.7% of them had 3+ (strong intensity at low power).

Where as the malignant lesions (FC and FVPC) had 57% of negative staining ,14% of them were 1+ and 28% of them had 2+ staining.

Fischer exact test was used to calculate and the P values obtained were less than 0.05.

This shows that the instensity of staining were signnificantly higher in follicular carcinoma and follicular variant of papillary carcinoma.

Table 14: The Extent of PSMA staining by immunohistochemistry in FTA, FC and FVPC

Extent score	Negative	Minimal	Moderate	Diffuse	Total	Fischer's exact test (df)	p value
Follicular Adenoma	34	1	0	1	36	10.04 (4)	0.023
Follicular carcinoma and Follicular variant of papillary carcinoma	9	0	2	2	14		

In the present study 86 % of the follicular adenoma had negative staining (0 to 9% of satined endothelial cells, 5.5 % had minimal staining (10 to 39% of stained endothelial cells ,1% had moderate staining (40 to 69% of stained endothelial cells) and 5% had diffuse staining (more than 70% of stained endothelial cells)

Where as for the malignant lesions (FC and FVPC) 64% showed negative staining and 14% had diffuse staining.

Fischer exact test was used to calculate and the P values obtained were less than 0.05.

This shows that the extent of staining were signnificantly higher in follicular carcinoma and follicular variant of papillary carcinoma.

PHOTOGRAPHS

PHOTOGRAPHS

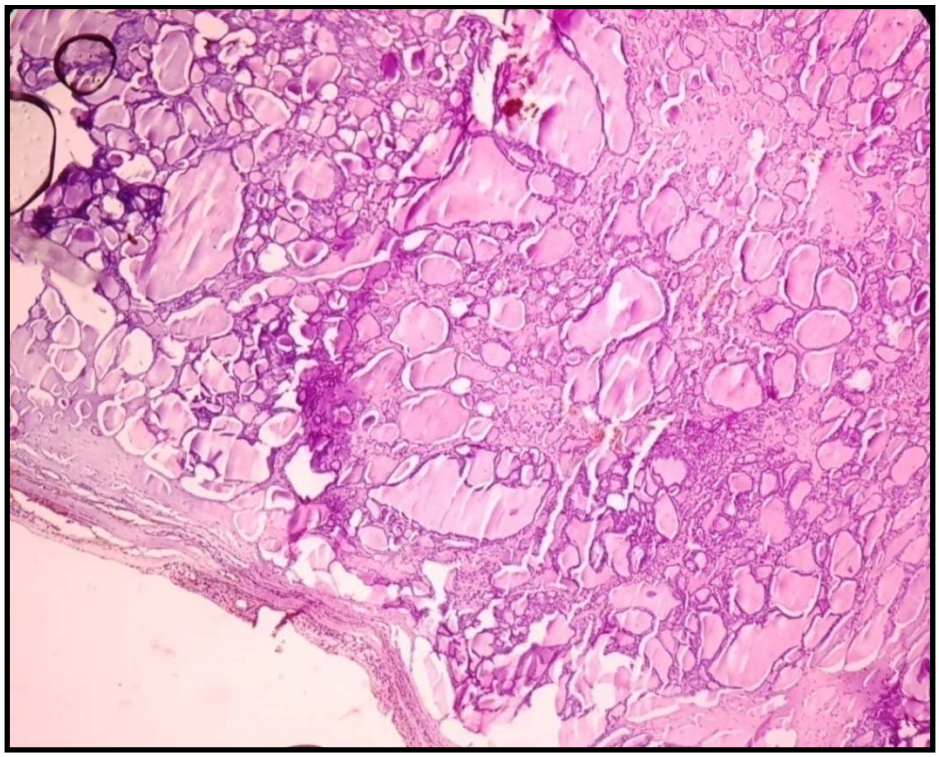


Figure 1 - Follicular adenoma – (H&E 100x) case 1581/18

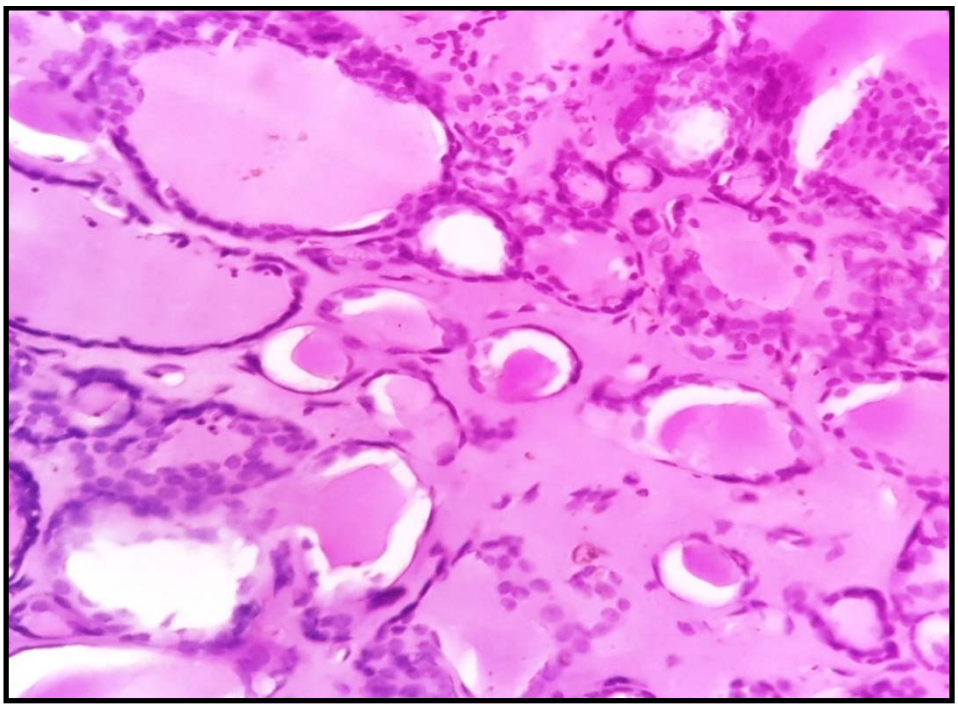


Figure 2- Follicular adenoma -normofollicular – (H&E 400x) case 2255/18

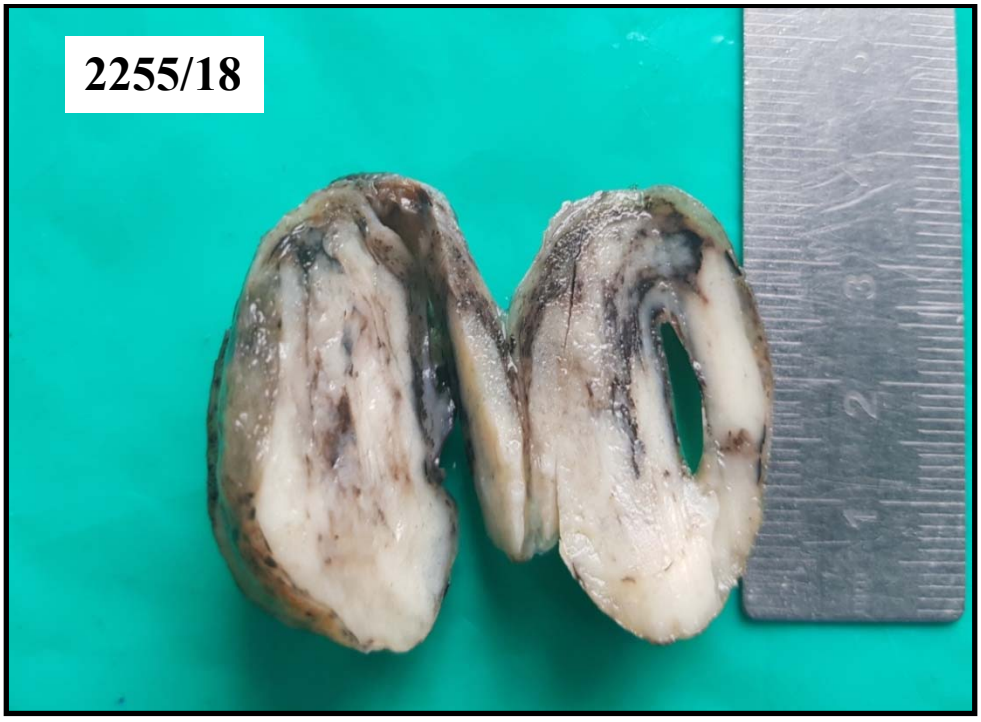


Figure 3 – Follicular adenoma: Well encapsulated single nodule with a thin capsule. Case 2255/18.

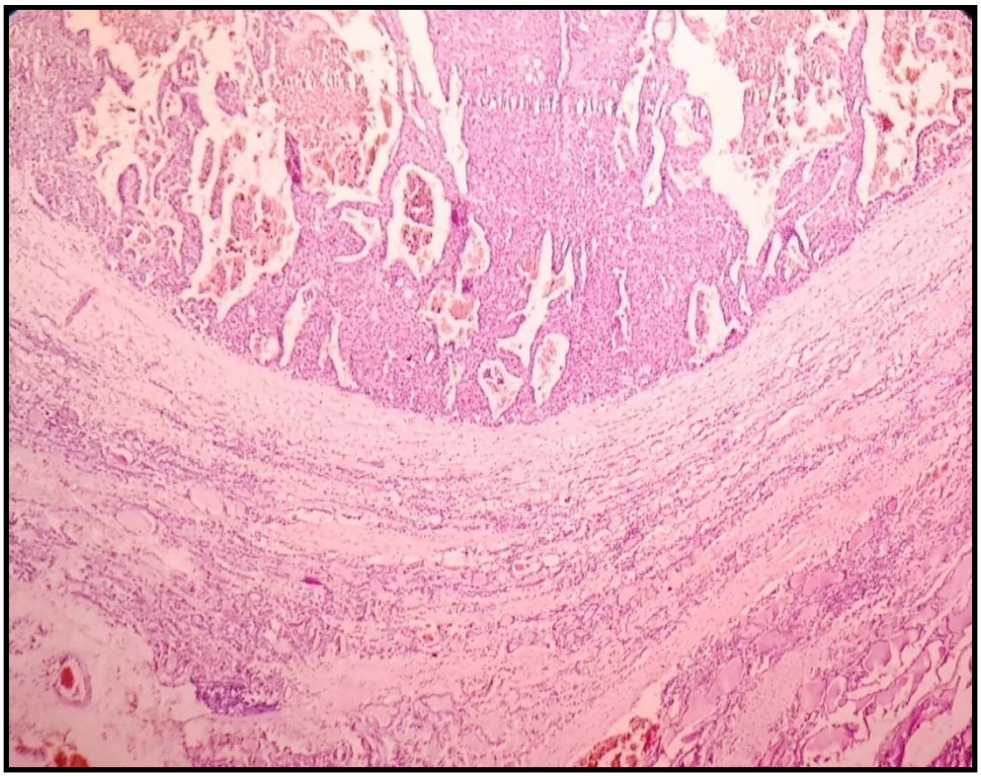


Figure 4 - Follicular adenoma –Hurthle cell variant (H&E 100x) case 674/18

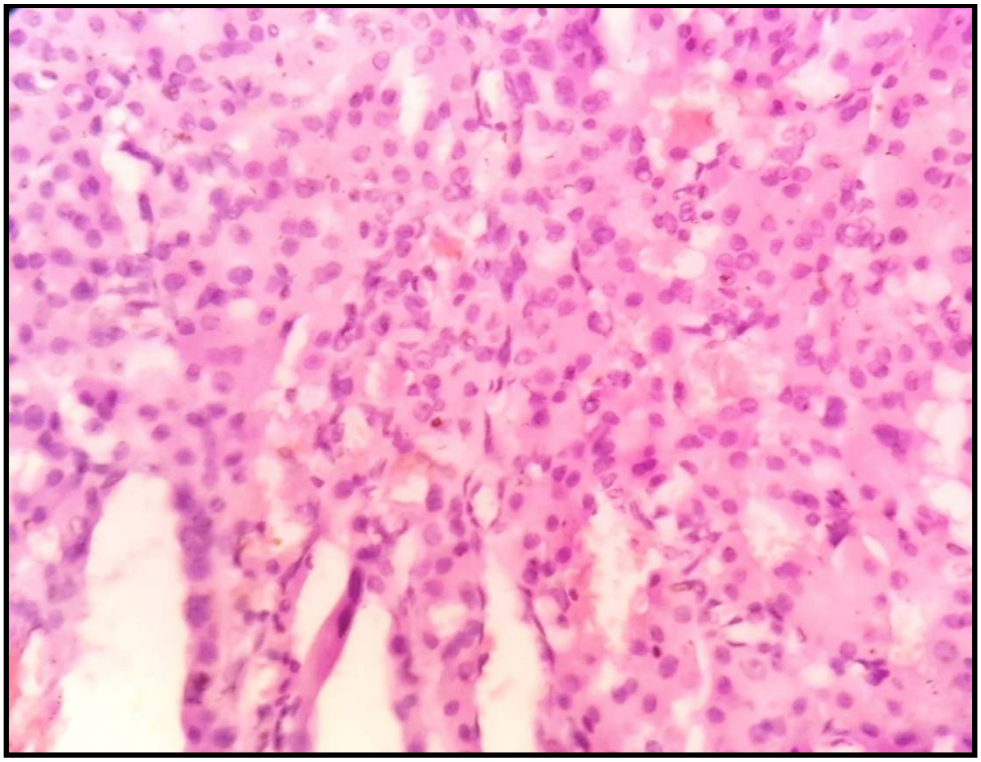


Figure 5- Hurtle cell variant of follicular adenoma (H&E 400x) case 2361/16

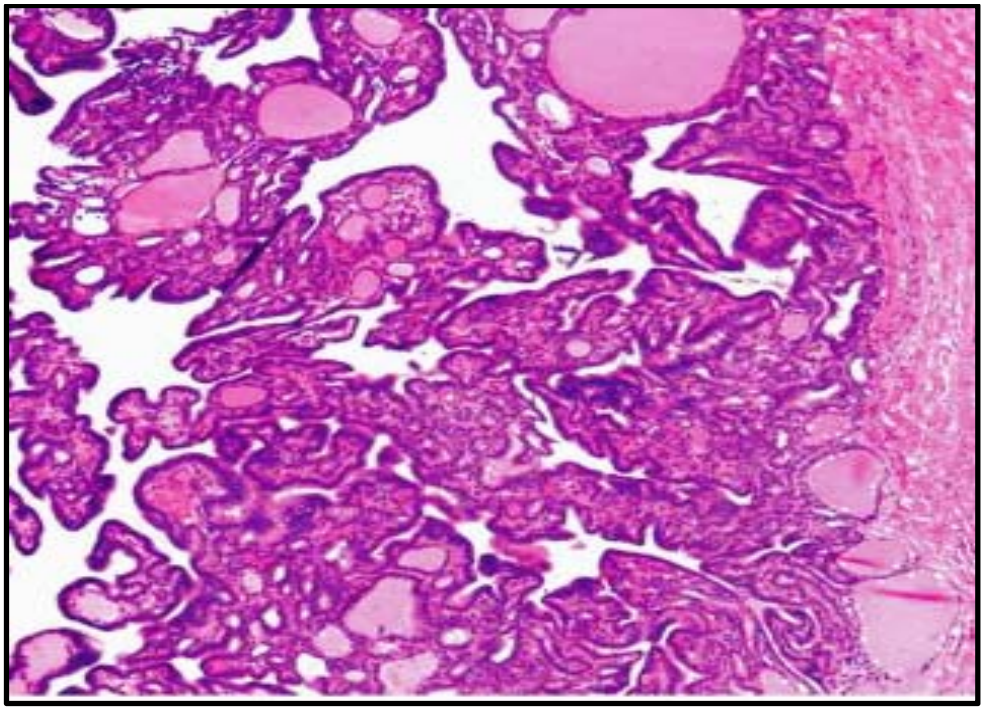


Figure 6-Follicular Adenoma with papillary hyperplasia (H&E 100x) case 529/17.

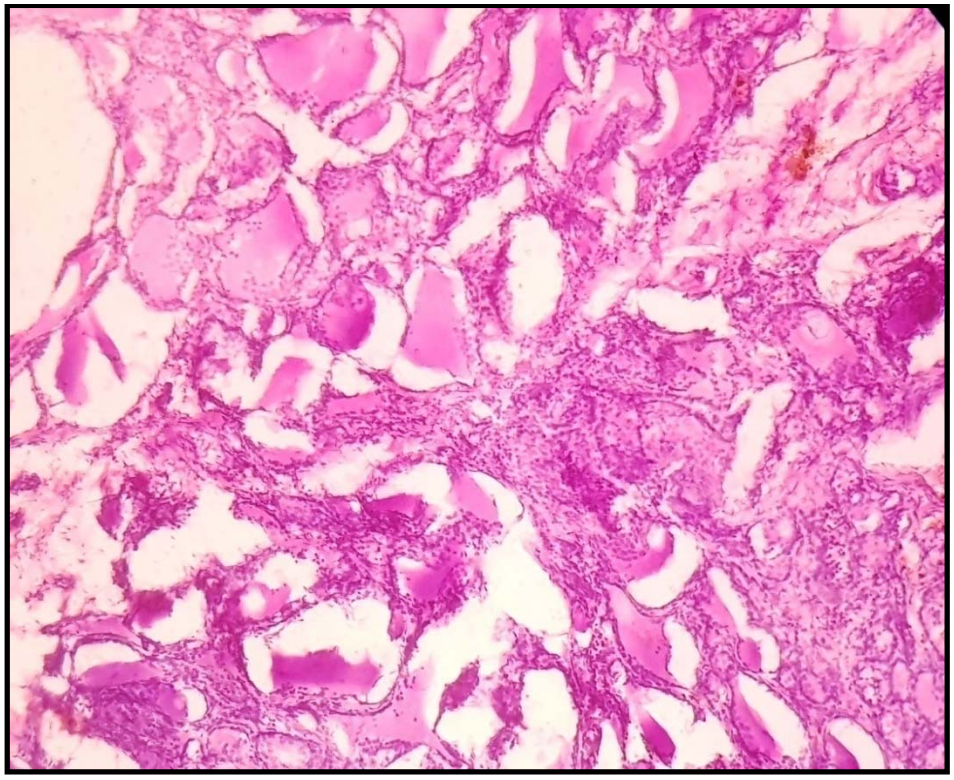


Figure 7–Follicular variant of papillary carcinoma (H&E 100X) case 1835/18

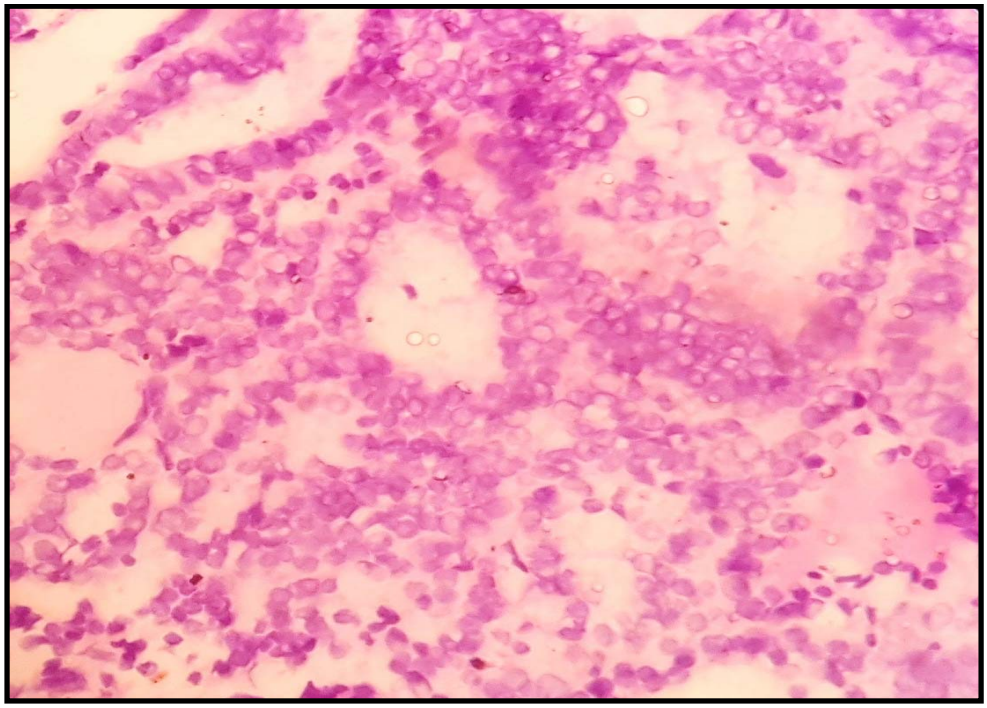


Figure 8 –Follicular variant of papillary carcinoma (H&E 400x) case 3270/16

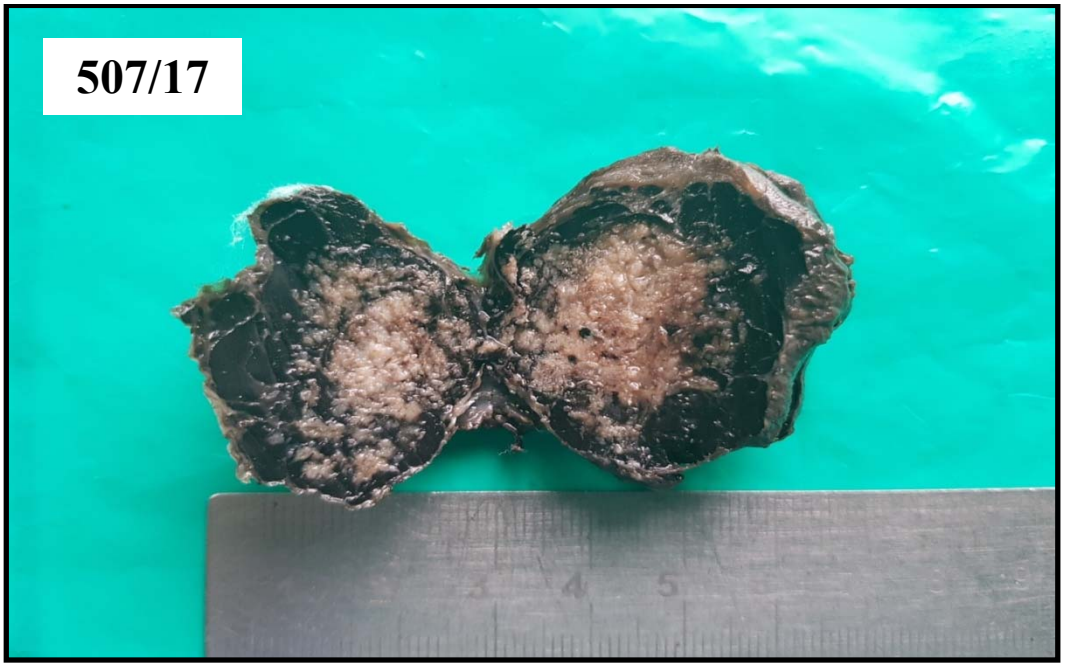


Figure 9 – Gross picture of follicular variant of papillary carcinoma. Case 507/17.

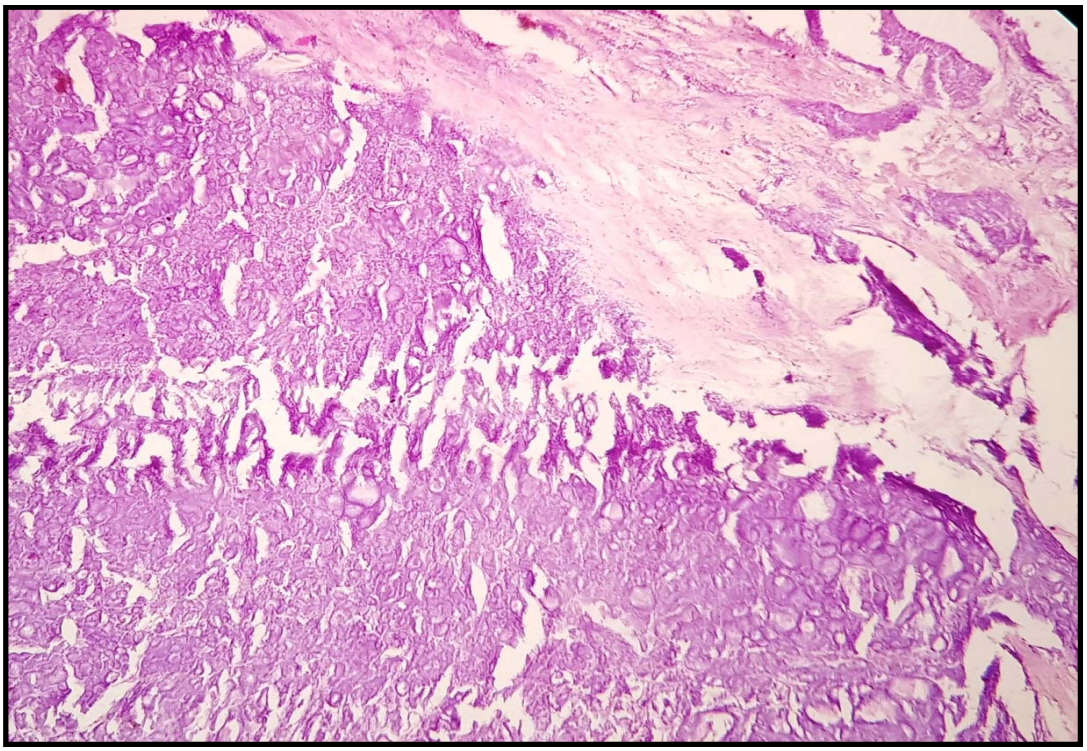


Figure 10 – follicular carcinoma thyroid (H&E 100x) case 1018/16

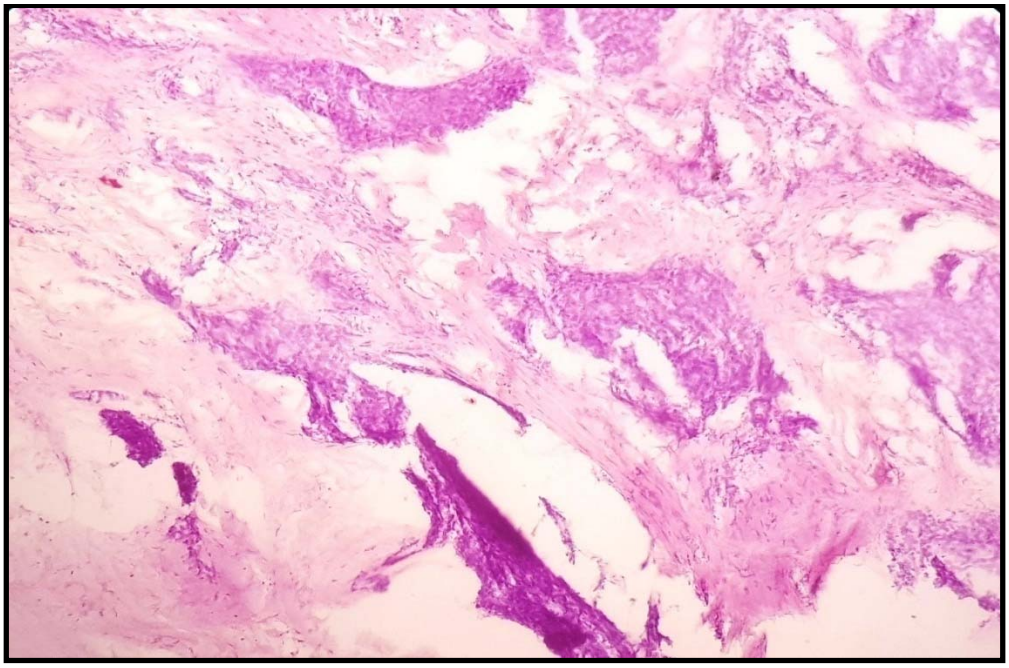


Figure 11- follicular carcinoma showing capsular invasion (H&E 400x) case 1018/16

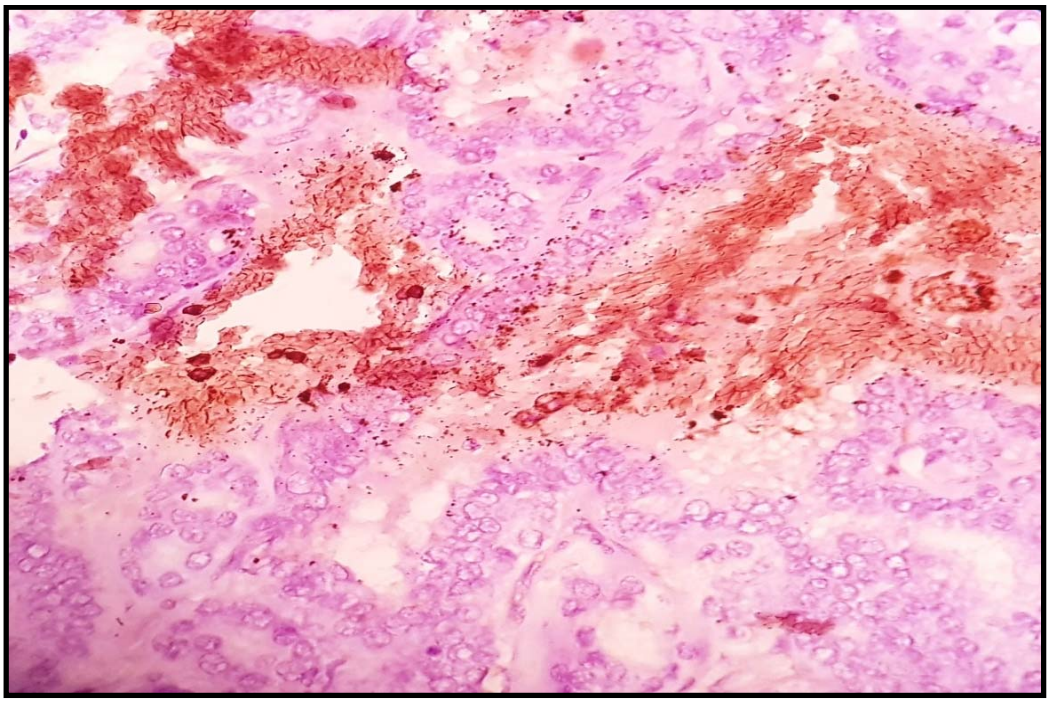


Figure 12 – Follicular variant of papillary carcinoma showing positive case staining for PSMA (400x).Case 1835/18.

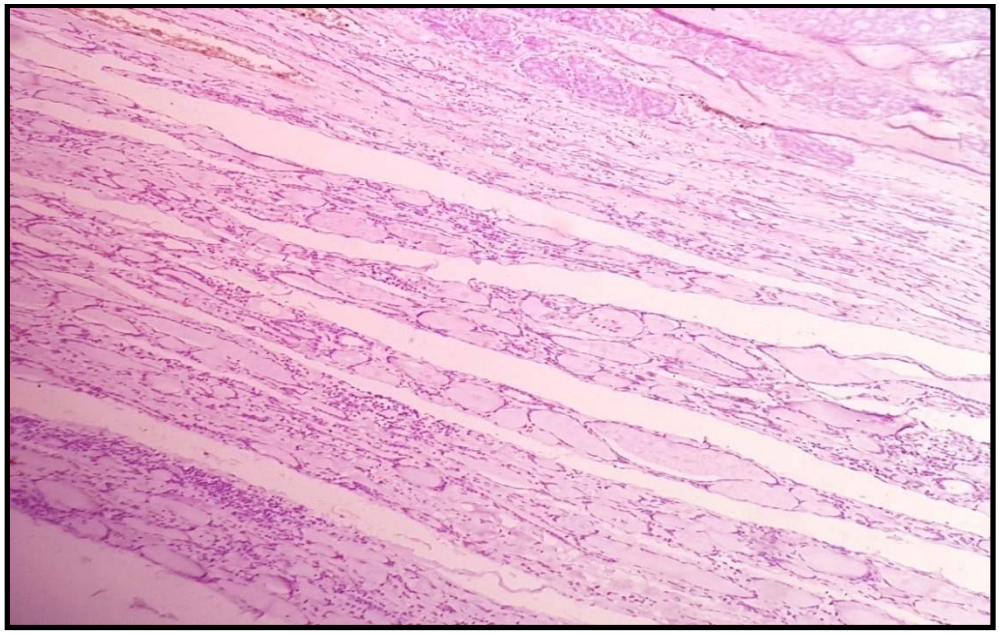


Figure 13- Follicular adenoma - PSMA staining negative. Case 3411/16.

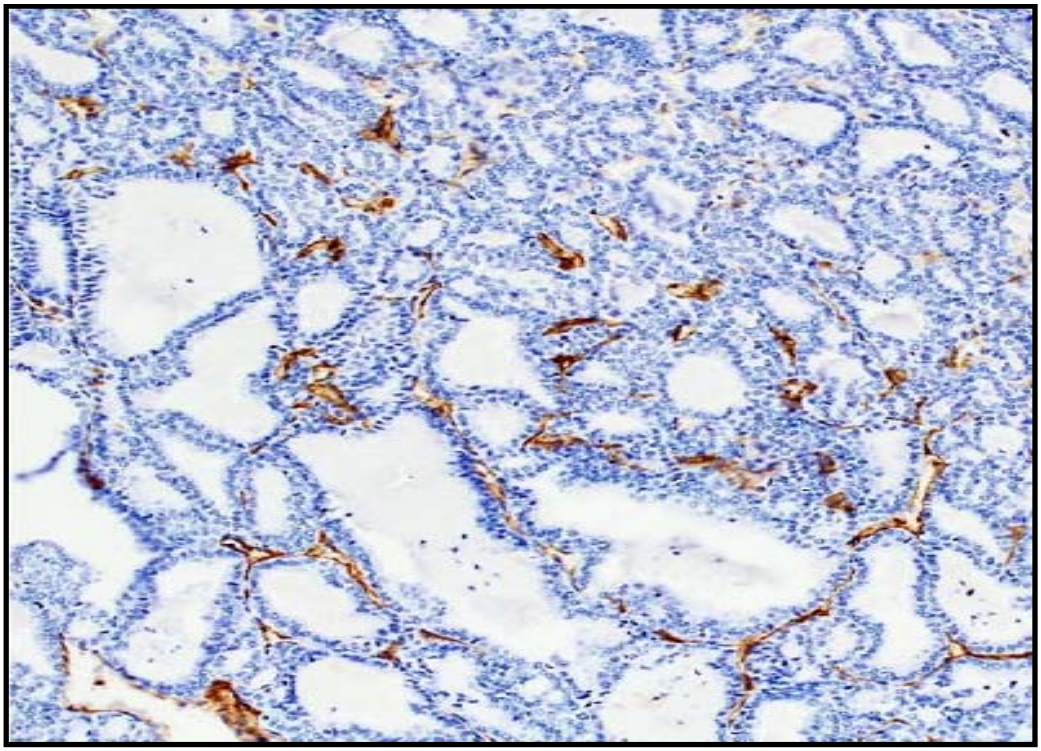


Figure 14- Follicular carcinoma - Positive PSMA staining.
Case 1018/16

DISCUSSION

DISCUSSION

Thyroid gland lesions having follicular growth pattern includes Follicular thyroid adenoma (FTA), follicular thyroid carcinoma (FTC) and follicular variant of Papillary Thyroid Carcinoma (FVPTC).Diagnosing the thyroid tumour pathology preoperatively would reduce the cost of management of thyroid nodules by eliminating diagnostic thyroidectomies which are being performed for benign thyroid nodules.

Even though FNAC is a widely recognised practice and a non expensive technique for diagnosing the lesion, we cannot differentiate between follicular adenoma and carcinoma in FNAC. Even in histomorphology, it is sometimes problematic for pathologist to diagnose a solitary encapsulated nodule with follicular histological pattern.

In this present study, out of 113 cases of thyroid follicular neoplasm, 95 cases were follicular adenomas, a case was follicular carcinoma and 17 cases were of follicular variant of papillary carcinoma. Thyroid follicular neoplasm showed an incidence of 7.4% which correlated with similar studies conducted in other institutions

Age distribution and sex distribution of the cases were studied.

Age incidence

Most of the cases of follicular adenoma were in the third decade. This correlated with the study conducted by Hiva saffer et al which also showed the highest incidence of follicular adenoma in the third decade of life [101] .The incidence of follicular carcinoma was common in fifth and sixth decade. In the study done by Christopher et al the highest incidence of follicular adenoma was in the third decade and follicular carcinoma was in the sixth decade of which correlated with our study [102]. The incidence of follicular variant of papillary carcinoma was in the third decade of life.

Table number 15 gives the age incidence comparative study

Table 15
Age Incidence Comparison Study

Sl.no.	Study	Age incidence
1	Hiva Saffar et al	Follicular adenoma -Third decade Follicular carcinoma –fourth decade Follicular variant of papillary carcinoma – fourth decade
2.	Christopher et al	Follicular adenoma- Third and fourth decade. Follicular carcinoma – sixth decade
3.	Present study	Follicular adenoma- Third decade Follicular carcinoma – sixth decade Follicular variant of papillary carcinoma – Third decade

Sex incidence

In the present study the gender incidence of thyroid follicular neoplasm showed that female outnumbered males with the female: male ratio of 7:1.In study done by Phitayakorn et al showed female: male ratio of 3 :1 and in the study done by Christopher et al showed female male ratio was 4 to 5 :1[102].

The tables 16 shows gender incidence comparative study

Table 16
Gender incidence comparative study

S.no	study	Gender incidence
1	Phitayakorn et al	F:M = 3 :1
2.	Christopher et al [103]	F:M =4 to 5:1
3.	Present study	F:M = 7:1

Presentation

In the present study the mode of clinical presentation was solitary nodule, which was about 71.6% .This correlates with the study done by Klonoff DC, et al which also showed the common type of clinical presentation as solitary nodule [104]

Behaviour of thyroid follicular neoplasm

In the present study the most common of the thyroid follicular neoplasm was benign tumour which constituted about 84% of the all lesions. The frequency of malignant lesions was 15.9%.In study done by

Roasi et al, the incidence of benign to malignant follicular neoplasm was 5:1[105].

Distribution of thyroid follicular neoplasm

- The incidence of follicular adenoma was 6% in the present study.
- Whereas the study done by Silverberg et al the incidence of follicular adenoma was 3% and another study done by Bisi et al the incidence was 4.3% .[106][107]

Table 17

Incidence of follicular adenoma comparative study

Sl.no	Study	Incidence
1	Silverberg et al	3%
2	Bisi et al	4.3%
3	Present study	6%

The incidence of follicular variant of papillary carcinoma and follicular carcinoma was about 1%, in the present study.

In the study conducted by S.K.G grebe et al, the incidence of follicular carcinoma was 9% and in the study conducted by xio –min et al the incidence of papillary carcinoma was about 9% of all papillary carcinoma. [108][109]

Variant of Follicular adenoma

In the present study the most common variant of follicular adenoma was normo follicular or simple variant which constituted about

85% of all follicular adenoma cases followed by follicular adenoma with papillary hyperplasia which was about 5% and the least common variant was follicular adenoma colloid variant which was about 2%.which correlates with the study conducted by Nikofov et al. [110]

PSMA IMMUNOQUANTITATION

An attempt has been made to study the expression of PSMA in thyroid follicular neoplasm, its intensity of distribution and its usefulness in differentiating the benign from malignant lesions.

In the present study, PSMA immunoquantitation revealed the following

- Follicular adenoma showed a low intensity of staining of about 16 %.
- Malignant follicular neoplasm (follicular carcinoma and follicular variant of papillary carcinoma) showed a comparatively a high intensity of 46%.
- Fischer's exact test was calculated. p value derived was 0.005 ($p<0.05$) and hence the correlation is strong and statistically significant.
- These results are comparable with studies by Andrey Bychkov et al and Hiva Saffar et al where they observed that the intensity of staining of PSMA were higher in follicular carcinoma and follicular variant of papillary carcinoma than follicular adenoma.[111,112]

SUMMARY

SUMMARY

In the present prospective study of 113 cases of thyroid specimens, the following results were obtained:

- Thyroid follicular neoplasms constituted about 7.4% of all thyroidectomy specimens.
- Benign tumour was more common than the malignant .Benign tumours constituted about 95% and malignant lesion constituted about 15%.
- The age range of thyroid follicular neoplasm was between 17 to 65 years .The most common age group involved was between 30 to 39 years which constituted about 35%.
- The average age of malignant thyroid follicular neoplasm was also between 30 to 39 years.
- The thyroid follicular neoplasm was more common in females than males with female male ratio of 7:1.
- The most common of the follicular neoplasm was follicular adenoma, which constituted about 84% followed by follicular variant of papillary carcinoma (15%) and follicular carcinoma (1%).

- The most common variant of follicular adenoma was simple or normofollicular variant which constituted about 81%, followed by follicular adenoma with papillary hyperplasia.
- The most common mode of clinical presentation was solitary nodule (71%).
- The most common surgical procedure done was hemi total thyroidectomy which was about 74%.
- PSMA immunoquantitation results revealed higher intensity of staining in follicular carcinoma and follicular variant of papillary carcinoma.
- Fischer's exact test was used and p value derived was 0.005 ($p<0.05$) and hence the correlation is strong and statistically significant.

CONCLUSION

CONCLUSION

Thyroid neoplasm represents one of the most common malignancies of the endocrine system. They pose a significant challenge to the pathologist and oncologists.

The histomorphological distinction between follicular carcinoma and follicular adenoma is one of a challenging task. Different diagnostic tools such as IHC and molecular profiling were tried to differentiate between benign and malignant follicular neoplasms or differentiating malignant tumour subtypes.

PSMA Expression in neovasculature can be used to distinguish benign and malignant follicular neoplasm by assessing the intensity and extent of staining of endothelial cells. PSMA-targeted radionuclide therapy can be used in advanced I^{131}-resistant/negative thyroid cancers.

ANNEXURES

ANNEXURE I

WHO CLASSIFICATION OF THYROID TUMOURS (2017)

TUMOURS OF THYROID GLAND

- Follicular adenoma
- Hyalinising Trabacular Adenoma
- Other encapsulated follicular pattered thyroid tumours
    - Follicular adenoma of Uncertain malignant potential
    - Well differentiated Tumours of Uncertain malignant potentaial
    - noninvasive follicular thyroid neoplasm with papillary like nuclear features
- Papillary thyroid carcinoma
    - Papillary carcinoma
    - Follicular variant of papillary carcinoma thyroid
    - Encapsulated variant of Papillary thytroid carcinoma
    - Papillary microcarcinoma
    - Columnar cell variant of PTC
    - Oncocytic variant of PTC
- Follicular thyroid carcinoma (FTC) NOS
    - Follicular thyroid carcinoma , minimally invasive

    - Follicular thyroid carcinoma ,,encapsulated angioinvasive
    - Follicular thyroid ,carcinoma widely invasive
- Hurthle (oncocytic) cell tumours
    - Hurthle cell Adenoma
    - Hurthle cell carcinoma
- Poorly differentiated thyroid carcinoma
- Anaplastic thyroid carcinoma
- Squamous cell carcinoma
- Medullary thyroid carcinoma
- Mixed medullary and follicular thyroid carcinoma
- Mucoepidermoid carcinoma
- Sclerosing mucoepidermoid carcinomawith eosinophilia
- Mucinous carcinoma
- Ectopic thymoma
- Spindle epithelial tumour with thymus like differentiation
- Intra thyroid thymic carcinoma
- Paraganglioma and Mesenchymal stromal tumours
    - Paraganglioma
    - Peripheral nerve sheath tumour(PNSTs)
        - Schwannoma
        - Malignant PNST

        - Benign vascular tumours
            - Hemangioma
            - Cavernous haemangioma
            - Lymphangioma
- Angiosarcoma
    - Smooth muscle tumours
        - Leiomyoma
        - Leimyosarcoma
        - Solitary fibrous tumours
    - Hematolymphoid tumours
        - Langerhans cell Histiocytosis
        - Rosai –dorfman disease
        - Follicular dentritic cell sarcoma
        - Primary thyroid lymphoma
    - Germ cell tumours
        - Benign teratoma
        - Immature teratoma
        - Malignant teratoma
    - Secondary tumours

ANNEXURE II

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

PRIMARY TUMOR (T)

TX Primary tumor cannot be assessed

T0 No evidence of primary tumor

T1 Tumor ≤2 cm greatest dimension limited to the thyroid

T2 Tumor >2 cm but not >4 cm limited to the thyroid

T3 Tumor >4 cm in greatest dimension limited to the thyroid or any tumor with minimal extrathyroid extension (e.g., extension to sternothyroid muscle or perithyroid soft tissues)

T4a Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve

T4b Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels *All anaplastic carcinomas are considered T4 tumors* Intrathyroidal anaplastic carcinoma–surgically resectable Extrathyroidal anaplastic carcinoma–surgically unresectable

REGIONAL LYMPH NODES (N)

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

DISTANT METASTASIS (M)

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

STAGE GROUPING

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

Papillary or Follicular (<45 years)

Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1

Papillary or Follicular (45 years and older)

Stage I	T1	N0	M0
Stage II	T2	N0	M0

Stage III	T3	N0	M0
Stage III	T1	N1a	M0
Stage III	T2	N1a	M0
Stage III	T3	N1a	M0
Stage IVA	T4a	N0	M0
Stage IVA	T4a	N1a	M0
Stage IVA	T1	N1b	M0
Stage IVA	T2	N1b	M0
Stage IVA	T3	N1b	M0
Stage IVA	T4a	N1b	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1
Medullary Carcinoma			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage III	T2	N1a	M0
Stage III	T2	N1a	M0
Stage III	T3	N1a	M0
Stage IVA	T4a	N0	M0
Stage IVA	T4a	N1a	M0

Stage IVA	T1	N1b	M0
Stage IVA	T2	N1b	M0
Stage IVA	T3	N1b	M0
Stage IVA	T4a	N1b	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Anaplastic Carcinoma

All anaplastic carcinomas are considered Stage IV

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

ANNEXURE III

PROFORMA

Name: Age / Sex:

IP No: Unit & Ward:

HPE No:

H/O Presenting illness:

Significant past history (if any):

Type of specimen:

Procedure done:

Details of any relevant imaging studies:

Details of any investigation for metastatic disease:

ANNEXURE IV

HAEMATOXYLIN AND EOSIN STAINING METHOD

1. Sections will be deparaffinised with xylene for 20 minutes.
2. Sections will be hydrated through descending concentrations (absolute alcohol,90%, 70%, 50%) of ethanol to water solutions.
3. Sections will be rinsed in distilled water.
4. Sections will be placed in Ehrlich haematoxylin stain for 20-30 minutes.
5. Sections will be rinsed with water.
6. Differentiation will be done by immersing the sections in 1% acid alcohol for 10seconds.
7. Sections will be rinsed with water.
8. Blueing will be done by keeping the sections in Scott's tap water for 2-10minutes.
9. Counterstaining will be done with 1% aqueous Eosin for 1-3 minutes.
10. Sections will be rinsed with water.
11. Sections will be dehydrated through increasing concentrations of ethanolsolutions (50%, 70%, 95%, absolute alcohol) and cleared with xylene.
12. Sections will be mounted with DPX.

ANNEXURE V

KEY TO MASTER CHART

SEX:

M - Male

F - Female

HPE NO – Histopathological Examination Number

Presentation

SNG –solitary nodular goitre

MNG –multinodular goitre

Surgical procedure

HT- hemi thyroidectomy.

TT- total thyroidectomy.

PSMA Intensity score

Score 0 :no reaction

Score 1+ : Faint reaction visible only at high power

Score 2+ : Moderate intensity at low power

Score 3+ : Strong reaction easily visible at low power

PSMA extent score

Percentage of stained endothelial cells (%)	Interpretation
0-9	Negative
10-39	Minimal
40-69	Moderate
≥70	Diffuse

ANNEXURE VI A

MASTER CHART OF HISTOPATHOLOGY OF THYROID FOLLICULAR NEOPLASMS

Sl No.	HPE NO.	AGE	SEX	PRESENTATION	SURGERY	DIAGNOSIS
1	613/16	35	F	SNG	HT	follicular adenoma with degenerative changes
2	631/16	55	F	MNG	TT	follicular adenoma -oncocytic variant with degenerative changes
3	717/16	30	M	SNG	HT	follicular adenoma with degenerative changes
4	765/16	60	F	MNG	TT	follicular adenoma
5	1018/16	65	F	MNG	TT	**follicular carcinoma**
6	1066/16	48	F	SNG	HT	follicular adenoma with degenerative changes
7	1033/16	45	F	SNG	HT	follicular adenoma with degenerative changes
8	1150/16	35	F	SNG	HT	follicular adenoma with degenerative changes
9	1226/16	39	F	SNG	HT	follicular adenoma
10	1376/16	25	F	MNG	tt	follicular adenoma with degenerative changes
11	1382/16	52	M	SNG	HT	follicular adenoma with degenerative changes
12	1485/16	19	F	SNG	ht	follicular adenoma with degenerative changes
13	1507/16	30	F	MNG	TT	follicular adenoma
14	1588/16	26	F	MNG	TT	follicular adenoma -fetal variant
15	1722/16	36	F	SNG	HT	follicular adenoma
16	1769/16	30	F	SNG	HT	follicular adenoma
17	1827/16	32	F	SNG	HT	follicular adenoma with degenerative changes
18	2068/16	21	F	SNG	HT	follicular adenoma
19	2177/16	55	F	SNG	HT	follicular adenoma

20	2227/16	38	F	SNG	TT	Follicular variant of papillary carcinoma
21	2242/16	49	F	MNG	TT	colloid adenoma
22	2361/16	35	F	SNG	HT	follicular adenoma -hurthle cell variant
23	2409/16	25	F	SNG	HT	follicular adenoma
24	2416/16	26	F	MNG	TT	follicular adenoma with degenerative changes
25	2429/16	40	F	SNG	TT	follicular adenoma with degenerative changes
26	2509/16	42	F	SNG	NEAR TOTAL	Follicular adenoma
27	2512/16	40	F	SNG	HT	Follicular variant of papillary carcinoma
28	2575/16	22	F	SNG	HT	follicular adenoma
29	2670/16	22	F	SNG	HT	follicular adenoma
30	2802/16	23	F	SNG	HT	follicular adenoma with degenerative changes
31	2937/16	30	F	SNG	HT	follicular adenoma
32	2979/16	31	F	SNG	HT	follicular adenoma
33	2986/16	15	F	SNG	HT	follicular adenoma
34	3004/16	46	M	MNG	TT	follicular adenoma
35	3112/16	27	F	MNG	TT	Follicular variant of papillary carcinoma
36	3269/16	34	F	SNG	HT	follicular adenoma
37	3270/16	30	F	MNG	TT	Follicular variant of papillary carcinoma
38	3272/16	45	F	MNG	TT	follicular adenoma
39	3411/16	50	F	SNG	HT	follicular adenoma
40	3668/16	33	F	SNG	HT	follicular adenoma
41	3808/16	48	F	SNG	HT	follicular adenoma
42	25/17	37	F	SNG	HT	follicular adenoma
43	314/17	24	F	SNG	HT	follicular adenoma

44	346/17	58	M	SNG	TT	follicular adenoma
45	504/17	33	F	MNG	TT	Follicular variant of papillary carcinoma
46	522/17	66	M	SNG	HT	follicular adenoma
47	529/17	32	F	MNG	TT	follicular adenoma with papillary hyperplasia
48	550/17	55	F	MNG	TT	Follicular variant of papillary carcinoma
49	656/17	37	F	SNG	HT	follicular adenoma
50	861/17	43	f	SNG	HT	follicular adenoma with degenerative changes
51	955/17	60	F	SNG	HT	follicular adenoma with degenerative changes
52	1005/17	36	F	SNG	HT	Follicular variant of papillary carcinoma
53	1117/17	32	F	SNG	tt	Follicular variant of papillary carcinoma
54	1217/17	40	F	SNG	HT	follicular adenoma with degenerative changes
55	1316/17	33	F	MNG	TT	Follicular variant of papillary carcinoma
56	1394/17	35	F	SNG	HT	follicular adenoma
57	1443/17	24	F	SNG	HT	follicular adenoma
58	1478/17	57	F	SNG	HT	follicular adenoma
59	1515/17	30	F	SNG	HT	follicular adenoma
60	1521/17	40	M	MNG	TT	follicular adenoma
61	1611/1`7	19	F	MNG	TT	follicular adenoma with papillary hyperplasia
62	1889/17	46	F	MNG	TT	Follicular variant of papillary carcinoma
63	1995/17	48	F	SNG	HT	follicular adenoma
64	2044/17	35	F	SNG	HT	foillicular adenoma
65	2089/17	28	F	SNG	HT	foillicular adenoma
66	2272/17	20	F	SNG	HT	foillicular adenoma
67	2315/17	45	F	SNG	TT	Follicular variant of papillary carcinoma

68	2507/17	47	F	SNG	HT	follicular adenoma
69	2548/17	37	F	MNG	TT	follicular adenoma
70	2911/17	27	M	SNG	HT	Follicular variant of papillary carcinoma
71	2953/17	55	M	SNG	HT	follicular adenoma with degenerative changes
72	3190/17	50	M	MNG	TT	follicular adenoma- colloid variant
73	3202/17	43	F	MNG	TT	follicular adenoma with degenerative changes
74	3360/17	55	M	SNG	HT	follicular adenoma -fetal variant
75	3539/17	36	F	SNG	HT	follicular adenoma
76	3682/17	35	F	MNG	TT	follicular adenoma
77	3708/17	33	F	MNG	TT	Follicular variant of papillary carcinoma
78	3801/17	60	F	SNG	HT	follicular adenoma with degenerative changes
79	40/18	38	F	MNG	TT	follicular adenoma
80	116/18	47	F	MNG	TT	follicular adenoma
81	431/18	30	F	SNG	HT	follicular adenoma
82	438/18	26	F	SNG	HT	follicular adenoma
83	480/18	46	F	SNG	HT	follicular adenoma
84	611/18	44	M	MNG	TT	follicular adenoma with degenerative changes
85	674/18	47	F	SNG	HT	follicular adenoma -Hurthle cell variant
86	759/18	23	f	SNG	HT	Follicular adenoma
87	899/18	30	F	SNG	HT	Follicular adenoma
88	1205/18	45	f	MNG	TT	follicular adenoma with degenerative changes
89	1207 /18	21	M	SNG	TT	follicular adenoma -microfollicular variant
90	1384/18	42	F	SNG	HT	follicular adenoma with degenerative changes
91	1405/18	35	F	SNG	HT	Follicular adenoma

92	1410/18	40	F	SNG	HT	Follicular adenoma
93	1423/18	61	M	MNG	TT	Follicular variant of papillary carcinoma
94	1448/18	42	F	SNG	HT	follicular adenoma with hashimaotos thyroiditis
95	1478/18	37	F	MNG	TT	Follicular adenoma
96	1581/18	55	F	SNG	HT	Follicular adenoma
97	1629/18	45	F	SNG	HT	Follicular adenoma
98	1`636/18	23	F	SNG	HT	Follicular adenoma
99	1784/18	29	F	MNG	TT	Follicular adenoma
100	1835/18	58	F	SNG	HT	Follicular variant of papillary carcinoma
101	2019/18	34	F	SNG	HT	follicular adenoma
102	2038/18	17	F	MNG	TT	Follicular variant of papillary carcinoma
103	2044/18	44	F	SNG	HT	follicular adenoma
104	2048/18	30	F	SNG	HT	follicular adenoma with papillary hyperplasia
105	2177/18	57	F	SNG	HT	follicular adenoma- microfollicular variant.
106	2195/18	57	F	SNG	HT	follicular adenoma with degenerative changes
107	2255/18	37	F	SNG	HT	follicular adenoma
108	2321/18	21	F	SNG	HT	follicular adenoma -colloid variant
109	2348/18	34	F	SNG	HT	follicular adenoma
110	2379/18	23	f	SNG	HT	folicular adenoma with papillary hyperplasia
111	2418/18	47	F	SNG	HT	follicular adenoma with papillary hyperplasia
112	2516/18	17	F	SNG	HT	follicular adenoma with degenerative changes
113	2648/18	38	M	SNG	HT	Follicular variant of papillary carcinoma

ANNEXURE VI B
IMMUNO HISTOCHEMISTRY MASTER CHART

SL.No	HPE NO.	AGE	SEX	PRESENTATION	Surgery	Diagnosis	PSMA Intensity	PSMA EXTENT
1	613/16	35	F	SNG	HT	follicular adenoma with degenerative changes	0	negative
2	631/16	55	F	MNG	TT	follicular adenoma -oncocytic variant with degenerative changes	0	negative
3	1018/16	65	F	MNG	TT	**follicular carcinoma**	3	diffuse
4	1226/16	39	F	SNG	HT	follicular adenoma	0	negative
5	1588/16	26	F	MNG	TT	follicular adenoma -fetal variant	0	negative
6	1769/16	30	F	SNG	HT	follicular adenoma	0	negative
7	2512/16	40	F	SNG	HT	follicular variant of papillary carcinoma	1	moderate
8	2575/16	22	F	SNG	HT	follicular adenoma	0	negative
9	2670/16	22	F	SNG	HT	follicular adenoma	0	negative
10	2802/16	23	F	SNG	HT	follicular adenoma with degenerative changes	0	negative
11	3112/16	27	F	MNG	TT	follicular variant of papillary carcinoma	0	negative
12	3269/16	34	F	SNG	HT	follicular adenoma	0	moderate
13	3411/16	50	F	SNG	HT	follicular adenoma	0	negative
14	504/17	33	F	MNG	TT	follicular variant of papillary carcinoma	0	negative
15	550/17	55	F	MNG	TT	follicular variant of papillary carcinoma	0	negative
16	955/17	60	F	SNG	HT	follicular adenoma with degenerative changes	1	negative
17	1005/17	36	F	SNG	HT	follicular variant of papillary carcinoma	2	moderate
18	1117/17	32	F	SNG	tt	follicular variant of papillary carcinoma	0	negative
19	1316/17	33	F	MNG	TT	follicular variant of papillary carcinoma	2	moderate
20	1611/1`7	19	F	MNG	TT	follicular adenoma with papillary hyperplasia	0	negative
21	1889/17	46	F	MNG	TT	follicular variant of papillary carcinoma	0	negative
22	1995/17	48	F	SNG	HT	follicular adenoma	0	negative

23	2044/17	35	F	SNG	HT	foillicular adenoma	0	negative
24	2089/17	28	F	SNG	HT	foillicular adenoma	0	negative
25	2272/17	20	F	SNG	HT	foillicular adenoma	0	negative
26	2315/17	45	F	SNG	tt	follicular variant of papillary carcinoma	0	minimum
27	2507/17	47	F	SNG	HT	follicular adenoma	2	diffuse
28	2548/17	37	F	MNG	TT	follicular adenoma	0	negative
29	2953/17	55	M	SNG	HT	follicular adenoma with degenerative changes	0	negative
30	3190/17	50	M	MNG	TT	follicular adenoma- colloid variant	0	negative
31	3360/17	55	M	SNG	HT	follicular adenoma -fetal variant	3	Diffuse
32	1207 /18	21	M	SNG	TT	follicular adenoma -microfollicular variant	1	diffuse
33	1384/18	42	F	SNG	HT	follicular adenoma with degenerative changes	0	negative
34	1405/18	35	F	SNG	HT	Follicular adenoma	0	negative
35	1410/18	40	F	SNG	HT	Follicular adenoma	1	negative
36	1423/18	61	M	MNG	TT	follicular variant of papillary carcinoma	0	negative
37	1478/18	37	F	MNG	TT	Follicular adenoma	0	negative
38	1581/18	55	F	SNG	HT	Follicular adenoma	0	negative
39	1638/18	23	F	SNG	HT	Follicular adenoma	0	negative
40	1835/18	58	F	SNG	HT	follicular variant of papillary carcinoma	2	diffuse
41	2019/18	34	F	SNG	HT	follicular adenoma	0	negative
42	2038/18	17	F	MNG	TT	follicular variant of papillary carcinoma	1	negative
43	2044/18	44	F	SNG	HT	follicular adenoma	1	negative
44	2177/18	57	F	SNG	HT	follicular adenoma microfollicular variant.	0	negative
45	2195/18	57	F	SNG	HT	follicular adenoma with degenerative changes	0	negative
46	2255/18	37	F	SNG	HT	follicular adenoma	0	negative
47	2321/18	21	F	SNG	HT	follicular adenoma -colloid variant	3	negative
48	2348/18	34	F	SNG	HT	follicular adenoma	0	minimum
49	2379/18	23	f	SNG	HT	folicular adenoma with papillary hyperplasia	0	minimum
50	2648/18	38	M	SNG	ht	follicular variant of papillary carcinoma	2	diffuse

ANNEXURE VII

LIST OF ABBREVAITIONS USED

FA	–	Follcular adenoma
FC	-	follicular carcinoma
FVPC	–	follicular variant of papillary carcinoma
CD	-	Cluster of Differentiation
FNA	–	Fine needle aspiration
WHO	–	World Health Organisation
FFPE	-	Formalin Fixed Paraffin Embedded
IHC	–	Immunohistochemistry
TNGM	-	Tumour Node Grade Metastasis

ANNEXURE – VIII

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ANNEXURE IX

INSTITUTION ETHICAL COMMITTEE

MADURAI MEDICAL COLLEGE
MADURAI, TAMILNADU, INDIA -625 020
(Affiliated to The Tamilnadu Dr.MGR Medical University, Chennai, Tamil Nadu)

Prof Dr V Nagaraajan MD MNAMS DM (Neuro) DSc.,(Neurosciences) DSc (Hons)
Professor Emeritus in Neurosciences, Tamil Nadu Govt Dr MGR Medical University
Chairman, IEC

Dr.M.Shanthi, MD.,
Member Secretary,
Professor of Pharmacology,
Madurai Medical College, Madurai.

Members

1. Dr.V.Dhanalakshmi, MD, Professor of Microbiology & Vice Principal, Madurai Medical College

2. Dr.Sheela Mallika rani, M.D., Anaesthesia , Medical Superintendent Govt. Rajaji Hosptial, Maudrai

3.Dr.V.T.Premkumar,MD(General Medicine) Professor & HOD of Medicine, Madurai Medical & Govt. Rajaji Hospital, College, Madurai.

4.Dr.S.R.Dhamotharan, MS., Professor & H.O.D i/c, Surgery, Madurai Medical College & Govt. Rajaji Hosptial, Madurai.

5.Dr.G.Meenakumari, MD., Professor of Pathology, Madurai Medical College, Madurai

6.Mrs.Mercy Immaculate Rubalatha, M.A., B.Ed., Social worker, Gandhi Nagar, Madurai

7.Thiru.Pala.Ramasamy, B.A.,B.L., Advocate, Palam Station Road, Sellur.

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.syed Abdullah Mohamed Ameen
Course	:	PG in MD., Pathology
Period of Study	:	2016-2019
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Histopathological analysis and study of expression of PSMA in thyroid follicular neoplasms.
Ethical Committee as on	:	13.04.18

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary | Chairman | Dean / Convenor

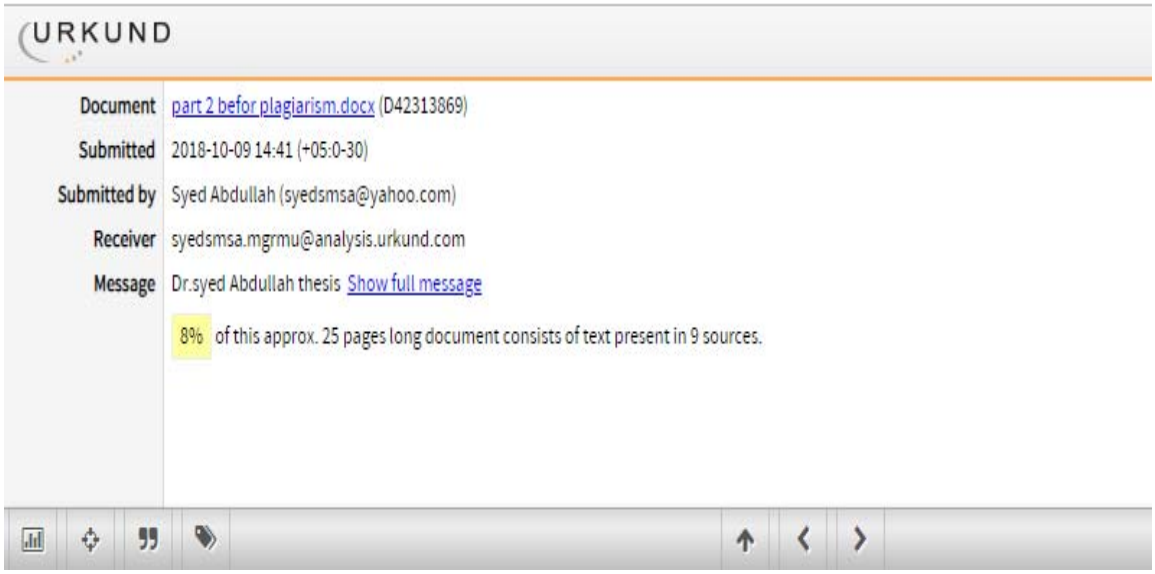
Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
CHAIRMAN
IEC - Madurai Medical College
Madurai

DEAN
Madurai Medical College
Madurai-20

16 APR 2018

ANNEXURE X

ANTI-PLAGIARISM CERTIFICATE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CERTIFICATE - II

This is to certify that this dissertation work titled **"HISTOPATHOLOGICAL ANALYSIS AND STUDY OF EXPRESSION OF PSMA IN THYROID FOLLICULAR NEOPLASMS"** of the candidate **Dr.SYED ABDULLAH MOHAMED AMEEN** with registration Number **201613102** for the award of **M.D.,** Pathology. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file includes pages from introduction to conclusion and the result shows **8** percentage of plagiarism in the dissertation.

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