“A STUDY OF ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION IN BENIGN AND MALIGNANT THYROID LESIONS BY IMMUNOHISTOCHEMISTRY”

By

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DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE IN

PATHOLOGY

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LIST OF ABBREVIATIONS

S.No.	Abbreviation	Expansion
1.	IHC	Immunohistochemistry
2.	ERs	Estrogen Receptors
3.	PRs	Progesterone Receptors
4.	H & E	Hematoxylin and Eosin
5.	PTC	Papillary Thyroid carcinoma
6.	FTC	Follicular Thyroid carcinoma
7.	MTC	Medullary Thyroid carcinoma
8.	FA	Follicular Adenoma
9.	n	Number of cases
10.	WHO	World Health Organization
11.	TNM	Tumor, Nodal, Metastasis
12.	DAB	Di- Amino Benzidine
13.	DPX	Distyrene Plasticizer Xylene
14.	HPF	High Power Field
15.	HRP	Horse Radish Peroxidase

ABSTRACT

Background:

The incidence of Thyroid diseases is seen to be higher in women as compared to men particularly between puberty and menopause. Women are seen to be more susceptible to the goitrogenic effect of iodine deficiency. Frequency of thyroid carcinoma is seen to be higher in women as compared to men (approximately 3 times more). Thyroid tissue is known to be influenced by several hormones, growth factors and steroids. Estrogen has a role in affecting functions of various target tissues like that of breast, uterine endometrium and myometrium, bone, thyroid etc. It acts by regulating cell proliferation by binding to specific receptors: Estrogen Receptors (ERs). Progesterone too has its role in cellular proliferation and function of female reproductive tissues through a hormonally regulated transcription factor: Progesterone Receptors (PRs). There are only few studies done using both ER and PR expression in various thyroid lesions both men and women and none in our geographic area. Also studying sex steroid receptor effects on thyroid cell is a potential tool to a better understanding the pathogenesis of thyroid diseases and to develop targets to its treatment.

Objective of the study:

To study the Estrogen and Progesterone receptor expression in benign and malignant thyroid lesions and their comparison in males and females.

Methods:

The study was carried at The Department of Pathology, R.L.Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar, during the period of 01-01-2015 to 30-09-2016. The study included 65 cases of various thyroid lesions out of which 18 were Malignant and 47 were Benign lesions.

Immunohistochemistry was done using antibodies against Estrogen and progesterone receptors. Proportion and Intensity of positive cells were documented and Total Immunoscore was calculated. Estrogen and Progesterone expression was correlated with nature of lesions, age, gender and histopathological type. Statistical correlation was done using Chi-square test of Fischer's exact test and Mann Whitney U test. A p value of less than 0.05 was considered significant.

Results:

Out of 65 cases studied, Benign lesions were 72.3% and Malignant 27.7%. Male: Female ratio was 1:33. The most common age group was 30-50 years. Majority of the Benign lesions (42.6%) had a lower ERs with a Total score of 2, whereas, majority of the Malignant tumors (33.3%) had a higher with a Total score of 4. Similarly higher Total score of PRs (2) was seen in majority of the Malignant lesions (55.6%) as compared to Benign, majority of which (61.7%) showed Total score of 0. Among the cases it was seen that there was a positive co-relation between expression of ERs between the Genders and between Malignant and Benign thyroid lesions. The correlation was significant with the p value less than 0.05, whereas PRs expression was statistically significant between Benign and Malignant thyroid lesions but not statistically significant between Genders.

Conclusion:

The results of this study showed that expression of ERs is higher in malignant thyroid lesions and also in females as compared to males. There was a significant positive correlation between total

score of ER expression in females and malignant thyroid lesions, unlike PR whose expression was not statistically significant.

When compared with histological type of malignant lesion it was seen there were more cases of PTC and expression of ERs was statistically significant. Also, larger thyroid masses were mostly benign whereas smaller nodules were malignant. Hence, it can be concluded that ERs plays a significant role in the pathogenesis of thyroid lesions in females more as compared to males and PRs might have an indirect effect.

Keywords : Estrogen receptor, Progesterone receptor, Immunohistochemistry, Thyroid cancers.

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INTRODUCTION

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Thyroid diseases are among the commonest endocrine disorders worldwide; India too, is no exception. It has been estimated that about 42 million people in India suffer from thyroid diseases. Thyroid disorders are the most common among all the endocrine diseases in India and hyperthyroidism and hypothyroidism are more frequent in women. Hypothyroidism refers to any state that results in a deficiency of thyroid hormone, including hypothalamic or pituitary disease and generalized tissue resistance to thyroid hormones, and disorders that affect the thyroid gland directly. Clinically hyperthyroidism, also called as thyrotoxicosis, is caused by the effects of excess thyroid hormone.[1]

Thyroid cancers are three times more common in women than in men. There are four types mainly- Papillary, Follicular, Medullary and Anaplastic. The cause of thyroid cancer is unknown, but certain risk factors have been identified and they include a family history of goiter, exposure to high levels of radiation and certain hereditary syndromes.[2]

It is seen that thyroid cancers are relatively common in our geographic area, Kolar. It is more common in females (79%) than in males (21%) and, histologically it is seen that more cases are that of Papillary carcinoma (70%) followed by medullary carcinoma (15%). Thyroid cancers are among the top ten cancers in the studies done in the southern India, important cities being Bangalore, Hyderabad and Thiruvananthapuram.[3]

The clinical evaluation of thyroid nodule is a common problem confronting the clinicians. The vast majority of such nodules are benign, but such a thyroid swelling harboring malignancy demands prompt and accurate diagnosis. The natural history of thyroid carcinomas allows the surgeon to perform a more prolonged and thoughtful preoperative workup and evaluation. Worldwide, the prevalence of a palpable thyroid nodule in community is about 12.2%.[4]

Estrogen has a role in affecting functions of various target tissues like that of breast, uterine endometrium and myometrium, bone, thyroid etc. It acts by facilitating cell proliferation by binding to specific Estrogen Receptors(ERs).[5] Estrogen is known to have a role in thyroid cell proliferation and Estrogen mediated thyroid cancer cell proliferation involves ER-alpha and ER-beta transcriptional and non-genomic signaling events.[6] Progesterone also has its role in cellular proliferation and function of female reproductive tissues through a hormonally regulated transcription factor-Progesterone Receptors (PRs) and which in turn are also regulated by ERs.[7]

ERs and PRs are a potential targets for site specific therapy in Breast carcinomas and female genital tract carcinomas, and targeted therapy with Tamoxifen is proven.[8] Though their expression has been studied in other tumors in India, there are a very few studies demonstrating ER and PR expression in Thyroid lesions. Studying sex steroid receptor effects on thyroid cell is also a potential tool to a better understanding of the pathogenesis

of thyroid diseases in women and to develop targets to its treatment and thus, reduce the mortality and morbidity associated with it.

Although numerous studies have been done to evaluate ER and PR expression in Thyroid in other countries, extensive review of literature revealed limited study has been done in India to evaluate the same. This is a time bound study of cases, which presented with thyroid lesions.

AIMS AND

OBJECTIVES OF THE

STUDY

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To study the Estrogen and Progesterone receptor expression in benign and malignant thyroid lesions and their comparison in males and females.

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

Andres Vesalius (1514-1564 A.D.), who is known as the "Father of Anatomy" gave the first description of thyroid gland. Pathological swellings in the anterior surface of the neck have been recognized since ancient times. The Greeks were the first to describe thyroid gland enlargements, referring to them as bronchoceles or hernias of the wind pipe. French term "GOITRE" is the origin of the word "Goiter", both originating from the Latin word "gutter", meaning throat. The close anatomical association of these glands with the larynx led to the name of thyroid (shaped like a shield) after the designation given by Galen to the thyroid cartilage.[9]

The name thyroid is derived from the Greek description of shield shaped gland in the anterior neck (Threoides).[10] In the late nineteenth century two surgeons revolutionized the treatment of thyroid disease – Theodor Billroth and Emil Theodor Kocher. As a result of pioneering developments in the understanding of thyroid physiology, Kocher received the Noble prize in 1909. The collar incision introduced by James Boeckel of Strasbourg was adopted widely. Payer recorded the first transplantation of thyroid in 1906. He transplanted a portion of the thyroid from a woman into the spleen of her myxoedemic daughter.[11]

EMBRYOLOGY: [12]

The thyroid gland arises as an out pouching of the primitive foregut around the third week of gestation. It originates at the base of the tongue in the vicinity of the foramen caecum. Endoderm cells in the floor of the pharyngeal anlage thicken to form the median thyroid anlage. During its descent the median anlage remains connected via Thyroglossal duct. Paired lateral anlage originates from the fourth branchial pouch and fuse with the median anlage at fifth week of gestation. Thyroid follicles are initially apparent by eight weeks and colloid formation begins by the eleventh week of gestation.

Development of Parafollicular cells: [13]

These cells lie in the Para follicular space and secrete Thyrocalcitonin. They are also known as C-cells. In 1960's the origin and function of these glands was clarified by Pierre and Pollock. It has been demonstrated that C cell are derived from the neural crest cells that migrate ventrally into the ultimobranchial body. Subsequently they migrate into the ventral part of the neck and are incorporated into the thyroid glands.

ANATOMY:

The thyroid gland is a brownish-red, highly vascular organ, situated anteriorly in the lower part of neck at the level of the fifth, sixth, seventh cervical and first thoracic vertebrate. It consists of two symmetrical lobes (right and left lobe) connected in the midline by an isthmus of gland tissue. The weight of the gland is variable 20-25 grams

and slightly heavier in females in whom it becomes enlarged at the time of stress like menstruation and pregnancy. It is ensheathed by the pre-tracheal layer of the deep cervical fascia. [14]

The lobes are approximately conical in shape, apex of each being directed upwards and laterally. The length of each lobe is about 5 cm, its transverse diameter is about 3 cm and its greatest anterior-posterior diameter is about 2 cm. A ligamentous band, called the lateral ligament of thyroid gland attaches the posterior medial aspects of each lobe to the cricoid cartilage. The lateral or superficial surface is convex. The surface of the gland external to the sheet of deep fascia is covered by sternothyroid muscle which is inserted into the oblique line on the lamina of the thyroid cartilage, which prevents the upper part of the lobe from extending forward into the strap muscles. More anteriorly it is covered by sternohyoid, superior belly of omohyoid and overlapped below by the anterior border of sternocleidomastoid. Medial surface is in relation with the larynx and trachea. Superior pole is in contact with thyropharyngeus part of inferior constrictor of the pharynx. The cricothyroid muscle lies between the gland, the posterior part of the lamina of thyroid cartilage and side of the cricoid cartilage. Below, it is related to the external and recurrent laryngeal nerve and trachea posteriorly. The postero- lateral surface is in relation to the carotid sheath and overlaps the common carotid artery. The anterior border is thin, descending obliquely and medially, and is related to the anterior branch of superior thyroid artery. The posterior border which is blunt and rounded is in between the posterior and medial surface and is closely related below to the inferior thyroid artery. [14]

The Isthmus:

It connects the lower part of the two lobes; it measures about 1.25 cm transversely and vertically. It usually extends anterior to the 2nd and 3rd tracheal rings and sometimes it may be placed at a higher or lower level. Anteriorly it is separated from sternocleidomastoid by the pretracheal fascia. More anteriorly it is covered by the sternohyoids, the anterior jugular veins, the fascia colli of neck and skin. An anastomosing branch which unites the two superior thyroid arteries runs along its upper border. The inferior thyroid vein leaves the gland at its lower border. Occasionally in some individual, the isthmus may be absent. [14]

The Pyramidal lobe:

Pyramidal lobe is often present which is conical in shape. From the upper part of the isthmus or from one of the either lobe more commonly the left and it ascends towards the hyoid bone. Occasionally it may be detached, or may present in two or more separate parts. Fibrous or fibro-muscular band sometimes descend from the body of the hyoid bone to isthmus of the gland on its pyramidal lobe, when it is termed as Levatores glandular thyroidae. [14]

Accessory thyroid gland:

Vestiges of the thyroglossal duct may persist between the isthmus of the thyroid gland

and foramen caecum of the tongue. It may give rise to accessory nodules or cysts of thyroid tissue, situated in or near the medium plane or even in the substance of the tongue.

BLOOD SUPPLY OF THE THYROID GLAND[15]:

The thyroid gland receives a dual blood supply from the superior and inferior thyroid arteries, which have abundant collateral anastomosis with each other, both ipsilaterally and contra-laterally.

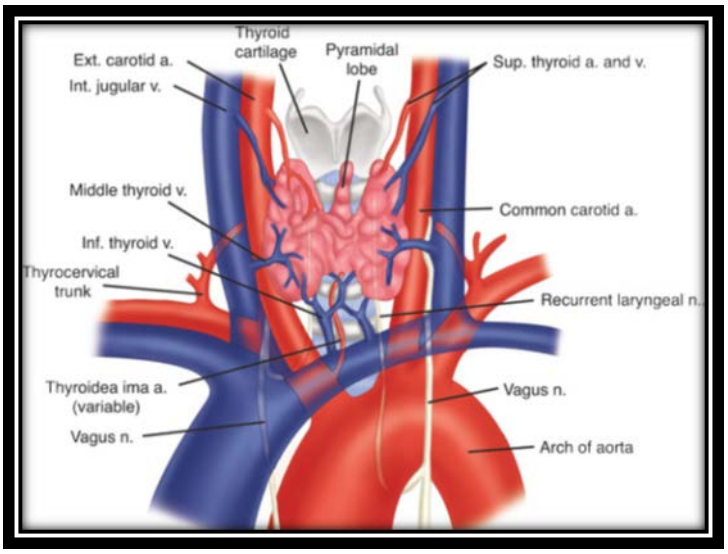


Figure 1: Photograph of blood supply of thyroid gland.[15]

Superior thyroid artery:

This is the first branch of the external carotid artery. In a small percentage of cases, it

may arise from the common carotid artery just before its bifurcation into the internal and external branches. Near the upper pole of the lateral lobe, the superior thyroid artery sends a small branch, the cricothyroid artery across the cricothyroid muscle towards the midline. It anastomosis with its opposite member in the midline on the surface of the median cricothyroid ligament.

Inferior thyroid artery:

This artery arises from the thyrocervical trunk, a branch of the first part of the subclavian artery at the level of the first rib. It ascends vertically for a short distance before turning medially, forming an arching loop and entering the tracheoesophageal groove. Most of its small branches penetrate the posterior aspect of the lateral lobe but there is a longitudinal branch that anastomoses with the superior thyroid artery near the superior pole.

Thyroid ima artery:

A variable third artery, thyroid ima artery arises from brachiocephalic artery or aortic ach and ascends anterior to trachea.

Venous drainage of the thyroid gland[15]:

Although two pairs of arteries supply the thyroid, three pairs of veins provide the venous drainage. The superior thyroid vein parallels the course of the superior thyroid artery on

the anterior surface of the thyroid as it ascends to become a tributary of the internal jugular vein. A middle thyroid vein usually the shortest of the three pairs has a direct lateral course from the surface of the thyroid to the internal jugular vein. Middle thyroid vein maybe absent in some individuals. The inferior thyroid vein lies on the anterior surface of the thyroid gland compared with the posterior position of the corresponding artery. It has an almost vertical downward course before entering the brachiocephalic vein.

LYMPHATIC DRAINAGE OF THE THYROID GLAND: [15]

Within the gland lymphatic channels occur immediately beneath the capsule and communicate between lobes through the isthmus. Lymph from the upper part of the gland reaches the deep cervical lymph nodes either directly or through the prelaryngeal nodes. Lymph from the lower part of the gland drains to lower deep cervical nodes directly, and also through the pretracheal and para tracheal nodes.

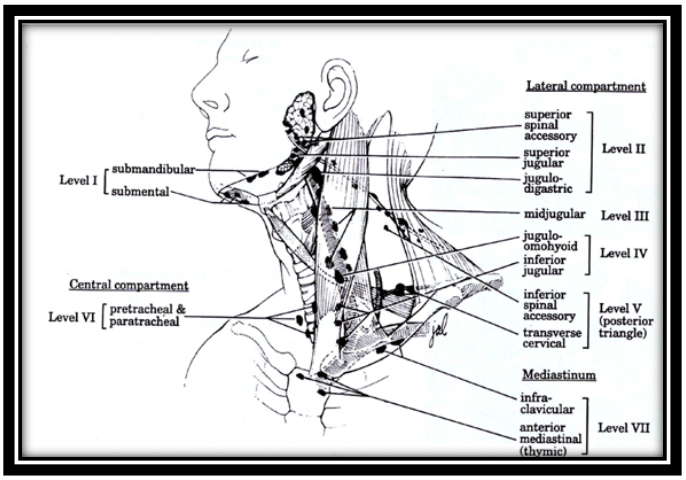


Figure 2: Diagrammatic representation of Lymph nodes of Neck[15]

Papillary carcinoma is commonly associated with adjacent nodal metastasis and medullary carcinoma has a strong predilection for metastatic lymphatic involvement, usually within central compartment.

NERVE SUPPLY OF THE GLAND: [16]

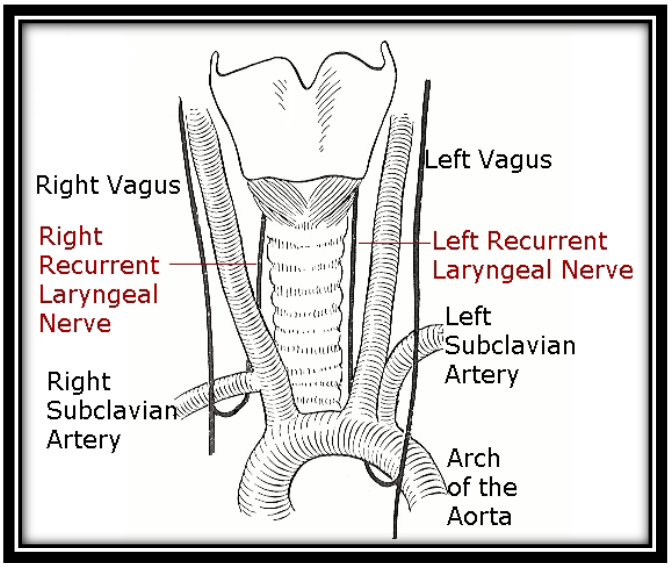


Figure 3: Diagrammatic representation of Nerve supply of Thyroid gland[16]

Recurrent laryngeal nerve (RLN):

Innervates the intrinsic musculature and provides sensory innervation to the glottis and subglottic larynx. The RLN arises from the vagus at the level of the subclavian artery on the right and at the level of aortic arch on the left. The nerves then turn superomedially to run towards the tracheo-oesophageal(TE) groove, giving off oesophageal and tracheal

branches. The RLN ascends in close relation with the trachea and oesophagus but not necessarily in the true TE groove. Unusually the non-recurrent laryngeal nerve can arise directly from the Vagus and pass directly into the thyroid, this non-recurrent anatomy is found in 1-1.5% of patients. This is usually associated with and Aberrant Right subclavian artery. Even more infrequently, there may be recurrent and non-recurrent laryngeal nerves.

Superior laryngeal nerve:

The SLN arises from the inferior vagal ganglion and descend inferiorly deep to carotid system. Posterior to internal carotid artery it branches into external branch and an internal branch. External branch supplies cricothyroid muscle. Internal branch is sensory to supraglottis.

HISTOLOGY: [17, 18]

Thyroid gland is enclosed by a dense connective tissue capsule and fibrous septa extends from capsule into the substance of gland dividing it into multiple lobules. Each lobule is made up of aggregate of 20-40 follicles.

Each follicle is morphological and functional unit of thyroid measuring about 200um in diameter with considerable variation in size and is lined by a layer of low cuboidal to flattened epithelium depending upon the activity of the follicle. The cytoplasm of each

cell is acidophilic or amphophilic and nucleus is round to oval /uniform and nucleolus is not prominent.

The follicle is filled with colloid, the quantity of colloid changes according to functional activity of gland. The colloid is scanty in hyper-functioning gland and dense abundant, homogenous and intensely eosinophilic in hypoactive glands. In adults some of the Follicular cells transform to large with deep abundant eosinophilic granular cytoplasm, referred to as Hurthle cells, oxyphilic cells or oncocytes. Ultra-structurally these granules are due to accumulation of mitochondria. Sparsely interspersed within the interfollicular spaces are minor endocrine component of gland called Parafollicular or 'C' cells. These cells appear larger and paler than follicular cells. They are polygonal and spindle shape cells containing granular or foamy cytoplasm with large eccentric nucleus with distinct nucleoli. [**Figure 4**].

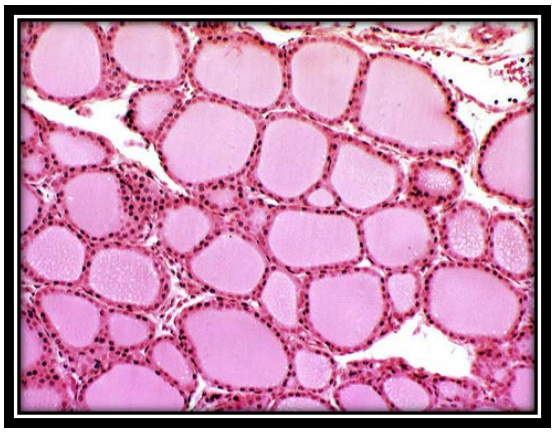


Figure 4: Microphotograph of histology of thyroid gland[17]

CYTOLOGY: [17, 18]

Normal aspirate: The normal thyroid is usually not sampled. Aspiration may yield epithelial cells, non-epithelial cells and non-cellular materials.

Normal structures

1. Follicular epithelial cells.
2. Colloid.
3. C-cells.
4. Cartilage.
5. Tracheal epithelium.
6. Skeletal muscle.

Grading of Thyroid diseases is done by Bethesda method in cytology.

Follicular epithelial cells are fragile and bare nuclei are common. Follicular cells are similar in Shape and size to that of normal lymphocytes. The nuclei are slightly oval to rounded with a smooth outline. The cytoplasm is fragile stains pale-blue or grey with May Grunwald Giemsa [MGG] stain with indistinct or fuzzy cell borders.

Colloid when thin on MGG stains violet/pink to blue/violet and forms a thin membrane-like film, which often cracks on slide when the colloid dries. When colloid is diluted with blood, it is hard to recognize and appear similar to that of blood serum, a protein-rich fluid. During processing colloid may be washed off the slide, but the crazy pavement' artifact remain on the slide suggesting presence of it. Thick colloid appears as darkly stained dense clumps of hyaline material dark blue/violet/magenta.

In Pap smears thin colloid appears pale green to orange, with cracking artifacts, thick colloid appears as variably dark green and orange. In the absence of blood, clean thin colloid is well shown both in MGG and in Pap smears. Thick colloid can be mistaken for collagenous fragments or amyloid. The blue/violet staining and hyaline texture makes colloid easier to identify in MGG than in Pap smears.

PHYSIOLOGY: [19, 20, 21]

The gland has two primary functions. The first is to secrete the thyroid hormones and second to secrete calcitonin, a hormone that regulates circulating levels of calcium.

Thyroid function is controlled by the thyroid-stimulating hormone of the anterior pituitary. The secretion of this hormone is in turn increased by Thyrotropin-releasing hormone from the hypothalamus and is also subject to negative feedback control by high circulating levels of thyroid hormones acting on the anterior pituitary and the hypothalamus.

Synthesis of Thyroid Hormones:

The primary hormone secreted by the thyroid is thyroxine (T4), along with much lesser amounts of triiodothyronine (T3). T3 has much greater biological activity than T4 and is specifically generated at its site of action in peripheral tissues by deiodination of T4.

Iodine Metabolism and transport:

The minimum daily iodine intake is 150 mcg in adults.

Iodide uptake is a critical first step in thyroid hormone synthesis. Ingested iodine is bound to serum proteins, particularly albumin. Iodide uptake is mediated by the $Na+/I^-$ - symporter, which is expressed at the basolateral membrane of thyroid follicular cells.

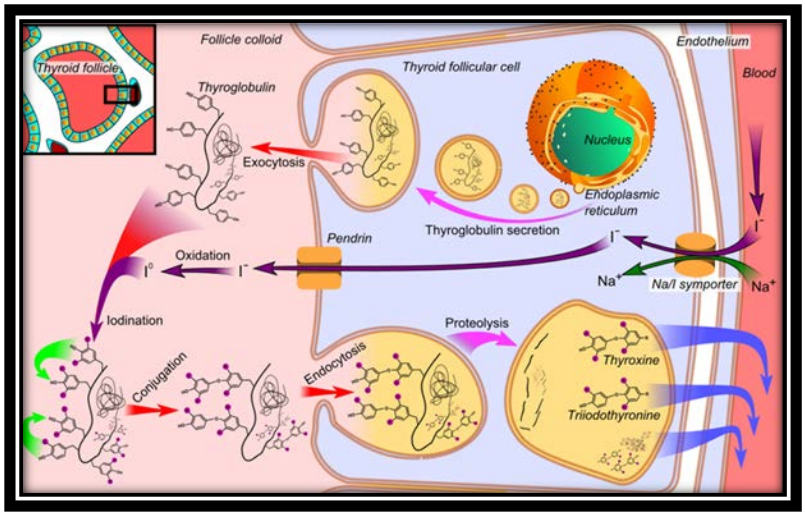


Figure 5: Diagrammatic representation of physiology of thyroid gland[17]

Organification, Coupling, Storage, Release:

After iodide enters the thyroid, it is trapped and transported to the apical membrane of thyroid follicular cells, where it is oxidized in an organification reaction that involves Thyroid peroxidase and hydrogen peroxide. The reactive iodine atom is added to tyrosyl residues within Thyroglobulin forming MIT and DIT. The iodotyrosines in Thyroglobulin are then coupled via an ether linkage in a reaction that is also catalyzed by Thyroid peroxidase. After coupling, Thyroglobulin is taken back into the thyroid cell, where it is processed in lysosomes to release T4 and T3. Uncoupled mono- and

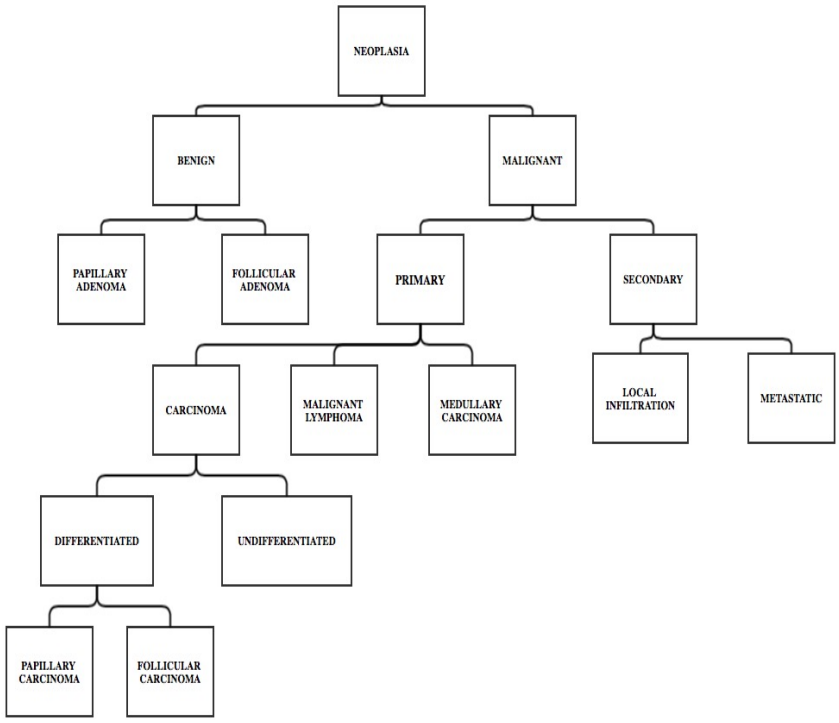
diiodotyrosines (MIT, DIT) are deiodinated by the enzyme dehalogenase, thereby recycling any iodide that is not converted into thyroid hormones.

Thyroid Hormone Transport:

T4 is secreted from the thyroid gland in about twentyfold excess over T3. Both hormones are bound to plasma proteins, including thyroxine-binding globulin, transthyretin and albumin.

The plasma-binding proteins increase the pool of circulating hormone, delay hormone clearance, and may modulate hormone delivery to selected tissue sites. The concentration of Thyroxin-binding globulin is relatively low (1–2 mg/dl), but because of its high affinity for thyroid hormones (T4 > T3), it carries about 80% of the bound hormones. Approximately 99.98% of T4 and 99.7% of T3 are protein-bound. The unbound hormone is thought to be biologically available to tissues. **[Figure 5].**

CLASSIFICATION OF PRIMARY THYROID TUMORS, MODIFIED FROM THE WHO CLASSIFICATION: [22, 23]



I. Tumors of thyroid follicular or metaplastic epithelium

1. Follicular adenoma
2. Follicular carcinoma [including Hurthle cell carcinoma]
   a. Minimally invasive
   b. Widely invasive
3. Papillary carcinoma [including Tall cell variant]
4. Columnar cell carcinoma [columnar cell variant of papillary carcinoma]
5. Mucoepidermoid carcinoma
6. Sclerosing mucoepidermoid carcinoma with eosinophilia
7. Mucinous carcinoma
8. Poorly differentiated thyroid carcinoma including insular carcinoma

9. Undifferentiated [anaplastic] carcinoma [including squamous cell carcinoma and carcinosarcoma]

II. Tumors showing C-cell differentiation

Medullary carcinoma

III. Tumors showing both follicular and C-cell differentiation

1. Collision tumor; follicular /papillary and medullary carcinoma

2. Mixed medullary and follicular cell carcinoma

IV. Tumors showing thymic or related branchial pouch differentiation

1. Ectopic thymoma

2. Spindle epithelial tumor with thymus like elements

3. Carcinoma showing thymus like element or intrathyroid thymic carcinoma

V. Tumors of lymphoid cells

1. Malignant lymphoma

2. Plasmacytoma

VI. Intrathyroid parathyroid tumors

1. Parathyroid adenoma

2. Parathyroid carcinoma

VII. Mesenchymal and other tumors

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

2. Paraganglioma

3. Teratoma.

BENIGN LESIONS: [24]

HASHIMOTOS THYROIDITIS:

Most common cause of hypothyroidism in areas where iodine levels are sufficient.

Macroscopic appearance-

Diffusely enlarged, capsule is intact, and the gland is well demarcated from adjacent structures. The cut surface is pale, yellow tan, firm, and somewhat nodular.

Microscopic appearance-

Extensive infiltration of parenchyma by a mononuclear inflammatory infiltrate composed of small lymphocytes, plasma cells, and well-developed germinal centers. The thyroid follicles are atrophic and are lined by epithelial cells having abundant eosinophilic, granular cytoplasm, termed Hurthle cells.

SUBACUTE (GRANULOMATOUS) THYROIDITIS:

Subacute thyroiditis, which is also referred to as granulomatous thyroiditis or De Quervain thyroiditis, occurs much less frequently than does Hashimoto disease.

Macroscopic appearance-

Unilaterally or bilaterally enlarged and firm gland, with an intact capsule. On cut section, the involved areas are firm and yellow-white and stand out from the more rubbery, normal brown thyroid substance.

Microscopic appearance-

Early is the active inflammatory phase, scattered follicles are disrupted and replaced by neutrophils forming micro-abscesses. Later, the more characteristic features appear in the form of aggregates of lymphocytes, activated macrophages, and plasma cells about collapsed and damaged thyroid follicles. Multinucleate giant cells enclose naked pools or fragments of colloid hence the designation granulomatous thyroiditis.

GRAVES' DISEASE:

Macroscopic appearance-

Gland is symmetrically enlarged because of diffuse hypertrophy and hyperplasia of thyroid follicular epithelial cells. On cut section, the parenchyma has a soft, meaty appearance resembling normal muscle.

Microscopic appearance-

Follicular epithelial cells are tall and more crowded than usual. Colloid within the follicular lumen is pale, with scalloped margins. Lymphoid infiltrates, consisting predominantly of T cells, with fewer B cells and mature plasma cells, are present throughout the interstitium; germinal centers are common.

MULTINODULAR GOITERS:

Macroscopic appearance-

Multilobulated, asymmetrically enlarged glands. On cut section, irregular nodules containing variable amounts of brown, gelatinous colloid are present.

Microscopic appearance-

Colloid-rich follicles lined by flattened, inactive epithelium and areas of follicular hyperplasia, accompanied by the degenerative changes.

BENIGN TUMOURS: [25]

An adenoma of the thyroid gland can be defined as a benign epithelial neoplasm with the neoplastic cells similar to the cellular morphology of adult or embryonic thyroid gland. Generally single, encapsulated nodule in a thyroid gland, compressing the surrounding tissue is accepted as adenoma.

If there is a tendency to form follicles it is called a follicular adenoma and those producing papillae are called papillary adenoma. In all differentiated carcinomas, the tumor tends to be TSH dependent.

FOLLICULAR ADENOMA:

This benign epithelial neoplasm has a tendency to form follicles.

Macroscopic appearance -

The typical follicular adenoma is solitary, round or oval, firm and has a well-developed capsule. When it arises in an otherwise normal gland, the encapsulation and contrast in texture to adjacent compressed gland are striking. The gross appearance varies somewhat with the amount of colloid present. However retrogressive changes, resulting from interference in blood supply leads to softening, cyst formation, hemorrhage and infarction with subsequent fibrosis and calcification usually can be located centrally. The lesions may be so firm in nature that they may be suspected to be of malignant origin.

Microscopic appearance-

Each individual follicular adenoma tends to have a consistent microscopic pattern within itself. For purposes of description only, there are six (possibly more) growth patterns, embryonal, foetal, simple, colloid, oxyphil and atypical.

a) Embryonal adenoma:

These type of adenoma are most poorly differentiated among the follicular group. They are composed of columns of small, closely packed thyroid cells often embedded in a considerable amount of edematous and fibrous stroma. There is little follicle formation. Follicles are small and rarely contain colloid.

b) Foetal adenoma:

Foetal adenoma are slightly more differentiated and show miniature follicle formation of a "foetal" type containing little or no colloid cyst formation and hemorrhage are most common.

c) Simple adenoma:

Simple adenoma is made up of well differentiated thyroid tissue with well-developed follicles. There is however, definite encapsulation.

d) Colloid adenoma:

Colloid adenoma contains excessive colloid within greatly distended follicles.

e) Oxyphilic adenoma:

Composed of predominantly a large cell with a granular acidophilic cytoplasm- is now regarded as variant of follicular carcinoma.

f) Atypical adenoma:

It shows a microscopic picture of more cellularity than other adenoma. The cells and cellular architecture are bizarre. Cells may be elongated or even spindle shaped with irregular nuclei and mitosis. There may be capsular or vascular invasion. If no invasion is found they are benign.

Follicular adenoma may appear in any group but are most common during middle age. They are several times more common in females than males.

Progression of adenomas:

1. It may remain stationary in size and produce no symptoms and signs other than a palpable mass.
2. It may slowly increase in size, gradually producing pressure on adjacent structures or becoming disfigured.
3. It may undergo retrogressive changes with hemorrhage into the tumor resulting in such an alarming increase in its size that the patient seeks immediate medical advice.
4. It may become hyper functioning which may produce clinical hyperthyroidism.
5. It may become malignant, but is probably an infrequent occurrence.

PAPILLARY ADENOMA :

It is a benign epithelial neoplasm producing papillae and has a tendency to cyst formation. The contrasting feature from follicular adenoma is formation of cyst. Typical cysts, filling with brown watery fluid vary in its size from a few millimeters to a centimeter or more. Lining walls present papilliferous process growing in fronds or grape like cluster. Cut surface may show shaggy appearance due to innumerable small papillae.

Microscopic appearance-

The typical cell is cuboidal or columnar often resembling thyroid epithelium, cellular and nuclear pleomorphism is slight and mitosis is rare. The individual cells are arranged in single layers and connective tissue stalks project into the lumen of various sized cyst spaces. Frequently some of the cells form a follicle which may contain colloid. There may be foci within a papillary adenoma that resembles follicular adenoma or normal thyroid tissue. The tendency to cyst formation is variable, many cysts are microscopic. Larger cysts are often multiloculated and contain haemosiderin from old or recent hemorrhage.

The course of these tumors is similar to that of follicular adenoma. Papillary adenoma often has foci of follicular pattern.

MAGLIGNANT TUMORS: [25]

PAPILLARY CARCINOMA:

Macroscopic appearance-

Largest tumors and have ill-defined borders, although some show partial encapsulation. Cysts of varying sizes are common in larger tumors filled with brown watery fluid unless hemorrhage occurs. Papillae project from the inner lining of the cyst. Fibrosis is common. The variants include columnar, tall cell variant, diffuse sclerosing, follicular variant of papillary, hurthle cell and microcarcinoma.

Microscopic appearance-

The tumor epithelium is arranged on fibro-vascular stalks which often project into cystic spaces. Typically, the epithelium is single layered, but multi- layering does occur. Tumor cells are cuboidal with a homogenous cytoplasm surrounding a central ovoid nucleus. Mitosis is extremely rare. It has a tendency to invade vein and lymphatics.

Papillary carcinoma is the commonest type of thyroid cancer. They may invade through the thyroid capsule into the soft tissue, intra thyroidal lymphatic spread leads to multiple foci in the some lobe or less commonly in both lobes usually giving a multinodular appearance.

FOLLICULAR CARCINOMA:

Follicular carcinomas are slow growing and often seen to have originated from pre-existing benign tumor. Follicular carcinomas are often indistinguishable from adenomas, since their invasive nature is not extensive. Retrogressive changes like hemorrhage are common.

Microscopic appearance-

Some tumors are solid and form only small follicles while some form well differentiated follicles. Follicular carcinoma in which invasion is moderate is termed invasive, and prognosis depends upon the histologic pattern.

Follicular carcinomas are capable of metastasizing by blood and lymphatic's and may invade the local tissue extensively.

MEDULLARY CARCINOMA:

Medullary carcinoma maybe sporadic, familial, or may occur in combination with adrenal pheochromocytoma and hyperparathyroidism (due to hyperplasia) – MEN IIa (Sipple's Syndrome). When familial form is associated with mucosal neuromas, Marfanoid habitus, the syndrome is referred to as MEN II B.This type of tumor arises from the parafollicular or C cells often at the age of 40 years. A carcinoma of moderate degree of malignancy, the cells of which grow in solid cluster surrounded by a dense stroma.

Microscopic appearance-

Tumor cells are clustered into solid irregular groups separated by a hyaline, amyloid containing stroma. Cells of tumor are round or polyhedral and have eosinophilic granular cytoplasm. Nuclei are hyperchromatic and frequently binucleate, Mitosis is sparse.

UNDIFFERENTIATED CARCINOMA:

Undifferentiated carcinoma form neither papillary nor follicular structure. It may be either small cell carcinoma or giant cell carcinoma.

a) **Small cell carcinoma:**

A highly malignant carcinoma.

Microscopic appearance-

Composed of small cell. It may be of two types- compact and diffuse. Compact type has a uniform appearance of small closely packed strands or cluster with frequent mitosis. The diffuse type often shows abortive follicles and solid clumps of tumor cells. Most cells show atypical mitosis, stroma is scanty and blood vessel invasion is common.

a) Giant cell carcinoma:

Giant cell carcinoma is a highly malignant and differentiated tumor composed of cells larger than the usual follicular cells which may be giant or spindle forms and hence is termed giant cell carcinoma. Pleomorphism and multinucleated cells are common. Identifiable epithelial structure may be difficult to find.

Table 1: TNM Classification of Thyroid Tumors: [26]

T	Primary tumor (Multifocal tumors are designated(m). The diameter of the largest determines the classification
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor 2cm or less in greatest dimension, limited to the thyroid
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to thyroid
T3	Tumor more than 4 cm in greatest dimension, limited to the thyroid or any tumor with minimal extrathyroid extension(e.g. Extension to sternohyoid muscle or perithyroid soft tissue)
T4a	Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissue, larynx, trachea, esophagus, or recurrent laryngeal nerve.
T4b	Tumor invades prevertebral fascia or encase carotid artery or mediastinal vessels.
All Anaplastic(undifferentiated) tumors are considered T4	
T4a	Intrathyroid Anaplastic carcinoma-surgically resectable
T4b	Extra-thyroidal Anaplastic carcinoma-surgically unresectable

Regional Lymph nodes(N) Regional nodes are central compartment, lateral cervical and upper mediastinal Lymph node.	
NX	Regional nodes cannot be assessed
N0	No regional lymph nodes metastasis
N1	Regional lymph node metastasis
N1a	Metastasis to level VI(pretracheal, paratracheal, and prelayngeal/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

Table 2: Staging of Thyroid Tumors: [26]

Papillary and follicular thyroid cancer (age < 45y):			
Stage	T	N	M
I	Any T	Any N	M0
II	Any T	Any N	M1
Papillary and follicular; differentiated (age ≥ 45y):			
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1	N1a	M0
	T2	N1a	M0
	T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	AnyT	AnyN	M1

MEDULLARY CARCINOMA			
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1	N1a	M0
	T2	N1a	M0
	T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a,b	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
IVB	T4b	AnyN	M0
IVC	AnyT	AnyN	M1
UNDIFFERENTIATED(ANAPLASTIC) CARCINOMA(All are considered Stage IV)			
IVA	T4a	AnyN	M0
IVB	T4b	AnyN	M0
IVC	AnyT	AnyN	M1

IMMUNOHISTOCHEMISTRY:

Immunohistochemistry is a revolutionary diagnostic tool for the pathologist. Despite the presence of various diagnostic criteria, there is overlapping among different entities and dissimilarities among the same entity. This when compounded with subjective disparity among the pathologists makes reproducibility of diagnosis difficult[27]. This prompted the development of special staining technique to stain cells of particular lineage, marking the beginning of histochemistry in the mid nineteenth century. Francis Vincent Raspard was the first botanist to use immunohistochemistry. Advent of aniline dyes revolutionized IHC from 1862 to 1929[28.] The origin of IHC techniques lies in the pioneering work of Albert Coons, starting in 1941. He described his first attempts to label antibodies directly with fluorescent isocyanate. Later the indirect technique was introduced by Nakane and Pierce in 1966, in that an unlabeled antibody is followed by second antibody or substrate. Various stages of development of IHC from peroxidase antiperoxidase (1970), Alkaline phosphatase labeling (1971), biotin technique (1977, 1979) and to two layer dextrin polymer technique (1993) carries both advantages and disadvantages for each techniques.[29]

DETECTION SYSTEM:

Subsequent to the development of specific antibodies to the antigens, next step for the immunochemist was to develop techniques to visualize the antigen-antibody reaction complex.

Two methods employed for this:

l) Direct method

2) Indirect method

DIRECT METHOD:

In this the primary antibody is conjugated directly to the label. Most popular direct conjugates are those which are labelled with a fluorochrome, horse radish peroxidase and alkaline phosphatase. The advantage of this method is that it is simple to use as it requires one application of reagent followed by appropriate chromogen substrate solution.[29]

The current techniques is Enhanced polymer one step staining method-Epos is a new direct technique reported by Pluzek et al in 1993,[30] in which a large number of primary antibody molecules and peroxidase enzymes are attached to a dextran polymer "back bone". This is rapid, used for frozen sections and is sensitive to demonstrate small amounts of antigen.

INDIRECT METHOD:

This is a two-step method in which labeled secondary antibody reacts with the antigen bound to primary antibody. Further increase in sensitivity was achieved with the introduction of peroxidase enzyme complex. Subsequent development resulted in Avidin-Biotin Complex method and is the most widely used method.[29]

A dextran polymer conjugate -two step visualization systems is a new indirect system based on dextran technology employed in the Epos system. This offers greater sensitivity than the traditional indirect system. It is less time consuming than the three stage Avidin - Biotin system.[29]

IHC PROCEDURES:

Antigen retrieval in immunohistochemistry (ar-ihc) is done by any of the following technique-

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION:

Pre-treating formalin-fixed, routinely processed paraffin sections with proteolytic enzymes to unmask certain antigenic determinants was described by Huang et al (1976).[31] Before the advent of heat pretreatment, proteolytic digestion was an essential requirement in antigen retrieval. The commonly used enzymes are trypsin and protease. Enzyme digestion breaks down formalin cross-linking and unmasks the antigenic sites. It

is used in demonstrating immunoglobulins and complements in renal biopsies, but it has problems like over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new and revolutionary technique. Microwave oven heating retrieves many antigens thought previously to be either lost or destroyed by routine histological processing techniques. It involves boiling of cleaned formalin -fixed paraffin sections in various solutions and allows rapid and uniform heating. Antibodies and such as the proliferation markers anti-Ki67 and MIB-1 which are unstable in frozen section work well after heat pre-treatment on paraffin wax sections.[29]

PRESSURE COOKER ANTIGEN RETREIVAL:

Replacing the microwave oven with the Pressure cooker has proved to have some advantages. Microwaving of larger numbers of slides using bigger containers does suffer from inconsistencies. Miller et al in 1995[32,] compared and proved that pressure cooking method does not suffer from such inconsistencies also it is less time consuming.

PITFALLS OF HEAT PRE-TREATMENT:

Care should be taken not to allow the sections to dry after heating, as this destroys antigenicity. Damage of nuclear details is seen in poorly fixed tissue. Fibres and fatty tissues tend to detach from the slides while heating Not all antigens are retrieved by heat pre-treatment and also some antigens show altered staining pattern with some primary antibodies e.g. PGP9.5.[33]

HORMONE RECEPTORS:

The steroid receptors, androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor, mineralocorticoid receptor and progesterone receptor (PR), belong to nuclear receptor super-family group of transcription factors activated by the binding of a hormone ligand.[34] Among these, ER, PR and AR are receptors and ligands, estrogens, progestins and androgens have been defined by their role in reproductive function and are hence known as sex steroid receptors which undergo a conformational change upon hormone binding and cause separation of the receptor from its cytoplasmic chaperone protein and exposure of its localization sequences. Subsequent homo-dimerization occurs and there is binding of the receptor to steroid response elements on the promoter regions of target genes, recruiting and interacting with coactivators, corepressors or other transcription factors to regulate gene expression and activate various intracellular pathways.[35]

ESTROGEN RECEPTORS:

Walker D (1999) described that ER are regarded as cytoplasmic receptor in unliganded state. Since they are steroid receptors they do not require membrane bound receptors for their activation. During activation ER rapidly diffuses into the cytoplasm and migrates from cytosol to nucleus, then dimerises and subsequently it binds into HREs (Hormone Response Elements). The DNA receptor complex activates P13K pathway and induces cell proliferation. Two hypotheses have been proposed for its action. One hypothesis

states that ER induces transcription activity by alternative RNA splicing of Estrogen receptor alpha subunit thereby inducing rapid uncontrolled proliferation and accumulation of genetic mutation. The other hypothesis states that it acts by producing by genomic waste.[36, 37]

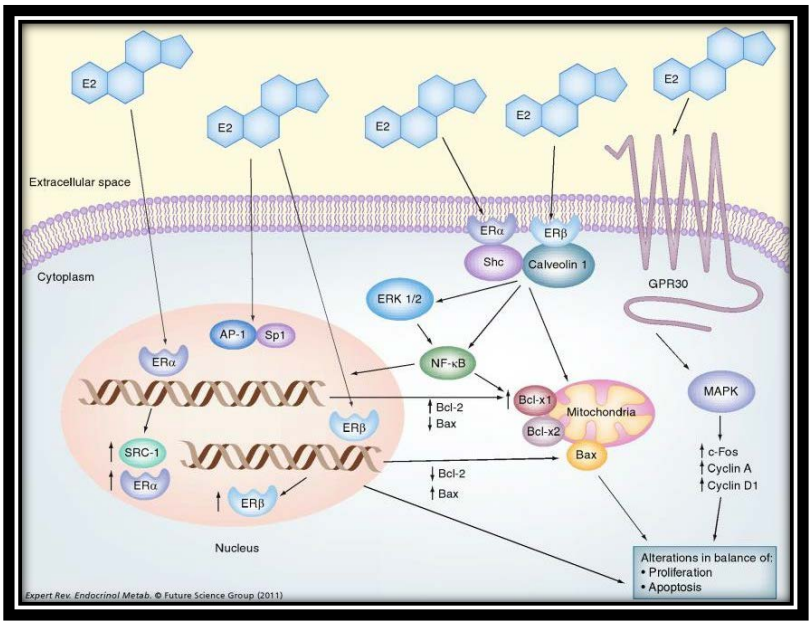


Figure 6: Diagrammatic representation of Estrogen receptor mechanism

Two distinct ERs, ERα and ERβ, which are coded by separate genes and have somewhat different tissue distributions, are known. ERα is expressed in the female reproductive tract, uterus, vagina and ovaries, while ERβ expressed in the prostate, ovaries and lungs.[38,39] Both ER isoforms are also expressed to a lesser extent by other tissue types, including thyroid tissue. The activity, functions and regulation of the two receptors are

also different from each other, and ERα transcriptional activity is inhibited by ERβ.[40] The responses of the different ERs to various estrogenic compounds are also different. The major ligands of ERs are the endogenous estrogens E1, E2 and estriol (E3).[41] E2 binds both receptors with equal affinity, while E1 and E3 preferentially bind to ERα and ERβ, respectively.

PROGESTRONE RECEPTOR:

Progesterone receptor is an intracellular steroid receptor that specifically binds to progesterone. PRs is encoded by a single gene PGRIlq22 and regulated by ERs. [42]

Estrogen receptor is always necessary to induce Progesterone receptor. Binding of hormone to the receptor induces structural changes whereby inducing cell proliferation. Estrogen and Progesterone receptor share a common structural and functional organization. WHO consensus development conference(1980) found that Progesterone receptor expression is a predictive marker. Hence it is estrogen regulated: most Progesterone receptor positive carcinomas are Estrogen receptor positive also.[43, 44]

Clarks et al (1988)[45] demonstrated Progesterone receptor by immunohistochemistry in formalin fixed paraffin embedded section. It is seen visually in the same cells which are positive for Estrogen receptor.[46]

PRs are activated by the endogenous hormone progesterone and exist in two isoforms, PR-A and PR-B. PR-A and PR-B share many common structural domains but are

functionally distinct. PR-B largely mediates the stimulatory actions of progesterone and PR-A acts as a repressor of PR-B-mediated transcription, as well as acting as a transcriptional inhibitor of PR-B and other steroid receptors[47, 48]. Expression of both PR isoforms can be induced by estrogen,[49] while progesterone decreases PR expression in most target tissues.[50] PRs are expressed not only by the female reproductive tract but also in the testes, breast, neural tissues and by other tissue types.[49]

Estrogen and Progesterone receptors belong to super family protein. They are nuclear transcription factors that are involved in breast development, growth, differentiation and tumorigenesis[29, 36]. Estrogen receptor regulates the expression of other genes such as Progesterone & bcl2.[36] Thus Progesterone receptor is an indicator for intact Estrogen receptor functional pathway.

These hormone receptors are well established biomarkers in breast carcinoma and their assessment helps in predicting the response to endocrine therapy[36, 37]. Assessment of expression of ER and PR in thyroid will help to know about behavior of thyroid lesions and there by establish targeted therapies for thyroid cancers.

IHC SCORING- QUICK SCORING SYSTEM:

Estrogen and Progesterone receptors express nuclear positivity They are scored by the proportion of tumor cells showing positivity and intensity of the reaction. Both are summated to give a total score.[51]

THYROID CANCER WORLDWIDE SCENARIO:

Thyroid cancer is the most common endocrine cancer (approximately 1.0%–1.5% of all new cancers diagnosed each year[52], and its incidence has continuously increased in the last three decades all over the world. This trend is present on every continent except Africa[53], where detection is possibly low. The increasing prevalence is indicated by the annual percent change (APC) that in the USA was 2.4% from 1980 to 1997 and 6.6% from 1997 to 2009 (both genders). Based on recent data, thyroid cancer is the fifth most common cancer in women[54]. Only in few countries (Norway, Sweden) thyroid cancer incidence have decreased. During the past several decades, an increasing prevalence of thyroid cancer has been reported in many parts of the world. To date, no study has compared the trends in thyroid cancer incidence across continents. Briseis AK et al[53] had studied international patterns and trends in thyroid cancer incidence, 1973–2002. Briseis AK et al[53] examined data from cancer incidence in five continents (CI5) over the 30-year period 1973–2002 from 19 populations in the Americas, Asia, Europe, and Oceania. Thyroid cancer rates have increased from 1973–1977 to 1998–2002 for most of the populations except Sweden, in which the incidence rates decreased about 18% for both males and females. The average increase was 48.0% among males and 66.7% among females. More recently, the age-adjusted international thyroid cancer incidence rates from 1998 to 2002 varied 5-fold for males and nearly 10-fold for females by geographic region.

THYROID CANCER IN INDIA:

In India, thyroid tumors are the most common endocrine neoplasms. 1 to 5% of hyperfunctioning and 15 to 20% of hypofunctioning solitary nodules of all thyroid nodules coming to medical attention are carcinomas. In areas of iodine sufficiency, papillary carcinomas are the predominant variety. Different studies from India show a predominance of papillary malignancy followed by follicular malignancies. The overall prognosis for thyroid carcinoma is worse in endemic goiter regions, in comparison with regions with an adequate dietary iodine intake, perhaps because of the higher incidence of undifferentiated thyroid malignancies in iodine deficiency areas[54]. Even in regions with endemic goitres (and iodine deficiency), papillary neoplasms predominate over follicular cancers[55, 56]. Two large series of medullary carcinomas of the thyroid was reported from India[57, 58]. Male predominance was reported in MTC in contrast to differentiated thyroid malignancies. A study by Bal et al addressed the prevalence of thyroid malignancies in children. 85% of the 122 patients had papillary carcinoma of thyroid. The disease was found to be more aggressive and widespread in younger age groups (< or =10 years), with male preponderance and high mortality. Cervical lymph node involvement was seen in 66% of patients, and distant metastasis, mainly pulmonary, in 29%. In children less than 10 years of age, 75% of patients had distant metastasis at the time of presentation[59]. Although the distribution of malignancy according to the types is similar to world literature, the incidence of distant metastasis is significantly more than that reported from iodine sufficient areas of the world[60]. Epidemiological studies of cancer in India show an increased incidence of thyroid cancers in females in southwest coastal districts and also in Kerala[61, 62]. Studies from India show that thyroid malignancy presents at an advanced

stage compared to western literature. Incidence of thyroid malignancy in children and teenagers is increasing of late. Proactively profiling the cytology of thyroid nodules at first detection will lead to detection of thyroid malignancies at an earlier stage. The Indian Council of Medical Research established the National Cancer Registry Program, and the NCRP has collected the data of more than 3, 00,000 cancer patients between the periods from1984 to 1993[63]. Among these patients, the NCRP noted 5624 cases of thyroid cancer, comprising of 3617 females and 2007 males. The nationwide relative frequency of thyroid cancer among all the cancer cases was 0.1%–0.2%. The age-adjusted incidence rates of thyroid cancer per 100,000 are about 1 for males and 1.8 for females as per the Mumbai Cancer Registry, which covered a population of 9.81 million subjects. The histological types of thyroid cancer were studied in a Hospital Cancer Registry of 1185 “new cases” of thyroid cancer.[64]

MATERIAL

AND

METHODS

MATERIAL AND METHODS:

The study deals with the correlation of expression of ER and PR with various histological types in thyroid lesions and their expression in males and females.

A total of 65 cases of thyroid lesions were included in the study. All cases of thyroidectomy specimens received at department of Pathology, from the department of Otorhinolaryngology and Head and Neck Surgery, Sri RL Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College and Research Centre, Tamaka, Kolar during the period of January, 2015 to September, 2016 were included in the study.

Few cases of thyroid lesions were also retrieved from archives of department of Pathology from the year 2014.

Prior to the study ethical clearance was obtained from the institutional ethical board.

SAMPLE SIZE:

Sample size estimated based on prevalence of histological sections of thyroid tumors in the Department of Pathology, Sri Devaraj Urs Medical College.

Taking absolute error of 10% and Alpha at 0.001- Sample size n = 65

$n= (alpha)^2 pq/(d)^2$

$n= (1.96)^2 X 20.7 X (100-20.7)/(10)^2$

Absolute error d= 10%

Prevalence p= 20.7%

n= 65 at 95% confidence level expecting 10% non-compliance.

STUDY DESIGN: Laboratory observational study

INCLUSION CRITERIA:

1. Benign and malignant thyroid lesions in women and men in all age groups.

EXCLUSION CRITERIA:

1. Post Radiotherapy and Chemotherapy specimens.
2. Metastatic tumours in thyroid.
3. Recurrent lesions.

METHOD OF COLLECTION OF DATA:

SOURCE OF DATA:

1. All cases of Thyroidectomy (total and hemi-thyroidectomy) specimens of benign and malignant lesions received at department of Pathology from RL Jalappa hospital and research center during the period of January 2015 to October 2016.

2. Thyroidectomy (total and hemi-thyroidectomy) specimens from the archives of department of Pathology from the year 2014, previously diagnosed as benign and malignant lesions were included.

METHOD:

1. Thyroidectomy specimens received at department of Pathology, were analyzed and clinical data such as, name, age, history of present illness, personal history, family history, clinical examination and surgical details were obtained.
2. The specimens of thyroidectomy received were examined and the following details were noted- type of thyroidectomy, side (right/left), measurements, external appearance of thyroid, overlying capsule and is isthmus present its measurements, and tumor component were noted. The specimens were cut at intervals of 1cm and were allowed for fixation in 10% formalin. After adequate fixation, the tumor component was identified and details such as- size, shape, consistency, multicentricity, lobe were noted. Neck dissection specimen received were also grossed and the total number of lymph nodes retrieved, was noted.
3. Tissue bits were taken from the representative areas, processed, blocked and sections taken.
4. Cases of thyroid lesions from the year 2014 were retrieved from the archives of department of Pathology and above said findings were noted.
5. All slides were reviewed and histological parameters such as, benign or malignant, histopathological type, grading for carcinomas, lymph node status if present was noted.

6. Additional sections of 4um thickness were cut from these paraffin blocks and subjected to staining with ER and PR according to the standard protocols.

The details of the immunohistochemical markers used in the study are as follows:

Table 3: ER Antibodies

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Estrogen receptor	monoclonal	Mouse	Biogenex	Ready to use	Breast	Nuclear stain

Table 4: PR Antibodies

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Progesterone receptor	monoclonal	Mouse	Biogenex	Ready to use	Breast	Nuclear stain

IHC Procedure:

- Sections are 3-4mm thickness, floated on to organosialine coated slide and left on hot plate at 60^0C overnight.
- Deparaffinization using Xylene I and II—15 min each
- Dexylinisation using absolute alcohol I and II—1 min each
- Dealcoholisation using 90% and 70% alcohol—1 min each
- Tap water – 10 min wash.
- Distilled water- 5 min rinsing.
- Antigen Retrieval technique: Microwave at power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0 for 3 cycles.
- Transfer to TBS (Tris buffer solution pH- 7.2) - 5minutes x 3 times-wash.
- Peroxidase block- 30 minutes to block endogenous peroxidase enzyme.
- TBS buffer for 5 minutes washing for 3 times.
- Drain and cover sections with targeted antibody (primary)- 1hr and 30 minutes
- Super sensitive poly- HRP (secondary antibody)- 30 min
- TBS buffer- 5min x 3 times
- Color development with working color development solution (DAB) - 5-8 min
- TBS wash- 5min x 3 times
- Counter stain with Haematoxylin – 1 min.
- Tap water wash for 5 minutes.
- Dehydrate, clear and mount
- Mount with DPX.

Immunohistochemical analysis and counting:

Sections were first examined at low magnification (x40 and x100 magnification) using (Olympus CX 21i) microscope to identify areas of highest positivity (hotspot).

Areas of hotspots which had positivity of tumor cells were selected. 200 individual cells were counted under x 200 magnification and then average was taken.

1. **ER expression:** [51]
   - Estrogen showed nuclear staining of tumor cells.
   - Proportion score was calculated by counting proportion of cells stained.
   - Intensity score was calculated by estimating the intensity of cells stained.
   - Total score was calculated by adding the Proportion score and Intensity score.
   - A score between 0-8 was taken.
   - ER positive breast tissue sections from already known ER positive cases were taken as positive controls.

2. **PR expression:** [51]

- PR antibody stained the nucleus of cancer cells weakly.
- Proportion score was calculated by counting proportion of cells stained.
- Intensity score was calculated by estimating the intensity of cells stained.
- Total score was calculated by adding the Proportion score and Intensity score.
- A score between 0-8 was taken.
- PR positive breast tissue sections from already known PR positive cases were taken as positive controls.

Table 5: Proportion of stained cells/Proportion score (PS):[51]

Score	Proportion
0	None
1	1/100
2	1/100-1/10
3	1/10-1/3
4	1/3-2/3
5	>2/3

Table 6: Intensity of staining/Intensity score (IS):[51]

Score	Intensity
0	None
1	Weak
2	Intermediate
3	Strong

Total score was calculated by adding PS and IS ranging from 0-8

Score of 2 or more was considered positive

RESULTS

&

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test of Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data.

Continuous data was represented as mean and standard deviation. **Independent t test or Mann Whitney U test** was used as test of significance to identify the mean difference between two quantitative variables and qualitative variables respectively.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram and Scatter plots.

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

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data. EPI Info (CDC Atlanta), Open Epi, Med calc and Medley's desktop were used to estimate sample size, odds ratio and reference management in the study.

RESULTS:

Sixty five specimens with various thyroid lesions were studied during the period January 2015 to September 2016 in the Department of Pathology, Sri Devaraj Urs Medical College Tamaka, Kolar.

Immunohistochemistry was done for all 65 cases for ER and PR status. The following data was recorded and analyzed:

1. Age distribution
2. Sex distribution
3. Type of thyroid surgery done
4. Size of the tumor
5. Histopathological diagnosis
6. ER total score
7. PR total score

Table 7: Diagnosis of lesions in subjects :

		No. of cases	%
Benign or malignant	Benign	47	72.3%
	Malignant	18	27.7%
	Total	65	100.0%

Majority 72.3% were diagnosed to have benign lesions and 27.7% were diagnosed to have malignant lesions.

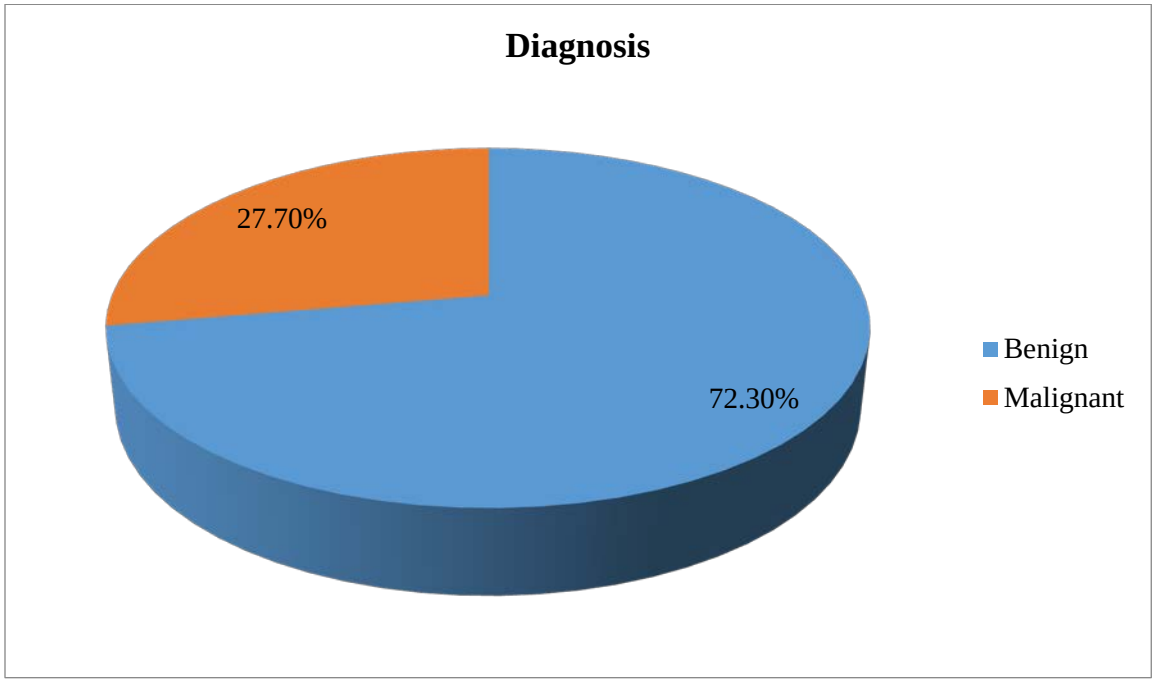


Chart 1: Pie diagram showing Diagnosis of lesions in subjects

Table 8: Mean age of subjects in the study:

	Diagnosis				P value
	Benign		Malignant		
	Mean	SD	Mean	SD	
Age	40.2	11.5	33.8	7.8	0.035*

Mean age of subjects with benign lesions was 40.2 ± 11.5 years and in Malignant lesion was 33.8 ± 7.8 years. This difference in age distribution between benign and malignant lesions was statistically significant.

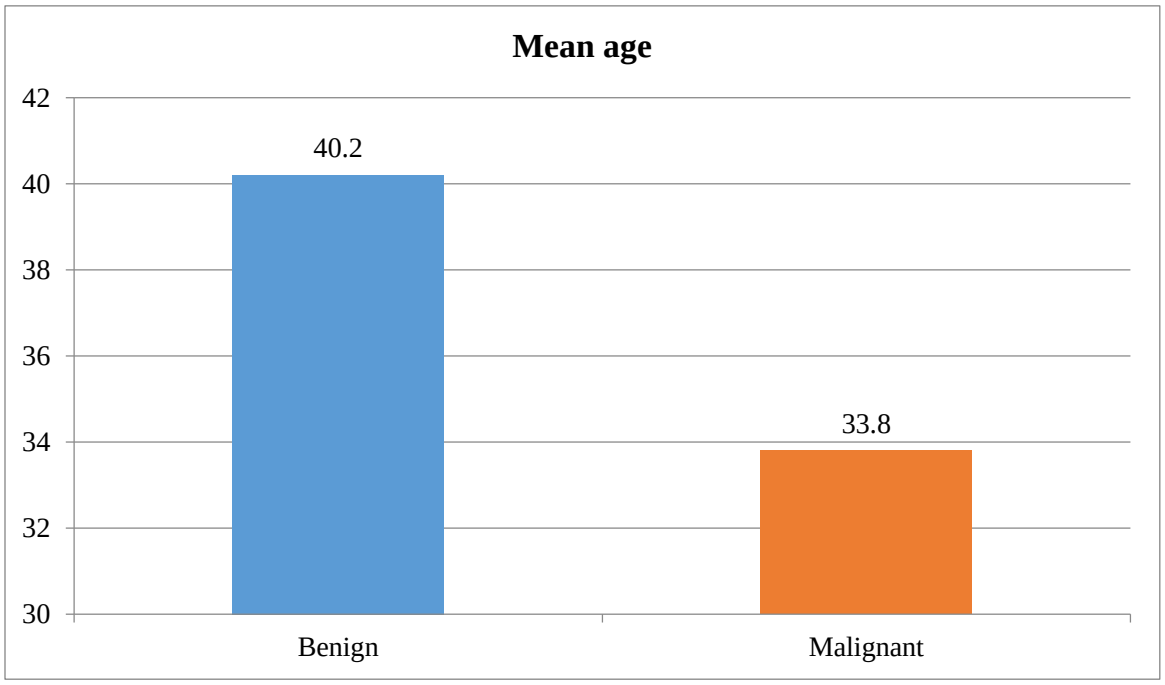


Chart 2: Bar diagram showing Mean age of subjects in the study

Table 9: Gender distribution of subjects with respect to diagnosis:

		Diagnosis			
		Benign		Malignant	
		No. of cases	%	No. of cases	%
Sex	Female	36	76.6%	14	77.8%
	Male	11	23.4%	4	22.2%
	Total	47	100.0%	18	100.0%

$\chi 2 = 0.01$, df = 1, p = 0.919

Among subjects with benign lesions, 76.6% were females and 23.4% were males, among subjects with malignant lesions 77.8% were females and 22.2% were malignant lesions. There was no significant difference in gender distribution between diagnoses.

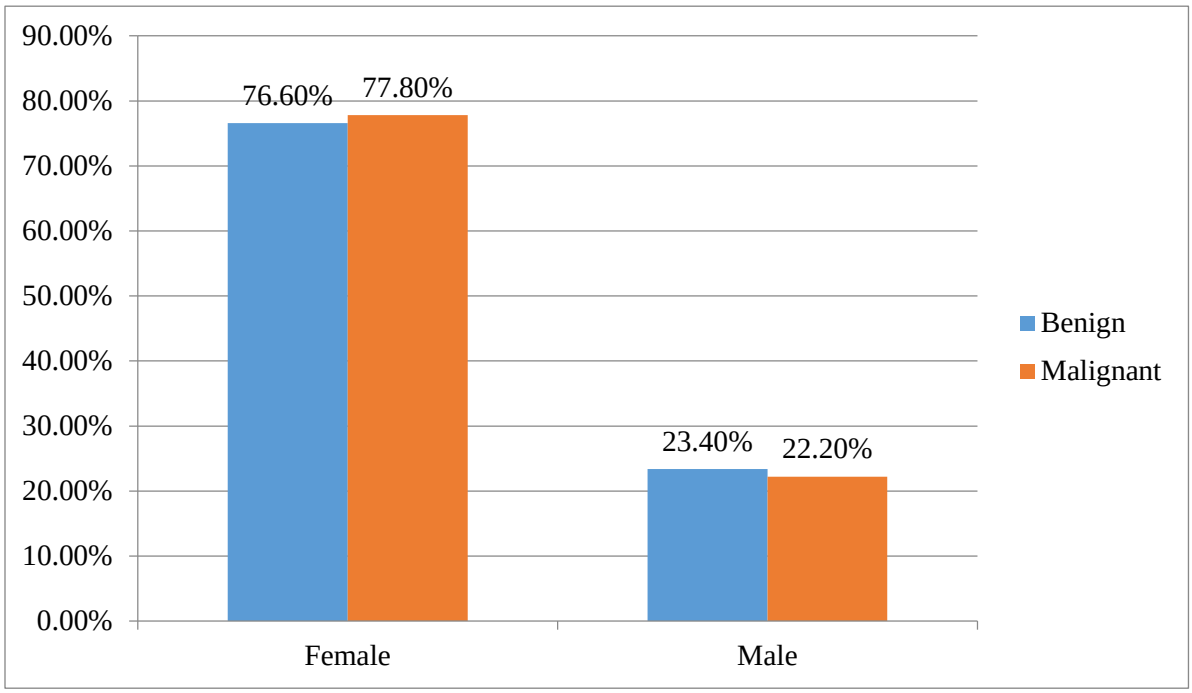


Chart 3: Bar diagram showing Gender distribution of subjects with respect to diagnosis

Table 10: ER score comparison between Benign and Malignant lesions :

ER		Benign or Malignant			
		Benign		Malignant	
		No. of cases	%	No. of cases	%
Total score	0	13	27.7%	0	0.0%
	1	1	2.1%	0	0.0%
	2	20	42.6%	1	5.6%
	3	10	21.3%	3	16.7%
	4	0	0.0%	6	33.3%
	5	3	6.4%	4	22.2%
	6	0	0.0%	4	22.2%
	7	0	0.0%	0	0.0%
	8	0	0.0%	0	0.0%

$\chi 2 = 40.158$, df = 6, $p < 0.001$*

In benign lesions ER score was lower compared to malignant lesions. Majority of subjects(42.6%) in benign lesions had score of 2 and majority with malignant lesions(33.3%) had a score of 4. This difference in ER total score between benign and malignant lesions was statistically significant.

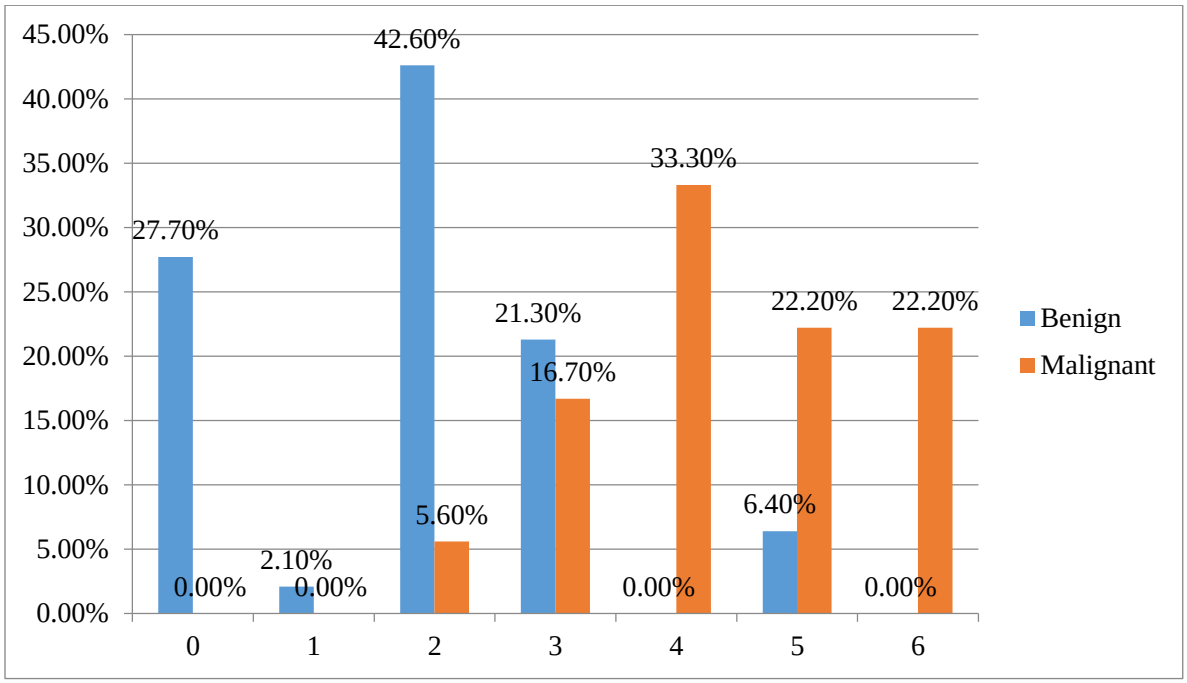


Chart 4: Bar diagram showing ER score comparison between Benign and Malignant lesions

Table 11: PR score comparison between Benign and Malignant lesions

PR		Benign or Malignant			
		Benign		Malignant	
		No. of cases	%	No. of cases	%
Total score	0	29	61.7%	0	0.0%
	1	0	0.0%	0	0.0%
	2	15	31.9%	10	55.6%
	3	3	6.4%	7	38.9%
	4	0	0.0%	1	5.6%
	5	0	0.0%	0	0.0%
	6	0	0.0%	0	0.0%
	7	0	0.0%	0	0.0%
	8	0	0.0%	0	0.0%

$\chi 2 = 24.54$, df = 3, $p < 0.001$*

In benign lesions PR score was lower compared to malignant lesions. Majority of subjects(61.7%) in benign lesions had score of 0 and majority with malignant lesions(55.6%) had a score of 2. This difference in PR total score between benign and malignant lesions was statistically significant.

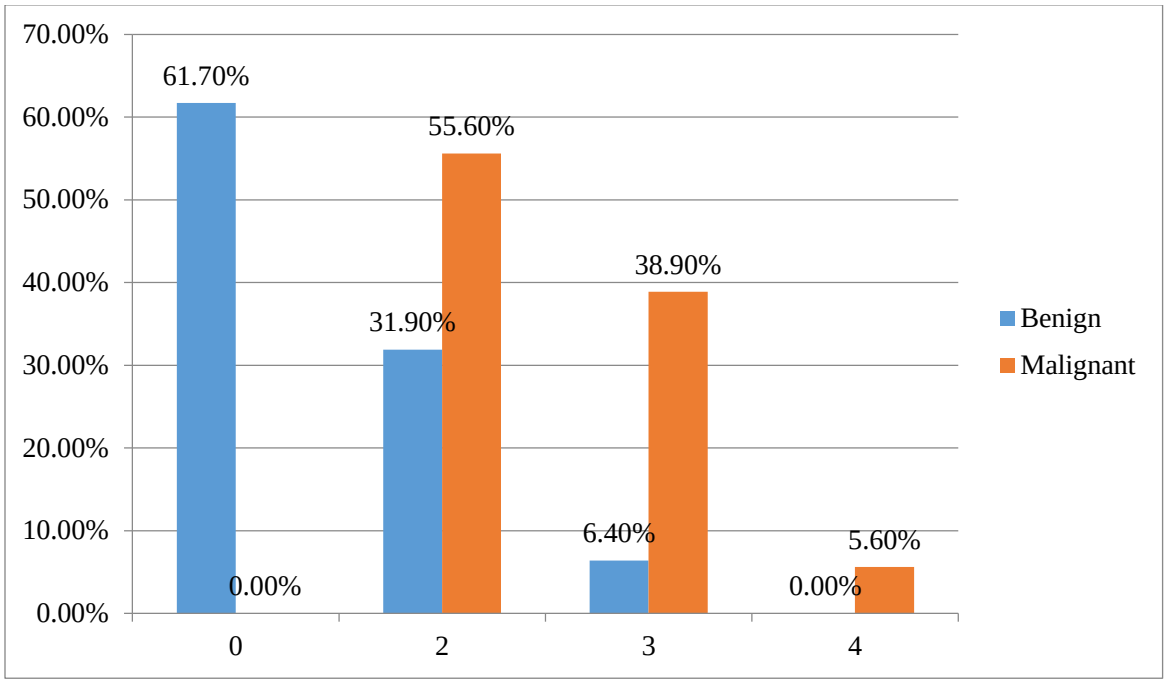


Chart 5: Bar diagram showing PR score comparison between Benign and Malignant lesions

Table 12: ER score comparison between Gender:

		Gender			
		Female		Male	
		No. of cases	%	No. of cases	%
ER Total Score	0	6	12.0%	7	46.7%
	1	0	0.0%	1	6.7%
	2	20	40.0%	1	6.7%
	3	9	18.0%	4	26.7%
	4	4	8.0%	2	13.3%
	5	7	14.0%	0	0.0%
	6	4	8.0%	0	0.0%
	7	0	0.0%	0	0.0%
	8	0	0.0%	0	0.0%

χ 2 = 18.324, df = 6, p = 0.005*

In Females ER score was higher compared to males. Majority of female subjects(40%) had ER score of 2 and majority of males(46.7%) had a score of 0. This difference in ER total score between females and males was statistically significant.

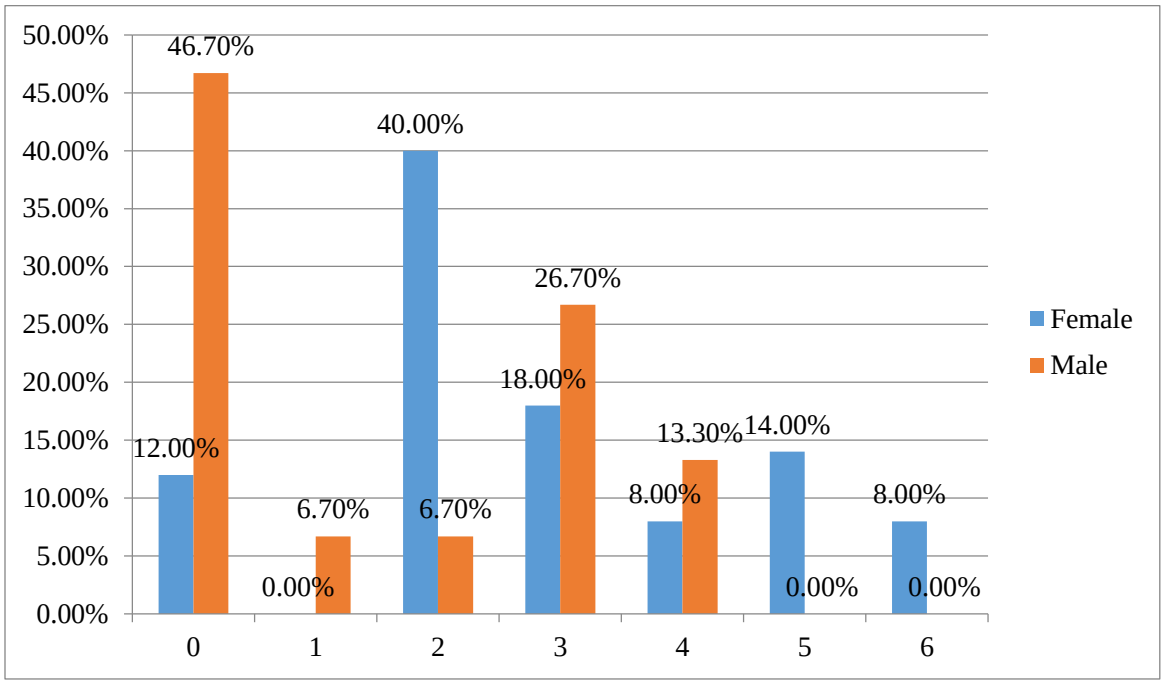


Chart 6: Bar diagram showing ER score comparison between sex

Table 13: PR score comparison between Genders:

		Gender			
		Female		Male	
		No. of cases	%	No. of cases	%
PR Total Score	0	20	40.0%	9	60.0%
	1	0	0.0%	0	0.0%
	2	19	38.0%	6	40.0%
	3	10	20.0%	0	0.0%
	4	1	2.0%	0	0.0%
	5	0	0.0%	0	0.0%
	6	0	0.0%	0	0.0%
	7	0	0.0%	0	0.0%
	8	0	0.0%	0	0.0%

$\chi 2 = 4.346$, df = 3, p = 0.226

In Females PR score was higher compared to males. Majority of female subjects had ER score of above 2 and majority of males had a score of 0. However this difference in PR total score between females and males was not statistically significant.

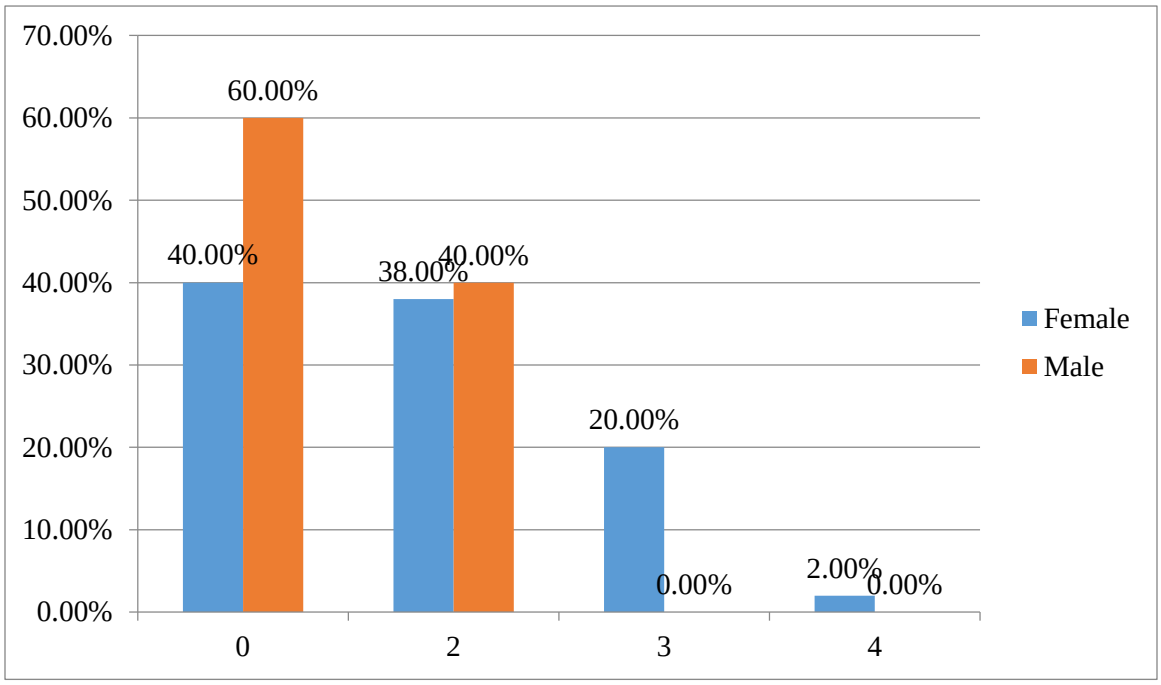


Chart 7: Bar diagram showing PR score comparison between sex

Table 14: Comparison of ER score between Genders in malignant subjects:

		Gender			
		Female		Male	
		No. of cases	%	No. of cases	%
ER Total score	2	1	7.1%	0	0.0%
	3	1	7.1%	2	50.0%
	4	4	28.6%	2	50.0%
	5	4	28.6%	0	0.0%
	6	4	28.6%	0	0.0%

$\chi 2 = 6.429$, df = 4, p = 0.169

In the malignant lesions majority of females had ER score above 4 and in males majority of them had ER score of 3 and 4 respectively. However there was no significant difference between genders with respect to ER score.

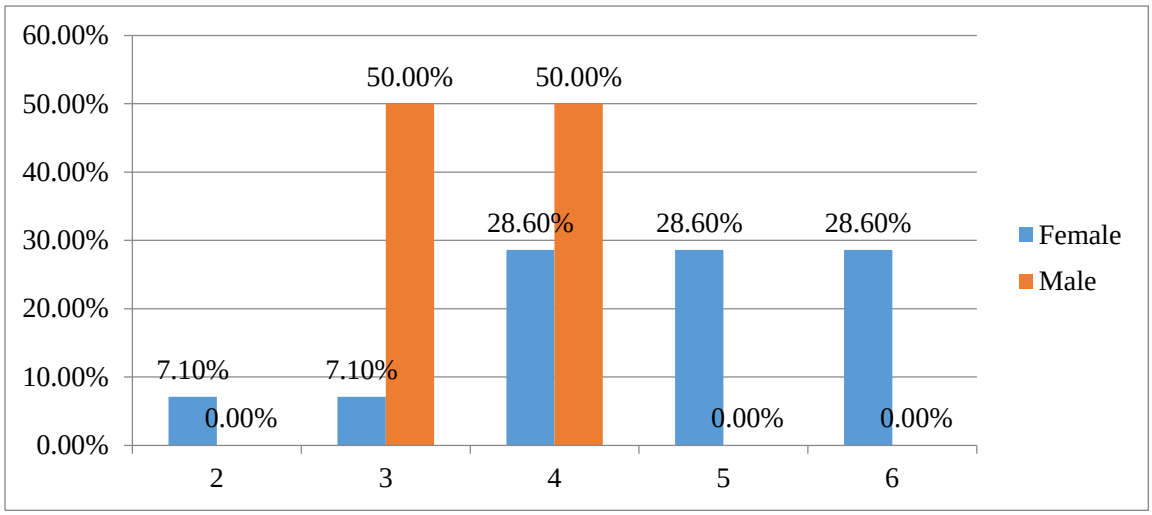


Chart 8: Bar diagram showing Comparison of ER score between genders in malignant subjects

Table 15: Comparison of PR score between Genders in malignant subjects :

		Gender			
		Female		Male	
		No. of cases	%	No. of cases	%
PR total score	2	6	42.9%	4	100.0%
	3	7	50.0%	0	0.0%
	4	1	7.1%	0	0.0%

$\chi 2 = 4.114$, df = 2, p = 0.128

In the malignant lesions majority of females had PR score above 3 and in males 100% of them had PR score of 0. However there was no significant difference between genders with respect to PR score.

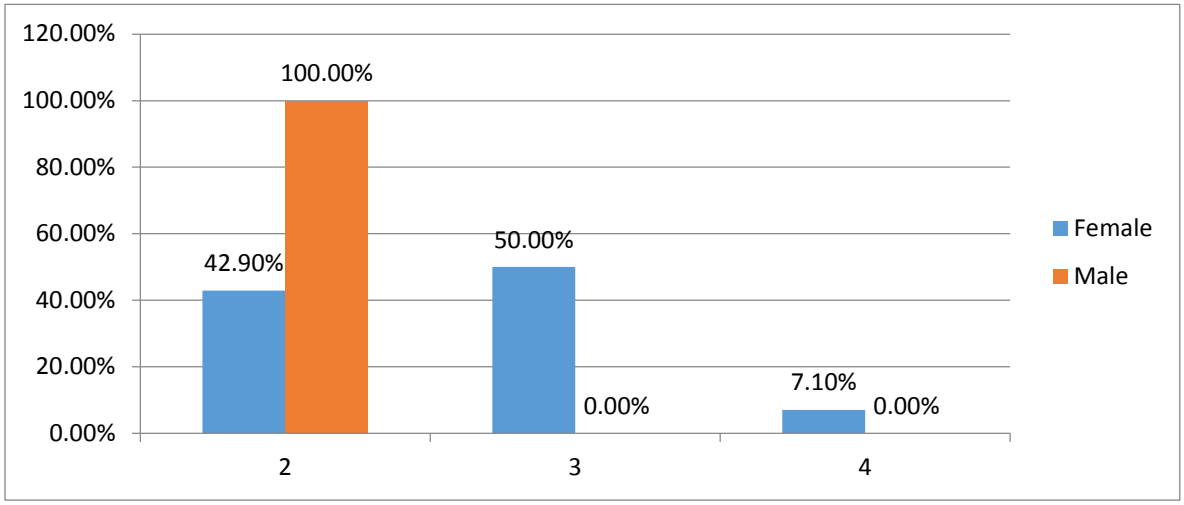


Chart 9: Bar diagram showing Comparison of PR score between genders in malignant subjects

Table 16: Total score of ER comparison between diagnoses:

		ER Total score		
		Percentile 25	Median	Percentile 75
Diagnosis	Benign	0	2	3
	Malignant	4	4	5
P value		<0.001*		

*Mann Whitney U test

Median ER score in benign lesions was 2 and in malignant lesions were 4. This difference in ER score between benign and malignant lesions was statistically significant.

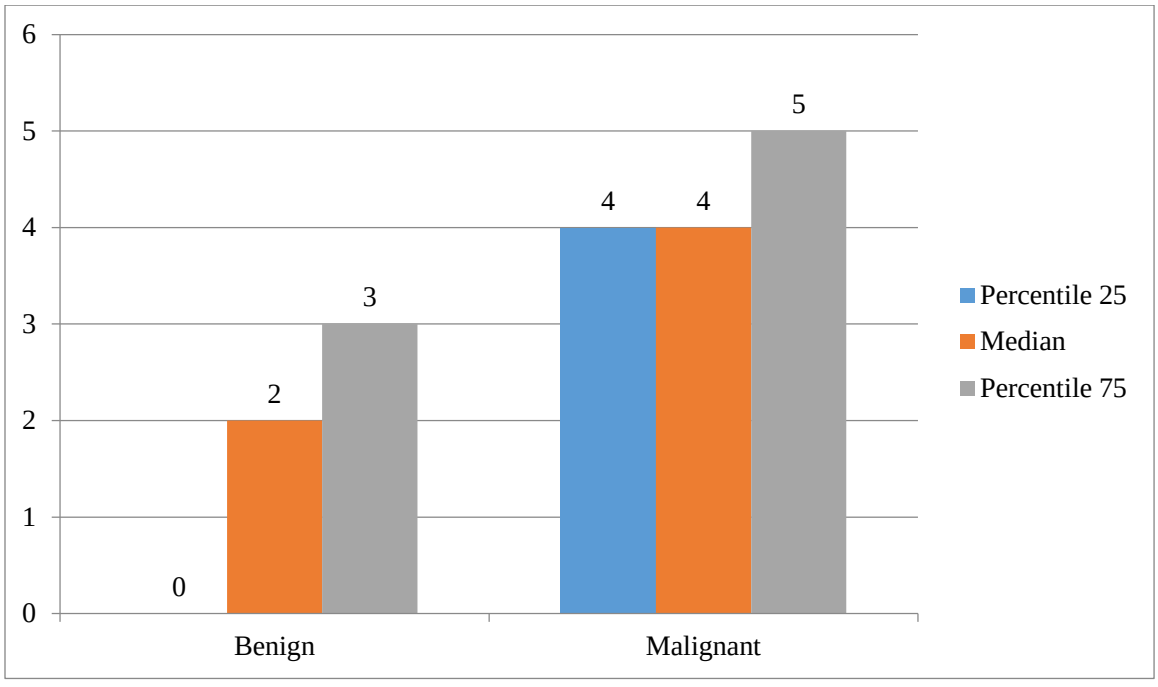


Chart 10: Bar diagram showing Total score of ER comparison between diagnoses

Table 17: Total score of PR comparison between diagnoses:

		PR Total score		
		Percentile 25	Median	Percentile 75
Diagnosis	Benign	0	0	2
	Malignant	2	2	3
P value		<0.001*		

Median PR score in benign lesions was 0 and in malignant lesions were 2. This difference in PR score between benign and malignant lesions was statistically significant.

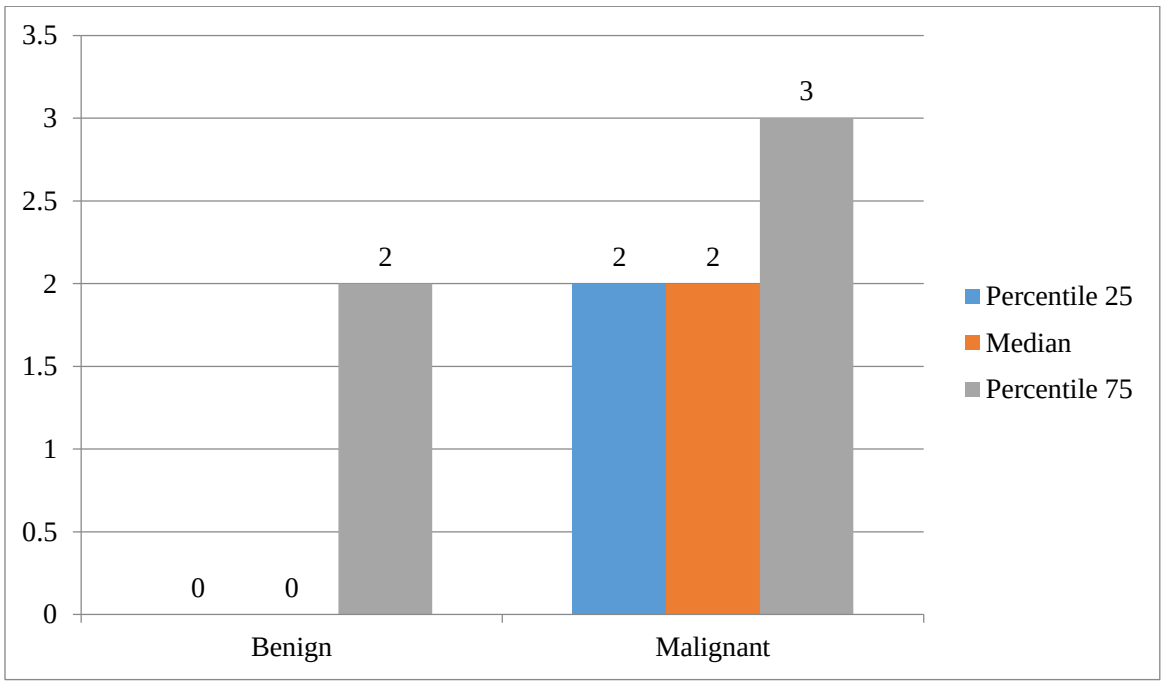


Chart 11: Bar diagram showing Total score of PR comparison between diagnoses

Table 18: Total score of ER comparison between Genders:

		ER Total Score		
		Percentile 25	Median	Percentile 75
Gender	Female	2	2	4
	Male	0	1	3
P value		0.03*		

Median ER Score among females was 2 and among males was 1. This difference in ER score between genders was statistically significant.

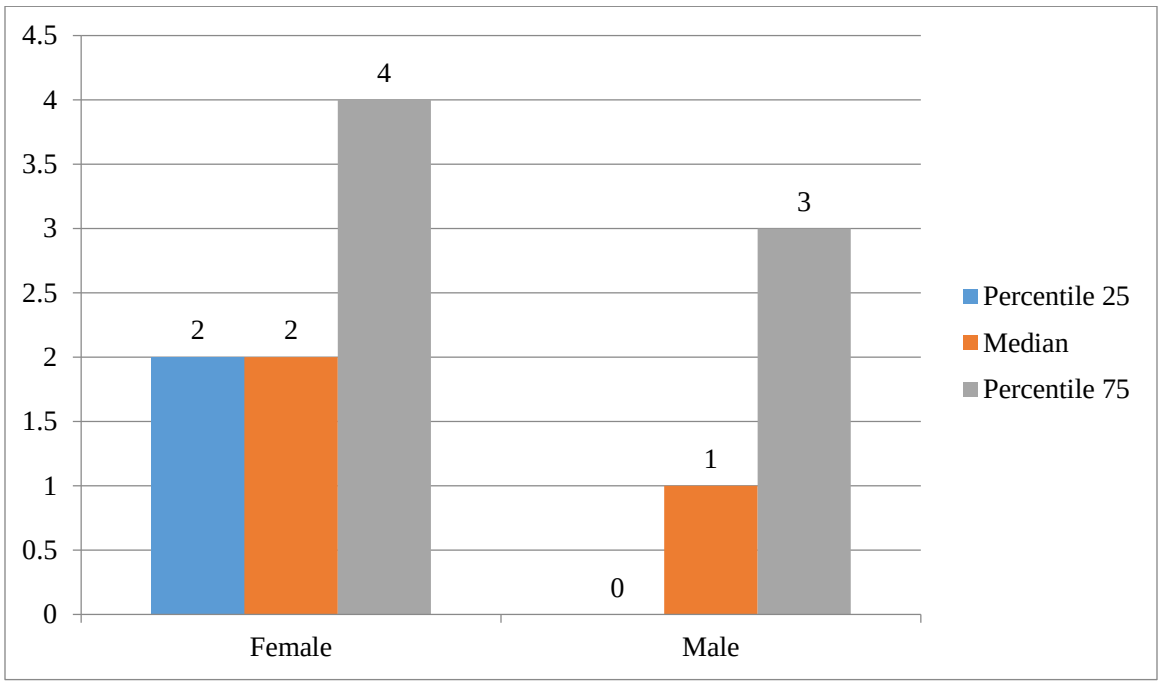


Chart 12: Bar diagram showing Total score of ER comparison between Genders

Table 19: Total score of PR comparison between Genders:

		PR Total Score		
		Percentile 25	Median	Percentile 75
Gender	Female	0	2	2
	Male	0	0	2
P value		0.068		

Median PR Score among females was 2 and among males was 0. However this difference in PR score between genders was not statistically significant.

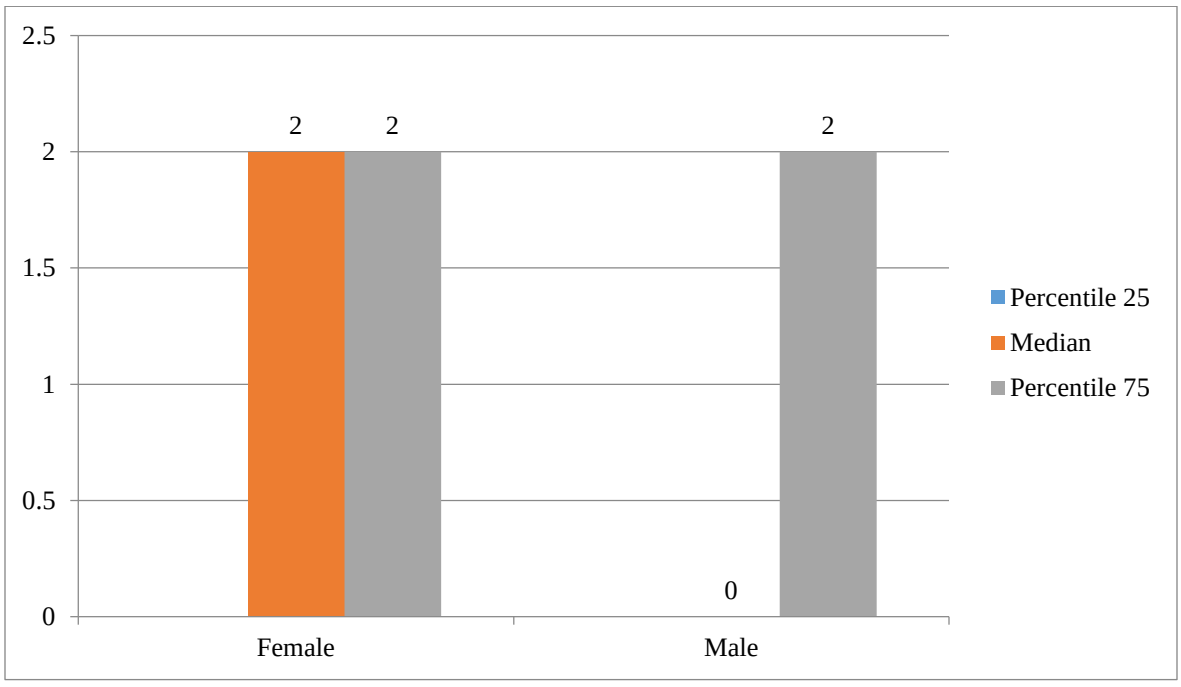


Chart 13: Bar diagram showing Total score of PR comparison between Genders

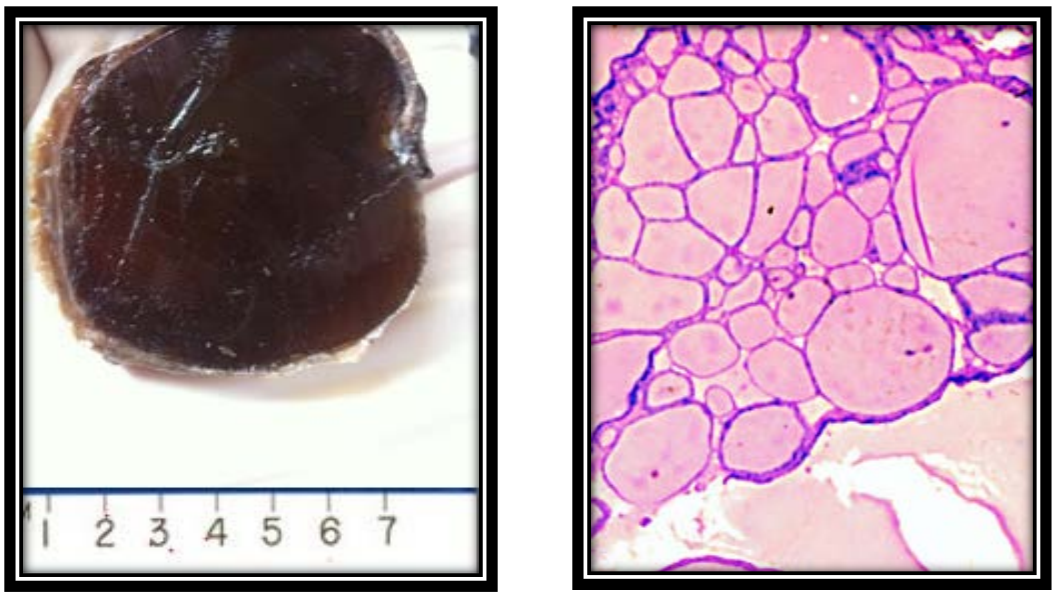


Figure 7-a) Gross image Colloid goiter showing diffuse enlargement of thyroid gland with grey brown colloid filled areas with peripheral rim of thyroid tissue. b) H & E stained tissue section of Colloid goiter showing varying sizes of thyroid follicles filled with colloid.

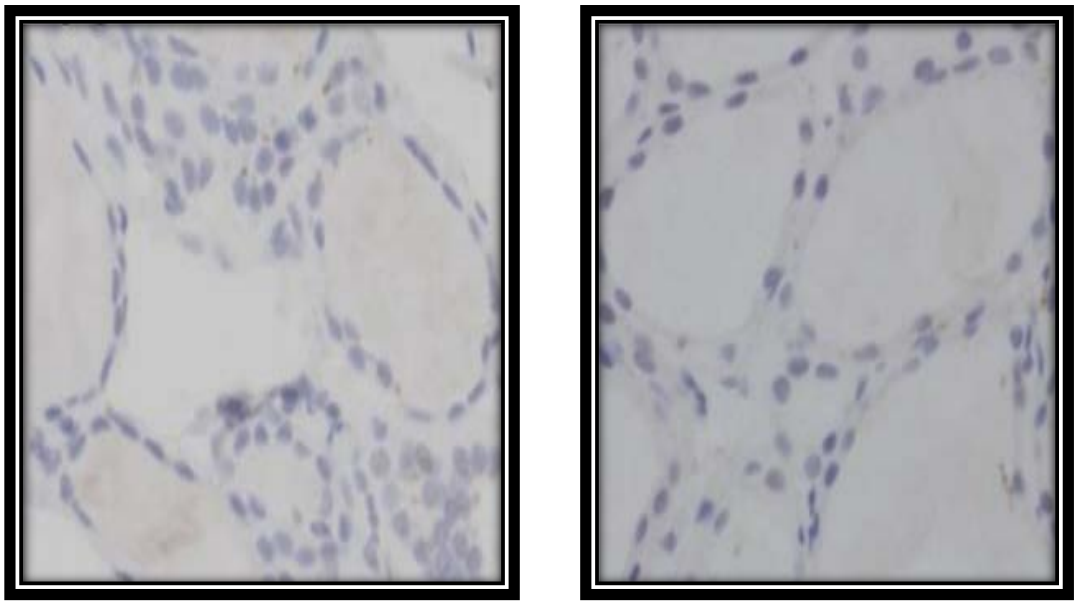


Figure 8- Immunohistochemistry a)- ER expression in colloid goitre. b)PR expression in colloid goiter.(Negative).

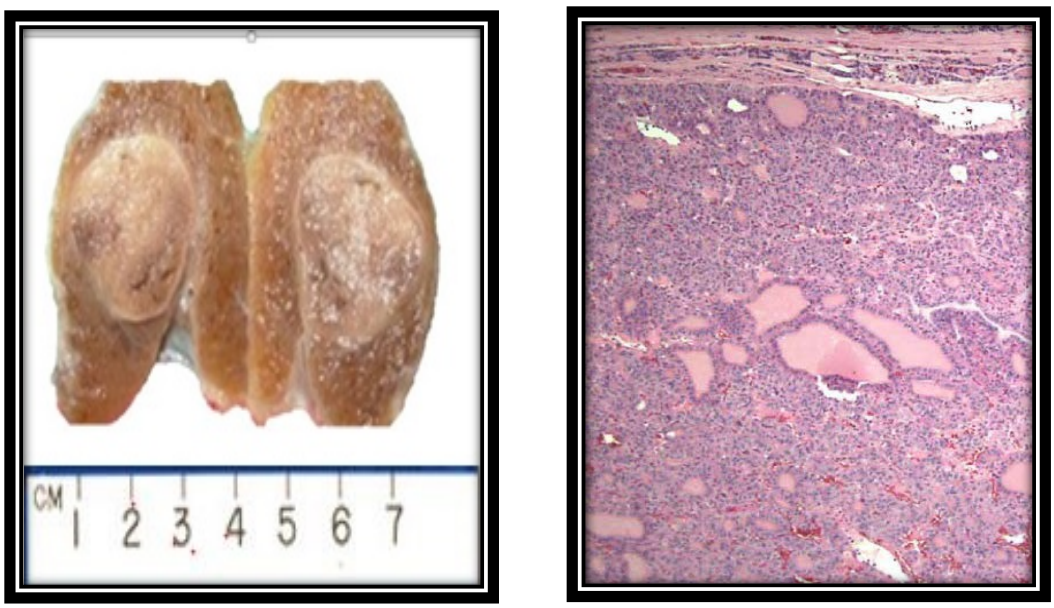


Figure 9-a) Gross image- follicular adenoma showing well circumscribed grey white nodule surrounded by grey brown colloid material. b) H & E stained tissue section of Follicular adenoma showing solid and microfollicular pattern.

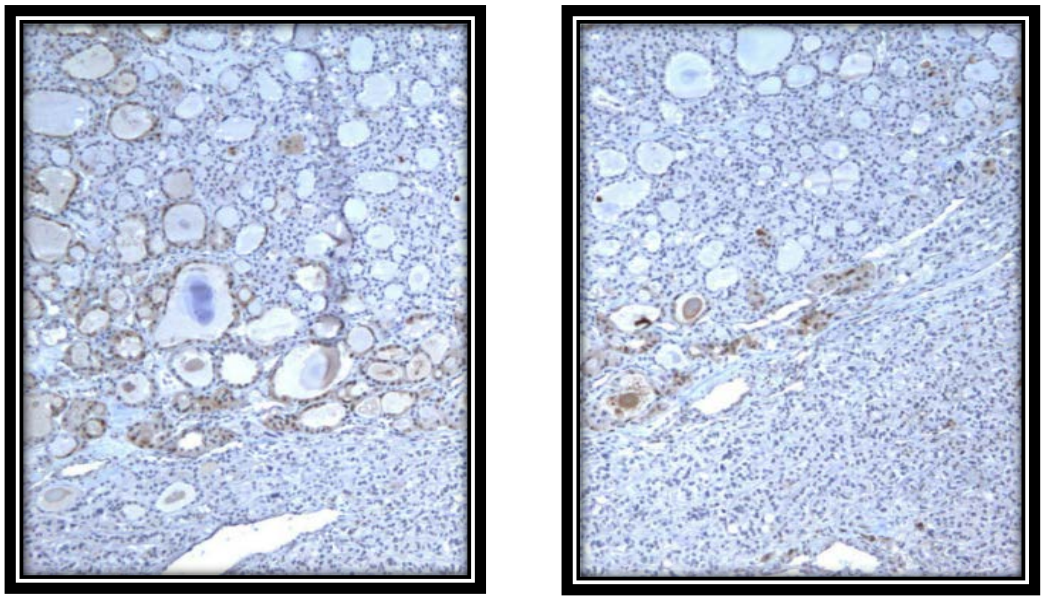


Figure 10- Immunohistochemistry a)- ER expression in Follicular adenoma . b)PR expression in Follicular adenoma showing focal positivity.

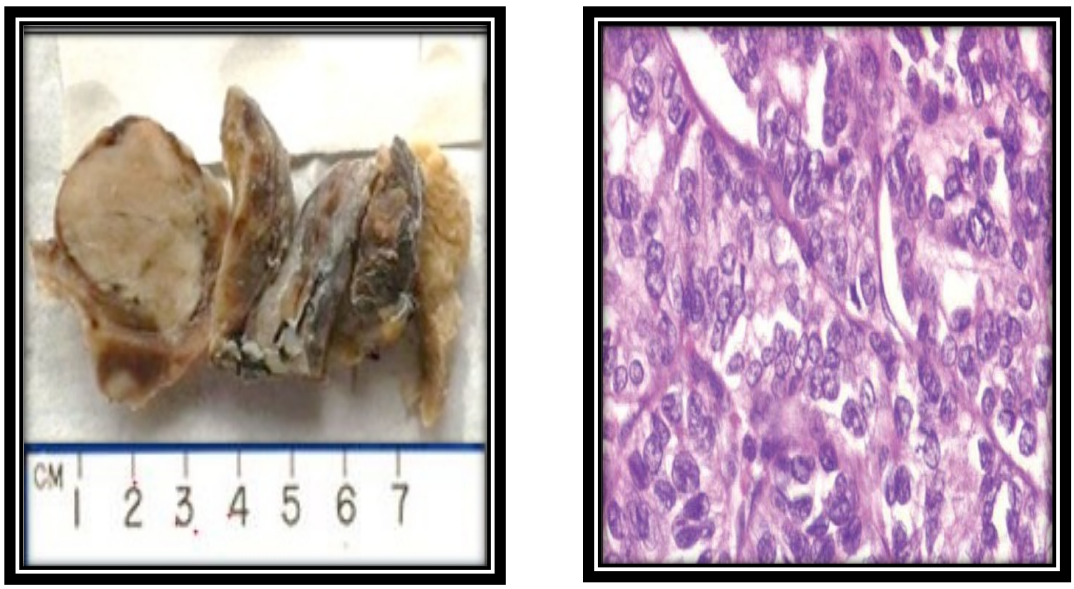


Figure 11-a)Gross image- showing papillary excrescences along with solid, firm grey white areas with brownish colloid filled areas in the periphery – Papillary carcinoma thyroid.b) H & E stained tissue section of PTC showing clearing of nucleus.

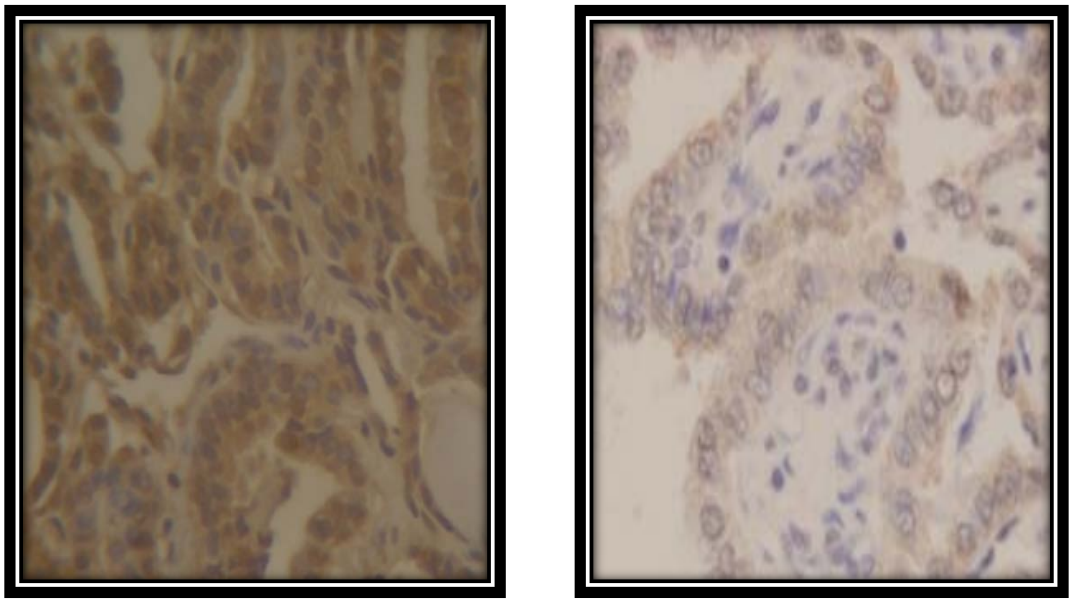


Figure 12- Immunohistochemistry a)- ER expression in PTC . b)PR expression in PTC.(Positive)

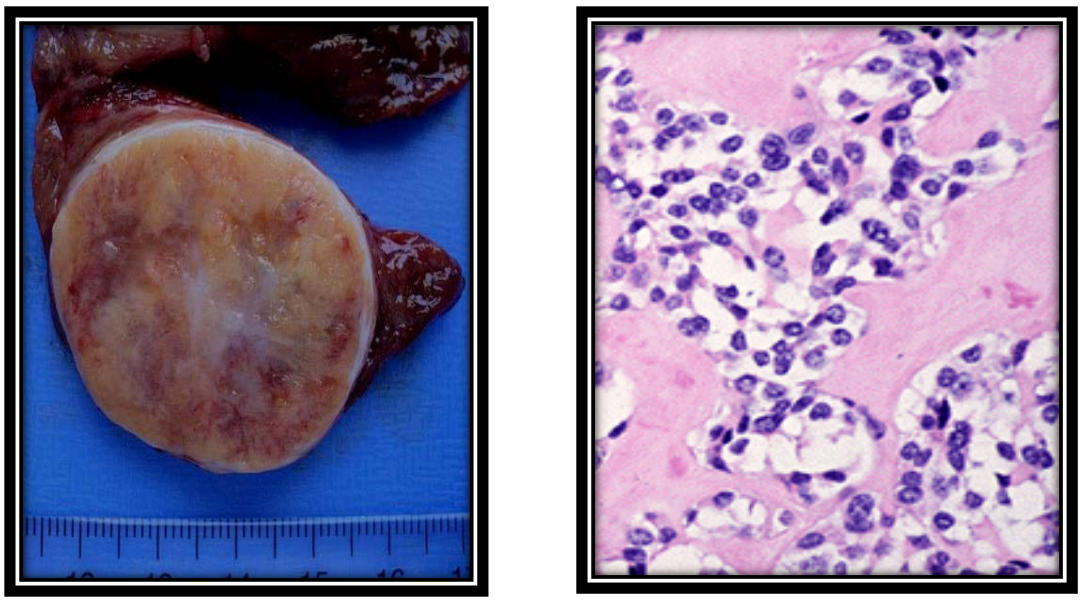


Figure 13-a) Gross image- showing a well circumscribed nodule with central stellate area-MTC. b) H & E stained tissue section of MTC showing abundant amyloid deposits.

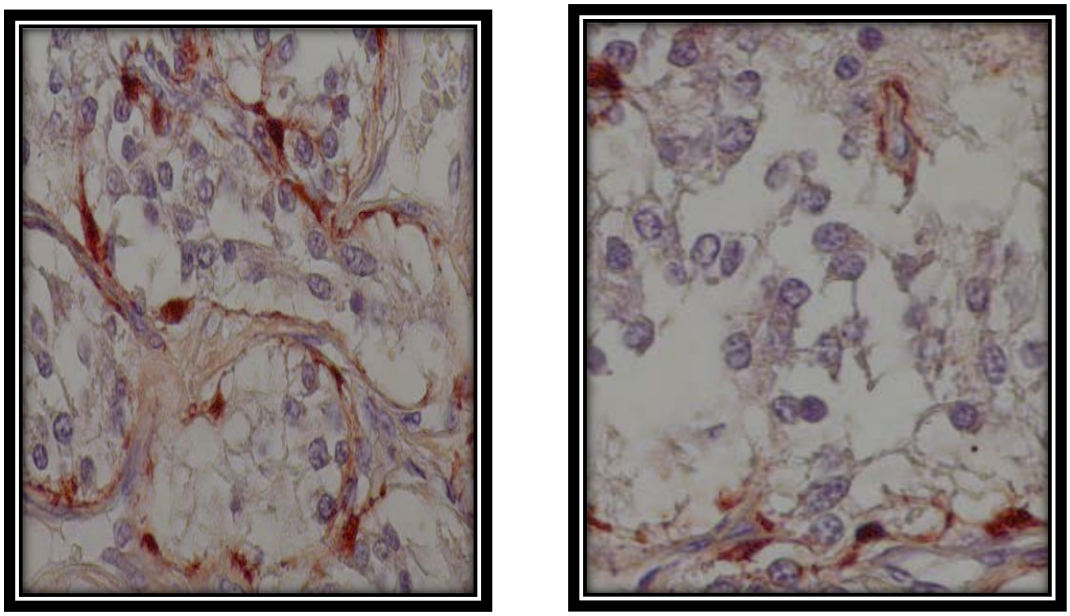


Figure 14- Immunohistochemistry a) ER expression MTC . b)PR expression in MTC(Negative with background staining).

DISCUSSION

DISCUSSION:

Thyroid disorders are common clinical problem, both benign and malignant disorders occurring in men and women in all ages and more commonly in females. The incidence of Thyroid diseases is seen to be higher in women as compared to men particularly between puberty and menopause and women are more susceptible to the goitrogenic effect of iodine deficiency. Thyroid cancer is overwhelmingly the most common type of endocrine malignancy, accounting for majority of deaths due to endocrine cancers. The majority of the patients with carcinoma of the thyroid have differentiated cancer varying in history from a pure papillary carcinoma to a follicular carcinoma and in most instances mixed papillary and follicular variants. Differentiated carcinoma of the thyroid gland is most prevalent in young adults with a female to male ratio of 2:1.

BENIGN LESIONS:

Table 20: Age distribution of Benign thyroid lesions: Comparison with other studies:

Study	**Present study**	**Bae JS et al**	**Norman J.**
Year of study	2016	2009	2016
No of benign cases	47	76	-
Mean age in years	40.2	54.24±9.79	45
Percentage of benign cases in mean age group	72.3%	76.8%	-

In the present study, Benign thyroid lesions were more common in middle age group. Mean age of subjects with benign lesions was 40.2± 11.5 years.

Bae JS et al(2009) reported in their study that majority of benign thyroid lesions were in the age group 44-64 years(76 cases).[65]

Norman J(2016) did extensive study on thyroid lesions and concluded that majority of benign thyroid lesions falls between the age group 20-70 years.[66]

In the present study, the highest incidence of benign thyroid lesions was in the age group of 28-52 years (72.3%). This finding is in concordance with other studies world-wide.

It is seen benign thyroid lesions are mostly seen in young to middle aged adults with peak age group of 20-50 years. Majority of cases have family history of goiter or clinical symptoms of hyperthyroidism or hypothyroidism. Majority of benign lesions tend to be "Warm" on thyroid scan.

Table 21: Gender distribution among Benign thyroid lesions: Comparison with other studies:

Study	**Present study**	**Bae JS et al**	**Chen D et al**
Year of study	2016	2009	2015
Number of cases	47(72.3%)	76(76.8%)	14(17.9%)
Number of males	11(23.4%)	12(15.8%)	4(28.6%)
Number of females	36(76.6%)	64(84.2%)	10(71.4%)
M:F ratio	1:33	1:5.3	1:2.5

Thyroid lesions are seen to be more common in females world-wide in the various studies conducted.

In a study done by Bae JS et al in 2009[65], 84.2% were females and rest were males out of 76 benign thyroid lesions studied. Male: Female ratio was 1:53.

Chen D et al in 2015, in their concluded Male: Female ratio as 1:2.5.[51]

In the present study, Male: Female ratio was 1:3.33 which is in concordance with other studies.

MALIGNANT LESION:

Table 22: Age distribution in Malignant thyroid tumours: Comparison with other studies

Study conducted	Present study	Bae JS et al	Zhang C et al
Year of study	2016	2009	2014
Number of malignant cases	18	23	70
Mean age group	33.8	52.73	42
Percentage of malignant cases in mean age group	27.7%	23.23%	30%

Thyroid carcinomas were more common between the age group of 25 to 41 years.

Bae JS et al, in a study published in 2009[65], concluded that majority of the thyroid carcinomas were seen in the age group of 34-72 years.

Zhang C et al in 2014, [67] studied 70 cases of differentiated thyroid carcinomas falling within age group of 22-76 years. They further classified them into two groups, one below

45 years which included 40 patients and other group more than or equal to 45 years which had 30 cases.

In the present study, the highest incidence of malignant thyroid tumors was in the age group of 25-41 years (27.7%). This finding is in concordance with other studies world-wide.

Table 23: Gender distribution among Malignant thyroid lesions: Comparison with other studies

Study	**Present study**	**Zhang C et al**	**Chen D et al**
Year of study	2016	2014	2015
Number of cases	18(72.3%)	70	14(17.9%)
Number of males	4(22.2%)	21(30%)	4(28.6%)
Number of females	14(77.8%)	49(70%)	10(71.4%)
M:F ratio	1:35	1:2.3	1:2.5

In a study done by Zhang C et al in 2014[67] 70% were females and rest males out of 70 thyroid cancers studied. Male: Female ratio was 1:23.

Chen D et al in 2015, [51] had concluded Male: Female ratio as 1:2.5.

In the present study, Male: Female ratio was 1:3.35 which is in concordance with other studies.

This difference in Gender proportion of Thyroid lesions could be due to Estrogen effect on thyroid differentiated and thyroid stem cells leading to various pathogenesis for different thyroid lesions. This can be better explained by the extensive research done by Xu S et al(2013)[68], in their research article they hypothesized that oestrogen has a supportive role in the propagation of thyroid stem/progenitor cells which may lead to the selection of a progeny of growth-prone cells with a decreased function. These cells may be the origin of hypo-functioning or non-functioning thyroid nodules in females.

Table 24: Comparison with size of lesions

Study	**Present study(2016)**	**Norman J(2016)**
Mean size of benign lesions	2.5x2 to 14x10cms	Larger
Mean size of malignant lesion	2x1 to 8x7cms	Smaller

In the present study it was seen that in benign thyroid lesions size ranged from 2.5x2 to 14x10cms and in malignant lesions the size ranged from 2x1 to 8x7 cm concluding that larger the size favoured benign lesions and smaller nodules were malignant.

Norman J in 2016[67] has done extensive research on thyroid tissue and concluded that large thyroid mass with other parameters favours possibility of the lesion being benign.

This in concordance with the worldwide literature that larger the thyroid tissue/nodule goes in favour of benign nature of the lesion.

This may be explained by the fact that malignant lesions grow faster and can have sudden increase in size, pressure effects, and associated lymphadenopathy compelling the patient to seek medical attention.

IMMUNOHISTOCHEMISTRY STATUS:

Table 25: ER score in benign and malignant thyroid lesions: Comparison with other studies:

Study	Present study	Zhang C et al	Chen D et al	Nadoushan JMR et al
Year of study	2016	2014	2015	2016
No of benign cases positive	35(66%)	Normal surrounding thyroid tissue	2(5%)	Normal surrounding thyroid tissue
No of malignant cases positive	18(34%)	15(21.4%)	38(95%)	43(46.7%)

In the present study, though total number of Benign cases showing ER positivity was higher (35/66%), they had a lower total score(Average of Total score=2), while total number of malignant cases showing ER positivity (18/34%) showed higher score (Average Total score=4) and this difference in total ER score between benign and malignant lesions was statistically significant (<0.001).

Chen D et al in the year 2015[51], did ER analysis on PTC and NTG tissue sections and had considered the score of 3 or more as positive. They concluded that, 38 cases (95%) of PTC showed strong immunopositivity for ER.

Nadoushan JMR, et al in 2016[69] did a study on only Papillary thyroid carcinomas concluded that the rate of ER and PR expression were 46.75% and 5.6%, respectively. Similar study done by Zhang C et al in 2014 [67], concluded that positive rates of ER and PR in tumour tissues of DTC patients were 21.4% (15/70) and 31.4 %(22/70), respectively, and no expression of ER or PR were found in normal thyroid tissue ($P < 0.01$). Both these studies were statistically significant ($p<0.05$).

Increasing number of studies are done to see for Estrogen's effect on tumorigenesis in human thyroid cells by ER-dependent or ER-independent mechanisms, by modulating cell proliferation and regulating the function of the thyroid. The evidence for these effects on thyroid function and growth regulation was reviewed by Santin and Furlanetto. [2]

The present study revealed that the expression of ER is increased in malignant lesions, compared with the expression in benign lesions which is in concordance with the other studies done world-wide supporting the role of Estrogen in the thyroid gland growth and proliferation and thus playing a role in pathogenesis of thyroid cancers.

Table 26: PR score in Benign and Malignant thyroid lesions: Comparison with other studies:

Study	**Present study**	**Memon GR et al**	**Chen D et al**
Year of study	2016	2000	2015
No of benign cases positive	18(50%)	0(0%)	1(3.3%)
No of malignant cases positive	18(50%)	0(0%)	29((96.7%)

In present study, total number of benign cases showing PR positivity was equal to total number of malignant cases (18) showing PR positivity but majority of benign lesions showed Average Total score of 0(29) and majority of malignant lesions (10) showed an Average Total score of 2 and this difference in total PR score between benign and malignant lesions was statistically significant (<0.001).

Chen D et al, in the year 2015[51], did PR analysis on PTC and NTG tissue sections and had considered the score of 3 or more as positive and concluded that 29 cases (45.3%) of PTC showed strong immunopositivity for PR.

Another study done by Memon GR et al in the year 2000[7], despite the consistently positive staining in control slides, in different age groups in both sexes, no positive

staining for PR was observed in the selected sample including surrounding normal tissues, concluding that the effect of progesterone on thyroid gland may be an indirect one.

It is a accepted fact that for a steroid hormone to have a direct effect on target organs, presence of specific, high affinity intracellular receptor is an important prerequisite. This concept is further supported by the findings that abundant amount of PR is present in target tissues. At least in breast tumour, PR status affects clinical behaviour, prognosis and response to hormonal therapy. In thyroid this relationship is illustrated by the presence of thyroid stimulating hormone receptors and their therapeutic importance in thyroid neoplasms.

PR status in diseased and surrounding normal thyroid tissue demonstration is needed because their presence is essential to implicate progestinic effect in biological evolution and therapy of thyroid lesions specially the differentiated Thyroid cancers similar to that of ERs expression in thyroid lesions.

In the present study, only few of the thyroid lesions showed PR positivity but, was not statistically significant concluding that, PR may have an indirect effect on thyroid lesions.

Table 27: ER score comparison between Gender: Comparison with other studies:

Study	**Present study**	**Magri F et al**	**Chen D et al**
Year of study	2016	2015	2015
No. of ER positive cases in females	44(86.3%)	78(85.7%)	21(55.3%)
No. of ER positive cases in males	7(13.7%)	13(14.3%)	17(44.7%)

In the present study, more number of females showed ER positivity and had higher score of ER as compared to males, majority of males showing ER total score of 0(46.7%).

Magri F et al in the year 2015[70] showed significant difference in the expression of ER between females and males i.e. 85.7% and 14.3% positivity respectively.

On the other hand, Chen D et al in 2015[51], did not observe much difference in the expression of ER between Males and Females.

The gender(female) predominance in the present study is in concordance with the findings of Magri et al.

Benign and malignant thyroid nodules are more prevalent in females than in males. Experimental data suggest that the proliferative effect of oestrogen responsible for this gender difference. Xu et al 2013[68], analysed both differentiated thyroid cells and thyroid stem and progenitor cells and concluded that they are targets of oestrogen action. Thyroid stem cells and progenitor cells expressed ERα (ESR1) and ERβ (ESR2) with eight times higher expression levels as compared with the differentiated thyrocytes. These cells may be the origin of hypofunctioning or non-functioning thyroid nodules in females.

Table 28: PR score comparison between Gender: Comparison with other studies

Study	**Present study**	**Memon GR et al**	**Chen D et al**
No of PR positive cases in females	30	0(n=40)	14
No of PR positive cases in males	6	0(n=10)	15

In the present study, more number of females showed PR positivity and had higher score of PR (Average Total score=2) as compared to males, majority of males showing PR total score of 0(46.7%).

Chen D et al in the year 2015[51] had not observed much difference in the expression of PR between Males and Females.

Memon GR et al in 2000[7] did a study on 50 thyroid lesions and found out that all the lesions were not positive for PRs.

These results were not in concordance with some of the other studies done worldwide.

Estrogen and progesterone indirectly affect the thyroid by influencing the pituitary TSH secretion via a mechanism of feedback inhibition.

Results of the current study showed that ER and PR expression could be detected in tumour tissue of thyroid cancers, ER having higher total score in malignant lesions as compared to benign and also higher in females as compared to males and was statistically significant with the p value of <0.05. On other hand, total score of PR expression was higher in malignant lesions and higher in females than males but was not statistically significant ($p > 0.05$).

As the total number of cases studied in this study are less the results were statistically not significant, but a in a larger study group it might turn out to be statistically significant as females though a lower average total score (2) still had higher score when compared with that of males (Average Total score=0).

Similarly studies by Clark et al (1985)[71] and Hampl et al (1985)[72,] found ER in both malignant and benign thyroid tumours, as well as in non-neoplastic thyroid tissue. Receptor content was higher in malignant tumours as compared with benign lesions.

A recent study by Rajoria et al in 2010[73], demonstrated an increase in adhesion, migration and invasiveness of the thyroid cell line Nthy-ori 3-1 when treated with E2. E2 also down regulated expression of β-catenin, a key protein involved in cell adhesion that the authors suggest may have a role in the adhesion and migration properties of thyrocytes.

Banu et al in 2002 found that sex steroids stimulated thyrocyte proliferation in a gender-specific manner. E2 had a stimulatory effect in thyrocyte proliferation in females but inhibited proliferation in males. While testosterone stimulated proliferation in both males and immature females, it impaired proliferation in adult females.[74]

The present study, laboratory observational study is a pilot study done to evaluate the expression of ER and PR in benign and malignant thyroid lesions and between males and females. However, more studies preferably multi-institutional and with larger samople size have to be done to validate the usefulness of ER and PR as a prognostic marker and also to implement ER and PR expression as a site for targeted therapy.

CONCLUSION

CONCLUSION:

The results of this study showed that expression of ERs is higher in malignant thyroid lesions and also in females as compared to males. There was a significant positive correlation between total score of ERs expression in females and malignant thyroid tumors as compared to Males and Benign thyroid lesions respectively. Whereas, PRs whose expression was not statistically significant.

When compared with histological type of malignant lesion it was seen there were more cases of PTC and expression of ER was statistically significant. Larger thyroid masses were mostly benign whereas smaller nodules were malignant. Hence, it can be concluded that ER plays a significant role in the pathogenesis of thyroid lesions and this role is more in females compared to males.

FURTHER SCOPE OF THE STUDY:

ER and PR expression is varied in thyroid lesions and their expression is relatively a new finding. More studies have to be done to evaluate the usefulness of ER and PR as a prognostic marker in thyroid cancers. Identification of their expression in a significant proportion could possibly have important therapeutic implications and in evaluating the role of site specific therapies in thyroid cancers, thereby modifying and being adjuvant to the treatment protocols which includes extensive thyroid surgeries and reducing the morbidity and mortality associated with invasive methods of treatment.

These Targeted therapies may give better disease free (recurrence free) survival and overall survival in future.

SUMMARY

SUMMARY:

A laboratory observational and descriptive study, to evaluate immunohistochemically expression of ERs and PRs in different thyroid lesions and comparison of their expression in males and females during the time period January 2015 to September 2016. Retrospectively few cases were also included from the archives of department of pathology from the year 2014. The following are the salient features noted:

1. A total of 65 cases were selected, out of which 47(72.3%) were benign and 18(27.7%) were malignant.
2. Male: Female ratio was 1:3.33.
3. Mean age of patients with benign lesions was 40.2 years and that with malignant lesions was 33.8 years.
4. Most common malignancy was Papillary thyroid carcinoma.
5. ER positivity was seen 78.5% cases out of which 86.3% being females and 13.7% males.
6. PR positivity was seen 55.4% cases out of which 83.3% being females and 16.7% males.
7. Statistically positive co-relation was seen between total scores of ER between Benign and Malignant thyroid lesions, higher Total score was seen in malignant cases (mean=4) as compared to Benign lesions(mean score=2) and higher Total scores of ER in females (mean=2)as compared to males(mean score=1).
8. Statistically, there was no correlation with the score of PR expression among the lesions and the different Genders.

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ANNEXURES

ANNEXURE-I

INFORMED CONSENT FORM

TITLE- A STUDY OF ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION IN BENIGN AND MALIGNANT THYROID LESIONS BY IMMUNOHISTOCHEMISTRY

I understand that I am free to withdraw from the study at anytime. I have read or it has been read to me and I understand the purpose of the study, the risk and benefits associated. I have had the opportunity to ask questions regarding various aspects of the study and my questions were answered to my satisfaction. I the undersigned agree to participate in this study and authorize for further testing on the surgical specimen and disclosure of my personal information for dissertation.

Subject name and signature/ Thumb impression DATE:

Parents / Guardians name / Thumb impression DATE:

Signature of the person taking consent DATE:

ANNEXURE II

Proforma

NAME: CASE NO:

AGE:

SEX: HOSPITAL NO:

PRESENTING COMPLAINT: CYTOLOGY NO:

CLINICAL DIAGNOSIS: BIOPSY NO:

USG FINDING:

THYROID FUNCTION TEST: T3: T4: TSH:

CYTOLOGICAL DIAGNOSIS:

HISTOPATHOLOGICAL FINDINGS-

MACROSCOPIC- SPECIMEN DIMENSIONS- RIGHT LOBE-

LEFT LOBE-

ISTHMUS-

TUMOR -

THYROID CAPSULE-

OTHERS-

MICROSCOPIC- TUMOR TYPE- ADENOMA

FOLLICULAR CARCINOMA

PAPPILARY CARCINOMA

MEDULLARY CARCINOMA

ANAPLASTIC CARCINOMA

OTHERS

EXTENSION INTO ADJACENT TISSUES:

LYMPH NODE STATUS:

TUMOUR STAGE:

IMMUNOHISOCHEMICAL STAINS-

ESTROGEN RECEPTOR(ER) STATUS:

PROGESTERONE RECEPTOR(PR) STATUS:

INTERPRETATION:

FINAL IMPRESSION:

ANNEXURE III

KEYS TO MASTER CHART

B	BIOPSY NUMBER
AGE	AGE IN YEARS
F	FEMALE
M	MALE
B	BENIGN
MA	MALIGNANT
STN	SOLITARY NODULE THYROID
NG	NODULAR GOITRE
NT	NODULAR HYPERPLASIA
MNG	MULTINODULAR GOITRE
HT	HASHIMOTOS THYROIDITIS
CN	COLLOID NODULE

FN	FOLLICULAR NEOPLASM
FA	FOLLICULAR ADENOMA
MFA	MIXED FOLLICULAR ADENOMA
CA	CARCINOMA
NOS	NOT OTHERWISE SPECIFIED
PCT	PAPILLARY CARCINOMA THYROID
FC	FOLLICULAR CARCINOMA
MCT	MEDULLARY CARCINOMA THYROID
TT	TOTAL THYROIDECTOMY
HT	HEMITHYROIDECTOMY
IHC	IMMUNOHISTOCHEMISTRY
PS	PROPORTION SCORE
IS	INTENSITY SCORE
TS	TOTAL SCORE

Case No	Biopsy no	Size of the lesion (cm)	Age	Sex	Nature of specimen Received	Clinical diagnosis	histopathological diagnosis	benign or malignant	Immunohistochemistry(IHC)					
									ER			PR		
									PS	IS	TS	PS	IS	TS
1	B-1794-14	3.5X2.5X2.5	22	F	H	STN	PTC	MA	3	1	4	1	1	2
2	B-1958-14	3.5X3X2	36	M	T	MNG	NH	B	0	0	0	0	0	0
3	B-2032-14	4X3X3	19	F	T	?CA	PTC	MA	3	2	5	1	1	2
4	B-2046-14	4X2X2	55	F	H	MNG	HT	B	1	1	2	0	0	0
5	B-2308-14	3X2X0.8	50	F	T	PTC	PTC	MA	4	2	6	2	1	3
6	B-2582-14	1.7X1.3X0.8	55	F	H	MTC	NH	B	1	1	2	0	0	0
7	B-2613-14	5X4X3.5	44	M	T	MNG	NH	B	0	0	0	0	0	0
8	B-2715-14	5X4X1.5	28	F	H	STN	FA	B	2	1	3	1	1	2
9	B-2742-14	4.5X4X3.3	52	F	H	MNG	MNG	B	1	1	2	0	0	0
10	B-2836-14	5X3X1.8	32	F	T	MNG	HT	B	1	1	2	0	0	0
11	B-2839-13	3X2.5X1	34	M	T	PTC	PTC	MA	2	1	3	1	1	2
12	B-2885-14	4X3X2	45	F	T	FN	HT	B	1	1	2	0	0	0
13	B-3076-14	5X4X3	40	F	T	HT	HT	B	1	1	2	0	0	0
14	B-3221-14	5x3x1.4	28	F	H	MNG	NG	B	0	0	0	0	0	0
15	B-11-15	3X2X1	27	F	T	?CA	PTC	MA	4	2	6	2	2	4
16	B-149-15	2X2X1	32	F	T	PTC	PTC	MA	3	2	5	1	2	3
17	B-232-15	3.2X1X1	33	M	H	NG	PTC	MA	2	2	4	1	1	2
18	B-275-15	2.3X1.6X1	30	F	T	MNG	NH	B	1	1	2	1	1	2
19	B-603-15	1.8X1.5X1	29	F	T	PTC	NOS	MA	2	1	3	1	1	2
20	B-624-15	3.2X3X2	38	F	T	MNG	HT PMC	MA	3	1	4	1	1	2
21	B-666-15	4X3.5X2.5	50	F	T	MNG	HT	B	1	1	2	1	1	2
22	B-746-15	3X2.2X2	45	F	H	HT	HT	B	1	1	2	1	1	2
23	B-841-15	3.5X2X1.5	22	M	H	STN	MFA	B	2	1	3	1	1	2
24	B-845-15	4X2X1	40	F	H	MNG	NH	B	1	1	2	1	1	2
25	B-959-15	3x2x1.8	24	F	H	CG	NH	B	1	1	2	0	0	0
26	B-963-15	3X1.5X1.2	40	M	H	STN	NH	B	1	1	2	0	0	0
27	B-1171-15	4.5X2X1	32	F	H	CN	PTC	MA	3	1	4	2	1	3
28	B-1187-15	5X4.5X2.5	45	M	H	MNG	NH	B	0	0	0	0	0	0
29	B-1243-15	4.5X2.8X2.5	70	F	T	?CA	MFA	B	3	2	5	1	1	2
30	B-1314-15	4.5X4X3.4	17	F	T	MNG	HT	B	1	1	2	0	0	0
31	B-1478-15	2X2X1	45	F	T	?CA	MNG	B	0	0	0	0	0	0
32	B-1579-15	4X3X0.8	35	F	T	MTC	HT	B	2	1	3	1	1	2
33	B-1599-15	4X3.5X2	25	M	T	MNG	NH	B	0	0	0	0	0	0
34	B-1655-15	2X1.5X1.2	43	F	H	FN	CN	B	0	0	0	0	0	0
35	B-1758-15	2X1X1	35	F	H	CG	NH	B	1	1	2	0	0	0
36	B-1837-15	5.2X3X1.5	45	M	T	MNG	NH	B	0	0	0	0	0	0
37	B-1873-15	5.5X4.5X3	37	F	H	CG	PTC	MA	4	2	6	2	1	3
38	B-1994-15	1.8x1.7.5x0.7	30	F	H	?CA	NH PMC	MA	3	1	4	2	1	3
39	B-2305-15	1.1X0.9X0.5	29	F	H	STN	NH	B	1	1	2	0	0	0
40	B-2333-15	6X6X3	50	F	T	?CA	NH HT	B	2	1	3	1	1	2
41	B-2357-15	5X5X4	40	M	T	?CA	HT PMC	MA	2	2	4	1	1	2
42	B-2381-15	3X3X3	32	F	H	STN	NH HT	B	2	1	3	1	1	2
43	B-2506-15	4X2X1	60	F	H	CG	NH	B	1	1	2	0	0	0
44	B-2561-15	3x1.5x1	25	F	H	STN	FA	B	3	2	5	2	1	3
45	B-2613-15	5X4X3	30	F	H	STN	PTC	MA	4	2	5	1	1	2
46	B-2694-15	4X3X3	30	F	H	PTC	PTC	MA	3	2	5	2	1	3
47	B-2817-15	4X3X2.3	62	M	H	CG	NG	B	0	0	0	0	0	0
48	B-2871-15	5.6X2.5X2	55	F	H	MNG	NH	B	1	1	2	0	0	0

49	B-2878-15	4X3.3X2.5	37	F	T	FC	NH	B	1	1	2	0	0	0
50	B-3196-15	4.8X3.6X2.3	45	F	T	MNG	HT	B	2	1	3	1	1	2
51	B-3276-15	2.5X1.5X1.2	45	M	H	MNG	MNG	B	0	0	0	0	0	0
52	B-07-16	4X2.5X2	30	F	H	STN	NG HT	B	2	1	3	1	1	2
53	B-215-16	1.0X1.0X0.5	48	F	H	CG	NH	B	1	1	2	0	0	0
54	B-364-16	3X2.5X2	53	M	T	MNG	NH	B	1	1	1	0	0	0
55	B-425-16	2.6X2.1X1	40	F	T	MNG	PTC	MA	4	2	6	2	1	3
56	B-661-16	1.2X0.5X0.5	32	F	H	?CA	NH	B	1	1	2	1	1	2
57	B-677-16	1.0X0.8X0.4	46	M	H	CG	PMC	MA	2	1	3	1	1	2
58	B-679-16	8X5X4	40	F	T	MNG	NG	B	0	0	0	0	0	0
59	B-796-16	5.5X4X3	33	F	T	?CA	NH	B	2	1	3	1	1	2
60	B-1008-16	4.5X4X2	30	F	H	MNG	NG	B	0	0	0	0	0	0
61	B-1023-16	3.5X3X2	28	F	T	MNG	NG	B	0	0	0	0	0	0
62	B-1176-16	3X2X2	48	F	T	MNG	HT NH	B	2	1	3	2	1	3
63	B-1341-16	2X2X2	35	F	T	FN	HCA	B	3	2	5	2	1	3
64	B-1413-16	3X4X1	45	M	H	STN	MFA	B	2	1	3	1	1	2
65	B-1957-16	8X5X4	40	F	T	MTC	MTC	MA	1	1	2	1	1	2