CYTOLOGICAL EVALUATION OF THYROID LESIONS BY NUCLEAR MORPHOLOGY AND NUCLEAR MORPHOMETRY

BY

DR.R YASHASWINI, MBBS

Dissertation submitted to the

Sri Devaraj Urs Academy of Higher Education and Research,

Tamaka, Kolar, Karnataka

DOCTOR OF MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF

DR. T N SURESH
PROFESSOR

DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"CYTOLOGICAL EVALUATION OF THYROID LESIONS BY NUCLEAR MORPHOLOGY AND NUCLEAR MORPHOMETRY**" is a bonafide and genuine research work carried out by me under the direct guidance of **Dr.T N Suresh**, Professor, Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

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MEMBER SECRETARY **PRINCIPAL**

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DATE: **SIGNATURE OF THE CANDIDATE**

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LIST OF ABBREVIATIONS

SL NO	ABBREVIATION	EXPANSION
1	FNAC	FINE NEEDLE ASPIRATION CYTOLOGY
2.	H&E	HEMATOXYLIN & EOSIN
3.	PAP	PAPANICOLAOU STAIN
4.	MGG	MAY GRUNWALD AND GIEMSA STAIN
5.	DPX	DISTYRENE PLASTICIZER XYLENE
6.	HPE	HISTOPATHOLOGICAL DIAGNOSIS
7.	BSRTC	BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY
8.	NG	NODULAR GOITRE
9.	CG	COLLOID GOITRE
10.	HG	HYPERPLASTIC GOITER
11.	FN	FOLLICULAR NEOPLASM
12.	HTA	HYALINIZING TRABECULAR ADENOMA
13.	PTC	PAPILLARY THYROID CARCINOMA
14.	INCI	INTRA NUCLEAR CYTOPLASMIC INCLUSION

LIST OF ABBREVIATIONS

SL NO	ABBREVIATION	EXPANSION
15.	MMND	MEAN MINIMAL NUCLEAR DIAMETER
16.	MMND	MEAN MAXIMUM NUCLEAR DIAMETER
17.	MNP	MEAN NUCLEAR PERIMETER
18.	MNA	MEAN NUCLEAR AREA
19.	MAR	MEAN AXIS RATIO
20.	MNC	MEAN NUCLEAR COMPACTNESS
21.	MSHF	MEAN SHAPE FACTOR
22.	MNS	MEAN NUCLEAR SIZE

ABSTRACT

INTRODUCTION

Fine needle aspiration (FNA) of the thyroid gland has proven to be an important cost-effective method for diagnosing patients with thyroid nodules. Thyroid cytopathology practice requires communication and collaboration among pathologists and clinicians. Therefore consistent diagnostic terminology is important and hence the new Bethesda System for Reporting Thyroid Cytopathology was developed which classifies thyroid lesions into 6 categories and gives implied risk for malignancy and management protocol in each category. Though the system gives specific criteria for each category, diagnostic challenges arise.

Using nuclear morphometry, we can quantify number of parameters such as those related to nuclear size and shape. The evaluation of nuclear morphometry is not well established in thyroid cytology

OBJECTIVE OF THE STUDY

1. To classify thyroid lesions on FNAC using Bethesda system
2. To assess nuclear features in thyroid FNAC using morphometry
3. To evaluate the significance of nuclear parameters in improving the prediction of thyroid malignancy

MATERIAL AND METHODS

The present study was undertaken at department of pathology, RL Jalappa Hospital and RC, Kolar.120 FNAC cases of thyroid lesions with proved histological diagnosis were subjected to the study. All cases were stained with routine stains H&E, PAP and Giemsa and looked into cytomorphological features and then categorized under Bethesda system.

Computerised Nuclear morphometry was done on 81 cases which had confirmed cytohistological correlation, using Olympus BX-41 research microscope with aperio computer software. 100 nuclei from each case were outlined and values were then converted to micrometer measurement. The system automatically displayed four parameters – Area, Perimeter, Minimum Nuclear Diameter and Maximum Nuclear Diameter. These parameters were saved in the excel sheet and later were used to calculate the other four parameters – Axis ratio, Compactness, Shape factor and Nuclear size.

RESULTS

In the present study, thyroid lesions were common in female with M:F ratio of 1:5 and most commonly involved age group of 40-60 yrs. Under Bethesda system, 73(60.83%) were category 2.14(11.6%) were category 3, 3(2.5%) were category 4, 8(6.6%) were category 5 and 22(18.3%) were category 6- malignant. The implied malignant potential in each category were within range described by Bethesda system. On applying nuclear morphometry, the parameters related to size – Minimal Nuclear Diameter , Maximal Nuclear Diameter , Nuclear Perimeter and Nuclear Area was higher in malignant group compared to non- neoplastic and benign group. A statistically significant correlation was obtained between the morphometric size parameters among malignant group and non neoplastic and benign group.

CONCLUSION

The Bethesda system is a very useful standardized system of reporting thyroid cytopathology in improving communication between cytopathologists and clinicians. Nuclear morphometry by computerized image analysis can be utilized as an additional tool in the differential diagnosis of thyroid follicular lesions on FNA smears.

KEYWORDS: Fine needle aspiration, Bethesda system, Computer nuclear morphometry.

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INTRODUCTION

The thyroid gland is the body's largest endocrine gland. It produces thyroid hormone, which controls the rate of metabolism and calcitonin produced by parafollicular or C cells, controls calcium metabolism. Thyroid disorders are common clinical problem. Thyroid disorders both benign and malignant occur in men and women in all ages and more common in females.

In worldwide the prevalence of a palpable thyroid nodule in community is about 12.2%.[1] The cause for increase in incidence and prevalence of thyroid cancer is uncertain. Thus detection of this condition through screening test plays a major role.

Fine needle aspiration cytology originally used by Martin and Ellis at New York for thyroid nodules offers clear advantages to patients and is been the first line of investigation in screening thyroid nodules. The technique is minimally invasive, simple, produces a speedy result and is cost effective. FNAC has been able to categories many benign and malignant lesions and thereby guide therapeutic protocols.[2]

To address terminology related to thyroid FNAC the NATIONAL CANCER INSTITUTE first developed BETHESDA SYSTEM in 2007. Bethesda system categorized thyroid lesions under 6 diagnostic categories. This reporting system for thyroid FNAC will facilitate effective communication among pathologists, surgeons, radiologists and endocrinologists.[3, 4]

However it is still difficult to establish precise diagnosis of thyroid follicular lesions by cytology. Differentiating benign thyroid adenoma from malignant follicular neoplasm on cytology was identified as a major contributor to the high false negative results. This concern specifically is for intermediate category where rate of malignancy is reported at 40%.[5, 6, 7] Further evaluation of

these lesions are necessary to improve the diagnostic accuracy and to the betterment in the treatment regimen for the patients.

In this study thyroid FNAC were classified using Bethesda system and cytology diagnosis is correlated with histopathological diagnosis.

Computerized nuclear morphometry is a cost effective, objective and reproducible tool for evaluation of nuclear features. It is a scientific tool to evaluate cellular changes and it can enhance the interpretation of morphological features by the transformation of pathological changes in cells to a quantitative form. Using nuclear morphometry; we can quantify a number of parameters such as those related to nuclear size and shape. Evaluation of these parameters has facilitated the diagnosis and management of different neoplasm in other systems. [9, 10, 11]

The evaluation of nuclear morphometry is not well established in thyroid cytology. Therefore the aim of the study is to evaluate the nuclear features in cytological evaluation of thyroid lesion.

AIMS AND OBJECTIVES

1. To classify thyroid lesions on FNAC using Bethesda system
2. To assess nuclear features in thyroid FNAC using morphometry
3. To evaluate the significance of nuclear parameters in improving the prediction of thyroid malignancy

REVIEW OF LITERATURE

EMBRYOLOGY [12, 13, 14]

In the embryo, at 3–4 weeks of gestation, the thyroid gland appears as an epithelial proliferation at the base of the tongue at a point of foramen cecum. The thyroid then descends as a bilobed diverticulum through the thyroglossal duct. Over the following few weeks, it migrates to the base of the neck, anatomical site. By then it has acquired a small median isthmus and two lateral lobes. The thyroid begins to function at approximately the end of the third month, at which time the first follicles containing colloid become visible. Follicular cells produce the colloid that serves as a source of thyroxine and triiodothyronine The portion of the thyroid containing the parafollicular cells / C cells are derived from the neural crest joins the primordial thyroid gland during its descent and serve as a source of calcitonin

ANATOMY [14]

The thyroid gland, is the largest endocrine glands found in the anterior side of the neck, below the thyroid cartilage and extending upto fifth or sixth tracheal ring .The butterfly-shaped gland is composed of two lobes connected via the isthmus. Each lobe measures about 5 cm long, 3 cm wide, 2 cm thick and weighs around 25grams. The gland is slightly heavier in females, which enlarges during menstruation and pregnancy.

The thyroid derives its arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk. The venous blood is drained via superior thyroid veins, and inferior thyroid veins.

Lymphatic drainage passes to deep cervical and the pre- and parathracheal lymph nodes.

Sympathetic innervation of the thyroid gland is provided by fibers from the superior and middle cervical sympathetic ganglia. Parasympathetic fibers are derived from the vagus nerve and reach the gland via branches of the laryngeal nerves. **[Figure 1]**

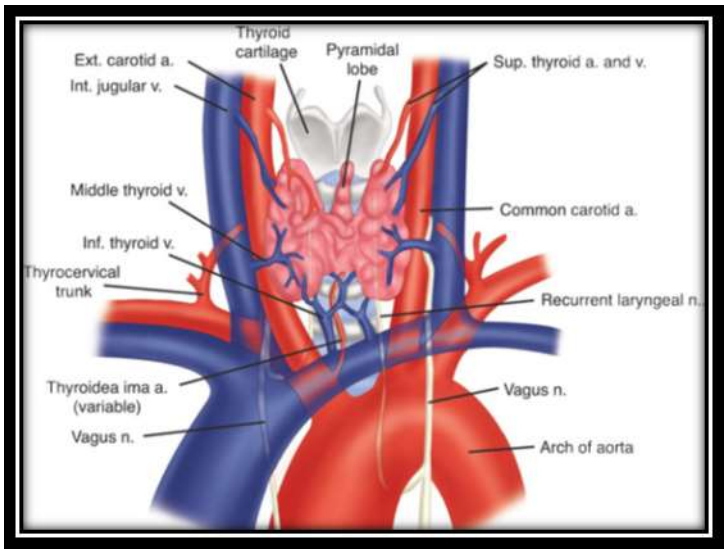

Figure 1: Microphotograph of anatomy of thyroid gland (Source: Netters atlas of anatomy)

HISTOLOGY [15,16]

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

Each follicle is morphological and functional unit of thyroid. Each follicle measures about 200um in diameter with considerable variation in size. Each follicle is lined by layer of low cuboidal to flattened epithelium depending upon the activity of the follicle. The cytoplasm of each cell is acidophilic or amphophilic and nuclei is round to oval /uniform and nucleoli 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 hyperfunctioning 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 are referred to as Hurthle cells, oxyphilic cells or oncocytes. Ultrastructurally 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 2**]

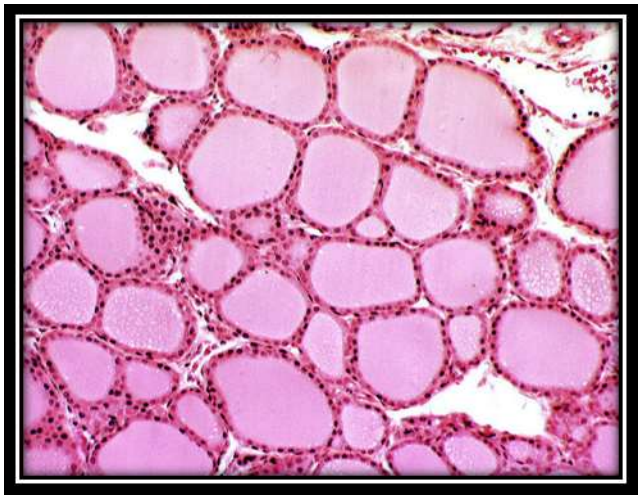

Figure 2: Microphotograph of histology of thyroid gland (Source: Inderbir singh)

CYTOLOGY[15,16]

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.

Follicular epithelial cells are fragile and bare nuclei are common. Follicular cells are similar in Shape and size 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] 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 fluids. During processing colloid may be washed off the slide, but the crazy pavement' artifact remain on the slide suggesting presence of it. Thick colloid appear as darkly stained dense clumps of hyaline material darkly blue/violet/magenta.

In Pap smears thin colloid appear pale green to orange, with cracking artifacts, thick colloid appear 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.

Collagen has a fibrillary structure and Amyloid stains dense magenta. C-cells resemble medullary carcinoma cells and are difficult to identify smears without the help of immunostaining for calcitonin. The finding of C-cells on smears may indicate significant C-cell hyperplasia.

If accidental puncture of trachea occurs during procedure, the patient may cough and air will be aspirated. This may result in a small number of tracheal epithelium- ciliated columnar cells with mucus clumps in the smear.

Cartilage appears as brilliant magenta flecks with fibrillary edges. Skeletal muscle fragments in MGG appear as straps of dark-blue material with multiple, pale ovoid nuclei. Cross-striation is distinguishable with high magnification.

PHYSIOLOGY [17, 18, 19]

The gland has two primary functions. The first is to secrete the thyroid hormones and 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.

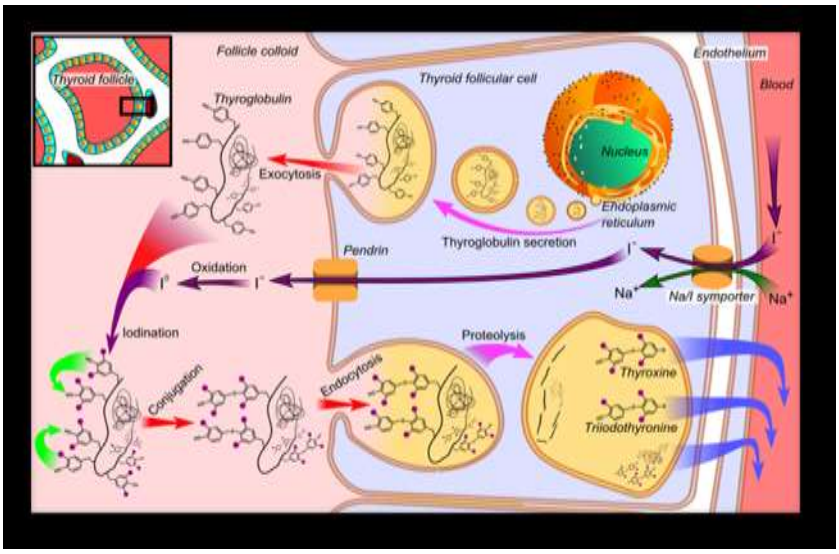

Figure 3: Microphotograph of physiology of thyroid gland (Source: Guyton's physiology)

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 3]**

CLASSIFICATION OF PRIMARY THYROID TUMORS, MODIFIED FROM THE WHO CLASSIFICATION [20, 21]

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
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.

FINE NEEDLE ASPIRATION CYTOLOGY

Virchow the father of cellular cytology published cellular pathology in 1855, later in 1869 Klebest described the technique of replacing intracellular water by molten paraffin wax. This technique has gained popularity over cytology because of its superior results.[22]

Cytology took its momentum in the nineteenth century due to work done by Thiersch and Waldeyer. Their contributions for development of human cytology was very important. In 1885 and 1867 they proposed the epithelial origin of carcinoma of skin and breast on cytology. Their critical observations were important for the development of diagnostic cytology as they formed the basis for recognition of precancerous epithelial abnormalities. This made cytological technique, an acceptable diagnostic tool. [23]

The introduction of aspiration cytology in twentieth century is attributed to surgeon Hayer Martin, Edward Ellis and Ewing's for cancer and Allied diseases. They in 1927 studied 1400 cases at Memorial hospital,Newyork,USA and advocated aspiration by using needle of thicker calibre (18-gauge).Professor Duggeon and Patrick from Great Britain in 1927 proposed the needling of tumor as a means of rapid microscopic diagnosis. [22, 23, 24]

FNAC has been practiced in Scandinavian Countries for more than four decades. Initial scepticism of pathologists and clinicians with regard to FNA gradually diminished and by the seventies this technique gained acceptance in the United States and the United Kingdom. Today it is practiced worldwide. [25]

In comparison with older, methods of preoperative morphological evaluation such as core biopsy, cytology has the advantage of being more rapid, less traumatic , less expensive and sampling is also more representative due to ease of several needle passes .complications are practically non-existent and diagnostic accuracy is better than the core biopsy. [25]

Cytology is an excellent method for the study of inflammatory and autoimmune thyroid lesions especially their natural history, that may be better understood by sequential cytological monitoring. The association of primary lymphoma of thyroid with hashimotos thyroiditis is well known and cytological monitoring has been shown to be of value in early detection of the lymphoma and prompt treatment. Autoimmune thyroid lesions usually are diffuse goiter that may not present clinical or biochemical features of altered thyroid function. They occasionally present as cold thyroid nodules leading to a clinical suspicioun of malignancy. In both situations, FNA cytology is of great value. [26, 27, 28]

Given the pivotal role played by FNA in the work-up of a thyroid nodule, it is important to ensure that this tool is providing reliable data. Most of the reported data showed sensitivity and specificity of thyroid FNA ranges between 80% and 100%. [29, 30]

ln a hospital based study of 469 patients found malignancy in 179 cases, out of which 147 cases were that of papillary carcinoma. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 82%, 100%, 100%, 90% and 93% respectively. [29] A study in Islamabad involved 327 cases of thyroid FNACs, out of which 230 were categorized as benign, 64suspicious for malignancy and 15 as malignant lesions. Subsequently 69 patients underwent thyroidectomy revealing an accuracy of 76.2%. Positive and negative predictive values were 50% and 94% respectively. [30] In other study, under diagnosis of malignancy was noted due to aspiration from the cystic area in thyroid. [31] It may be very difficult to sample the small lesion without ultrasound guidance. In such scenario ultrasound guide FNAC helps in obtaining the representative sample and in improving the diagnosis.

Though FNAC is been the procedure of choice in thyroid lesions, few of the cytological features overlap among thyroid lesions. In literature diagnostic features for PTC is been well

defined and include papillary tissue fragments, nuclear grooves, nuclear inclusions, psammoma bodies and others. [32, 33] Rupp et al [34], studied seventeen of 20 papillary carcinomas (85%) and less than 25% cases of other thyroid lesions showed nuclear groove. In contrast, one study 12 reported, nuclear groove in PTC (38%), follicular adenoma/carcinoma (10%), nodular goiter (22.5%), Hashimoto's thyroiditis (14%) and medullary carcinoma (16%).[35] Despite well-defined cytological features in FNA smears, diagnostic difficulties exist resulting in lower diagnostic accuracy either due to aspirate from non -representative area or due to overlap of cytological features between the lesions.

A key factor in the application of FNAC is consistency among reports. As interpretation of thyroid FNAC evolved, a number of systems were developed to categorize the results.

BETHESDA REPORTING SYSTEM

The majority of thyroid nodules are benign but all warrant investigation to rule out malignancy. A solitary nodule in the thyroid could be a colloid cyst, a dominant hyperplastic nodule in a multinodular goiter, a follicular adenoma or carcinoma or any other type of thyroid malignancy. Early detection and treatment of malignant nodules is associated with excellent outcomes. Hence it is necessary to provide reliable data and to communicate with an otolaryngist and surgeon for appropriate approach. This arouse a need for a development of reporting system in thyroid cytology.

Several classification schemes have been suggested by various authors based on personal/institutional experiences and clinical organizations. Despite, this until a decade back, there was no standardized terminology for FNA reporting. In UK during 2002, a system was proposed by Dr. P Cross and his colleagues in association with The Royal College of

Pathologist. Thy1−5 system was therefore proposed, similar to the C1−5 system used in the Breast Screening programme by the British Thyroid Association (BTA)/Royal College of Physicians (RCP). [3, 36]

Table 1: The THY system for reporting thyroid FNAC by Royal college of Pathologist [36]

Thy 1 category	Non diagnostic for cytology diagnosis
Thy 2 category	Non neoplastic
Thy 3 category	Neoplasm possible
Thy 4 category	Suspicious for malignancy
Thy 5 category	Malignant

A recent survey of pathologist and clinicians on diagnostic terminology and cytopathology reporting of thyroid FNA, showed a discord between these two groups. This was because there was much confusion regarding terminology used by different laboratories and countries.

On October 22 and 23, 2007, the National Cancer Institute (NCI) hosted "The NCI Thyroid Fine Needle Aspiration (FNA) State of the Science Conference," a two-day gathering in Bethesda, Maryland with intention of formulating internationally acceptable guidelines for reporting of thyroid cytopathology. The two-day "live" conference was attended by 154 registrants, comprised of pathologists, endocrinologists, surgeons, and radiologists, gave the opportunity to present their conclusions and debate over controversial areas in thyroid pathology. By the end of this conference a reporting system was finalized which was easy to understand and apply in clinical practice, and to reduce the intra- and inter-observer reproducibility between the various categories. The discussions and conclusions regarding terminology and morphologic criteria were than summarized by Baloch et al and formed the framework for the book on BSRTC. [3, 36]

Bethesda system streamlined the assessment and reporting of thyroid aspirates and alleviates the inter-observer variability of this procedure. This reporting system will facilitate effective communication among pathologists, surgeons, radiologists and endocrinologists. The Bethesda system differs from other systems of reporting in that each category is linked to evidence based clinical management protocol.

Table 2: BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY (BSRTC) [3]

CATEGORY 1	Non diagnostic or unsatisfactory
CATEGORY 2	Benign
CATEGORY 3	Atypia of undetermined significance or Follicular lesion of undetermined significance
CATEGORY 4	Follicular neoplasm or Suspicious for follicular neoplasm
CATEGORY 5	Suspicious for malignancy
CATEGORY 6	Malignant

THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY [3]

I. Non diagnostic or unsatisfactory

- Cyst fluid only
- Virtually acellular specimen
- Other (obscuring blood, clotting artifact, etc)

II. Benign

- Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc).
- Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context.
- Consistent with granulomatous (sub-acute) thyroiditis.
- Other

III. Atypia of undetermined significance or Follicular lesion of undetermined significance.

IV. Follicular Neoplasm or suspicious for a follicular neoplasm

Specify if Hurthle cell (oncocytic) type

V. Suspicious for malignancy

- Suspicious for papillary carcinoma
- Suspicious for medullary carcinoma
- Suspicious for metastatic carcinoma
- Suspicious for lymphoma

VI. Malignant

- Papillary thyroid carcinoma
- Poorly differentiated carcinoma
- Medullary thyroid carcinoma
- Undifferentiated (anaplastic) carcinoma
- Squamous cell carcinoma
- Carcinoma with mixed features (specify)
- Metastatic carcinoma

- Non Hodgkin lymphoma
- Others

Table 3: Implied risk of malignancy for Bethesda diagnostic category and recommended clinical management [3]

Diagnostic category	Risk of malignancy (%)	Usual management
Category 1		Repeat FNA with USG
Category 2	0-3	Clinical follow up
Category 3	5-15	Repeat FNAC
Category 4	15-30	Surgical lobectomy
Category 5	60-75	Surgical lobectomy/ near total thyroidectomy
Category 6	97-100	Near total thyroidectomy

Adequacy of the sample [3]

- Minimum of 6 groups of follicular cells with at least 10 cells per group.
- Exceptions:

1) Solid nodules with atypia
2) Solid nodules with inflammation- Thyroiditis
3) Colloid nodules

Category 1: Non-diagnostic/ unsatisfactory (ND/UNS) [3]

This category is similar in both BSRTC and THY systems and include

- Smears with Fewer than 6 groups of follicular cells
- Poorly prepared, poorly stained or obscured follicular cells
- Smears containing cyst fluid only with cyst macrophages and without abundant colloid

Category 2: Benign category [3, 12, 16, 37, 38]

This is the most common category in thyroid lesion

Simple colloid goiter

The combination of a diffusely enlarged gland and normal cytological appearances suggest simple colloid goiter. The cytological material obtained is usually thick and smear show diffuse background of colloid with variable cellularity, with follicular cells in monolayered sheets and honeycombing pattern. Globular masses of colloid with superimposed follicular cells called colloid globi are characteristic feature. The follicular cells are small and uniform with moderate amount delicate cytoplasm, and indistinct cell margins. The nucleus is round to oval and inconspicuous nucleoli.

Nodular goiter

Long standing simple goiter becomes transformed to multinodular goiter. They produce most extreme thyroid enlargement and frequently mistaken for neoplastic disease. Cytological features show abundant colloid and small number of both involutional and hyperplastic follicular cells. Follicular cells of involutional type show small round dark nuclei and fragile feathery cytoplasm. Many bare nuclei are seen. The nuclei are larger and show moderate anisokaryosis. Hyperplastic follicular cells are larger, with abundant vacuolated cytoplasm, sometimes with evidence of hypersecretions in the form of 'fireflares'. Larger follicles will form the monolayered sheets of epithelial cells, with honeycomb pattern and edges show frayed. Small follicles may appear as spherical cell cluster resembling multinucleated giant cells. Variable number of histiocytes, old blood, debris, and fragments of hyalinised stroma are due degenerative changes. Psammoma bodies, nuclear grooves and inclusions may also be seen occasionally.

Diffuse Toxic goiter [Graves' disease]

It is autoimmune disorder characterized by diffuse goiter, hyperthyroidism and exophthalmus. It is most commonly seen in women 20-50yrs of age. The thyroid enlargement and its hyperfunction are initiated by immunoglobin antibodies like TSH receptor antibody, thyroid stimulating immunoglobulin and thyrotropin-binding inhibitor immunoglobulin.

Cytological features are numerous follicular cells dispersed or in cluster, discrete to moderate anisonucleosis, marginal cytoplasmic vacuoles and blood stained smear with little or no colloid. Fire flares or marginal vacuoles are a common finding in a hyperplastic cells.

Hashimoto's thyroiditis [struma lymphomatosa]

It is an autoimmune disease which affects women more frequently than men and may be associated with hypothyroidism, euthyroidism and occasionally hyperthyroidism. Anti-microsomal and antithyroglobin antibodies are significantly elevated in 90% of HT. Three anatomically entities corresponds to different stages of the lesion. Cytological features show abundant lymphocytes and plasma cells with a scanty colloid. Follicular cells vary considerably in number and morphology.Atypical nuclear pattern with variation in size and shape in both follicular and Hurthle cell. Also seen are macrophages, multinucleated cells and sometime epitheloid cells.

Category 3 and 4: Follicular lesion of undetermined significance/atypia of Undetermined significance and Follicular neoplasm/Suspicious for follicular neoplasm

Follicular neoplasm-Benign

Follicular neoplasm may be benign or malignant. The cytological difference between follicular adenoma and carcinoma is very difficult, and the diagnostic criteria for follicular carcinoma not based on cellular characteristics but on other features such as capsular and vascular invasion.

Follicular adenoma [16, 38, 39]

It is defined as a benign encapsulated tumor that shows evidence of follicular cell differentiation. It is commonly seen in euthyroid females and present as solitary nodule in the neck. They may present as 'hot' or 'cold' nodule on radio –isotope scan. Architectural and cytological features are different from those of the surrounding gland, which usually shows signs of compression.

Adenoma may exhibit variety of patterns singly or in combination of normofollicular [simple], macrofollicular [colloid] microfollicular [foetal], trabecular or solid [embryonal] pattern.

It is a cellular often bloody smear, with moderate to high cellularity, uniform follicular cells in acinar, microfollicular, syncytial or honey comb pattern, synticial aggregates with nuclear crowding and overlapping is seen. Scanty or no colloid is seen. Micro follicles are characteristics of follicular neoplasm but may be found focally in multinodular goiter. In Pap and H & E smears show the cells with pale cytoplasm with poorly defined limits. The chromatin appears finely granular and evenly distributed. The nuclear membrane is thin and smooth and nucleoli when present are small and uniform. Fire flare appearance may be seen in variable proportion of cells and Hurthle cell change is occasionally present. Small blood vessels with adherent epithelial cells can be found any type of follicular neoplasm

Variants of follicular adenoma [16, 20, 37, 38]

1a] **Hyalinising trabecular adenoma**

It is term given to peculiar type of adenoma exhibiting a prominent trabecular arrangement and equally prominent hyaline appearance. The hyaline is present both in the cytoplasm of tumor cells and in the extracellular space. It is always easily confused with medullary or papillary carcinoma. On cytology, aspirate is bloody with cohesive tissue fragments. The follicular cells are arranged singly and cohesive aggregates, radially oriented around lumpy polymorphous extracellular material. The cells are round, oval or polyhedral with delicate, blue, non-granular cytoplasm. Cell borders are indistinct. Nuclei is eccentrically placed with fine chromatin, with nuclear grooves, intracytoplasmic inclusions and even psammoma bodies are seen. Amorphous, extracellular hyalinised matrix is seen which is congo red negative.

1b] Hurthle cell neoplasm

Hurthle cell represent an adaptive mechanism of the follicular cells of thyroid characterized by accumulation of mitochondria in the cytoplasm due to a primary alteration in mitochondrial DNA. The proliferation of mitochondria is the cause of their large size, eosinophilic and granular cytoplasm. Hurthle cell neoplasms constitute 5% of thyroid tumors. They may be benign or malignant. They are solid, homogenous, brown tumors with numerous regressive areas. Smears are cellular with little or no colloid. Cell are arranged in cohesive clusters and isolated oncocytes. Cells are large polygonal with granular cytoplasm and nuclei with prominent nucleoli. Prominent vascularization and absence of lymphocytic infiltrate and filamentous cellular debris. Ordinary follicular cells are usually fairly scarce of absent. The nucleus is large and generally eccentric, either single or double and sometimes pleomorphic. The nucleolus is prominent and has a cherry red appearance when smear are stained with Pap stain.

The distinction between benign and malignant Hurthle cell neoplasm is very difficult. Tumors with clearcut capsular and vascular invasion are designated as Hurthle cell carcinoma.

1c] **Atypical adenoma with bizarre cells**

They focally composed of extremely pleomorphic cells with presence of huge hyperchromatic nuclei usually in clusters. Unaccompanied by other features of malignancy.

1d] **Follicular adenoma with metaplastic stroma**

It is rare type of follicular adenoma interspersed with mature fat cells.

Category 6: Malignant tumor

Follicular carcinoma [16, 20, 40]

They account about 10-25% of the thyroid malignancy. Peak incidence of Follicular carcinoma is in 5th-6th decade with threefold female preponderance. These tumors are prevalent in areas of iodine deficiency. Other causes are treatment with radio-active iodine, low dose external therapeutic radiation. Follicular carcinomas are encapsulated tan or pink tumors that involve one or more lobes. It appears as slow growing mass that tends to metastasize via blood to distant organs, usually bones and lung. Follicular carcinoma has a microfollicular, macrofollicular, normofollicular or trabecular pattern. The criteria for malignancy rely on demonstration of capsular or vascular invasion or metastases at a distance.

Cytological features are show moderate to high cellularity within the cell groups, predominance of microfollicles or poorly formed follicular structures, disarray and crowding of cells in a cell groups numerous isolated cells, moderate to large nuclear size, chromatin

somewhat irregularly distributed, and prominent nucleolus or macronucleolus. Miller et al and Kini reported that by using several of these criteria 70 to 82% of follicular carcinoma can be diagnosed. Most of the authors are content to use cytology to select cellular follicular neoplasm for follow-up or surgical excision and leave the diagnosis of malignancy to histological assessment of capsular and vascular invasion.

Papillary carcinoma [16, 20]

It is malignant epithelial tumor showing evidence of follicular cell differentiation and characterize by nuclear features, it accounts for 75-80% of thyroid malignancy. It occurs most coomonly in females.

On cytology, highly cellular smear in a background usually devoid of blood or colloid and cells are arranged in papillary clusters, monolayered sheets with distinct anatomical border and focally nuclear crowding / overlapping. These papillae are with or without fibrovascular core; cells have dense blue cytoplasm with defined cell margins and enlarged ovoid nuclei. The nuclei show finely granular or powdery chromatin with multiple nucleoli. Intranuclear inclusions, nuclear grooves and psammoma bodies seen. Squamous metaplasia are also seen. There is presence of cystic degeneration macrophage, along with multinuclear giant cells and lymphocyte. Scanty, viscus, stringy colloid called chewing gum colloid seen. It is important to remember that no single cytological feature is diagnostic of papillary carcinoma and that a diagnosis of Papillary carcinoma is made upon intergration of various cytomorphological features.

Variants of papillary carcinoma

1) Cystic papillary carcinoma

2) Follicular variant of papillary carcinoma
3) Solid variant/ conventional variant
4) Encapsulated variant
5) Diffuse sclerosing variant
6) Tall cell variant
7) Oncocytic variant
8) Columnar cell variant
9) Warthin tumor like variant
10) Macrofollicular variant
11) Trabecular variant
12) Papillary carcinoma with lipomatous stroma
13) Variant with exuberant nodular fasciitis like stroma
14) Dedifferentiated Papillary carcinoma
15) Variant of spindle cell metaplasia

Undifferentiated or anaplastic carcinoma [12, 16]

It comprises of 2-5% of all thyroid carcinoma however it may account to 30-40% in places where goiter is endemic, commonly see in female and mean age of incidence is 70yrs. Modes of presentation is rapid enlargement of thyroid with longstanding goiter. Recent rapid enlargement of thyroid in a patient with recurrent well differentiated thyroid carcinoma, with regional / metastatic tumor. Cytology show necrotic background with dissociated or clustered malignant cells, spindle shaped, giant, squamous and poorly differentiated malignant cells in varying combination with prominent nuclear pleomorphism, multinucleation and mitosis.

Medullary carcinoma [16, 23]

Malignant tumor showing parafollicular C–cell differentiation which characteristically secrete calcitonin, but also a variety of other peptide products. It consists of 2-8% of all thyroid tumors, 70-80% of medullary carcinoma occur sporadically, while rest are familial. Most patient present with thyroid mass, pain and dysphagia hoarseness of voice and cervical lymphadenopathy. It invades locally, metastasize to cervical and mediastinal nodes and also distant organs. Cytologically show cellular smear in background of blood with dispersed cells and some clustering, variable cell pattern with plasmacytoid, spindle and small cell, moderated anisokaryosis, scattered large nuclei and bi and multinucleate forms. Uniform stippled chromatin and presence of amorphous pink background material called amyloid. Some of the cells show coarse granularity.

Poorly differentiated carcinoma [12, 38]

These carcinoma show limited evidence of structural follicular cell differentiation, and portray histologic and biologic features intermediate between well differentiated thyroid carcinoma with undifferentiated carcinoma. Usually occur in middle aged female, most such tumor exhibit insular growth pattern called insular carcinoma. Cytology show cellular smear with no colloid/scanty colloid. Cells are arranged in monolayered sheets, cluster, trabeculae follicular and dissociated pattern, small uniform cell with scanty pale cytoplasm and high N: C ratio. Necrosis is often present in background. Less common feature are grooving, intranuclear inclusion, foci of Hurthle cell, clear cell, and anaplastic cell with nuclear hyperchromasia, coarse chromatin and psammoma bodies.

BETHESDA SYSTEM

After introduction of Bethesda it was adapted by many researchers. Bethesda system is not just a classification system but also goes into great detail about cytological criteria for categorizing the findings on each FNAC. The system also details the risk of malignancy for each category, based on large patient series, as well as the suggested management for each FNAC results.[3]

In a study on 4966 thyroid aspirates, the reported malignancy rates in each category was category 1- 4.2%;category 2- 0%; category 3- 41.2%; category 4- 46.4%; category 5- 48.4%; and category 6 - 78.4%. These results were same as with other studies. [41, 42]

In a study of 528 cases, in which 403 cases were diagnosed as Bethesda 2 and 67 were Bethesda 3 while 22 cases were categorized as either malignant or suspicious for malignancy (Bethesda 6 and 5). Histopathologic correlation was done in 61 cases. For Bethesda 5 and 6 categories, 100% concordance was found, however for Bethesda 2 category, 5 out of 45 cases were found to have malignant diagnosis. In Bethesda 3 category, 66.7% cases were benign while 33.3% turned out to be malignant. [43] These finding were similar to other studies. [44, 45]

According to researchers, the malignancy rates in category 2 can further reduced by following strict criteria for adequacy and repeat FNAC from solid areas in cases of cystic lesion, as this is helpful because they improve the diagnostic efficiency of thyroid FNA and avoid unnecessary surgery for benign non neoplastic thyroid lesions. [46, 4]

The frequency of category 3 interpretations should be in the range of approximately 7% of all thyroid. [3] Category 3 is a heterogeneous category, due to which represent a benign lesion with the degree of cellular or architectural atypia like focal nuclear enlargement/clearing or

microfollicular pattern in a scanty smear which warrants repeat FNAC not sufficient to diagnose follicular neoplasm. There remain differences between observers in using this category. [48]

In other study, the malignancy rate in category 4 was found to be 46.4%. [44] The majority of cases in category 4, on HPE are adenomas, but 20 to 30% are carcinomas. [26] Patients with a diagnosis of "Follicular Neoplasm" should be referred for operative exploration. Usually a lobectomy is performed followed by histologic examination for capsular and vascular invasion. Although, the reporting system is associated with high prognostic potential and improved reproducibility, few of the thyroid lesions pose diagnostic difficulty and lie within grey zones due to the pathologist's subjective factors. In view of improving the clinical value, it nuclear pleomorphism can be quantified by measuring nuclear features.

COMPUTERISED NUCLEAR MORPHOMETRY

Morphometry refers to the quantitative analysis, a concept that encompasses size and shape. Computerized morphometry evaluates cellular changes and it can enhance the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form. Recent years have seen the development of computer equipment for use in quantitative microscopy and are now routinely used in research laboratory practice.

Baak et al.,[49] introduced nuclear morphometry for prognostication of breast cancer. Subjective grading has been successfully used for breast cancer but, by applying quantitative methodology, standardization and accuracy of grading can still be promoted. [50, 51, 52] Morphometry of nuclei and nucleoli may be helpful in diagnostic and prognostic evaluations, and improve the sensitivity and specificity of cytological diagnosis. [53, 54] Morphometric analysis

has been used as an objective way in prognostic factor in soft tissue, [55] skin [56] and in prognostic factor of survival in urothelial carcinoma. [57]

Nuclear morphometry has been used by an author to evaluate the prognostic value of the colorectal cancer in 90 patients. It was noticed, patients with greater nuclear diameters had a significant poor prognosis than the patients with cells of smaller diameter.58 Similar studies were done by Ikeguchi et al in nasopharyngeal carcinoma.[59] In other study nuclear parameters like nuclear area, axis minor, diameter minor, radius minor, perimeter area in oligodendroglioma and found it to be statistically significant with regard to grading of oligodendrogliomas. [60]

In one study in breast lesion, they found mean nuclear diameter of 10.26, mean nuclear perimeter of 30.02, mean nuclear area of 54.54. They compared these parameters with histological grade of breast tumor and found that nuclear diameter and area were higher in grade 3 than in grade 2 and grade 1. [61] This finding was similar to other study. [62] Other researcher observed that the morphometric parameters like nuclear diameter and area were almost equal between lymph node positive and negative groups. [63] In contrast, study conducted by other authors found significant correlation between morphometric parameters and lymph node status. [61, 62]

Morphometry for thyroid lesions was introduced by Luck et al [64] and Gundersen et al [65] where estimation of nuclear size was performed using test system and ruler. This was associated with high interobserver variability and hence was not universally accepted.

Though the cytological diagnosis following FNAC gives the diagnosis of the thyroid lesion, the problem still persist in accuracy among follicular lesion. Morphometry has been described for two decade now and usefulness of this technique complement cytological diagnosis and provide useful information.

The ImageScope software interface by Aperio is user friendly and takes shorter time to understand. In image scope, although the image analysis tools require significant initial time to familiarize, once the tools and methods have been understood and acquired, the process of image analysis is straightforward. Once set up, Whole slide image (WSI) examination consumes the most significant time in manual delineation of the individual cell groups. This requires focused attention and patience, with judgment related to the element of morphology to be evaluated.

An author, studied 119 subjects of thyroid FNAC cases to look for the nuclear parameter among thyroid lesions and found that the mean areas and perimeters of follicular adenoma nuclei were significantly larger than nuclei from multinodular goitres. [66]

In other study, 10 cases of adenomatoid goiter, 10 case of follicular neoplasm, 4 cases of medullary carcinoma and 2 cases of anaplastic carcinoma, and 10 case of papillary carcinoma. A minimum of 10 nuclei per case under 200× magnifications,photo were captured and nuclear parameters were measured. Coefficient of variation of the nuclear area (NACV) value showed significant differences between non neoplastic lesion like adenomatous goiter and neoplastic lesion like follicular neoplasm and in papillary carcinoma and author also noted higher mean nuclear diameter and perimeter in anaplastic carcinomas compared to follicular neoplasms. [67]

Murata et al, measured 100 cells in PAP smear of 39 benign and malignant thyroid tumor cases. Gray-level image analysis was performed and data included seven parameters for nuclear size, four parameters for nuclear shape, and 16 parameters for nuclear chromatin patterns. They concluded, nuclei of papillary carcinoma were larger size, more irregular shape, and higher contrast of chromatin pattern than those of the benign group. The follicular carcinomas have larger nucleus and more monotonous chromatin pattern than those of the benign group.[68]

In other study, nuclear parameters like coefficient of variation of the nuclear area, nuclear perimeter was evaluated and this showed significant differences among follicular carcinomas, adenomas and adenomatous goiters. [69] Whereas Kaur et al, reported that nuclear features were not helpful in differentiation of follicular carcinomas from adenomas.[70]

Computerized morphometry is a scientific tool to evaluate cellular changes and it can enhance the interpretation of morphological features by the transformation of pathological changes in cells to a quantitative form. Nuclear morphometry in combination with other objective prognostic criteria, can improve the evaluation of the lesions and patient's prognosis, and possibly predict response to therapy.

This study focuses on the role of classification of thyroid lesions under Bethesda system and evaluation of the nuclear parameters by computerised nuclear morphometry.

Study period: The study comprised of 120 patients who presented clinically with thyroid swelling to R L Jalappa Hospital and Research Centre, Tamaka, during January 2010- May 2015. Patient who underwent both FNAC and surgery were included in the study to facilitate cytohistological correlation.

All the patients are clinically examined in detail according to the proforma and careful local examination of the thyroid gland was done to judge the location of swelling before doing the aspiration. A detail of the procedure was explained to the patient in their own language and consent was taken.

Aspiration of the thyroid lesion was done with the patient lying in a supine position and the neck hyperextended with pillow under the shoulder so as to make the thyroid swelling appear prominent. Under aseptic precaution 23 gauge needle with syringe holder is inserted into lesion and to and fro movement performed quickly. Under negative pressure material gets collected in the needle, after collection of material negative pressure was released, needle was removed along with syringe holder, the material is spread over clean slide and smears are prepared**. [Figure 4]** Smears are fixed in 95% ethyl alcohol and stained with H & E and PAP. Air dried smear were stained with giemsa.

Figure 4: Photograph of FNAC Technique

Surgical specimens were fixed with 10% formalin and detailed gross examination was done according to standard protocol and sections were taken from the representative areas for paraffin sections and stained by H &E.

INCLUSION CRITERIA:

1. All thyroid lesions with FNAC and histopathology diagnosis.
2. Adequate FNAC sample: minimum of 6 group of well visualized follicular cluster with at least 10 cells/ group except for in colloid goiter where in abundant colloid in presenve of few follicular cells was considered satisfactory for interpretation.

EXCLUSION CRITERIA:

1. Patient on anti-thyroid medication, wherever relevant history available.
2. Patient who have undergone chemotherapy and radiotherapy in head and neck region.
3. Category 1 of Bethesda system

STAINING PROCEDURE FOR FNAC SAMPLES

A) RAPID PAP STAINING:

Step 1: Fix smear immediately in 95% methanol - 1 min

Step 2: Immerse in hematoxylin solution - 3 mins

Step 3: Wash in running tap water

Step 4: Dip in OG – 6 and EA – 50 solution for 3 min

Step 5: Wash in running tap water

Step 6: Clear in 2 changes of xylene, 2 minutes each

Step 7: Mount with DPX medium

Morphology of cells in PAP stain: The nuclei stain blue and cytoplasm is pink or green depending on the cells.

B) HEMATOXYLIN AND EOSIN STAINING:

Step 1: Fix smear immediately in 95% methanol - 1 min

Step 2: Immerse in hematoxylin solution - 3 mins

Step 3: Wash in running tap water

Step 4: Dip in acid alcohol

Step 5: Wash in running tap water

Step 6: Dip in eosin stain

Step 7: Wash in running tap water

Step 8: Clear in 2 changes of xylene, 2 minutes each

Step 9: Air dry and mount with DPX

Morphology of cells in H&E stain: The nuclei stain blue and cytoplasm is pink

C] GIEMSA STAINING PROCEDURE

Step 1: Allow the smear to air dry

Step 2: Stain with May-Grünwald Giemsa working solution for 20 minutes

Step 3: Wash with clean buffered water for 2 minutes

Step 4: Wash in running water

Step 5: Dry the slides in upright position at room temperature

Step 6: Mount the slides with a coverslip using DPX

Morphology of cells in Giemsa stain: The nuclei stain blue and cytoplasm is pink

THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY [3]

- Non diagnostic or unsatisfactory
- Cyst fluid only
- Virtually acellular specimen
- Other (obscuring blood, clotting artifact, etc)
- Benign
- Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc).
- Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context.
- Consistent with granulomatous (sub-acute) thyroiditis.
- Other
- Atypia of undetermined significance or Follicular lesion of undetermined significance.
- Follicular Neoplasm or suspicious for a follicular neoplasm
    i. Specify if Hurthle cell (oncocytic) type
- Suspicious for malignancy
- Suspicious for papillary carcinoma
- Suspicious for medullary carcinoma
- Suspicious for metastatic carcinoma
- Suspicious for lymphoma
- Other
- Malignant
    - Papillary thyroid carcinoma
    - Poorly differentiated carcinoma

- Medullary thyroid carcinoma
- Undifferentiated (anaplastic) carcinoma
- Squamous cell carcinoma
- Carcinoma with mixed features
- Metastatic carcinoma
- Non Hodgkin lymphoma

- Others

Cytological diagnosis was correlated with histopathological diagnosis

For computerized nuclear morphometry cytology slides stained with H&E, PAP smear are taken. Computerized Nuclear morphometry was done by using photos captured under Olympus CX-41 research microscope, an average of 5–10 microscopic fields, at magnification x400 were captured for each case. At least 100 nuclei were analyzed per case. Cells in uniform sheets with no overlapping and cells with intact whole nuclei from the actual lesion, with nuclear characteristics are considered .Nuclei of stromal cells or background cells were avoided. Single cells were not included because the cellular borders are not always apparent or the nuclei are "stripped," rendering them noncontributory These cells are outlined using the Sketch command by the computer mouse in the Aperio – Image analyzer software and four parameters were measured – Area, Perimeter, Minimum Nuclear Diameter and Maximum Nuclear Diameter. These parameters were saved in the excel sheet and later were used to calculate the other four parameters. Measurements were calibrated in terms of micrometer, using a NOW micrometer slide before performing measurements in the software. Calibration was checked and the reliability was confirmed by repeated measurements of a control group and of known cell

dimension, RBC in present study. Person doing nuclear morphometry is blinded for histopathology diagnosis.

For nuclear morphometric analysis the FNAC cases which correlated with histopathological diagnosis was taken.

Measured Parameters (μm)

- Mean Minimal Nuclear Diameter (Mmnd)
- Mean Maximum Nuclear Diameter (MMND)
- Mean Nuclear Perimeter (MNP)
- Mean Nuclear area (μm^2) (MNA)

Calculated Parameters (μm)

- Mean Axis ratio (MAR) = Mmnd/MMND.
- Mean nuclear Compactness (MNC) =MNP^2 / MNA
- Mean Shape factor (MSHF) = $4 \times \pi \times MNA / MNP^2$
- Mean Nuclear size (MNS) = $2 \times (MNA/\pi)^{0.5}$

The mean axis ratio (MAR) is the ratio between the shortest and the longest diameter of the nuclei. The mean nuclear shape factor (MSHF) is an indicator of nuclear shape irregularity.

The nuclear parameter were correlated with histopathological diagnosis.

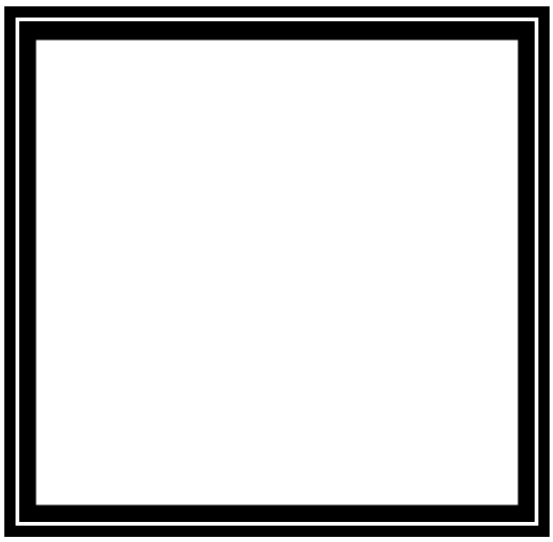

Figure 5: Schematic representation of nuclear diameter

Figure 6: Schematic representation of nuclear are and perimeter

STATISTICAL ANALYSIS

For sample sample size calculation,

Formula, $n = (\alpha)^2 pq/(d)^2$ $n = (3.29)^2 \text{ X } 12.2 \text{ X } 87.8 / 10^2$

Sample size n= 120 at 99.9% confidence level expecting 10% non-compliance with absolute error of 10% and alpha at 0.001.

For result analysis:

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.

The following assumptions on data is made, Assumptions:

1. Dependent variables should be normally distributed.

2. Samples drawn from the population should be random, cases of the samples should be independent.

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Post-hoc Tukey test has been used to find the pairwise significance.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical software**:** The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc

OBSERVATION AND RESULTS

During the study period of present study, 1115 FNAC of thyroid lesions were performed in the institution, among these the 120 cases which satisfied inclusion criteria were included for the present study.

Age:

FNAC was done on 120 patients with thyroid lesions, the age distribution of the patients was analyzed.The youngest patient in our study was 22 yrs and oldest patient 60yrs of age.In our study it has been observed that the occurance of thyroid lesions were more in the age group of 41-50yrs, i.e., 50cases (41.6%), and least common in population <30 yrs of age (8.3%).

Sex:

From the total number of cases the sex distribution of thyroid aspirates showed more percentage of females - 83% whereas the males were only 17%. Male to female ratio was found to be 1:5

Table 4: Age and sex wise distribution

Age in Years	Sex		Total	Percentage (%)
	Male	Females		
<20	00	00	00	00
21-30	00	10	10	8.3
31-40	12	26	38	31.6
41-50	5	45	50	41.6
51-60	03	19	22	18.3
Total	20	100	120	100

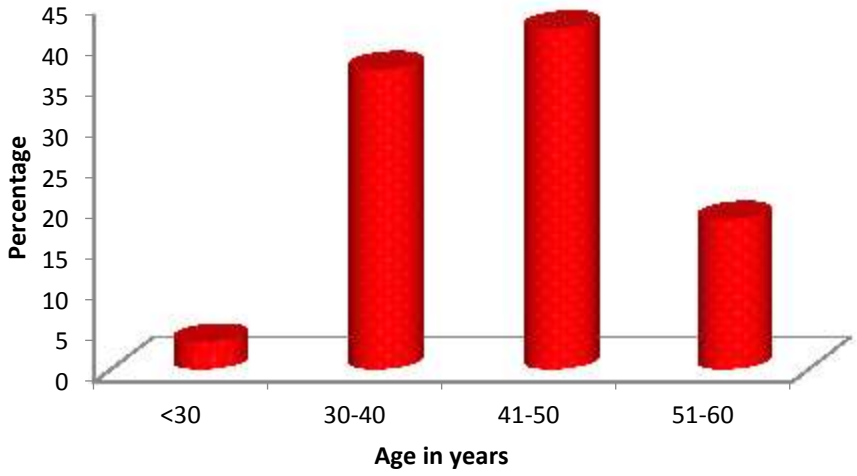

Chart 1: Column diagram of age distribution

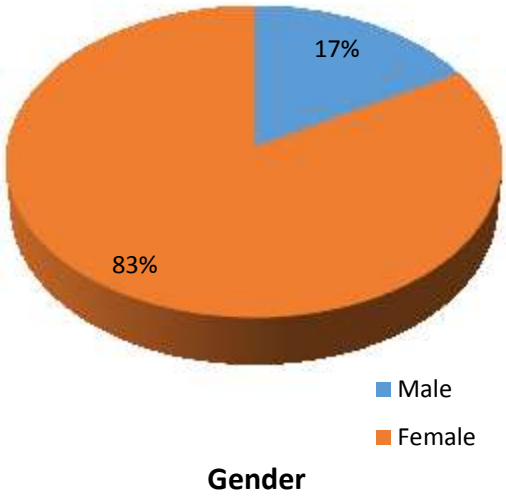

Chart 2: Pie diagram of gender distribution

Clinical features:

Out of 120 patients in the present study, 82 patients (68.3%) presented with thyroid swelling. Duration ranged from 15 days to 3 years. Pain during swallowing was present in 16 patients (16%). Among 120 patients, 22 came for routine checkup (18%).

Table 5: Presenting complaints

Presenting complaints	Number of cases	Percentage (%)
Thyroid swelling	82	68.3
Pain during swallowing	16	16
Routine check up	22	18

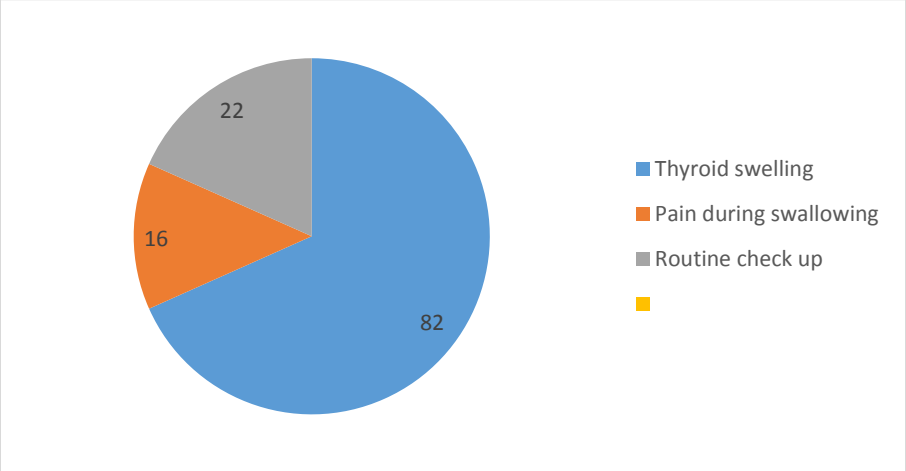

Chart 3: Pie diagram of presenting complaints

TABLE 6: Thyroid function status based on thyroid function test

THYROID STATUS	NO OF PATIENTS
Euthyroid	23
Hyperthyroid	10
Hypothyroid	2

Out of 120 patients, biochemical values of T3, T4 and TSH was available in 35 cases. 23 cases were Euthyroid, 10 were hyperthyroid and two patients were in hypothyroid state.

FNAC smears were analyzed using Bethesda system

Table 7: Cytological diagnosis of thyroid lesions as per Bethesda system

Categories	Number of cases	Percentage (%)
Category 2	73	60.83
Category 3	14	11.6
Category 4	3	2.5
Category 5	8	6.6
Category 6	22	18.3
Total	120	100

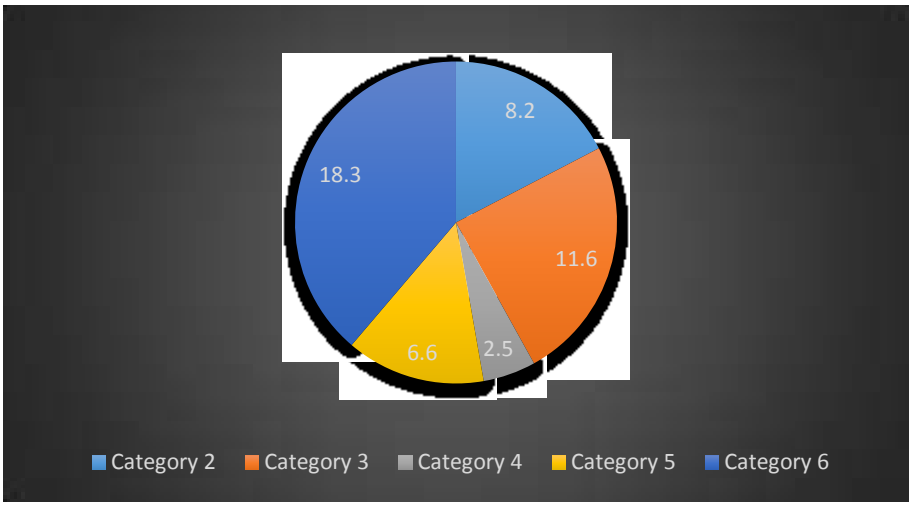

Chart 4: Pie diagram of cytological diagnosis under Bethesda system

Category 2 was the most common lesion constituting upto 60%, followed by category 6 which was 18%.

Table 8: Cytological diagnosis under Category 2 [Figure 7, 8]

CATEGORY 2	Total no (n)	Percentage (%)
Nodular goiter	29	39.7
Colloid goiter with degenerative changes	22	30.1
Lymphocytic thyroiditis	20	27.3
Granulomatous thyroiditis	1	1.3
Hyperplastic goiter	1	1.3
Total	73	100

Among category 2, colloid/ nodular goiter is the predominant diagnosis constituting about 69%.

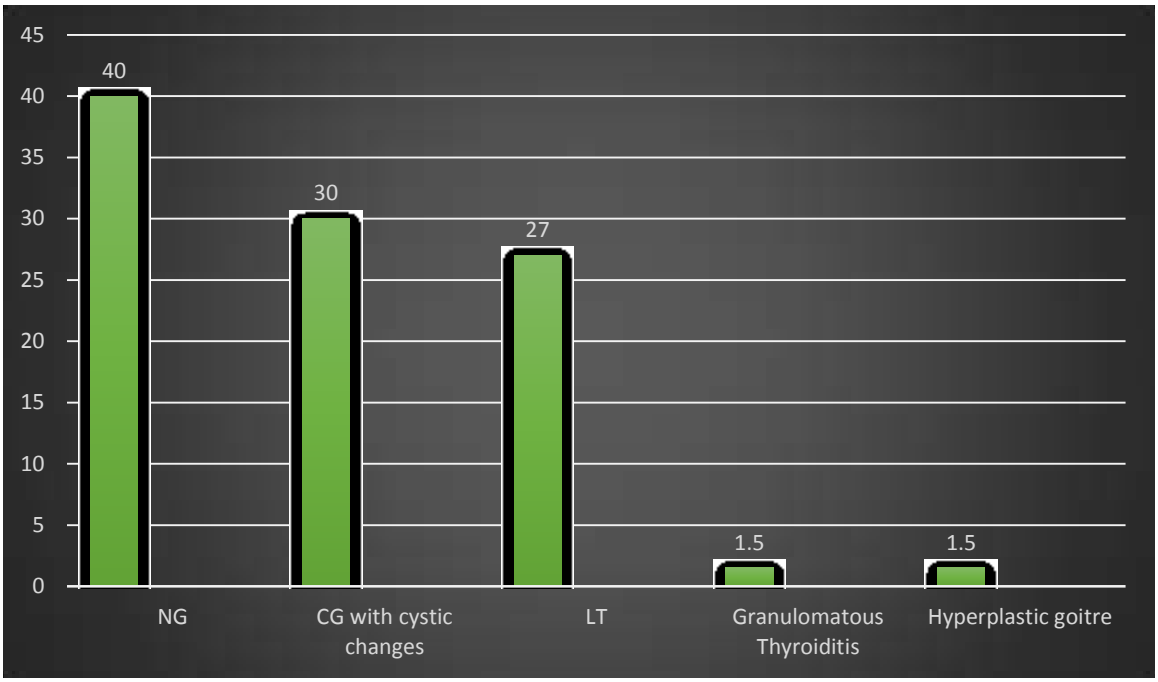

Chart 5: Column diagram of cytological diagnosis under Bethesda category 2

Table 9: Cytological features of different lesions in category 2

Features	Number of cases (n=73)	Percentage (%)
Scant / absent colloid	21	28.7
Moderate / abundant colloid	58	79.4
Follicular cells in singles and cluster	65	89.0
Follicular cells in microfollicles, papillary	8	10.9
Cyst macrophages	22	30.1
Lymphocytes	18	24.6
Multinucleated giant cells	8	10.9
Oncocytes	7	9.58
Fire flares	4	5.47
Granulomas	1	1.36
Nuclear groove	2	2.73
Nuclear inclusion	0	-
Nuclear pleomorphism	26	35.61

Among category 2, majority of cells were arranged in cluster and singles (89%) with background having abundant thin and thick colloid. Among 22 cases of colloid goiter with degenerative changes showed cyst macrophages in 18 cases, multinucleated giant cells in 8 cases and 2 cases showed oncocytic changes. Of 20 cases of lymphocytic thyroiditis, lymphocytes were seen impinging onto the thyroid follicular cells in 15 cases, and all cases showed polypmorphous lymphocytic population in the background along with scant colloid and 5 cases showed oncocytic changes. Of one case of granulomatous thyroiditis, an ill - defined granuloma was noted along with scattered lymphocytes and thin scant colloid. Of one case of hyperplastic goiter, patient had clinical symptoms of hyperthyroidism and with cytological features of well-

defined fire flares in the giemsa stains along the periphery of the clustered thyroid follicular cells.

Table 10: Cytological features in category 3 & 4 [Figure 9 &10]

Features	Number of cases (n=17)	Percentage (%)
Moderately cellular	14	82.3
Highly cellular	3	17.6
Microfollicles	13	76.4
Follicles in clusters	15	88.2
Nuclear pleomorphism	9	52.9
Scant/ absent colloid	15	88.2
Moderate / abundant colloid	2	11.7

Of 17 cases in category 3 and 4, 14 of the cases had moderate cellularity with cells were arranged in microfollicles with about ten follicular cells in each of them and few of them in sheets. Cells were predominantly arranged in microfollicles in 13 cases with dispersed isolated cells. Cells were normal sized to slightly enlarge with scant to moderate amount of cytoplasm with enlarged, hyperchromatic nuclei and inconspicuous nucleoli. Colloid was thin and scant in majority of cases

Cytological features in category 5

8 cases of suspicious for malignancy were reported in our study. Out of the 8 cases, 5 smears showed moderate to marked cellularity. Cells were arranged in sheets, singles and ill-defined papillae. Cytoplasm was scant to moderate with mild anisonucleosis, nuclei showed finely dispersed chromatin. All 8 cases had occasional nuclear grooves and 3 cases showed intranuclear cytoplasmic inclusions.

Table 11: Cytological features in category 6 [Figure 11, 12 &13]

Features	Number of cases (n= 22)	Percentage (%)
Scant / absent colloid	4	18.18
Follicular cells in papillary	12	54.44
Follicular cells in singles and cluster	8	36.36
Cellular swirls	14	63.36
Nuclear groove	15	68.18
Nuclear inclusion	13	59.12
Nuclear pleomorphism	13	59.12
Multinucleated giant cells	4	18.18
Psamomma bodies	1	4.54

Among 22 cases of papillary carcinoma, 12 cases are arranged in papillary pattern and 14 cases had cellular swirls pattern having about 50–200 tumor cells concentrically arranged , with ovoid nuclei, the long axes of which were oriented perpendicular to the radius of the swirl. [**Figure 11]** Fifteen cases showed transpolar nuclear grooves. 13 cases showed intanuclear cytoplasmic inclusion and nuclear pleomorphism with mild anisonucleosis and dispersed chromatin. Multinucleated giant cells was seen in 4 cases and were larger with diverse shape, dense cytoplasm and more nuclei compared those seen in colloid goiter.

Table 12: Histological diagnosis of Thyroid lesion

HPE diagnosis	Total Number (n)	Percentage (%)
Non neoplastic lesion	65	54.6
Neoplastic lesion	55	46.4
Total	120	100

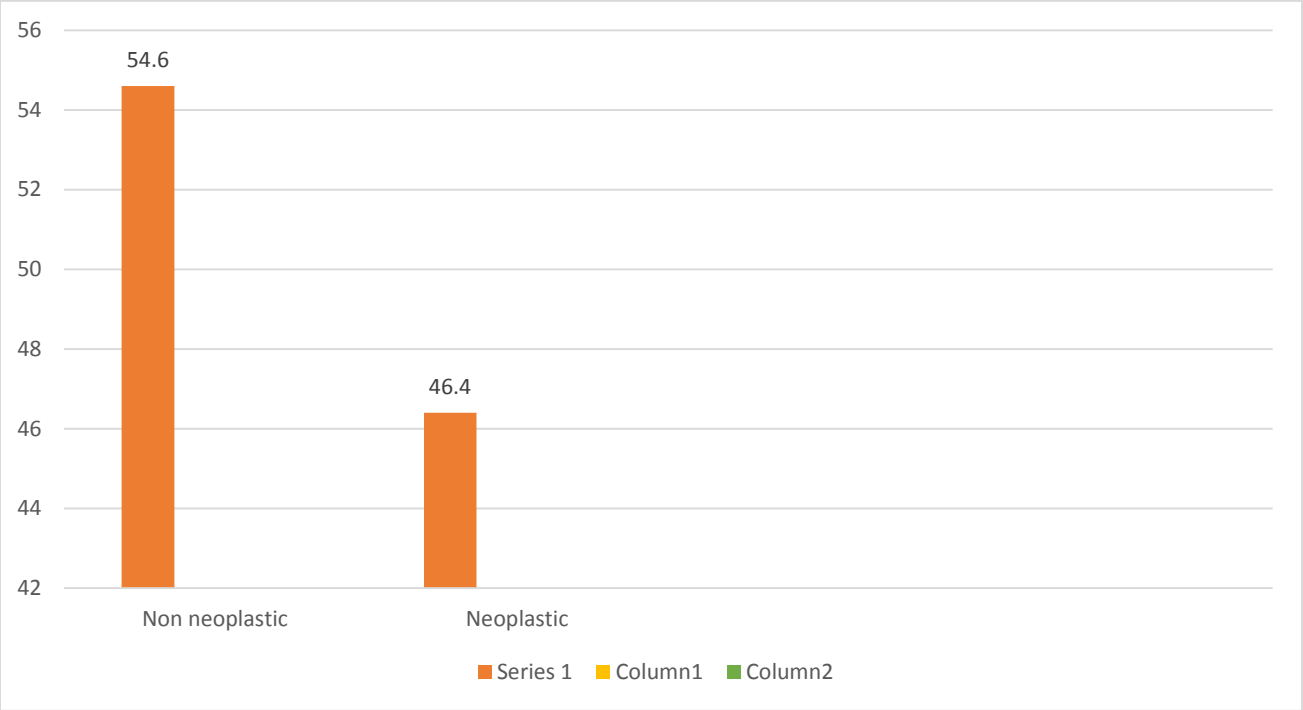

Chart 6: Column diagram of histopathological diagnosis of Thyroid lesion

On histopathological examination, 54.6% cases showed non neoplastic lesion and 46.4% turned out to be neoplastic lesions.

Table 13: Histological diagnosis of non-neoplastic Thyroid lesion [Figure 14]

Lesion	Total number(n)	Percentage (%)
Nodular goiter/ colloid goiter	27	41.5
Multinodular goiter	10	15.3
Nodular hyperplasia	6	9.2
Hashimotos thyroiditis	22	33.8
Total	65	100

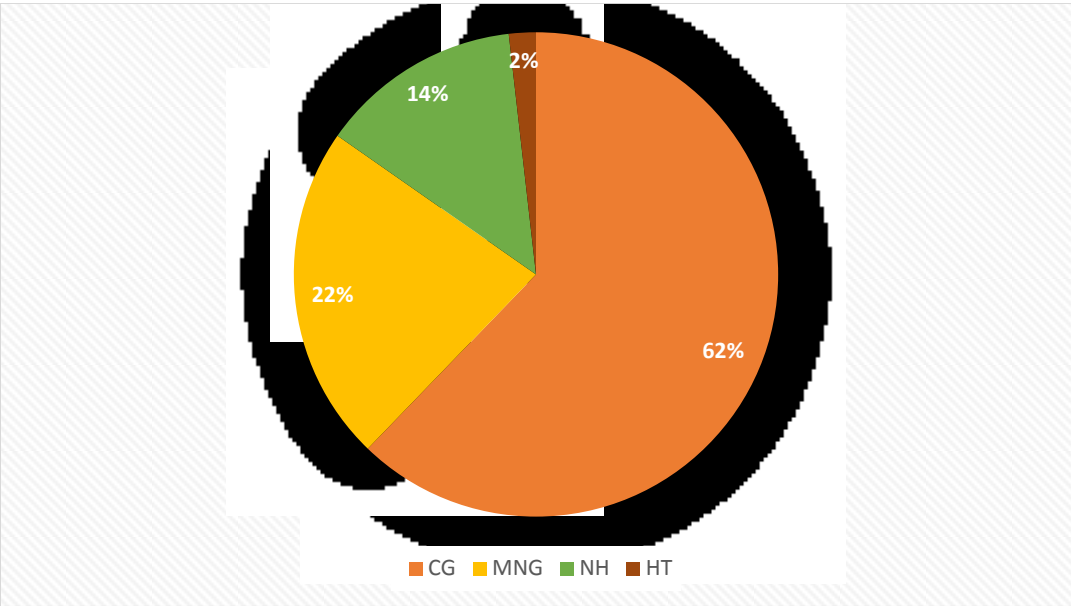

Chart 7: Pie diagram of histological diagnosis of non-neoplastic lesion

Among 65 cases of non - neoplastic lesion nodular goiter was predominant with 27 cases (41.5%), followed by hashimotos thyroiditis in 22 cases (33.8%).

Table 14: Histological diagnosis of neoplastic Thyroid lesion [Figure 15, 16]

Lesion	No of cases	Percentage (%)
Follicular adenoma	24	42.8
Follicular carcinoma	1	1.78
Hyalinising trabecular adenoma	1	1.78
Papillary carcinoma	29	53.5
Total	55	100

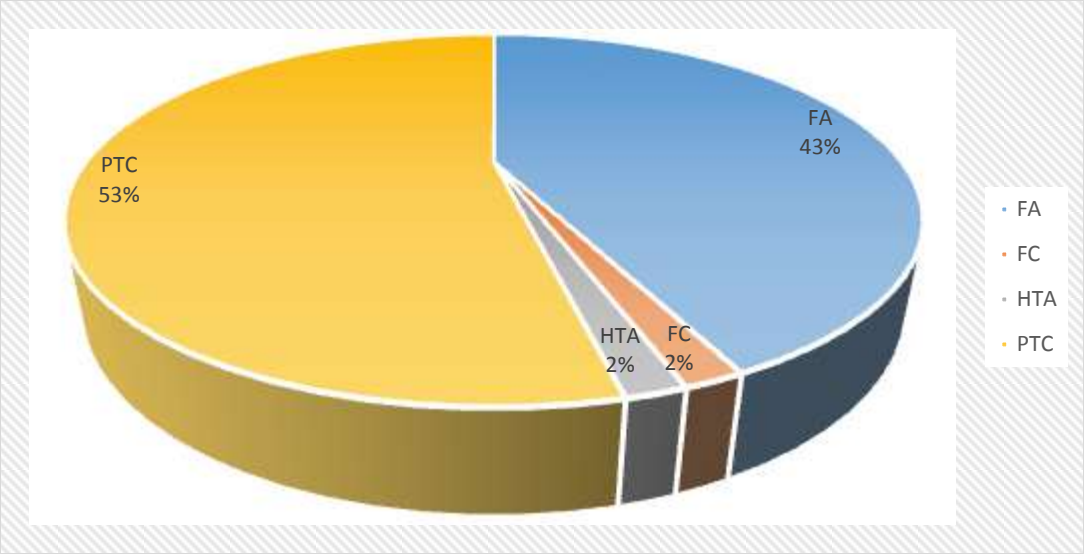

Chart 8: Pie diagram of histopathological diagnosis of Neoplastic lesion

Out of 56 neoplastic cases on HP 24 cases were of follicular adenoma (42.8%), one case each of hyalinizing trabecular adenoma and follicular carcinoma and most common being 29 cases of papillary carcinoma (53.5%)

Table 15: Histological variants of papillary thyroid carcinoma

PTC variants	Total number (n)
Conventional	22
Micropapaillary	4
Follicular variant	2
Tall cell variant	1

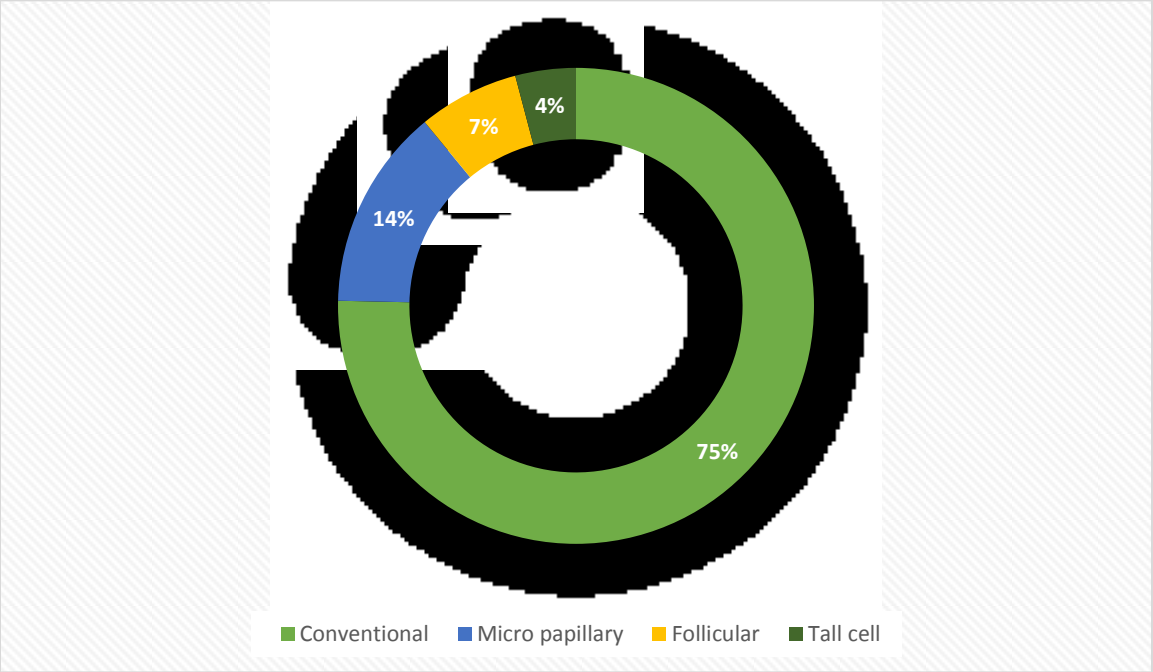

Chart 9: Pie diagram of histopathological diagnosis of Variants of Papillary carcinoma

Among the variants of papillary carcinoma, conventional type was most common, followed by micropapillary variant

Table 16: Correlation of Cytologic diagnosis with final histology, with incidence of malignancy in each Bethesda category

Cytology diagnosis	Number (n)	%	HPE	Frequency (n)	%	Incidence of malignancy (%)
Category 2 CG - 51 LT – 20 Granulomatous-1 HG -1	73	60.83	Non neoplastic CG LT Granulomatous NH Neoplastic FA FC PTC	 32 19 0 3 14 1 4	 43.8 26.0 - 4.1 19.1 1.3 5.4	6.7
Category 3	14	11.6	Non neoplastic CG LT Granulomatous NH Neoplastic FA PTC	 2 0 0 3 7 2	 14.2 - - 21.42 50 14.2	14.2
Category 4	3	2.5	Non neoplastic CG LT Granulomatous	 1 	 33.3 	

			NH			----
			Neoplastic			
			FA	2	66.6	
			PTC	0		
Category 5	8	6.6	Non neoplastic			
			CG	1	12.5	50
			LT	1	12.5	
			Neoplastic			
			FA	1	12.5	
			HTA	1	12.5	
			PTC	4	50	
Category 6	22	18.3	NG	2	9.09	
			LT	1	4.54	86.36
			PTC	19	86.36	

Among 73 cases in category 2, 54 cases correlated with the histopathological diagnosis with diagnostic accuracy of 73.97%. In category 3, seven cases of 14 cases correlated with histopathological diagnosis with 50 % diagnostic accuracy, and low diagnostic accuracy can be explained due to the fact that, category 3 is a heterogeneous category, which reflects the difficulty in the cytological diagnosis of the follicular lesions of thyroid. It includes cases in which the cytological findings are not of a benign lesion yet the degree of cellular or atypia is not sufficient to render an interpretation of follicular neoplasm/suspicious for a follicular neoplasm or suspicious for malignancy. In such scenario, repeat FNA and correlation with clinical and radiological findings might help in categorizing them further into other specific category. In category 4, among three cases, two on HPE showed follicular adenoma, thereby showing 66.6% correlation. In category 5, four case on HPE was diagnosed as papillary carcinoma. In category

6, 19 cases correlated with the HPE diagnosis of papillary carcinoma thyroid with diagnostic accuracy of 86.36%.

Table 17: Cytology and histopathology disparity

Category	Total (n)	Disparity case (n)	Diagnosis on histopathology
Category 2	75	19	14–follicular adenoma,1-follicular carcinoma,4–papillary carcinoma
Category 3	14	2	2- papillary carcinoma
Category 4	3	1	1 – Lymphocytic thyroiditis
Category 5	8	4	1 – colloid goiter, 1- lymphocytic thyroiditis, 1- follicular adenoma, 1- Hyalinizing trabecular adenoma
Category 6	22	3	2 – Nodular goitre,1- Lymphocytic thyroiditis

Of 75 cases in benign lesion category 2, 56 belonged to non- neoplastic lesion on HPE. 14 cases on HPE turned out to be follicular adenoma, among these 10 cases had admixture of follicular adenoma along with colloid goiter in surrounding tissue. Cytology of these cases showed macrofollicular arrangement along with thin colloid. One case of follicular carcinoma, on cytology shoed cells were arranged in microfollicular and macrofollicular pattern with moderate nuclear pleomorphism and moderate colloid with background of polymorphous population of lymphocytes, impinging onto follicular cells. Of 4 cases diagnosed as papillary carcinoma, 3 were micro papillary carcinoma type and none of them showed nuclear features of papillary carcinoma in cytology and other case was conventional papillary carcinoma with surrounding colloid goiter. The miss of diagnosis in these 19 cases were due to aspiration from non- representative areas and this can be avoided by ultrasound guided aspiration.

Out of 14 cases in category 3, 2 cases which turned out to be follicular variant of papillary carcinoma on HPE, and on cytology these cases showed macrofollicular and microfollicular arrangement with occasional nuclear grooves.

Among 3 cases in category 4, one case turned out to be lymphocytic thyroiditis on HPE, which on cytology showed follicular cells in clusters and microfollicular arrangement with lymphocytic background.

Among 8 cases in category 5, papillary carcinoma was confirmed in 4 cases. In view of high cellularity, nuclear groove and occasional nuclear inclusion suspicious of malignancy was given in another 4 cases.

Out of the 22 cases of papillary carcinoma, two turned out to be nodular goiter and one as lymphocytic thyroiditis. These 3 cases on cytology, showed moderate cellularity and cells arranged in clusters and ill-defined papillae. At focal areas, these cells showed intranuclear cytoplasmic inclusions and nuclear grooves which had led to the cytological diagnosis of papillary carcinoma.

Table 18: Summary of sensitivity and specificity in present study

		Histopathology		
		Neoplastic	Non Neoplastic	Total
Cytology	Neoplastic	35	10	45
	Non neoplastic	21	54	75
	Total	56	64	120

Sensitivity – 62.5 %

Specificity – 84.38 %

Positive predictive value – 77.78 %

Negative predictive value – 72%

Accuracy – 74.16%

The low sensitivity is mainly due to aspiration from non representative areas. Use of ultrasound guidance will improve the sensitivity of thyroid FNAC.

COMPUTERIZED NUCLEAR MORPHOMETRY

Computerized morphometry is an objective computer image analysis to estimate the chosen parameters in every individual cells. In present study we have used this application in evaluating the thyroid lesions

Among 120 study cases, the cases which had 100% histocytological correlation and cases with minimum of 100 nuclei for nuclear morphometry i.e, 81 cases were included for analysis. This was divided into three group

1. Non neoplastic group
2. Benign neoplastic group
3. Malignant group group

Under non neoplastic group 54 cases of cytohistologically diagnosed colloid goiter, lymphocytic thyroiditis, hyperplastic goiter were considered. Under benign neoplastic category, 8 cases of cytohistologically diagnosed follicular adenoma (follicular neoplasm)

cases were considered and under malignant group 19 cases of papillary carcinoma was considered.

For nuclear morphometric analysis H&E and PAP stained cytological smears was selected and microphotographs was captured and analysis was performed as mentioned earlier**. [Figure 17, 18]**

Table 19: Group of lesions for nuclear morphometric analysis

Groups	Total number of cases (n)
Non neoplastic Colloid goiter Lymphocytic thyroiditis Hyperplastic goiter	54 32 18 4
Benign - neoplastic Follicular adenoma	8
Malignant Papillary carcinoma	19
Total	81

Table 20: Nuclear morphometric parameters in non-neoplastic group

Variables	Micrometer (μm) ± SD
Mean Maximal Nuclear Diameter, μm	6.90±0.24
Mean Minimal Nuclear Diameter, μm	6.76±0.23
Mean Nuclear Perimeter, μm	23.90±0.31
Mean Nuclear Area, μm2	81.73±5.11
Mean Axis Ratio	0.97±0.01
Mean Nuclear Compactness	6.66±0.36
Mean Shape Factor	1.87±0.08
Mean Nuclear Size	9.84±0.33

The maximum nuclear length of cells in non-neoplastic cases varied from 6.70 (μm) to 7.14 (μm) and the minimum nuclear length varied from 6.53 (μm) to 6.99 (μm) with SD of 0.23 for both parameter. Mean Nuclear area was 81.73 (μm) and mean nuclear perimeter was 23.90(μm). Calculated parameters like Mean Axis Ratio was 0.97, Mean Nuclear Compactness was 6.66, Mean Shape Factor was 1.87 and Mean Nuclear Size varied from 9.51 to 10.17 with the SD of 0.33.

Table 21: Nuclear morphometric parameters in benign neoplastic group

Variables	Micrometer (µm) ± SD
Mean Maximal Nuclear Diameter, µm	7.37±0.12
Mean Minimal Nuclear Diameter, µm	7.23±0.10
Mean Nuclear Perimeter, µm	24.62±0.20
Mean Nuclear Area, µm2	84.07±2.61
Mean Axis Ratio	0.98±0.01
Mean Nuclear Compactness	7.48±0.28
Mean Shape Factor	1.75±0.07
Mean Nuclear Size	10.11±0.16

The maximum nuclear length of cells in benign neoplastic cases varied from 7.25 (µm) to 7.49 (µm) and the minimum nuclear length varied from 7.13 (µm) to 7.36 (µm) with SD of 0.12 and 0.10 respectively for both parameter. Mean Nuclear area was 84.07 (µm) and mean nuclear perimeter was 24.62(µm). Calculate parameters like Mean Axis Ratio was 0.98, Mean Nuclear Compactness was 7.48, Mean Shape Factor was 1.75 and Mean Nuclear Size varied from 9.97 to 10.27 with the SD of 0.16.

Table 22: Nuclear morphometric parameters in malignant group

Variables	Micrometer (µm) ± SD
Mean Maximal Nuclear Diameter, µm	8.67±0.76
Mean Minimal Nuclear Diameter, µm	8.50±0.76
Mean Nuclear Perimeter, µm	27.04±1.32
Mean Nuclear Area, µm2	114.26±18.99
Mean Axis Ratio	0.98±0.00
Mean Nuclear Compactness	8.96±0.69
Mean Shape Factor	1.58±0.18
Mean Nuclear Size	12.00±1.07

The maximum nuclear length of cells in malignant group cases varied from 9.43 (µm) to 7.91 (µm) and the minimum nuclear length varied from 7.74 (µm) to 9.26 (µm) with SD of 0.76 for both parameter. Mean Nuclear area was 114.26 (µm) and mean nuclear perimeter was 27.04(µm).

Table 23: Comparison of mean nuclear diameter in different group

	Non neoplastic	Benign neoplastic	Malignant group
MMND (Mean Maximal Nuclear Diameter, µm)	6.90 ± 0.24	7.37±0.12	8.67 ± 0.76
Mmnd (Mean Minimal Nuclear Diameter, µm)	6.76 ± 0.23	7.23 ± 0.10	8.50 ± 0.76

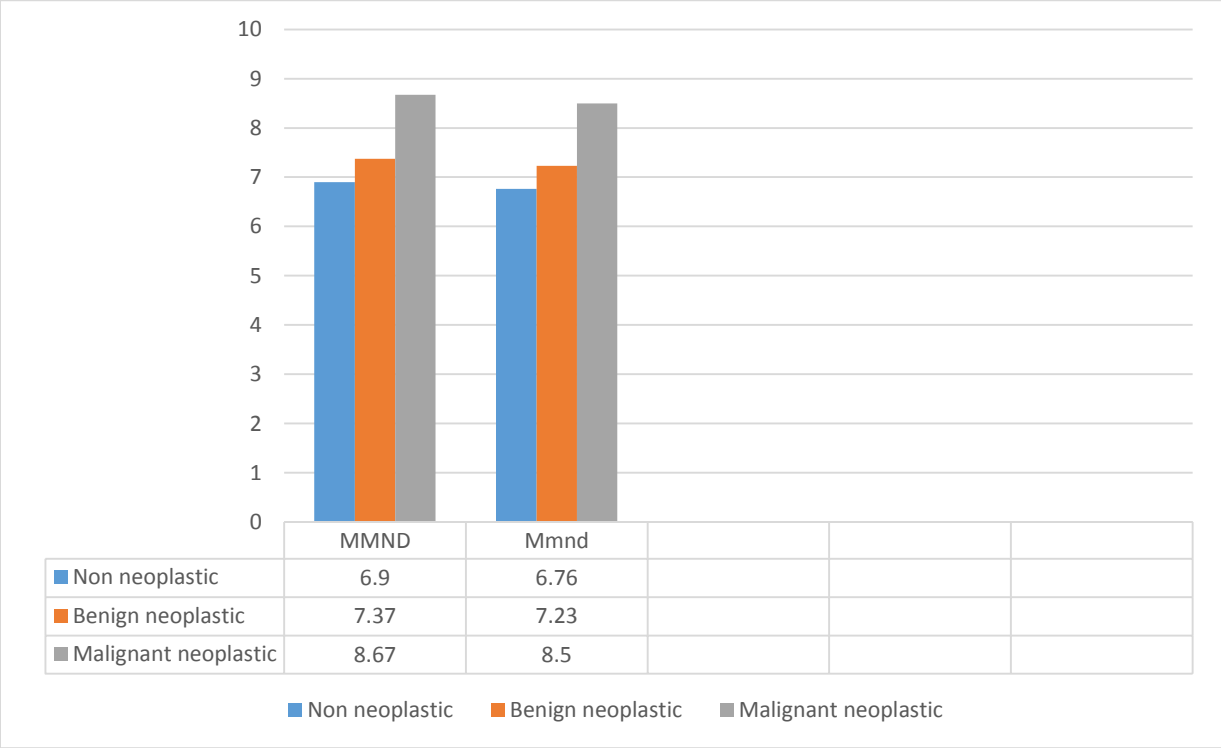

Chart 10: Column diagram of mean nuclear diameter in different group

The mean minimal and maximal diameter are higher in malignant group compared to that of non - neoplastic group. The SD of mean minimal and maximal diameter of both non neoplastic and malignant category was higher compared to that of benign neoplastic lesion. There by supporting the features of monomorphism of follicular cells in benign category is common compared to non - neoplastic and malignant category.

Table 24: Comparison of mean nuclear perimeter and area in different group

	Non neoplastic	Benign neoplastic	Malignant group
MNP (Mean Nuclear Perimeter, µm)	23.84 ±0.31	24.62 ±0.20	27.04 ±1.32
MNA (Mean Nuclear Area, μm^2)	81.73 ±5.11	84.07 ±2.61	114.26 ±18.99

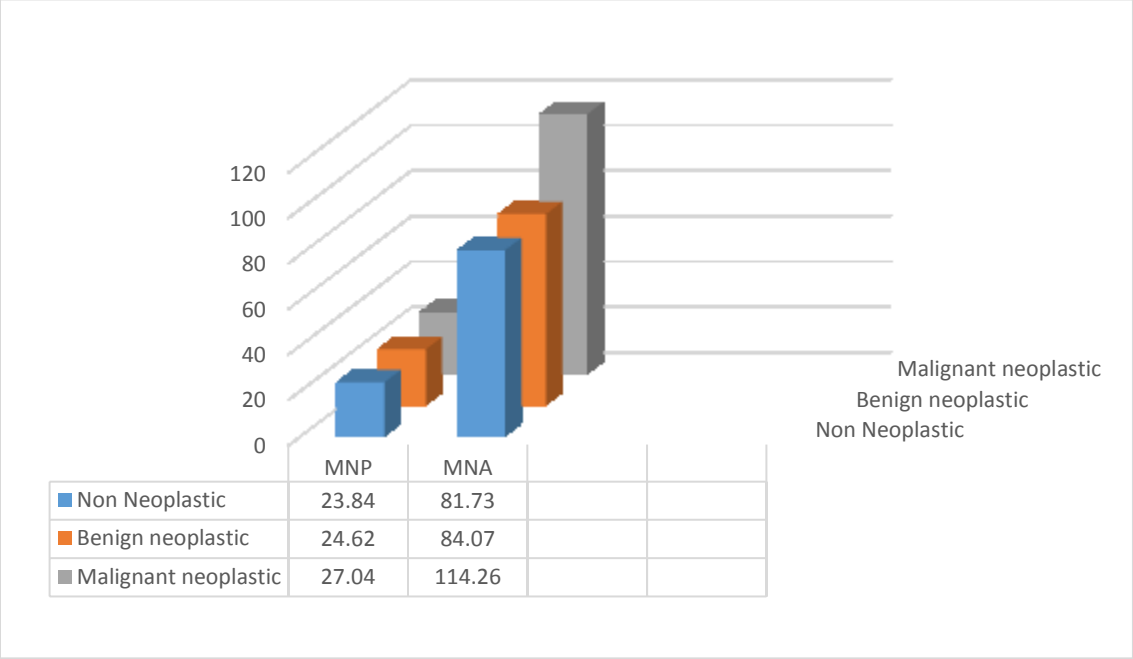

Chart 11: Column diagram of mean nuclear perimeter and area in different group

The mean nuclear perimeter and area are higher in malignant group compared to that in non - neoplastic and benign neoplastic group. The SD of mean nuclear area among non-neoplastic, benign and malignant group group were 5.11, 2.61. 18.99 respectively. The higher mean nuclear area in malignant group suggest the enlarged nuclear size, which is a feature of malignant lesions.

Table 25: Comparison of calculated mean nuclear parameter in different group

	Non neoplastic	Benign neoplastic	Malignant group
MAR (Mean Axis Ratio)	0.97 ±0.01	0.98±0.01	0.98±0.01
MNC (Mean Nuclear Compactness)	6.66±0.36	7.48±0.28	8.96±0.69
MSHF (Mean Shape Factor)	1.87±0.08	1.75±0.07	1.58±0.18
MNS (Mean Nuclear Size)	9.84±0.33	10.11 ±0.16	12.00±1.07

The mean nuclear axis ratio and shape factor did not vary among the groups. Mean nuclear size and mean nuclear compactness was higher in malignant group group compared to other groups.

Table 26: Correlation between mean value nuclear diameters in various lesions of non -neoplastic group

Parameters	Colloid goiter	Lymphocytic thyroiditis	Hyperplastic goiter
Mmnd (Mean Minimal Nuclear Diameter , μm)	6.63	6.86	6.91
MMND (Mean Maximal Nuclear Diameter , μm)	6.78	7.00	7.06

Among the lesions in non -neoplastic group, nuclear diameter was higher in hyperplastic goiter than those of other lesions. Although the values were higher among hyperplastic goiter, there was no significance of parameters within the various lesions.

Table 27: Correlation between mean value nuclear perimeter and area in various lesions of non-neoplastic group

Parameters	Colloid goiter	Lymphocytic thyroiditis	Hyperplastic goiter
MNP (Mean Nuclear Perimeter , μm)	23.68	23.94	24.05
MNA (Mean Nuclear Area, μm^2)	72.35	77.09	78.44

Mean nuclear area is higher in hyperplastic goiter compared to other lesions and this could be due to increased activity of follicular cells in hyperplastic goiter thereby suggesting the variation in nuclear area.

To find out utility of nuclear morphometry in distinguishing between variants of papillary carcinoma, morphometric analysis was performed on one case of tall cell variant of PTC, which was diagnosed as suspicious for papillary carcinoma in cytology.

Table 28: Nuclear Morphometric analysis among variants of PTC

Parameters	Conventional	Tall cell
Mmnd (Mean Minimal Nuclear Diameter , µm)	8.50	8.64
MMND (Mean Maximal Nuclear Diameter, µm)	8.67	8.82
MNP (Mean Nuclear Perimeter, µm)	27.02	28.52
MNA (Mean Nuclear Area, μm^2)	114.26	120.71

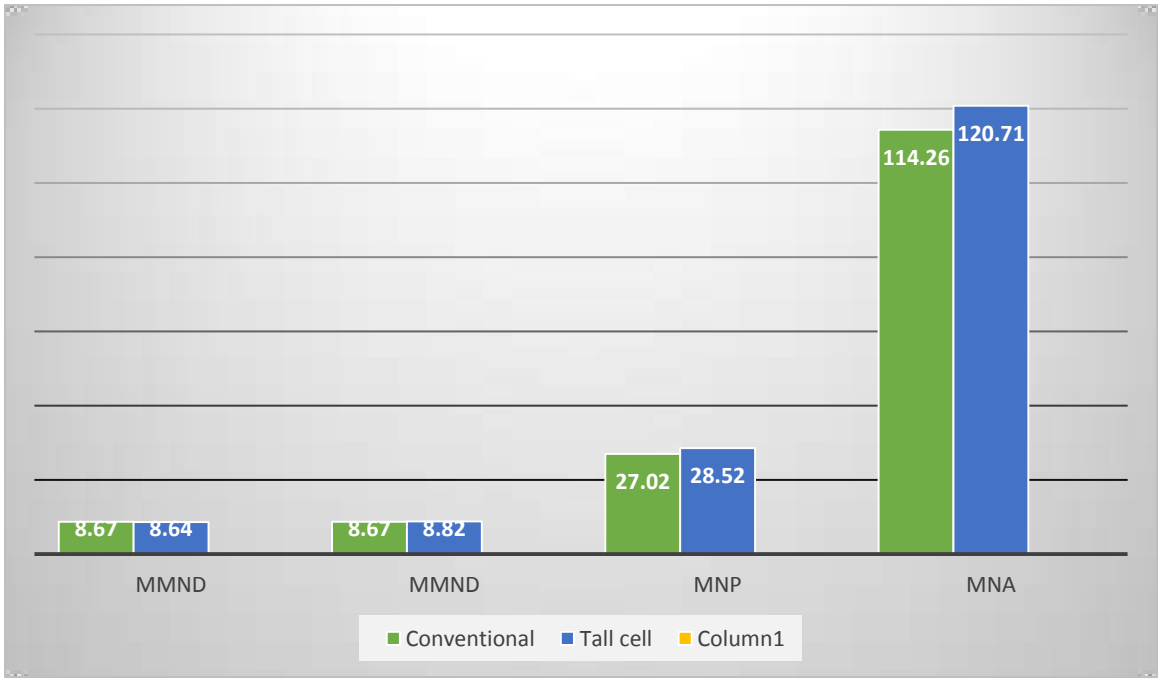

Chart 12: Column diagram of nuclear parameters in variants of PTC

Among the variants of papillary carcinoma thyroid, tall cell variant by its definition showed increased nuclear diameter compared other variants. Among the variants, tall cell variant of PTC had higher nuclear perimeter compared to other variants

Table 29: Pairwise Comparison between Non Neoplastic and Malignant Group.

Variables	Non neoplastic	Malignant group	p value
Mean Maximal Nuclear Diameter, µm	6.90±0.24	8.67±0.76	<0.001**
Mean Minimal Nuclear Diameter, µm	6.76±0.23	8.50±0.76	<0.001**
Mean Nuclear Perimeter, µm	23.84±0.31	27.04±1.32	<0.001**
Mean Nuclear Area, µm2	81.73±5.11	114.26±18.99	<0.001**
Mean Axis Ratio	0.97±0.01	0.98±0.00	0.989
Mean Nuclear Compactness	6.66±0.36	8.96±0.69	<0.001**
Mean Shape Factor	1.87±0.08	1.58±0.18	<0.001**
Mean Nuclear Size	9.84±0.33	12.00±1.07	<0.001**

Nuclear diameter, perimeter and area showed statistically significant difference when compared between the two groups. Among calculated parameters. Mean axis ratio did not show any statistical significance between groups, all other remaining parameters had good statistical correlation.

Table 30: Pairwise Comparison between Benign and Malignant group Group

Variables	**Benign neoplastic**	**Malignant group**	**p value**
Mean Maximal Nuclear Diameter, µm	7.37±0.12	8.67±0.76	<0.001**
Mean Minimal Nuclear Diameter, µm	7.23±0.10	8.50±0.76	<0.001**
Mean Nuclear Perimeter, µm	24.62±0.20	27.04±1.32	<0.001**
Mean Nuclear Area, µm2	84.07±2.61	114.26±18.99	<0.001**
Mean Axis Ratio	0.98±0.01	0.98±0.00	0.676
Mean Nuclear Compactness	7.48±0.28	8.96±0.69	0.007
Mean Shape Factor	1.75±0.07	1.58±0.18	0.001
Mean Nuclear Size	10.11±0.16	12.00±1.07	<0.001**

Nuclear diameter, perimeter and area showed statistically significant difference when compared between the benign and malignant group group. Among calculated parameters, mean nuclear size was statistically significant between benign and malignant group group.

In order to evaluate the use of nuclear morphometry in improving diagnostic accuracy of thyroid cytology, the nuclear parameters were applied in cytohistological disparity. 9 cases were considered here out of 30 cases, because in other cases the aspirates were from non representative areas and applying morphometry on these lesions does not give any valuable information.

Table 31: Nuclear morphometric parameters in False negative cases in category 3

	Cytological diagnosis	HPE diagnosis	Mmnd(µm)	MMND(µm)	MNP(µm)	MNA (µm)
Case 1	Non neoplastic	Malignant	7.43	7.62	25.62	88.28
Case 2	Non neoplastic	Malignant	7.52	7.65	25.82	89.10

Among 9 cases, 2 cases which was diagnosed as non neoplastic lesion under Bethesda category 3, turned out to be papillary carcinoma in histopathology. The mean maximal nuclear diameter was 7.62 µm and 7.65 µm, mean minimal nuclear diameter was 7.43 µm and 7.52 µm for each case respectively. The mean nuclear perimeter was 25.62 µm and 25.82 µm respectively. Mean nuclear area was 88.28 μm^2 and 89.10 µm respectively.

The nuclear parameters in 2 false negative cases are closer to the nuclear values observed in neoplastic group.

Table 32: Nuclear morphometric parameters in False Positive cases

	Cytological diagnosis	HPE diagnosis	Mmnd(µm)	MMND(µm)	MNP(µm)	MNA(µm)
Case 1	Category 5-Suspicious for neoplasia	Non neoplastic	7.22	7.39	24.48	85.84
Case 2	Category 5-Suspicious for neoplasia	Non neoplastic	7.15	7.32	24.18	85.21
Case 3	Category 5-Suspicious for neoplasia	Benign neoplastic	7.24	7.32	24.52	86.12
Case 4	Category 5-Suspicious for neoplasia	Benign neoplastic	7.20	7.29	24.48	85.89
Case 5	Category 6-Malignant	Non neoplastic	7.12	7.29	24.12	84.42
Case 6	Category 6-Malignant	Non neoplastic	7.08	7.26	23.97	82.80
Case 7	Category 6-Malignant	Non neoplastic	7.16	7.32	24.15	85.25

Among 9 cases, 7 cases which was diagnosed as suspicious for malignancy and malignant lesion under Bethesda category 5 and 6, turned out to be colloid goiter and lymphocytic thyroiditis in histopathology. The mean maximal nuclear diameter ranged from 7.29

μm to 7.39 μm, mean minimal nuclear diameter ranged from 7.08 μm to 7.24 μm. The mean nuclear perimeter ranged from 23.97 μm to 24.52 μm. Mean nuclear area ranged from 82.80 μm^2 to 86.12 μm^2.

The nuclear parameters in 7 false positive cases were closer to the nuclear values of mean maximal nuclear diameter, mean minimal nuclear diameter, mean nuclear perimeter and mean nuclear area observed in non neoplastic group that is, 6.90±0.24 μm, 6.76±0.23 μm, 23.84±0.31 μm, 81.73±5.11 μm^2 respectively.

Nuclear morphometry is useful in improving the diagnostic accuracy of thyroid cytology in 9 cases.

PHOTOGRAPHS

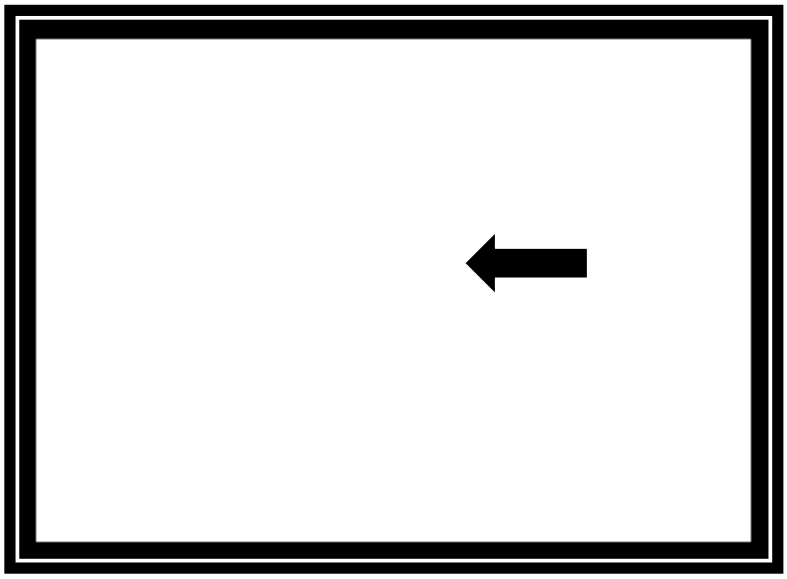

Figure 7: Microphotograph showing thyroid follicular cells in small clusters and singles against background of thin and thick colloid – Giemsa 100X, Nodular goiter

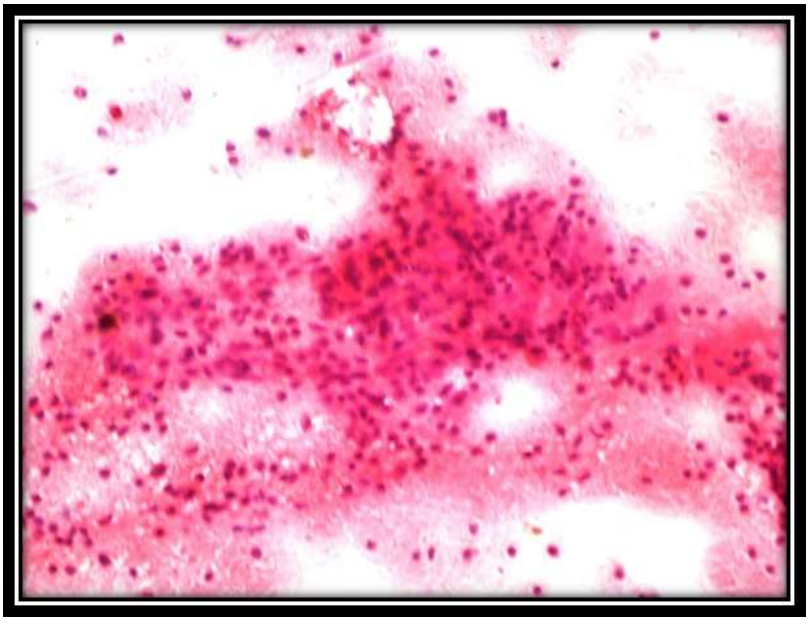

Figure 8: Microphotograph showing thyroid follicular cells admixed with lymphocytes and background show thin colloid H & E , 100 X– Lymphocytic thyroiditis.

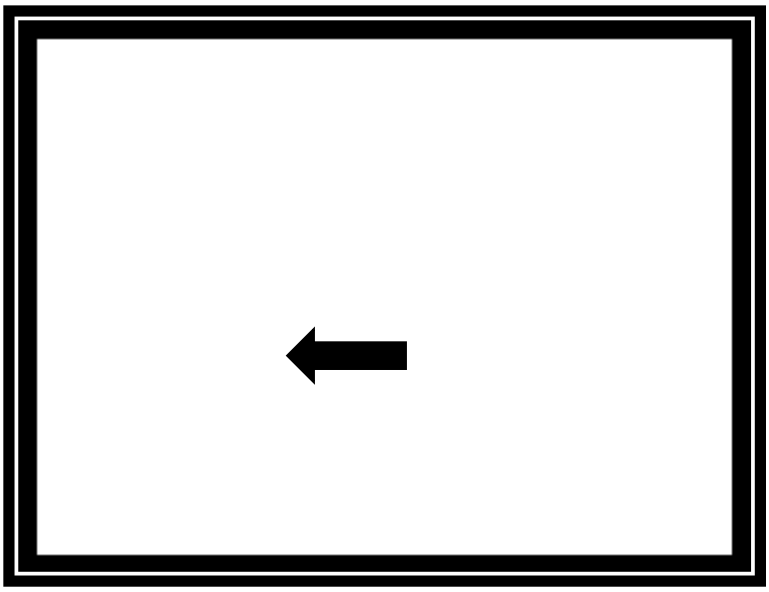

Figure 9: Microphotograph showing follicular neoplasm H&E 400X

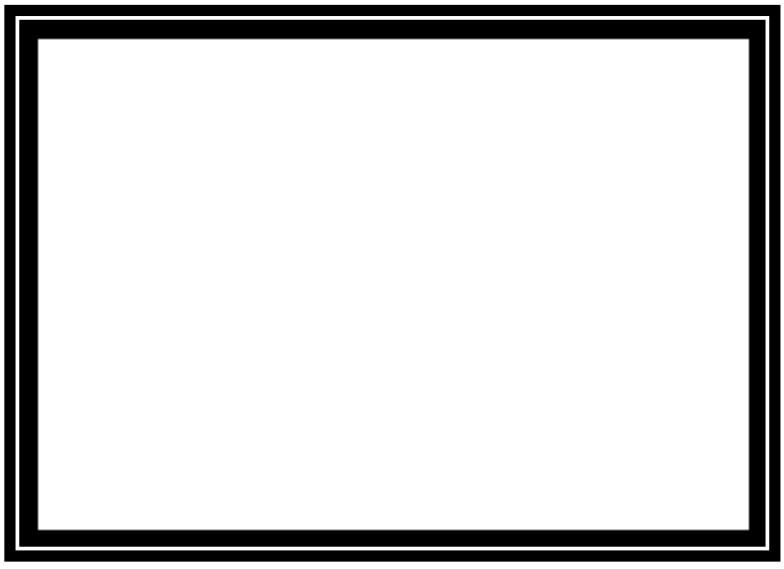

Figure 10: Microphotograph showing nuclear pleomorphism in follicular neoplasm. Giemsa 400X

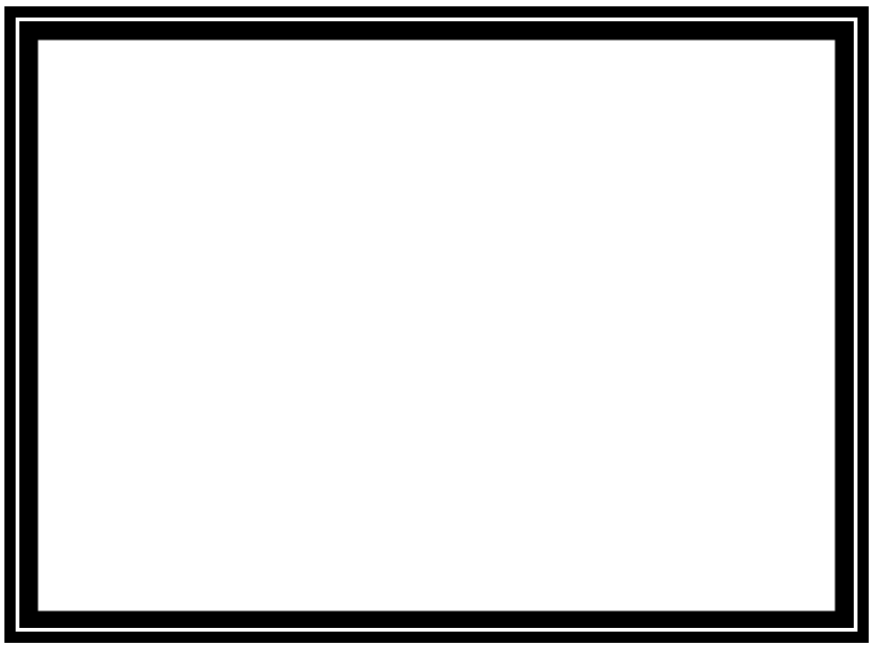

Figure 11: Microphotograph showing Cellular swirls .PAP X 400

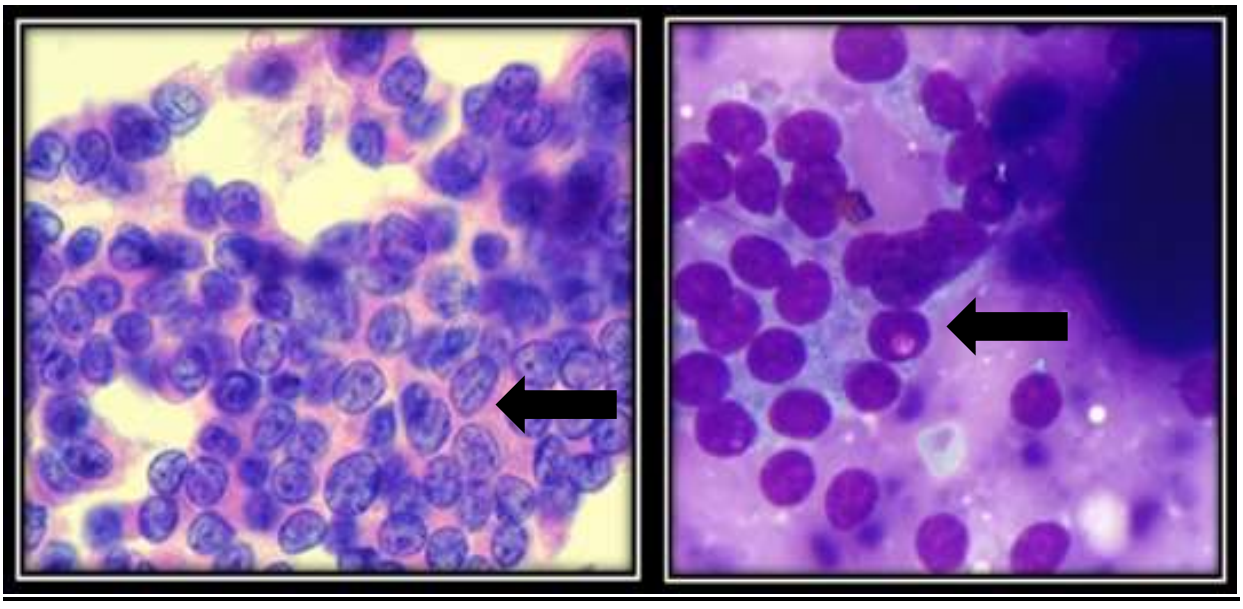

Figure 12 and 13: Microphotograph showing nuclear groove and nuclear inclusion. Giemsa 400X

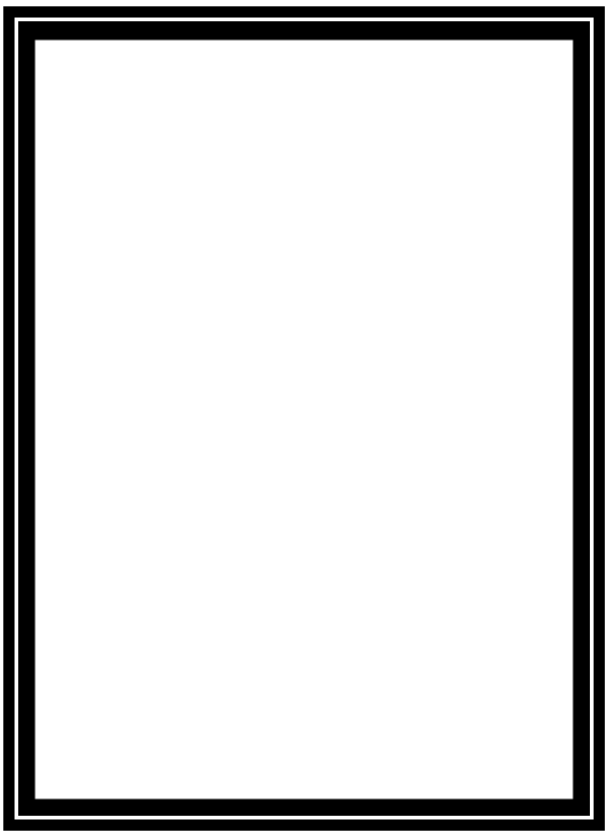

Figure 14: Photograph – Colloid goiter showing diffuse enlargement of thyroid gland with grey brown colloid filled areas with peripheral rim of thyroid tissue.

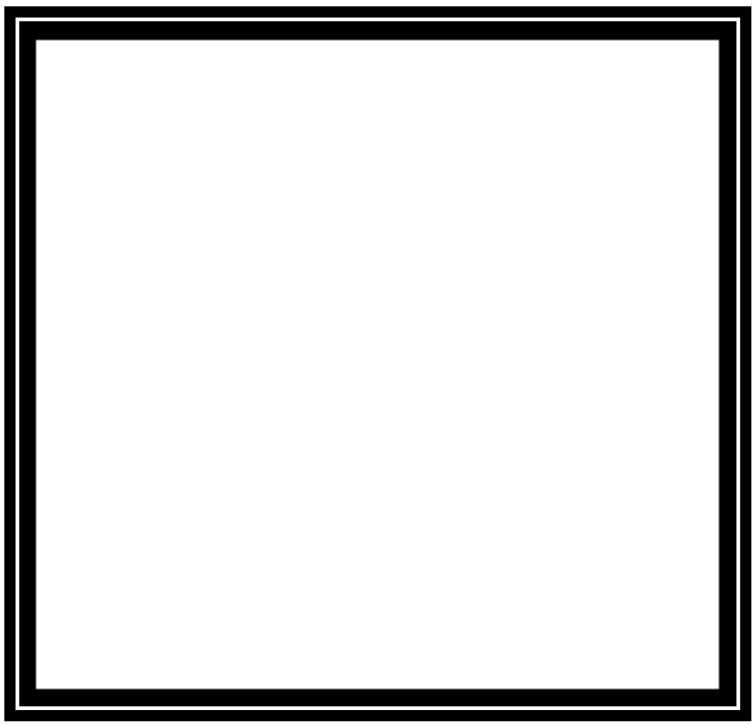

Figure 15: Photograph of follicular adenoma showing well circumscribed grey white nodule surrounded by grey brown colloid material

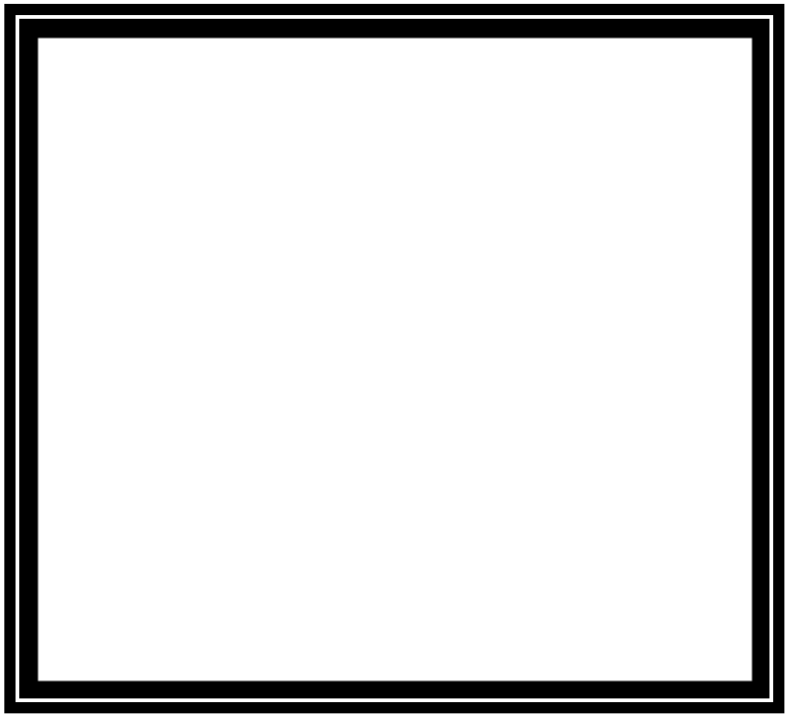

Figure 16: Photograph showing papillary excrescences along with solid, firm grey white areas with brownish colloid filled areas in the periphery – Papillary carcinoma thyroid.

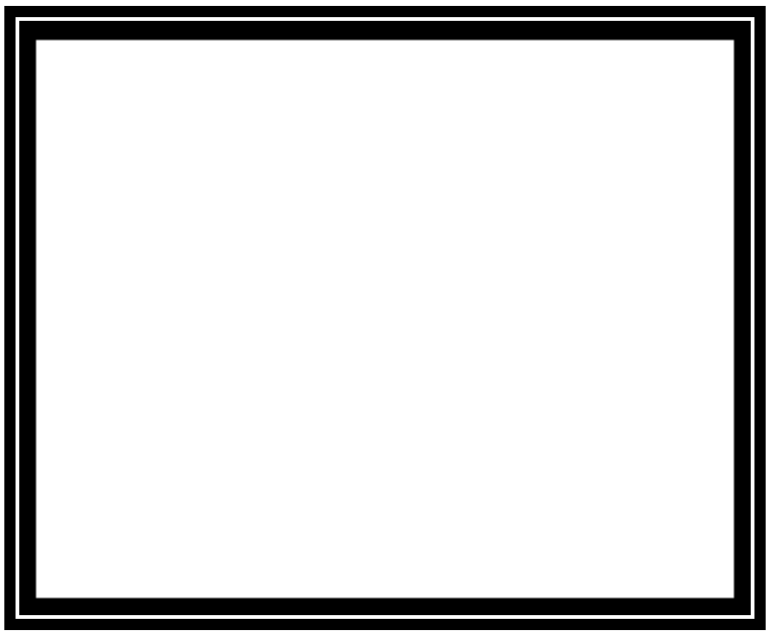

Figure 17: Photograph showing image analysis of nuclear morphometry – Nuclear length

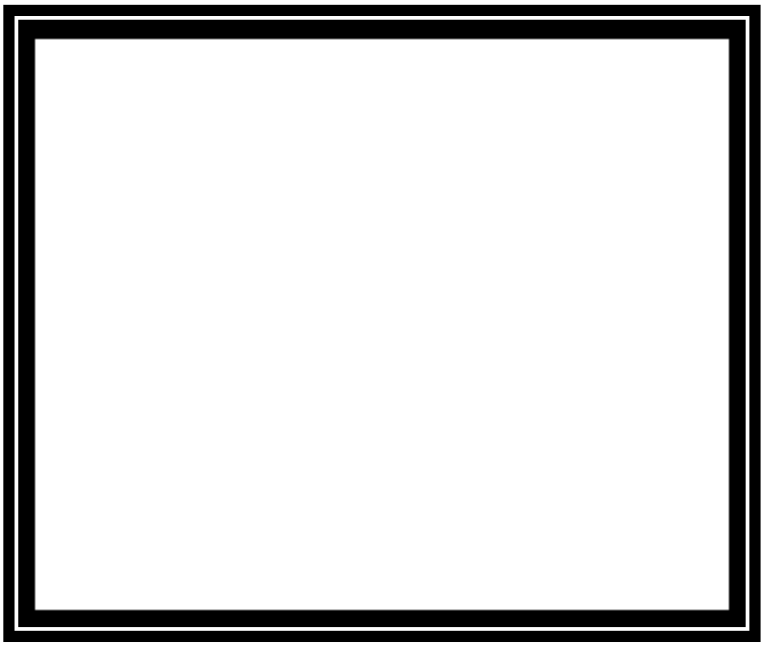

Figure 18: Photograph showing image analysis of nuclear parameters- nuclear diameter, perimeter and area

FNAC is a safe, simple and inexpensive technique and plays an important role in the diagnosis of thyroid lesions. The present study was conducted to know its accuracy in the diagnosis of thyroid neoplasm. Various parameters were compared with the results obtained by various studies.

Table 33: Comparison of age in present study with other studies:

Age (Years)	Tarrar et al[71]	Abdullah et al [72]	Robbani et al [73]	Present study
<20	5%	8%	16%	0%
21-30	25%	38%	28%	8%
31-40	40%	28%	26%	32%
41-50	18.34%	14%	16%	42%
51-60	8.33%	6%	10%	18%

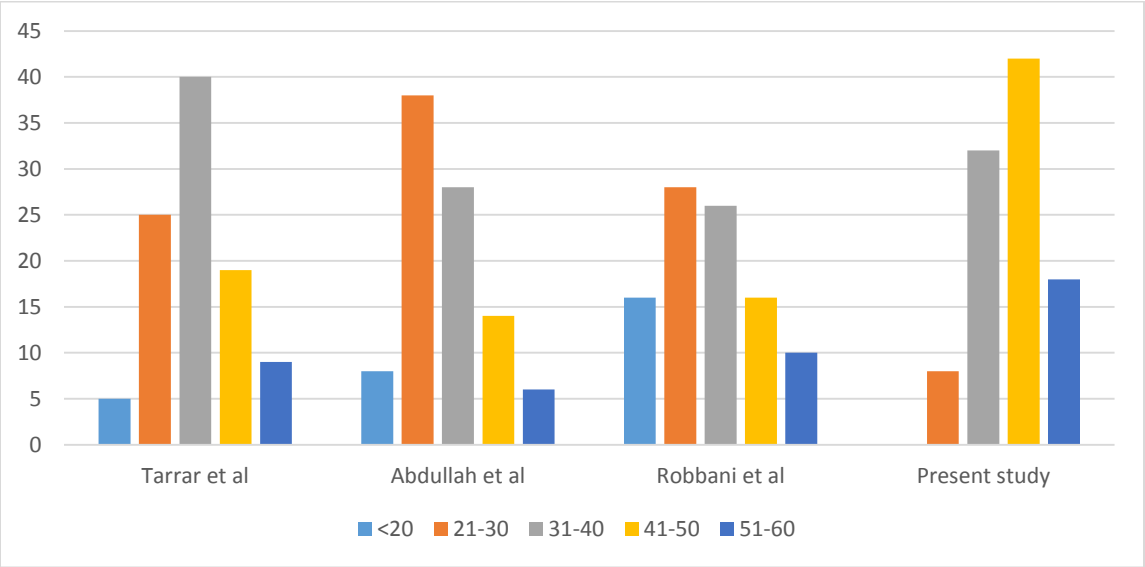

Chart 13: Comparison of age with other studies

Tarrar et al[71] study showed highest incidence in 4th decade accounting for 40% of cases. Abdullah et al[72] study showed highest incidence in 3rd decade. In our study majority of cases are in 5th decade, this could be due to selection criteria of only operated cases in present study.

Table 34: Comparison of sex ratio in present study with other studies

AUTHORS	Year of publication	Total number of cases (n)	Males	Females	M:F
Das DK et al[74]	2004	448	69	379	1:1.52
Sanjay Jogai et al[75]	2005	192	57	135	1:2.4
Gupta C et al[76]	2010	500	85	415	1:5
Present study	2015	120	20	100	1:5

Majority of study showed female preponderance in thyroid disease. The effects of female gonadal hormones like prolactin and estrogen action and X chromosome inactivation on thyroid gland and immune system greatly contribute to the female predilection of thyroid disorder.

Terminology for thyroid FNA has varied significantly among the pathologist and clinicians

To address terminology and other issues related to thyroid FNA, the National Cancer Institute (NCI) hosted the "NCI Thyroid FNA State of the Science Conference'' and introduced Bethesda system. A uniform reporting system for thyroid FNA will facilitate effective communication among cytopathologists, endocrinologists, surgeons, radiologists, and other health care providers. It also facilitates cyto-histologic correlation for thyroid diseases. The Bethesda System for Reporting Thyroid Cytopathology recommends 6 general diagnostic categories

Table 35: Comparison of percentage of distribution of FNA diagnoses according to Bethesda system among published studies

Diagnostic category	Present study (%)	Yassa et al (%)[28]	Yang et al (%)[26]	Nayar et al (%)[80]	Mario et al (%)[81]
Year of publication	2015	2007	2007	2009	2015
Category 2	65.43	66	64.6	64	23
Category 3	11.6	4	3.2	18	2
Category 4	2.5	9	11.6	6	17
Category 5	7.5	9	2.6	2	37
Category 6	11.6	5	7.6	5	16

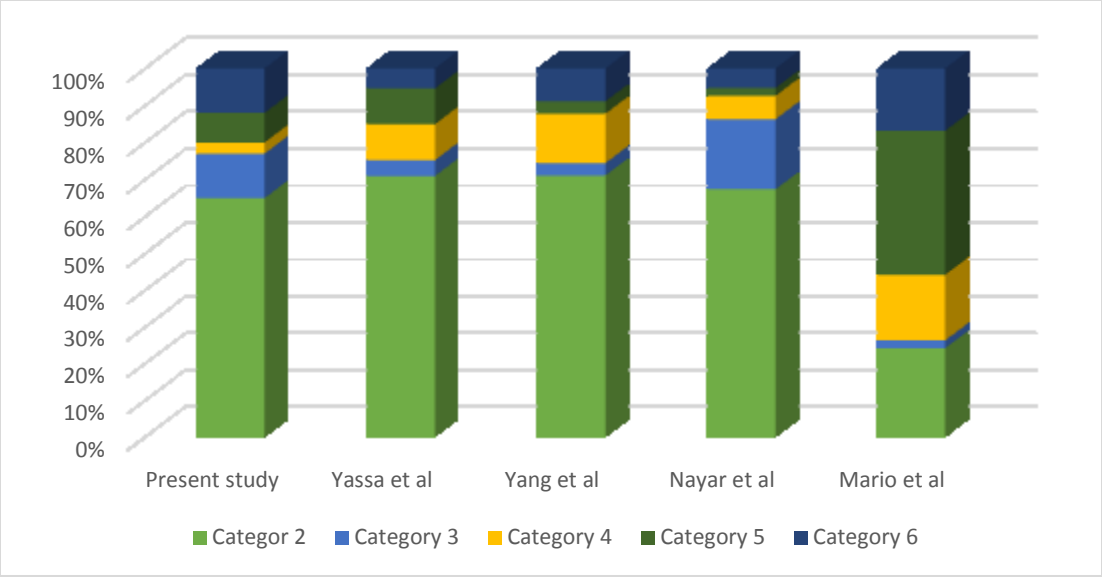

Chart 14: Comparison of cytological diagnosis under Bethesda system in various study

With above data, category 2 has the predominate number of cases in all the above study with distribution of 60-65%, followed by category 6. These finding correlated with the present study.

However the study by other author, showed higher incidence in category 5 and 6 compared to all other studies, as this study was conducted in an oncology hospital in Columbia.[81]

CATEGORY 2	Present study (%)	Jo et al[41] (%)	S K Monda et al[82] (%)
Nodular goiter	68.8	74	71
Lymphocytic thyroiditis	24.2	7.81	21.7
Granulomatous thyroiditis	1.2	-	1.11
Hyperplastic goiter	6.3	16	10

Table 36: Comparison of lesions in Bethesda category 2 system among published studies

In present study colloid /nodular goiter was predominant among the category 2, which was similar to other studies.

In the category 2, thyroid follicular cells were arranged in microfollicles and papillary structures in 10% cases and nuclear pleomorphism was seen in 32% and nuclear grooves were identified in 2 cases.

Bakhos et al[31] observed nuclear groves in 8% of cases and these consisted of suboptimal material and under diagnosis of papillary carcinoma due to cystic degeneration. This was supported by other study[83], where in due to the geographic miss of the needle localization may lead to lower accuracy and suggested that FNAC under ultra sound guidance, in cases of such lesion might help in identifying these lesions.

Table 37: Comparison of lesions in Bethesda category 3 system among published studies

	Present study, n (%)	Jo et al[41] (%)	Bongiovanni et al[84] (%)	Bohacek et al[85] (%)
Category 3	14 (11.6)	101 (3.4)	220 (6.3)	8 (0.8)

In the present study, there were 11.6% of cases which belonged to category 3, which was same as incidence seen in other studies. Among 14 cases of category 3 in present study, 7 cases were of follicular adenoma, 3 case of hyperplastic goiter and 2 case each of nodular goiter and papillary carcinoma on histopathology. In other study by shagufta et al, only 2 cases were diagnosed in category 3 and when correlated with HPE, one case turned out to be medullary carcinoma. Category 3 is a heterogeneous category, which reflects the difficulty in the cytological diagnosis of the follicular lesions of thyroid. The recommended management for category 3 is based on clinical correlation and require repeat FNA after an appropriate interval (22). A repeat FNA usually help in definitive diagnosis.

Table 38: Comparison of lesions in Bethesda category 4 system among published studies

	Present study, n (%)	Jo et al[41] (%)	Bongiovanni et al[84] (%)	Bohacek et al[85] (%)
Category 4	3 (2.5)	298 (10.0)	6 (2.4)	72 (3.8)

In the present study, the incidence of category 4 was 2.5%, which was similar to other studies except in study by Jo et al[41] who had higher incidence in category 4 in their study. Among 3 cases of category 4, one case turned out to be lymphocytic thyroiditis on HPE.

The criteria for reporting under Bethesda category 4 are significant alteration in the follicular cell architecture, characterized by cell crowding, micro follicles, and dispersed isolated cells and scant or absent colloid. The criteria for FN Hurthle cell type/suspicious for a FN

Hurthle cell type are a sample consisting exclusively of hurthle cells, usually little or no colloid or virtually no lymphocytes or plasma cells.[3]

The distinction between Follicular Neoplasm and Nodular goiter is the most common differential diagnostic problem in thyroid lesion on FNAC. A Microfollicle focus in a Colloid nodule looks identical in smears to microfollicular neoplasm.[3]

In cytology, follicular adenoma and follicular carcinoma have identical cytological features with follicular cell arranged in macrofollicular structure with high colloid content. There is uniform nuclear enlargement of follicular cells in follicular carcinomas compared to nuclear enlargement in follicular adenoma. However this is subjective in each case, and the definitive mode of differentiating these lesions is by HPE with follicular carcinoma showing capsular and vascular invasion, in contrast to well capsulated follicular adenoma lesion.[16, 20]

The false negative rate of FNA in the diagnosis of Follicular neoplasm may be 30% or more because of inability to recognize the normofollicular neoplasm.

Table 39: Comparison of cytological features of papillary carcinoma in present study with other study

Features	Tseng et al[86]	Chandanwale[87]	Present study
Papillary structures	76.3%	52.4%	54.4%
Monolayered sheets	100%	58.2%	80%
INCIs	31.6%	82.3%	59.12%
Nuclear grooves	53.3%	64.7%	68.18%
Nuclear pleopmorphism	56.7%	60.5%	54.44%
Psammoma bodies	2.6%	5.8%	4.6%

The cytological features which were observed in papillary carcinoma were similar to those observed in other study.[86] The percentage of cases which showed papillary structures, monolayered sheets and moderate cytoplasm were comparable with those observed in other study.[86] Intranuclear cytoplasmic inclusions and nuclear grooves were observed in more cases in the present study. One of the cases in the present study showed psammoma bodies and in 2.6% of cases in other study.[86]

In a study by Szporn et al[88] in 2006, cellular swirls was seen in seventeen of 100 FNAs (17%) of papillary carcinoma. No cases diagnosed as Hashimoto's thyroiditis, nodular goiter, or follicular neoplasm contained these structures. Hence it was concluded that cellular swirls are a finding that is highly specific to PTC. Kumar et al, [89] found cellular swirls in all the four cases of PTC in their study and remarked that it is a novel finding and when present are highly specific for PTC. In the present study, cellular swirls are seen in 66.6%of PTC cases and none in other cytological cases, thus implying its specificity to papillary carcinoma.

Out of the 14 cases of papillary carcinoma, 1 turned out to be nodular goiter and 1 as lymphocytic thyroiditis. These 2 cases on cytology, showed moderate cellularity cells arranged in clusters and ill-defined papillae. At focal areas, these cells showed intranuclear cytoplasmic inclusions and nuclear grooves which had led to the diagnosis of papillary carcinoma. In many other literatures, [26, 35, 74] nuclear groove and intranuclear inclusion were found in 3.4% of other lesions like nodular goiter, hyalinizing trabecular adenoma, follicular adenoma and medullary carcinoma. 4- 12% of False positive rate of were also noted in other studies[41, 90] and diagnosis of PTC was given on cytology, due to moderate cellularity, with nuclear groove and nuclear pleomorphism

Table 40: Diagnostic accuracy of FNAC in thyroid lesions compared with other studies

	Sensitivity (%)	Specificity (%)
Yang et al[26]	94	98
Mamon et al[30]	61	99
Gupta et al[76]	80	86
Present study	62.5	84.38

The sensitivity and specificity of present study is comparable with other studies. [30,76] The diagnostic accuracy of FNAC can be improved by adapting ultrasound guided FNAC thereby aspirating the representative sample.

Comparison of Percentages of Follow-up Malignancy Rates in different category of Bethesda system

Table 41: Comparison of Percentages of Follow-up Malignancy Rates in category 2 among Published Studies.

	Present study % (n=120)	Her-Juing Wu H et al[42] % (n=221)	Jo VY et al[41] % (n=892)	Lee K et al[90] % (n=905)	Bethesda system[3]
Year of publication	2015	2011	2010	2010	2009
Category 2	6.7	3	6.1	0	0-3%

The increased risk in present study is due to 2 of papillary carcinoma present as cystic degeneration and aspiration from such lesion did not reveal any feature of carcinoma in FNAC and rest 3 cases were of micropapillary carcinoma and missed due to aspiration from non-representative areas. Ultra sound guided FNAC help in providing representative sample in these cases.

Table 42: Comparison of Percentages of Follow-up Malignancy Rates in category 3 and 4 among Published Studies.

	Present study % (n=120)	Her-Juing Wu H et al[42] % (n=221)	Jo VY et al[41] % (n=892)	Lee K et al[90] % (n=905)	Bethesda system[3]
Year of publication	2015	2011	2010	2010	2009
Category 3 and 4	14.2	29.8	15.7	14.2	15- 30

The overall percentage of category 3 and 4 in this analysis was 11.6 %, with a 14.2% risk of malignancy. In other studies which were categorized as Follicular lesion of undetermined significance(FLUS) found a malignancy rate of 22% .[44, 45] In study, 11 cases (1.95%) were of this category out of which 7 cases were papillary carcinoma and 4 were follicular adenoma. This finding is compatible with rates given for thyroid FNAs in Bethesda system. [91] In Bethesda system, only repeat FNAC is advised in follicular lesion of undetermined significance (FLUS) category along with that a multidisciplinary approach to the management of cases diagnosed as FLUS including ultrasound, clinical workup and thyroid scan in addition to repeat FNAC.[3]

Table 43: Comparison of Percentages of Follow-up Malignancy Rates in category 5 among Published Studies.

	Present study % (n=120)	Her-Juing Wu H et al[42] % (n=221)	Jo VY et al[41] % (n=892)	Lee K et al[90] % (n=905)	Bethesda system[3]
Year of publication	2015	2011	2010	2010	2009
Category 5	50	48.2	54.5	60.5	60- 75

The incidence of malignancy in category 5 was 50% in present study, which is comparable with other studies. The treatment advised in this category is to surgical excision of the lesion due to increased risk of carcinoma.

Table 44: Comparison of Percentages of Follow-up Malignancy Rates in category 6 among Published Studies.

	Present study % (n=120)	Her-Juing Wu H et al[42] % (n=221)	Jo VY et al[41] % (n=892)	Lee K et al[90] % (n=905)	Bethesda system[3]
Year of publication	2015	2011	2010	2010	2009
Category 6	86.36	48.2	54.5	60.5	97-100

The incidence of malignancy in present study is comparable with other studies and are within the range advised by Bethesda. [3, 41, 42, 90] The treatment advised in this category is to surgical excision of the lesion.

COMPUTERIZED NUCLEAR MORPHOMETRY:

Although, the reporting system is associated with high prognostic potential and improved reproducibility, few of the follicular lesions of thyroid lie within grey zones due to the pathologist's subjective factors. In this view quantification of nuclear parameters will help in improving the diagnosis. Computerized morphometry is an objective computer image analysis to estimate the choosed parameters in every individual cells.

Doroto et al[92] studied thyroid aspirates from histologically confirmed cases of follicular adenoma and correlated the nuclear parameters like nuclear volume and nuclear perimeter with the age of the patient. They found no parameters correlated with age of the patient.

Similar to the present study, an author studied 44 giemsa stained FNAC cases and for morphometry pictures were captured at 200X magnification analyzed using aperio slide scanner. It was noted that the malignant category thyroid FNA had higher nuclear diameter compared with non neoplastic cases and the N/C ratio was > 0.5 in malignant cases and <0.5 in benign cases.[93] The results are very similar to the present study where the mean minimal and maximal nuclear diameter are higher in papillary carcinoma when compared to non- neoplastic lesion and follicular neoplasm.

In present study the nuclear parameters are measured and is tabulated in micrometer i.e, (1pixel=0.0502 micrometer). The nuclear diameter, nuclear area and nuclear perimeter which identify the nuclear size variations are 6.96 µm ±0.24, 81.73 µm ±5.11, 23.84 µm ±0.31 respectively for non - neoplastic group and 8.67 µm ±0.76, 114.26 µm ±18.99, 27.04 µm ±1.32 respectively for malignant group. These parameters show statistically significant difference between the two groups. Karslioglu et al, studied 112 FNAC cases in PAP stain, 100 cells were counted and values in pixels were converted to micrometer (1 pixel _ 0.206612 mm). The

Minimum diameter, maximum diameter and nuclear perimeter were significantly different across benign and malignant groups.[94] This finding was in agreement with other study and concluded that nuclear perimeter and area are two important parameters which help in distinguishing the benign and malignant category.[95]

In present study, the mean minimal and maximal nuclear diameter among the benign and malignant group showed statistically p value<0.001, but when the maximum/ minimum diameter ratio was considered it did not show any statistically higher values. Whereas in other study of 25 FNAC cases, with 200 cells, maximum/ minimum diameter of malignant category was greater than that of adenomatoid goiter.[96]

An author studied 36 FNAC cases at 200× magnifications and 10 nuclei per case were measured. The coefficient of variation of the nuclear area along with mean nuclear diameter and perimeter showed significant differences between all the groups, which is similar to our study where the nuclear area showed significant difference among benign – adenomatoid goiter, follicular lesion and malignant papillary carcinoma. They also noted the circular rate/perimeter was significantly higher with adenomatous goiter than with follicular neoplasm and in papillary carcinoma.[67] This was in contrast to present study where in nuclear perimeter was higher among malignant group. This might be due to the difference in the methodology wherein other author, have used circular rate for a perimeter measurement of 10 nuclei in only 10 cases.

Nagashima et al [69]also evaluated variation in nuclear size by using NACV which showed significant differences among follicular carcinomas, adenomas and adenomatous goiters.

Table 45: Comparison of nuclear morphometry result with other study.

	Methodology	Methodology	Lesions	Parameters (μm)	Present study	Rajesh et al
	Present study	Rajesh et al[97]				
Total no of cell/case	100	100	Non neoplastic lesion	N perimeter	23.90	22.67
				N area	81.73	34.85
Magnification	400X	400X	Benign neoplastic	N perimeter	24.62	27.58
				N area	84.07	48.48
1 pixel value	0.0502	0.446	Papillary carcinoma	N perimeter	27.04	27.91
				N area	114.26	49.16

In a study by Rajesh et al [97] H&E stained 40 FNAC aspirates were considered and 100 cells per case was captured at 400X for morphometry and measured values in pixels were converted to micrometer with value of 1 pixel = 0.446 micrometer. They found that convex nuclear area and perimeter of follicular hyperplasia were much lower than that of follicular neoplasm and follicular variant of papillary carcinoma. However these parameters overlap between benign neoplastic lesion – follicular adenoma and papillary carcinoma. In present study nuclear parameters like nuclear diameter, nuclear perimeter and area had statistically significant difference among non -neoplastic and malignant group and between non neoplastic and benign malignant group. Though in both study the methodology followed is similar, the difference exist in measurement of convex area by rajesh et al[97] and in present study, we have considered the complete area of a cell.

In contrast to all the above studies, back in In 1990, La Rosa GL et al [98] classified follicular thyroid lesions into three groups (goiter, adenoma and follicular carcinoma) and studied 100 to 200 thyroid cells, for nuclear areas (mean, maximum and minimum area). Mean values of nuclear areas showed significant differences among the three groups, but a

considerable overlap occurred in the size distribution of cell nuclei. They concluded that measurements of cell nuclei in fine needle aspirates do not improve the accuracy cytological examination in follicular thyroid lesions. This default in overlap of values can be explained due to use of planimetric type of morphometric analysis back then. Now with development of image analysis and morphometric software, nuclear morphometry has slowly gained its value.

In present study, the mean nuclear areas and perimeters of cells for papillary carcinomas (114.26 μm 2, 27.04 μm) were significantly larger than those of nodular goiter in benign group (81.73 μm^2,23.84 μm)which was similar to other study wherein 25 thyroid cells were evaluated. The mean nuclear areas and perimeters of cells from follicular (50.1 pm2, 26.1 pm) and papillary carcinomas (57.1pm2, 27.8pm) were significantly larger than those from multinodular goiter (43.6pm2, 24.2pm) and the mean areas and perimeters of follicular adenoma nuclei were significantly larger than nuclei from multinodular goiter.[66]

In the present study within the variants of PTC, all the cases showed larger nucleus with higher nuclear area of 114.26±18.99$\mu m^2$with irregular shape and nuclear groove. This was in concordance with observation of an author on 39cases, wherein they concluded, nuclei of papillary carcinoma were larger size, more irregular shape, and higher contrast of chromatin pattern than those of the benign group.[68] The follicular carcinomas have larger nucleus and more monotonous chromatin pattern than those of the benign group which was similar to the present study.

In the present study, among the variants of papillary carcinoma, tall cell variant showed higher nuclear diameter, perimeter and area of 8.82 μm, 28.52 μm, 120.71 μm^2 respectively when compared to conventional variant which had 8.67 μm, 26.64 μm, 114.26 μm^2 respectively. Though the values are higher in tall cell variant there was no statistical significance

noted. This could be because only one case of tall cell variant observed in the study and this can be improved with larger number of cases. Dina et al[99] studied 5 cytology cases of tall cell variant of papillary carcinoma and 14 cases of conventional papillary carcinoma and found that nuclear area and nuclear diameter was higher in tall cell variant compared to conventional PTC. Sofia Asioli et al [100], studied histological sections of variants of PTC and observed that oncocytic variant of PTC had higher nuclear parameters and these findings correlated with vascular and capsular invasion which are the poor prognostic factors and therefore concluded cell size assessment could thus be proposed as an inexpensive parameter for predicting prognosis and planning therapy. Though the methodology is different in both studies, the point of importance is few variants like tall cell, oncocytic variant of papillary carcinoma have poorer prognosis and a simple method of nuclear morphometry will help in identifying these lesions and in their outcome.

Nuclear morphometry is helpful in cases which are of diagnostic challenge on cytology due to the overlapping cytological features. On applying morphometry in 9 such cases in present study, it was found that 2 false negative cases on cytology had nuclear parameters values like mean maximal nuclear diameter - 7.62 and 7.65, mean minimal nuclear diameter - 7.43 and 7.52,mean nuclear perimeter- 25.62 and 25.82 and mean nuclear area - 88.28 and 89.10 in each case respectively. These morphometric parameter are comparable with nuclear parameters of malignant group.

When morphometry applied on 7 false positive cases the values for each parameter ranged from mean maximal nuclear diameter - 7.29 to 7.39, mean minimal nuclear diameter - 7.08 to 7.24, mean nuclear perimeter - 23.97 to 24.52 and mean nuclear area - 82.80 to 86.12. These morphometric nuclear parameter are comparable with morphometric parameters of non

neoplastic group.Whenever there is dilemma in diagnosing the lesion on cytology to various categories under Bethesda system, An adjunct of nuclear morphometry along with cytological features can be helpful.

Nuclear morphometry on histopathological slides have become far more popular and applied in many different lesions. In a study on 48 histopathological thyroid specimen, 100 nuclei were analyzed and computed in micrometer. The neoplastic group (FA, FC, FVPC) showed significantly higher mean values of nuclear parameters related to size like nuclear area, nuclear perimeter, Maximum diameter, Minimum Diameter, nuclear size) when compared to the non-neoplastic group.[101] These finding were similar to those with other studies.[102]

Delia et al, studied 40 thyroid cases on histopathology, and 50 cells were counted. In nodular goiter, the nuclear diameter and the nuclear area were 95.4pixels, 6253.3pixels, in follicular adenoma the nuclear diameter and the nuclear area were 97.9 pixels, 6625.3 pixels and in follicular carcinoma the nuclear diameter and the nuclear area were 108.9 pixels and 8087.0 pixels. They concluded that nuclear area and the nuclear diameter, in the cases of follicular carcinoma, are significantly larger than in the case of any other type of thyroid nodular diseases.[103]This results were in agreement with other study.[104]

There is no cut off measurement which differentiate between non neoplastic, benign and malignant groups. This is because researchers have used different methodology for calculating nuclear parameters. Multicentric study using standardized method may help in inclusion of nuclear morphometry in diagnostic cytology.

SUMMARY

This study entitled "Cytological evaluation of thyroid lesions by nuclear morphology and nuclear morphometry" was carried out at RLJH, kolar from 2010 to may2015. It included 120 FNAC cases of thyroid lesion which had histopathological correlation.

The salient features of the study were:

1. Age range of the patients was between 30- 60 yrs and more common in female, with female to male ratio being 5:1
2. All the FNAC cases were categorized according to Bethesda system.
3. On FNAC, out of the 120 cases, 73(60.83%) were category 2,14(11.6%) were category 3, 3(2.5%) were category 4 , 8(6.6%) were category 5- SFM and 22(18.3%) were category 6- malignant.
4. In category 2, nodular goiter was the most common lesion comprising of 69% and in category 6 papillary carcinoma was commonest lesion comprising of 86.8%.
5. On histopathology, non neoplastic was 46.6% and neoplastic lesion was 54.6%.among non neoplastic lesions, nodular goiter (41.5%) was commonest lesion and among neoplastic lesions papillary carcinoma (53.5%) was the commonest lesion.
6. On histopathological correlation, the malignancy rates in category 2 was 6.7%, category 3 was 14.2%, category 5 was 50% and category 6 was 86.6%. Our findings are within the limits proposed by Bethesda system.
7. The sensitivity and specificity in the present study was 62.5% and 84.3% respectively.

8. The most common diagnostic pit fall in present study was due to aspiration from non representative area in 30 cases.
9. Computerised Nuclear morphometry was done on 81 cases which correlated both on cytology and histopathology. For morphometry, 100 nuclei per case from fixed smears was considered. All was classified under three groups – Non neoplastic, Benign and malignant group.
10. Photographs was captured at 400X and parameters was analysed using software. 4 morphometric parameters, i.e, Minimal Nuclear Diameter, Maximal Nuclear Diameter, Nuclear Perimeter and Nuclear Area were measured.
11. Other 4 parameters such as Axis Ratio, Nuclear Compactness, Shape Factor and Nuclear Size were calculated from the above parameters.
12. The mean Minimal Nuclear Diameter, Maximal Nuclear Diameter in non neoplastic, benign neoplastic and malignant group was 6.76 μm and 6.90 μm, 7.23 μm and 7.37 μm, 8.50 μm and 8.67 μm respectively.
13. The mean nuclear perimeter and mean nuclear area in non neoplastic, benign and malignant group was 23.84 μm and 81.73 μm^2, 24.62 μm and 84.07 μm^2, 27.04 μm and 114.26 μm^2 respectively.
14. The malignant group showed higher Minimal Nuclear Diameter, Maximal Nuclear Diameter, Nuclear Perimeter and Nuclear Area compared to non- neoplastic group.
15. The malignant group showed higher Minimal Nuclear Diameter, Maximal Nuclear Diameter, Nuclear Perimeter and Nuclear Area compared to benign neoplastic group.

16. Among the calculated nuclear parametrs, mean nuclear size was highest in malignant group group (12.00 μm) followed by benign neoplastic group (10.11 μm) and non neoplastic group (9.84 μm).
17. The nuclear parameters like Minimal Nuclear Diameter, Maximal Nuclear Diameter, Nuclear Perimeter and Nuclear Area in tall cell variant of papillary carcinoma was 8.64 μm, 8.82 μm,28.52 μm and 120.71 μm^2 . These parameters was higher in tall cell variant compared to conventional papillary carcinoma.
18. The morphometric nuclear parameter of 7 false positive cases was comparable with morphometric parameters of non neoplastic group and parameter like mean maximal nuclear diameter ranged from - 7.29 to 7.39 μm, mean minimal nuclear diameter - 7.08 to 7.24 μm, mean nuclear perimeter - 23.97 to 24.52 μm and mean nuclear area - 82.80 to 86.12 μm^2 .
19. The morphometric nuclear parameters of 2 false negative cases had mean maximal nuclear diameter - 7.62 μm and 7.65 μm, mean minimal nuclear diameter - 7.43 μm and 7.52 μm,mean nuclear perimeter- 25.62 μm and 25.82 μm and mean nuclear area - 88.28 μm^2 and 89.10 μm^2 in each case respectively and these morphometric parameter were comparable with nuclear parameters of malignant group.
20. Nuclear morphometry was useful in 9 cases (7 false positive and 2 false negative) and helped in Bethesda categorization.

Nuclear morphometry can be used in objective diagnosis of thyroid lesions. The present study focused on the quantitative criteria for nuclear grading in thyroid lesions and by this means to improve the consistency and the accuracy of the diagnosis. Quantitation of nuclear parameters is a powerful tool that may be an adjunct to other ancillary test in diagnosis.

CONCLUSION

Our study further confirms the utility of Bethesda system of reporting in the clinical management of thyroid swelling. This standardized system of reporting gives risk of malignancy in each category. Although there are diagnostic challenge in thyroid cytology, ultrasound guided FNAC, nuclear morphometry and clinical features helps in improving the diagnostic accuracy.

Nuclear size parameters (Mmnd, MMND, MNP, MNA and MNS) were significantly different in non neoplastic and neoplastic group particularly mean nuclear diameter, mean nuclear perimeter and mean nuclear area. Nuclear morphometry is useful especially in diagnostic dilemma cases. It improves diagnostic accuracy of thyroid cytology in detecting the malignancy. Nuclear morphometric parameters can be used as an ancillary diagnostic tool in thyroid lesions.

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PROFORMA

NAME: **CASE NO:**

AGE/SEX:
HOSPITAL NO:

PRESENTING COMPLAINT:
CYTOLOGY NO:

CLINICAL DIAGNOSIS:
BIOPSY NO:

THYROID FUNCTION TEST: T3: T4: TSH:

CYTOLOGY:

BETHESDA CATEGORY:

I IV

II V

III VI

CYTO-MORPHOLOGICAL FEATURE:

NUCLEAR MORPHOMETRIC PARAMETERS:

MEAN NUCLEAR SIZE:

MEAN NUCLEAR PERIMETER:

MEAN MINIMAL NUCLEAR DIAMETER:

MEAN MAXIMAL NUCLEAR DIAMETER:

HISTOPATOLOGICAL DIAGNOSIS:

IMPRESSION:

KEY TO MASTER CHART

Column A- Hospital number

Column B- Age

Column C- Sex

0-male, 1- female

Column D- Histopathology number

Column E- Histopathology diagnosis

NG- nodular goiter, CG- colloid goiter, NH- nodular hyperplasia, HT/LT- hashimotto/Lymphocytic thyroiditis, HTA- hyalinizing trabecular adenoma,FA- follicular adenoma, FC- follicular carcinoma, PCT- papillary carcinoma, Micro PCT- micropapillary carcinoma thyroid, FV-PCT- follicular variant papillary carcinoma, tall PCT- tall cell variant papillary carcinoma

Column F – Cytology number

Column G- Cytology diagnosis

CG- colloid goiter, NH- nodular hyperplasia, HT/LT- hashimotto/Lymphocytic thyroiditis, FN- follicular neoplasm, PCT- papillary carcinoma, susp pct- suspicious of papillary carcinoma thyroid

Column H – cellularity

0- Low, 1-moderate, 2- high

Column I- Arrangement, Singles, 1- cluster, 2- follicles, 3- papillary

Column J- Nuclear groove

0-absent, 1-present

Column K- Nuclear inclusion

0-absent, 1-present

Column L- Nuclear pleomorphism

0-mild, 1-moderate, 2- severe

Column M – Colloid

0-scant, 1-moderate, 2- abundant

Column N- Bethesda category, CAT- Category

Column O- Other features, MNG- Multinucleated giant cell

Column P- MMND**-** Mean Maximum Nuclear Diameter, µm

Column Q- Mmnd- Mean Minimal Nuclear Diameter, µm

Column R- MNP- Mean Nuclear Perimeter, µm

Column S- MNA-Mean Nuclear area, μm^2

Column T- MNA-Mean Axis ratio µm

Column U- MNC-Mean nuclear Compactness

Column V- MSHF- Mean Shape factor

Column W- MNS- Mean Nuclear size

HOSPITALNUMBER	AGE	SEX	HPENUMBER	HPEDIAGNOSIS	CNUMBER	CDIAGNOSIS	CELLULARITY	ARRANGEME	NGROOVE	NINCLUSION	NPLEOMORP	COLLOID	BETHESDACA	OTHERFEATURES	MMND	Mmnd_A	MNP	MNA	MAR	MNC	MSHF	MNSFinal
78126	35	1	2942/14	CG	2467/14	CG	1	0,1	0	0	0	2	CAT 2		6.97278	6.78704	23.51368	75.56543	0.973362	7.316747	1.693471	9.811305
78028	38	0	2748/14	CG	2376/14	CG	0	0	0	0	0	2	CAT 2		6.4256	6.28002	22.63518	117.7295	0.977344	4.351937	2.462773	12.24638
831608	56	1	1803/12	CG	1652/12	CG	1	0,1	1	0	1	0	CAT 2	ONCOCYTES	7.10832	6.89748	23.97552	77.76839	0.970339	7.391506	1.725257	9.953292
909657	40	0	1066/13	CG	887/13	CG	1	0,1	0	0	0	1	CAT 2		6.99788	6.86736	23.94038	89.25796	0.981349	6.421184	1.883741	10.66323
98231	35	1	198/15	CG	221/15	CG	1	0,1	0	0	0	2	CAT 2	MNG	6.86736	6.76696	23.82492	114.2073	0.98538	4.970145	2.281881	12.0618
99924	38	1	994/15	CG	815/15	CG	1	0,1,2	0	0	0	2	CAT 2	CYST MACROPHAGES	6.71676	6.61636	23.6693	110.2097	0.985052	5.083362	2.227079	11.84882
1001087	45	1	915/14	HT	662/14	LT	1	0,1	0	0	0	1	CAT 2	ONCOCYTES	7.04808	6.95772	24.01568	78.65844	0.987179	7.332372	1.714744	10.01009
1019800	50	1	3024/14	LT	2721/14	LT	0	0	0	0	0	1	CAT 2		6.93764	6.76696	23.83496	79.3293	0.975398	7.161356	1.734135	10.05268
72391	45	1	2836/14	LT	2524/14	LT	1	0,1	0	0	0	1	CAT 2	LYMPHOCYTES	7.10832	6.89748	24.00564	69.77555	0.970339	8.258922	1.552598	9.42794
761369	35	0	1427/12	LT	677/12	LT	1	1	0	0	0	1	CAT 2		7.01796	6.93262	24.04078	63.71333	0.98784	9.071243	1.428442	9.009078
810720	50	1	1624/12	LT	1762/12	LT	1	0,1,2	0	0	1	0	CAT 2	LYMPHOCYTES, ONCOCYTES	7.18864	7.06314	24.11106	78.43545	0.982542	7.411741	1.698499	9.995888
813696	48	1	1574/12	LT	1241/12	CG	1	1,3	0	0	1	1	CAT 2	LYMPHOCYTES	6.94768	6.74688	23.81488	78.88174	0.971098	7.189858	1.726884	10.02429
823581	35	1	1506/12	LT	1461/12	LT	1	0	0	0	0	2	CAT 2		6.92258	6.83724	23.93536	78.43545	0.987672	7.304114	1.703148	9.995888
829776	45	1	1584/12	LT	1597/12	LT	1	0,2	0	0	1	0	CAT 2	LYMPHOCYTES	7.03302	6.86736	23.90524	81.35896	0.976445	7.023941	1.76959	10.18047
714864	50	1	2383/11	LT	2184/11	LT	1	0,2	0	0	0	1	CAT 2	LYMPHOCYTES	7.04306	6.9025	23.99058	80.67956	0.980043	7.133752	1.754404	10.13788
588205	30	1	944/10	LT	802/11	LT	2	1,3	0	0	1	0	CAT 2	LYMPHOCYTES	6.87238	6.73684	23.80484	79.66579	0.980278	7.113096	1.743694	10.07398
567214	50	1	127/10	LT	119/10	LT	1	0,1	0	0	0	1	CAT 2		6.93262	6.82218	23.89018	82.8942	0.98407	5.771225	2.048878	10.27608
572988	55	1	406/10	LT	1564/10	LT	1	0,1	0	0	0	1	CAT 2	LYMPHOCYTES	7.0531	6.93764	24.02572	85.77148	0.98363	5.903923	2.019384	10.4529
631122	50	1	1033/10	LT	2269/10	LT	1	1,3	0	0	1	1	CAT 2	LYMPHOCYTES	7.07318	6.92258	23.99058	81.81348	0.978708	7.034879	1.775729	10.20887
1043	38	0	1203/14	LT	1071/14	LT	1	0,1	0	0	1	1	CAT 2	LYMPHOCYTE	7.05812	6.94768	24.04078	90.63057	0.984353	4.390767	2.47635	10.7449
59405	48	1	2555/14	LT	2259/14	CG	1	0,1	0	0	0	1	CAT 2		6.98784	6.84728	23.90524	81.13218	0.979885	7.043574	1.76686	10.16627
99100	40	0	358/15	LT	280/15	LT	1	1,2	0	0	0	1	CAT 2	LYMPHOCYTES	6.84728	6.63644	23.7195	80.67956	0.969208	6.973448	1.767085	10.13788
99583	35	1	810/15	LT	452/15	LT	1	1,2	0	0	1	0	CAT 2	ONCOCYTES, LYMPHOCYTES	6.9276	6.83724	23.9203	74.15048	0.986957	7.71648	1.63558	9.719013
98484	48	1	986/15	LT	614/15	LT	1	0,1	0	0	0	1	CAT 2		7.04808	6.93764	24.03576	91.33622	0.98433	4.536947	2.398663	10.78665
70282	40	1	3221/14	NG	2501/14	CG	1	0	0	0	0	2	CAT 2		7.03804	6.86736	23.61408	84.53882	0.975749	5.49174	2.122806	10.37751
898375	45	1	1369/13	NG	915/13	CG	1	0	1	0	1	1	CAT 2	FIRE FLARE	7.14848	6.98784	24.12612	75.45612	0.977528	7.714016	1.644285	9.804205
677232	38	0	1021/12	NG	835/12	CG	1	0,2,1	0	0	0	2	CAT 2	CYST MACROPHAGE, MNG	6.91756	6.7519	24.00564	88.75976	0.976052	4.47555	2.43028	10.63343
832340	60	1	1793/12	NG	1666/12	CG	1	0,1	0	0	0	2	CAT 2		6.4758	6.30512	23.38316	78.10157	0.973643	7.000784	1.746103	9.97459
833519	28	1	1716/12	NG	1699/12	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGE	6.6013	6.4256	23.49862	79.55355	0.973384	6.94105	1.771306	10.06688
841604	25	1	1830/12	NG	1856/12	CG	1	0,1,2	0	0	1	2	CAT 2	CYST MACROPHAGES	6.66656	6.45572	23.51368	78.5469	0.968373	7.039019	1.748869	10.00299
856380	38	0	1945/12	NG	2191/12	CG	1	0,1	0	0	1	0	CAT 2	LYMPHOCYTES	7.06816	6.85732	24.05584	66.45338	0.97017	8.708111	1.47559	9.20076
629667	40	1	1983/12	NG	2220/12	CG	1	0,1,2	0	0	0	2	CAT 2		7.08824	6.9527	24.09098	76.223	0.980878	7.614176	1.663769	9.853901
849668	45	1	463/13	NG	346/13	CG	1	1,3	0	0	1	1	CAT 2		6.50592	6.46576	23.51368	85.07535	0.993827	5.877131	1.900183	10.41039
904901	52	1	870/13	NG	765/13	CG	0	0	0	0	0	1	CAT 2		6.96776	6.8021	23.88516	65.22846	0.976225	8.746195	1.46374	9.115568
909723	48	1	1001/13	NG	905/13	CG	1	0,1	0	0	0	2	CAT 2		6.70672	6.53604	23.59902	64.72144	0.974551	8.60478	1.473129	9.080071
898375	45	1	1369/13	NG	915/13	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGES, MNG	6.44568	6.36536	23.43336	73.7178	0.987539	7.448979	1.657727	9.690616
936975	45	0	1885/13	NG	1467/13	CG	0	0	0	0	0	2	CAT 2		6.42058	6.3001	23.38316	88.25272	0.981235	4.436188	2.405693	10.60301
876692	35	1	1954/13	NG	99/13	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGES	6.61636	6.48584	23.58898	76.40208	0.980273	4.906788	2.253301	9.865469
918782	45	1	1237/12	NG	1103/13	CG	1	0	0	0	0	2	CAT 2		6.86736	6.73684	23.88516	80.45372	0.980994	7.091044	1.751372	10.12368
949188	50	1	1937/12	NG	1684/13	CG	1	2,3	0	0	1	2	CAT 2		6.92258	6.74186	23.81488	82.61193	0.973894	6.865213	1.798428	10.25856
987532	52	1	34/14	NG	248/14	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGE	6.8021	6.66656	23.73456	82.61097	0.980074	4.915143	2.286484	10.2585
948718	55	1	393/14	NG	136/14	CG	2	0,1	0	0	1	2	CAT 2	CYST MACROPHAGE	7.06816	6.9527	24.10604	77.32527	0.983665	7.515023	1.684297	9.924894
1014132	40	1	948/14	NG	991/14	CG	2	0,1,3	1	0	1	0	CAT 2	MICROFOLLICLES	7.75088	7.5551	25.12008	76.88341	0.974741	8.207472	1.605721	9.896497
913114	48	1	1061/14	NG	969/14	CG	0	0	0	0	0	2	CAT 2	CYST MACROPHAGE	6.86736	6.64648	23.70946	81.92731	0.967836	6.86143	1.794428	10.21597
1013087	60	1	1077/14	NG	960/14	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGE	6.92258	6.75692	23.82492	91.65083	0.97607	6.193363	1.927269	10.80521
722	55	1	1082/14	NG	1063/14	CG	1	0,1	0	0	1	0	CAT 2		6.82218	6.68664	23.7697	78.24	0.980132	4.749437	2.342717	9.983426
1836	32	1	1098/14	NG	1093/14	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGE	6.78202	6.68664	23.7948	92.75976	0.985936	4.397278	2.445614	10.87039
4195	48	0	1169/14	NG	1185/14	CG	0	1	0	0	0	2	CAT 2		6.2499	6.20472	23.21248	79.55355	0.992771	6.773038	1.754973	10.06688
721	45	1	1508/14	NG	1049/14	CG	1	0	0	0	0	2	CAT 2	CYST MACROPHAGE	7.00792	6.84728	24.00564	79.55355	0.977077	7.24381	1.729193	10.06688
64200	40	1	2571/14	NG	2376/14	CG	1	0,1,2	0	0	0	2	CAT 2	CYST MACROPHAGES	6.94768	6.76696	23.80484	96.77888	0.973988	5.855311	1.927222	11.10339
790930	40	1	1238/11	NH	632/12	CG	1	0,1	0	0	1	2	CAT 2		7.01796	6.8523	24.05584	74.04219	0.976395	7.81559	1.618528	9.711914
821416	30	1	1568/12	NH	1411/12	CG	1	0,1	0	0	0	2	CAT 2	MNG	7.09828	6.95772	24.10604	75.23772	0.980198	7.723535	1.64608	9.790007
964226	48	1	446/14	NH	1942/14	CG	1	0,1,3	0	0	1	0	CAT 3	MICROFOLLICLES	7.04808	6.90752	24.47752	88.4859	0.980057	4.736884	2.354128	10.61701
106	30	1	1456/14	NH	1035/14	NH	1	0,1,2	0	0	0	1	CAT 2	ONCOCYTES	7.14848	7.03804	24.15624	72.64165	0.984551	8.032912	1.591347	9.619622
756654	50	1	2483/11	FA	2429/11	FN	0	1	0	0	0	1	CAT 3		7.00792	6.84226	23.91026	79.3293	0.976361	7.206676	1.735904	10.05268
570564	50	1	189/11	FA	293/10	FN	0	0,3	0	0	0	1	CAT 3		7.59024	7.44466	25.03474	75.56543	0.98082	8.29398	1.59866	9.811305
603978	40	1	1075/11	FA	1443/10	FN	1	0,3	0	0	0	1	CAT 3		7.65048	7.47478	25.05984	76.33287	0.977034	8.227067	1.627055	9.861
800865	35	1	1024/12	FA	950/12	FN	1	3,1	0	0	1	0	CAT 3		7.75088	7.62036	25.16024	64.82269	0.983161	9.765681	1.429611	9.087171
678859	25	1	1044/11	FA	336/11	FN	1	0,2	0	0	0	1	CAT 4	CYST MACROPHAGES	7.06816	7.08824	24.16628	80.2282	1.002841	7.279349	1.728301	10.10948
717463	55	1	2141/11	FA	1375/11	FN	1	0,1	0	0	0	1	CAT 3	ONCOCYTES	7.21876	7.03804	24.1211	91.89186	0.974965	6.331654	1.909375	10.81941
573644	52	1	345/10	FA	284/10	FN	1	1,3	0	0	0	0	CAT 4	MICROFOLLICULAR	7.11836	6.94768	24.52772	126.2031	0.976023	4.766991	2.338947	12.67944
796643	40	1	991/12	FA +LT	827/12	FN	0	0,3	0	0	1	0	CAT 3	MICROFOLLICLES	7.62538	7.45972	25.04478	78.21278	0.978275	8.019674	1.633943	9.981689
59210	52	1	325/14	PCT	196/14	PCT	2	1,2,3	1	1	2	2	CAT 6		8.52898	8.32316	26.38512	110.12	0.975868	8.875764	1.548878	11.844
60616	45	0	602/14	PCT	225/14	PCT	2	1,2,3	1	1	2	0	CAT 6		8.37838	8.17256	26.25962	112.01	0.975434	8.841696	1.523965	11.9452
732403	55	1	1841/11	PCT	1179/11	PCT	1	0,3	1	1	0	0	CAT 6		8.85528	8.67456	27.46944	126.4	0.979592	9.046056	1.575612	12.68933
805331	50	1	1120/12	PCT	1045/12	PCT	1	0,1,2	1	0	0	0	CAT 6	MICR0FOLLICLES	7.85128	7.66052	25.24056	118.65	0.975703	8.16877	1.615984	12.29417
796267	55	1	1169/12	PCT	1046/12	PCT	1	2,3	1	1	0	0	CAT 6	MNG	8.96572	8.8101	27.63008	118.01	0.982643	8.093989	1.706822	12.26096
709563	40	0	1168/11	PCT	1185/11	PCT	1	0,3	1	0	1	1	CAT 6	MICROFOLLICLES	7.93662	7.79104	25.3761	120.14	0.981657	7.970365	1.659554	12.37112
758133	48	1	2522/11	PCT	2501/11	PCT	2	0,1,2	1	1	1	0	CAT 6		9.15648	8.94564	27.77064	110.89	0.976974	10.55421	1.390356	11.88533
592780	45	1	1097/10	PCT	1055/11	PCT	2	1,2,3	1	1	1	0	CAT 6	MNG	9.00588	8.8603	27.74052	124.62	0.983835	10.65642	1.374076	12.59967
604747	45	1	1692/10	PCT	1660/11	PCT	1	1,2	0	0	1	1	CAT 6		6.83724	6.66656	23.76468	104.21	0.975037	7.000039	1.768867	11.52179
599510	60	0	1280/10	PCT	1309/10	PCT	2	2,3,1	1	1	1	0	CAT 6		9.05608	8.85528	27.72044	106.12	0.977827	9.824773	1.474072	11.6269

913429	30	0	1110/13	PCT	976/13	PCT	1	0,1,2,3	1	1	2	1	CAT 6	MNG	8.8603	8.65448	27.51964	115.68	0.976771	9.822173	1.465924	12.13932
576263	45	1	749/10	PCT	381/10	PCT	1	1,3	1	0	1	0	CAT 6		9.05608	8.92556	27.79072	117.21	0.985588	8.04188	1.717231	12.21933
771582	48	1	247/10	PCT	158/12	PCT	2	1,3	1	1	0	0	CAT 6		9.11632	8.94564	27.79072	118.45	0.981278	10.07428	1.44938	12.2838
782967	35	1	707/10	PCT	447/12	PCT	1	0,1	1	1	0	1	CAT 6	MNG	9.00086	8.85528	27.83088	120.22	0.983826	10.22056	1.436735	12.37524
835015	45	1	1751/12	PCT	1722/12	PCT	1	1,2,3	1	1	0	0	CAT 6		8.69966	8.57416	27.38912	118.21	0.985574	6.591699	1.960771	12.27135
906171	50	1	1049/12	PCT	800/13	PCT	1	1,2	0	0	0	0	CAT 6		8.74484	8.62436	27.44936	110.14	0.986223	10.17619	1.422019	11.84507
935497	55	1	1902/12	PCT	1657/13	PCT	2	2,3	1	1	1	1	CAT 6	PSAMOMMA BODIES	8.97576	8.76492	27.56984	105.28	0.97651	10.32601	1.413793	11.58079
18847	35	1	1576/14	PCT	1460/14	PCT	2	0,1,2,3	1	1	1	0	CAT 6		8.86532	8.67456	27.46944	106.46	0.978482	5.939044	2.097189	11.64551
99452	45	1	602/15	PCT	352/15	PCT	2	0,1,2	1	1	0	0	CAT 6		8.99584	8.87536	27.70036	108.12	0.986607	10.1837	1.428245	11.73595
926080	30	1	1416/13	LT	1231/13	SUSICIOUS	2	1,2	1	0	0	0	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
833085	45	1	1668/12	FA	1683/12	CG	1	0,1	0	0	1	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
690177	28	1	868/11	FA	624/11	CG	1	0,1,2	0	0	0	2	CAT 2	CYST MACROPHAGES, MNG	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
709571	50	1	1391/11	FA	1178/11	CG	1	1,2	0	0	0	2	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
709588	45	1	1182/11	FA	1181/11	CG	1	1,2,0	0	0	0	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
758072	35	1	2465/11	FA	2459/11	CG	1	1	0	0	0	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
567206	35	1	152/10	FA	120/11	LT	1	0,1	0	0	0	1	CAT 2	LYMPHOCYTES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
583654	30	1	837/10	FA	624/11	CG	1	0,1,2	0	0	0	1	CAT 2	MACROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
627399	50	1	2411/10	FA	2190/10	CG	1	1,3	0	0	1	1	CAT 2	LYMPHOCYTES, OXYPHILIC CELLS	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
925362	55	1	1385/13	FA	1221/13	LT	1	1,3	0	0	0	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
865812	35	1	55/13	FA	2393/13	LT	1	0,1,3	1	0	1	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
64230	50	1	2660/14	HTA	2426/14	SUSICIOUS	2	0,1,2,3	1	0	2	0	CAT 5	HURTHLE CELL	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
572981	50	1	373/10	FA	283/10	CG	1	1,3	0	0	0	1	CAT 2	CYST MACROPHAGE	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1013275	55	1	994/14	FA	1019/14	CG	1	0,1	0	0	0	2	CAT 2	CYST MACROPHAGE	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
2499	38	1	1126/14	FA	390/14	LT	1	0,1	0	0	1	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
98963	55	0	172/15	FA	166/15	SUSICIOUS	1	0,1	1	0	1	0	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
833841	32	1	1780/12	FA+NG	1777/12	LT	1	0,2	0	0	0	1	CAT 2	LYMPHOCYTES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
878863	40	0	961/13	FA+NG	235/13	CG	1	0	0	0	0	2	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
912595	32	1	1919/13	FC	956/13	LT	1	1,3	0	0	0	2	CAT 2	MNG	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
996397	48	0	653/14	HT	388/14	FN	1	1,3	0	0	0	1	CAT 4	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
795006	42	0	963/10	LT	819/12	PCT	2	0,1,2	1	0	0	0	CAT 6		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
77011	55	1	3013/14	MICRO PCT WITH NG	2607/14	CG	1	0,1	0	0	1	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
845129	35	1	1901/12	NG	2043/12	PCT	1	1,2	0	0	1	1	CAT 6	CYST MACROPHAGE	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
876571	45	1	211/13	NG	121/13	SUSICIOUS	2	0,1,3	1	0	1	2	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
910951	50	1	1180/13	NG	1124/13	PCT	1	0,1	1	0	1	1	CAT 6		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
20201	38	1	1683/14	NG	1485/14	FN	1	0,2	0	0	0	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
34730	55	1	1924/14	NG	1678/14	FN	1	0,1	0	0	1	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
62675	40	1	2540/14	NH	2336/14	FN	1	0,3	0	0	0	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
829755	50	1	1620/12	NH	1610/12	FN	1	0,2	0	0	1	1	CAT 3		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
713935	45	1	1658/11	PCT	1297/11	FN	2	0,1,2	0	0	1	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
653723	30	1	592/11	PCT	602/11	CG	1	0,2	0	0	1	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
646171	30	0	2507/10	PCT	2671/11	FN	1	0,1,2	1	0	0	1	CAT 3	MICROFOLLICLES	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
879569	45	1	376/13	PCT	214/13	CG	1	0,1	0	0	1	2	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
929632	30	0	1525/13	PCT	1347/13	SUSICIOUS	1	1,3	1	0	1	0	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
581309	55	0	669/10	PCT	580/10	SUSICIOUS	1	2,3,1	1	0	1	0	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
99820	55	1	835/15	PCT	518/15	SUSICIOUS	1	0,1,2	1	0	0	1	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
826589	48	1	1525/12	PCT - FV	1520/12	CG	1	0,1	0	0	0	1	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
75945	45	1	149/15	TALL CELL PCT	2594/14	SUSICIOUS	2	1,2	1	1	2	0	CAT 5		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
101285	52	1	2026/14	FN	2214/14	CG	1	1,2	0	0	0	2	CAT 2		#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!