COMPARATIVE ANALYSIS OF CYTOMORPHOLOGIC FEATURES OF THYROID LESIONS USING VARIOUS CYTOLOGICAL STAINING TECHNIQUES

DISSERTATION

SUBMITTED TO TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY,

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL,

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MAY 2018

CERTIFICATE

I hereby certify that this dissertation entitled **"COMPARATIVE ANALYSIS OF CYTOMORPHOLOGIC FEATURES OF THYROID LESIONS USING VARIOUS CYTOLOGICAL STAINING TECHNIQUES"** is a record of work done by **Dr.N.MEENAKSHI**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2015- 2018. This work has not formed the basis for previous award of any degree.

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I solemnly declare that this dissertation titled **"COMPARATIVE ANALYSIS OF CYTOMORPHOLOGIC FEATURES OF THYROID LESIONS USING VARIOUS CYTOLOGICAL STAINING TECHNIQUES"** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2015-2017 under the guidance of **Prof.Dr. J. SURESH DURAI, M.D**, Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in May 2018.

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CONTENTS

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Dear , Dr.N.Meenakshi, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
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CERTIFICATE - II

This is certify that this dissertation work title **"COMPARATIVE ANALYSIS OF CYTOMORPHOLOGIC FEATURES OF THYROID LESIONS USING VARIOUS CYTOLOGICAL STAINING TECHNIQUES"** of the candidate **Dr.N.MEENAKSHI**, with registration Number **201513306** for the award of **M.D.** Degree in the branch of **PATHOLOGY (III).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **4 PERCENTAGE** of plagiarism in the dissertation.

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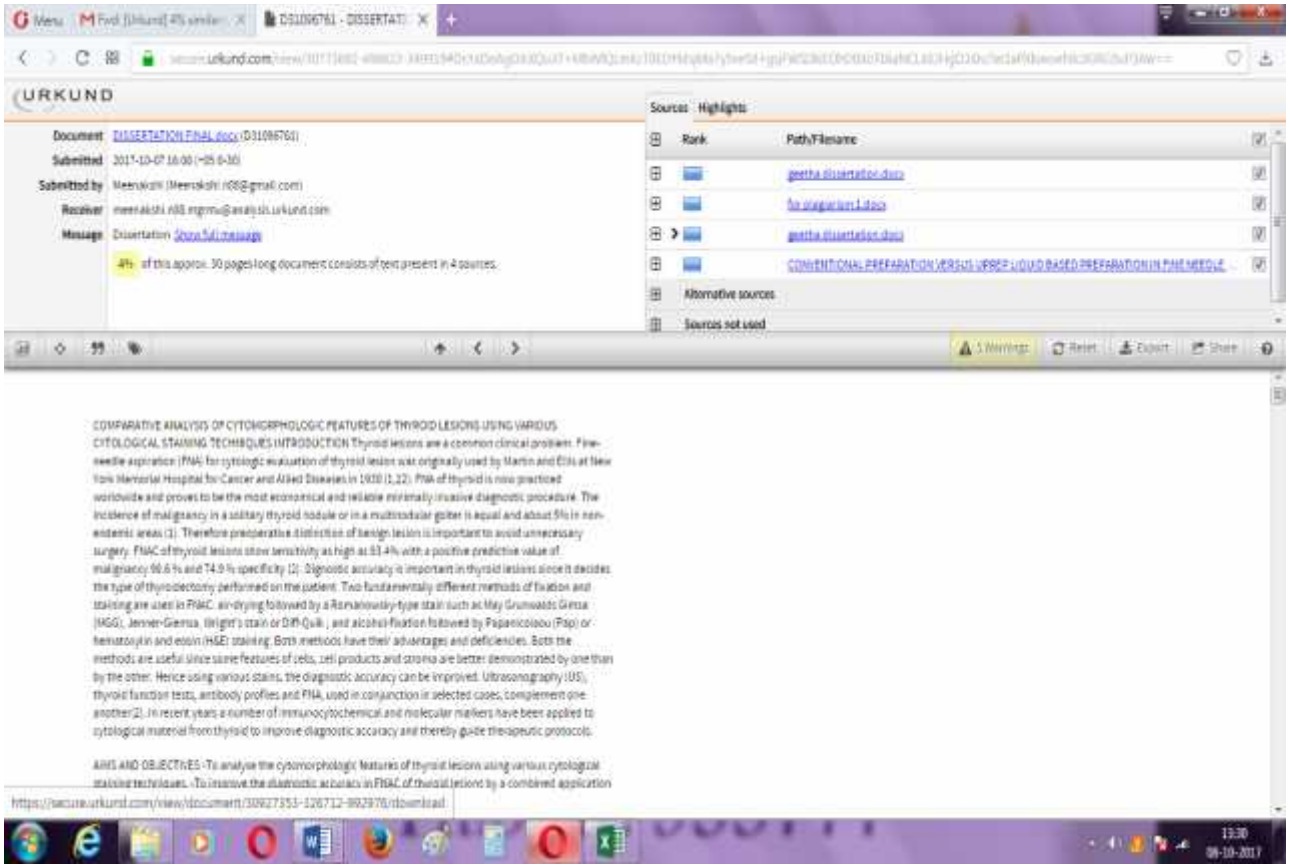
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COMPARATIVE ANALYSIS OF CYTOMORPHOLOGIC FEATURES OF THYROID LESIONS USING VARIOUS CYTOLOGICAL STAINING TECHNIQUES INTRODUCTION Thyroid lesions are a common clinical problem. Fine-needle aspiration (FNA) for cytologic evaluation of thyroid lesion was originally used by Martin and Ellis at New York Memorial Hospital for Cancer and Allied Diseases in 1930 (1,22). FNA of thyroid is now practiced worldwide and proves to be the most economical and reliable minimally invasive diagnostic procedure. The incidence of malignancy in a solitary thyroid nodule or in a multinodular goiter is equal and about 5% in non-endemic areas (1). Therefore preoperative distinction of benign lesion is important to avoid unnecessary surgery. FNAC of thyroid lesions show sensitivity as high as 93.4% with a positive predictive value of malignancy 96.6 % and 74.9 % specificity (2). Diagnostic accuracy is important in thyroid lesions since it decides the type of thyroidectomy performed on the patient. Two fundamentally different methods of fixation and staining are used in FNAC: air-drying followed by a Romanowsky-type stain such as May Grunwald Giemsa (MGG), Jenner-Giemsa, Wright's stain or Diff-Quik ; and alcohol-fixation followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining. Both methods have their advantages and deficiencies. Both the methods are useful since some features of cells, cell products and stroma are better demonstrated by one than by the other. Hence using various stains, the diagnostic accuracy can be improved. Ultrasonography (US), thyroid function tests, antibody profiles and FNA, used in conjunction in selected cases, complement one another(2). In recent years a number of immunocytochemical and molecular markers have been applied to cytological material from thyroid to improve diagnostic accuracy and thereby guide therapeutic protocols.
AIMS AND OBJECTIVES -To analyse the cytomorphologic features of thyroid lesions using various cytological staining techniques. -To improve the diagnostic accuracy in FNAC of thyroid lesions by a combined application
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ABBREVIATIONS

AUS – Atypia of Undetermined Significance

ATC – Anaplastic thyroid carcinoma

BFN – Benign follicular nodule

C cells – clear cells

CDX2 – Cluster of Differentiation X2

CK-19 – Cytokeratin 19

EA – Eosin Azure

FA – Follicular adenoma

FC – Follicular carcinoma

FN – Follicular neoplasm

FNA- Fine needle aspiration

FNAC – Fine needle aspiration cytology

FV-PTC – Follicular variant of papillary thyroid carcinoma

GD – Graves' disease

HCT – Hurthle cell tumour

H&E – Hematoxylin and eosin stain

HT – Hashimotos thyroiditis

HTT – Hyalinizing trabecular tumour

IHC – Immunohistochemistry

INCI – Intranuclear cytoplasmic inclusion

LBP – Liquid based preparation

MC – Medullary carcinoma

ND/UNS – Nondiagnostic or unsatisfactory

NG – Nodular goiter

OG 6 – Orange G 6

MGG – May Grunwalds Giemsa

Pap – Papaicolou stain

PAX8 – Paired box 8

PC – Papillary carcinoma

PCT – Papillary carcinoma of thyroid

PPARc – Peroxisome Proliferator activated recptor c

PTDC –Poorly Differentiated Thyroid Carcinoma

RAS – Rat Associated Sarcoma

T3 – Tri-iodothyronine

T4 – Tetra- iodothyronine or thyroxine

TCV – Tall cell variant

TSH – thyroid stimulating hormone

TTF 1 – Thyroid Transcription Factor 1

US – Ultrasound

UTC – Undifferentiated thyroid carcinoma

INTRODUCTION

Thyroid lesions are a common clinical problem. Fine-needle aspiration (FNA) for cytologic evaluation of thyroid lesion was originally used by Martin and Ellis at New York Memorial Hospital for Cancer and Allied Diseases in 1930 [1,22]. FNA of thyroid is now practiced worldwide and proves to be the most economical and reliable minimally invasive diagnostic procedure.

The incidence of malignancy in a solitary thyroid nodule or in a multinodular goiter is equal and about 5% in non-endemic areas[1]. Therefore preoperative distinction of benign lesion is important to avoid unnecessary surgery. FNAC of thyroid lesions show sensitivity as high as 93.4% with a positive predictive value of malignancy 98.6 % and 74.9 % specificity[2]. Dignostic accuracy is important in thyroid lesions since it decides the type of thyroidectomy performed on the patient.

Two fundamentally different methods of fixation and staining are used in FNAC: air-drying followed by a Romanowsky stain such as May Grunwalds Gimsa (MGG), Jenner-Giemsa, Wright's stain or Diff-Quik; and alcohol-fixation followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining. Both methods have their advantages and deficiencies. Both the methods are useful since some features of cells, cell products and stroma are better demonstrated by one than by the other.

Hence combining the morphological features of various stains can improve the diagnostic accuracy.

Ultrasonography (US), thyroid function tests, antibody profiles and FNA, used in conjunction in selected cases, complement one another[(2)]. In recent years a number of immunocytochemical and molecular markers have been applied to cytological material from thyroid to improve diagnostic accuracy and thereby guide therapeutic protocols.

AIM OF THE STUDY

To analyse the cytomorphologic features of thyroid lesions using various cytological staining techniques.

REVIEW OF LITERATURE

THYROID GLAND

It is a butterfly-shaped organ located anteriorly to the trachea at the level of the second and third tracheal rings. It has two lobes connected by the isthmus in the midline. Its bilaterality is significant because the presence of malignant cells on one or both sides can significantly alter the management of the patient, e.g., requiring more extensive surgery, such as bilateral neck dissections if there is local extension of the tumour[3].

HISTOLOGY AND FUNCTION

The thyroid gland is covered by thin capsule of connective tissue. It extends into the glandular parenchyma and divides each lobe into irregularly shaped and variably sized lobules. Follicles are the functional units of the thyroid gland. Each lobule consist of 20 to 40 follicles. Follicles are spherical and cyst like, between 0.02 and 0.9 mm in diameter. Follicles consist of a central colloid core surrounded by a single layered epithelium resting on a basal lamina. Follicular epithelial cells produce colloid. Colloid consists of an iodinated glycoprotein, inactive iodothyroglobulin, stored form of the active thyroid hormones, tri-iodothyronine (T3) and tetra-iodothyronine or thyroxine (T4). Sufficient iodothyroglobulin is stored extracellularly within follicles to regulate the

metabolic activity of the body for up to three months. Follicles are surrounded by a delicate connective tissue stroma, containing dense plexus of fenestrated capillaries, extensive lymphatic networks and sympathetic nerve fibres which supply the arterioles and capillaries. Some nerve fibres end close to the follicular epithelial cells.

Follicular cells may vary from squamous or low cuboidal to columnar cells, depending on their level of activity, which is controlled by circulating thyroid stimulating hormone (TSH). Resting follicles are large and lined by squamous or low cuboidal epithelium with abundant luminal colloid. Apical microvilli are short in resting cells, but elongate and often branch on stimulation by TSH. Follicles showing different levels of activity may co-exist.

Active follicular cells are highly polarized functionally. The secretion of TSH leads to endocytosis of colloidal droplets at the luminal epithelium. The hormone provokes the cells to extend cytoplasmic processes into the luminal colloid and sequester droplets of colloid. The iodinated thyroglobulin in the intracellular colloid droplets is degraded by follicular cell lysosomes, liberating T3 and T 4, which passes to the base of the cell and they are released.

The epithelial cells lining the acini are unique, in that they are bipolar, that is, they may secrete in either of two directions, into the

interior of the acini or into the vascular channels[4]. As Boyd so well puts it, "They have both a back and a front door". Secretion into the acinus may be considered as a process of storage, a provision for a time of greater need; while secretion in the reverse direction may be looked upon as meeting the current needs of the tissues.

In the colloid which is secreted into the acini there are two protein bodies: one is a nucleoprotein which contains no iodine but is high in phosphorus. This fraction has no known function. The other one is thyroglobulin, which is rich in iodine and from the molecule of which the essential thyroid hormone is probably split off as required[4].

The thyroid builds thyroglobulin when it is supplied with iodine and essential amino-acids. Under normal circumstances there is an approximate balance between the formation and release of hormone. The demand of the tissues for thyroid hormone varies with age, sex and season, and is increased by many physiological and pathological processes such as pregnancy, menstruation and infections.

C cells: thyroid parenchyma also contains C (clear) cells, so called because they have pale staining cytoplasm. C cells are members of the amine precursor uptake and decarboxylation (APUD) system of dispersed neuroendocrine cells. They produce peptide hormone calcitonin, which lowers blood calcium by inhibiting bone resorption and calcium recovery

from renal tubule ultrafiltrate. C cells populate the middle third of each lateral lobe of thyroid and are typically found scattered within thyroid follicles, lying inside the basal lumina but not reaching the follicle lumen. Occasionally they occur in clusters in the interfollicular stroma. Therefore it is also called as parafollicular cells.

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) OF THYROID LESIONS

INDICATION

The main indications of FNA in thyroid lesions are the following:

1. Evaluation of solitary thyroid nodules to distinguish benign from malignant neoplasm.
2. Evaluation of diffuse thyroid lesion to distinguish inflammatory or autoimmune lesions from nodular goiter
3. Confirmation and categorization of clinically obvious thyroid malignancy for example in case of anaplastic carcinomas preoperative palliative treatment may be required, and lymphoma and metastatic malignancy where surgery is usually not indicated.
4. To obtain material for ancillary tests
5. Evaluation of lesions detected by imaging, measuring 1–1.5 cm in diameter with features suspicious of malignancy[(2)].

FNA has been shown to be the safest and most accurate of diagnostic tools in thyroid . Its use has simultaneously diminished the number of surgeries done for benign lesions and increased the proportion of malignancies in surgically resected thyroids

Generally in thyroiditis, usual type of papillary carcinoma, medullary carcinoma, anaplastic carcinoma and high-grade lymphoma cytologic diagnosis is accurate. False negative reporting occur in cystic lesions harboring malignancy, low-grade or intermediate-grade lymphomas occurring in a background of Hashimoto's thyroiditis , anaplastic carcinoma with necrosis, focal involvement of the gland by thyroiditis and in cases with dual pathology where the dominant non-neoplastic lesion overlies or obscures a small carcinoma. False negatives have been shown to be minimized by using ultrasound guided fine needle aspiration. In fine needle aspiration cytology of thyroid, the term follicular neoplasm is used for the spectrum of follicular nodules because the diagnostic criteria for follicular carcinoma is based on capsular or vascular invasion which cannot be assessed on cytology and distant metastases.

The false positive rate can be reduced further by excluding indeterminate follicular lesions. Even though there is slight increase in accuracy achieved by core needle biopsy, follicular adenomas and follicular carcinomas cannot be distinguished, also fine needle aspiration is safe and easy when compared to core needle biopsy.

COMPLICATIONS OF THYROID FNAC

There are no contraindications to thyroid FNA. Local hemorrhage may be caused by needling, occasionally causing a hematoma in the anterior neck may lead to airway compression. Carotid hematoma is a very rare complication. Transient vocal cord paralysis, acute transient goiter, acute suppurative thyroiditis and chemical neuritis occur occasionally. Puncture of the trachea during needling usually causes coughing. Small amounts of blood may be coughed up but recovery is rapid. Needling may convert a hot nodule to a cold one and vice versa, therefore scans should be done before FNA. Post-FNA infarction is an uncommon complication and most reported cases have been Hurthle cell nodules, followed by PC and FNs. Hemorrhage, necrosis or infarction caused by needling may occasionally obscure the histological pattern of thyroid neoplasms. Cellular and vascular granulation tissue of organising hematoma or necrosis can mimic sarcoma or angiomatous tumors.

Fibrosis, papillary hyperplasia, calcification, cholesterol clefts, vascular thrombosis and capsular distortion simulating invasion are other worrisome histological alterations that occasionally follow FNAC[(2)]. Changes are, in general, proportionate to the size of the needle used and the number of needle passes. Post-needling alterations are generally less with the fine needle capillary sampling technique. Aggressive and repeated needling and using needles thicker than 22 gauge should be

avoided. In cases where needling is to be repeated for inadequate or inconclusive cytology, it is wise to allow an interval of a week to 10 days for any artifacts of initial needling to minimize. Cases of tumor implantation along the needle track have been documented rarely.

TECHNICAL CONSIDERATIONS

First examine the patient in sitting position. Then the patient should be made to lie supine with a pillow behind the neck for hyperextension, to make lesion more obvious. The fine needle capillary sampling technique is suitable in vascular structures like thyroid as it provides cellular material with minimal dilution by blood. Instruct the patient to not to swallow when needling. The lesion is needled with a fine needle , quickly and gently at different angles and points of entry. Conclude needling before or as soon as material appears at the hub of the needle, the needle then attached to an air-filled syringe, and material deposited and smeared on to clean glass slides. Half of the smears can be air-dried for Romanowsky staining, while the rest should be wet-fixed in alcohol for Papanicolaou (PAP) stain or H&E.

If the aspirate is scanty, air-drying with Diff-Quik or MGG stain is better as it ensures retention of 100% of cells on the slide[(2)]. Rapid smearing is important in bloody samples, as clotting of blood will entangle diagnostic cells and distort morphology. Slow drying of wet samples causes nuclear shrinkage and loss of cytological characteristics. A hair-

dryer can be used for rapid drying but should be avoided in samples that may be infectious, to avoid aerosols. Despite cost and compensation issues, bedside evaluation of a Diff-Quik or ultrafast PAP-stained smear is advantageous to ascertain adequate cellularity and representative sampling and to select cases for ancillary studies.

ULTRASOUND GUIDED FNAC

Published data indicate reduced non-diagnostic and false-negative rates with ultrasound (US) evaluation and US guidance[2]. Real-time ultrasound examination allows visualization of the needle within the lesion, hence facilitating accurate biopsy of small nonpalpable nodules [5]

Indications of US guided FNAC

Traditionally, the main indication for FNA biopsy of the thyroid has been the presence of a solitary nodule. The Society of Radiologists in Ultrasound suggested that FNA should be considered for a nodule 1.0 cm or more at the largest diameter if microcalcifications are present and for a nodule 1.5 cm or larger if the nodule is solid or if there are coarse calcifications within the nodule [6]. The American Association of Clinical Endocrinologists recommended FNA even for nodules smaller than 10 mm whenever clinical information or US features arouse suspicion about the presence of a malignancy [7].

The US features that are suggestive of malignancy include microcalcifications, marked hypoechogenicity, an irregular or microlobulated margin, a longitudinal dimension larger than the cross-sectional dimension, intrinsic vascularity, direct tumor invasion of adjacent soft tissue, and metastasis to one or more lymph nodes [8] .

In palpable nodules of nodular goiter, the cytologic sampling should be focussed on lesions characterized by suspicious US findings rather than on larger or clinically dominant nodules[6,70].

The presence of abnormal lymph nodes or thyroid extracapsular extension overrides the ultrasound features of thyroid nodule and should prompt US-FNA or biopsy of lymph node and or ipsilateral nodule[9]

FIXATION

Two most commonly used methods of fixation used in cytology are air dryring and wet fixation .

DRY FIXATION

Fixation by air-drying causes increase in size of the cell, because it causes both cytoplasm and nucleus, to flatten on the slide . It therefore appears larger than a cell fixed in ethanol. Nuclear enlargement and variation in nuclear size are exaggerated in air-dried smears. This enhances the difference between normal and abnormal cells . Optimal fixation of air-dried smears depends on rapid drying. This can be enhanced by using a hair dryer with moderate heat. Slow drying of thick bloody smears tends to

produce artifacts, in particular shrinkage of cells and nuclei, which may render diagnosis impossible.

WET FIXATION:

In smears that are wet fixed with alcohol the cells maintains its three-dimensional rounded shape . In wet fixed smears the size of the cell and nucleus is comparable to that of tissue sections. The smears should be immersed in alcohol immediately to prevent focal drying artifacts but there will be considerable cell loss during alcohol fixation. The main problem with wet-fixed smears is that highly cellular smears dry so quickly that drying artifacts can be difficult to avoid which may lead to difficulties in diagnosis.

STAINING METHODS USED IN CYTOPATHOLOGY:

Three staining methods are used routinely in thyroid cytopathology. Papanicolou stain is most popularly used. Romanowsky stains are used for rapid assessment of adequacy of aspirate. Hematoxylin and eosin stains are used for cell blocks as well as for staining smears.[10]

ROMANOWSKY STAINS

Romanowsky stains allow better estimation of relative cell and nuclear sizes, and superior visualization of cytoplasmic details, smear background elements and intercellular matrix components[11]

In Romanowsky stains, air drying leads to an apparent enlargement of cells and nuclei . Spreading cells onto the glass slide leads

to an increase in their apparent size proportional to the volume of the nuclei. The degree of perceived nuclear and cytoplasmic size increase due to air drying depends on the type of cell; it is smallest in mature squamous cells.

The apparent enlargement of cells and nuclei in air dried Romanowsky-stain preparations, compared to the cell and nuclear sizes in wet fixed Papanicolaou stained preparations, amplifies the cell and nuclear size differences within the specimen and permits more accurate evaluation of relative cell size as well as nuclear size and shape . In addition, uniform air drying of well prepared, evenly smeared cytological preparations stained with Romanowsky stains, makes it preferable for morphometry [13], since the unintentional focal air drying of wet fixed smears lead to nuclear size variability. Air drying also influences the structure of the nuclear chromatin, which becomes condensed and thus more hyperchromatic [14]. The accentuation of size and chromaticity differences between normal and malignant nuclei produced by Romanowsky stains is a useful feature for evaluating fine needle aspirates, especially when dealing with well differentiated malignancies [15]. Accurate exfoliative cytological diagnosis is dependent on high power evaluation of nuclear chromatin changes. By contrast, because they usually are very cellular and show many small true tissue fragments, fine needle aspirates frequently can be diagnosed under low power and nuclear chromatin detail is less important for diagnosis.

Romanowsky stains are essential for low power pattern-based diagnosis [16] owing to their better definition of cell cytoplasm and their accentuation of enlarged tumor cell nuclei.

Romanowsky stains enhance cytoplasmic detail, a useful feature for determining differentiation of neoplastic cells. Clearer cytoplasmic definition also enables better appreciation of the plasmacytoid appearance of certain neoplastic cells. Such plasmacytoid cells, defined by their eccentrically placed nuclei, are characteristic not only of plasma cell tumors , but also of neuroendocrine tumors of the pancreas, carcinoid tumour, medullary carcinoma of the thyroid [17]

Improved visualization of cytoplasmic granules may be valuable for fine needle aspirates of the thyroid and other organs. A common finding in fine needle aspirates of the thyroid is the presence of paravacuolar granules, which represent lysosomes containing hemosiderin or lipofuscin pigments [18] . Paravacuolar granules are more common in samples from normal thyroid than in those from colloid nodules [19] , but are found across the spectrum of thyroid pathology and thus are not specific for any pathology. Their presence, however, identifies the aspirate as coming from the thyroid and helps exclude aspirates of similar appearing cells from the parathyroids . Other types of granules potentially encountered in thyroid fine needle aspirates include the red cytoplasmic granules occasionally seen in neoplastic cells of medullary thyroid carcinoma [20] . These

granules can be helpful for cytological diagnosis of this otherwise difficult to diagnose malignancy.

Occasionally, fine needle aspirates of the thyroid may show follicular epithelial cells with marginal vacuoles, a phenomenon known as a " fire-flare " appearance [21] . Initially it was thought as a distinctive feature of hyperthyroidism but now it is considered as a nonspecific finding in both neoplastic and non-neoplastic disorders that could be identified only with Romanowsky stains [22] . In case of metastasis, the fire-flare appearance is helpful for identifying the primary tumor, usually follicular carcinoma of thyroid [23] .

In Romanowsky staining, amyloid stains deep blue with possible focal metachromasia [24,25] . Amyloid detection could be important for diagnosing medullary carcinomas of the thyroid [26] .

Colloid is a noncellular element whose identification is important for diagnostic purpose of thyroid FNAC. Both the amount of colloid and its quality are important in thyroid fine needle aspirates; the ratio of colloid to cells is one of the most important diagnostic criteria for differentiating between colloid nodules and potentially malignant thyroid neoplasms. While thick colloid can be identified easily on both Romanowsky and Papanicolaou stains, thin colloid may be extremely difficult to visualize in Papanicolaou stained smears. In Romanowsky stains, thin colloid is recognized easily owing to its characteristic folding

and cracking patterns, which imparts a mosaic like crackling [9] cracked glass or crazy pavement appearance [27]

Pathologists would be familiar with the Romanowsky stained appearance of hematolymphoid cells, so Romanowsky stains are better to wet fixed preparations for diagnosing hematolymphoid neoplasms. Certain characteristic features of hematolymphoid cells are not seen or are more difficult to recognize in Papanicolaou or hematoxylin and eosin stains. Although Romanowsky stains are mandatory for accurate interpretation of fine needle aspirates of primary lymph node conditions, many cytopathologists also use Papanicolaou stained smears for subtyping of lymphomas, because the better nuclear chromatin definition afforded by this stain aids in differentiating centroblasts from centrocytes.

The contrast between nuclear chromatin and nucleoli is variable, nucleoli are not always demonstrated well in Romanowsky stains. Wet fixed variations of Romanowsky stains have been recommended to overcome this shortcoming [20]; these offer nuclear chromatin and nucleolar detail similar to wet fixed Papanicolaou or hematoxylin and eosin stained preparations. As expected, however, the advantages of increased nuclear size and accentuation of nuclear size differences brought about by air drying are lost. In addition to the differences in the nuclear chromatin detail, wet fixed stains exhibit sharper nuclear outlines, a feature that is particularly useful for highlighting the nuclear contour abnormalities of

neoplastic cells. Nuclear grooves and pseudo-inclusions are more distinct in wet fixed preparations and unusual chromatin patterns, e.g., the " salt and pepper " chromatin in neuroendocrine neoplasms, are appreciated more easily. Many of these nuclear features, such as nuclear grooves in papillary carcinomas of the thyroid [29] and nuclear inclusions in both thyroid tumors and melanoma, also can be demonstrated in Romanowsky stained preparations, but they may be more difficult to identify

Further constraint is that Romanowsky stains, including the Diff-Quik stain commonly used in cytopathology, were created and intended for use with very thin, evenly smeared preparations. Typical fine needle aspirates commonly contain thicker tissue fragments and a thin smear may be impossible to make. Thicker tissue fragments, which may consist of three-dimensional cell groups or partially necrotic tissue fragments, frequently are poorly stained and difficult to visualize. In such cases, Papanicolaou or hematoxylin and eosin stains may reveal the cytological features of the individual cells within the three-dimensional cell clusters and may show well defined, individual, intact, non-necrotic cells. The technique for making good air dried smears from fine needle aspiration material is important. The optimal smearing technique usually requires some practice. Smears should be air dried relatively quickly (within 5 min) to avoid artifacts. Hand-held fans can be used successfully without

noticeable artifact formation to shorten the air drying time from about 3 min to about 1 min per smear in case of rapid on site evaluation [30] .

Romanowsky stained slides can be used for immunostains of certain lymphoid markers.

PAPANICOLOU STAIN

Papanicolaou (PAP) staining was first described by George Papanicolaou, the father of Cytopathology, in 1943 [31] .

Papanicolaou stain (PAP stain) is the most important stain utilized in the practice of Cytopathology. It is a polychromatic stain containing multiple dyes to differentially stain various components of the cells. The major components include hematoxylin, a basic dye which is a nuclear stain and two cytoplasmic stains EA 50 and OG 6 containing three acid dyes – light green, eosin and orange G.

With papanicolou stain cytoplasm stains eosinophilic, cyanophilic or orange. Keratin stains deep orange. Nucleus stains deep blue and nucleolus stains red.[32]

The colloid appear as fine film of variable colour , from gray-green to rose with PAP stain[9] . Colloid is cyanophilic to eosinophilic and orangish when mixed with blood. [10]

In PAP-stained smears, thin colloid stains pale green or orange, with cracking artifacts seen. Thick colloid appears as clumps of dark green or orange material[2].

Dependence on smearing technique is less when compared to air dried smears. Cellular architecture is very well maintained in wet fixed smears.[10] . Smears should be wet fixed rapidly. Immediate fixation is necessary since even slightest air drying lead to significant artifactual changes. But there will be a considerable loss of cells while fixing in alcohol.

Individual cells are usually clearly seen in Pap stained smears. Cell and nuclear size are comparable to tissue sections in wet fixed smears.

Cytoplasmic details are poorly demonstrated but nuclear details are excellently demonstrated[2] . Wet fixed stains exhibit sharper nuclear outlines, a feature that is particularly useful for highlighting the nuclear contour abnormalities of neoplastic cells. Nuclear grooves and pseudo-inclusions are more distinct in wet fixed preparations in case of papillary carcinoma of thyroid and unusual chromatin patterns, e.g., the " salt and pepper " chromatin in neuroendocrine neoplasms, are appreciated more easily[27]

Most cytopathologists believe that nuclear chromatin is better defined in wet fixed than in air dried preparations. Pap stained smears are very useful in differentiating follicular and hurthle cell lesions. Psammoma bodies are well visualised and presents variable morphology with multiple colours. Pap stained slides can be selected for cellularity, destained and used for immunostaing .[10]

HEMATOXYLIN AND EOSIN STAIN

Hematoxylin and eosin stain can be used in both air dried and wet fixed smears. It is used as a progressive stain for cytology.

This stain gives excellent nuclear details(10) . Mostly it is similar to papanicolou stain, in most of the parameters. Dependence on smearing technique is moderate. Immediate fixation is needed to prevent drying artifacts. There will be considerable cell loss during wet fixation. Celullar architectural features are well visualised. Hematoxylin and eosin stain provides crisp and excellent nuclear features such as chromatin, nuclear membrane and nucleoli. This stain demonstrates cytoplasmic details as well. Keratinisation is well demonstrated and oncocytic changes are readily identified using hematoxylin and eosin stain.

Psammoma bodies are well visualised and appear as basophilic, concentric lamallated structure. Colloid will be eosinophilic.[10] . H&E stained smears are very useful in differentiating follicular and hurthle cell lesions. But its use in hematologic malignancies and lymphoproliferative diseases is not as specific as Romanowsky stains and its application in immunostains is not known.

PROCEDURE FOR CYST FLUIDS

The method for cytopreparation is determined by the gross quality of cyst fluid .

If the specimen is clear , it should be centrifuged at 2500 rpm for 10 minutes . if the sediment is visible make a smear or wet film preparation. If the specimen is poorly cellular , a cytospin preparation is useful[10] .

- If the specimen or sediment is grossly bloody, the saponin technique may be used to hemolyse red cells.

Saponin technique :

Saponin is an enzyme that lyses red blood cells. A saponin solution is useful in processing grossly bloody specimens. Excess saponin may destroy the cellular component of the specimen . hence it should be used cautiously.

The bloody specimen should be suspended in 30 ml of balanced salt solution. Then 5 drops of saponin solution is added and agitated gently for 1 minute. 15 drops of calcium gluconate solution is added to stop the action of saponin enzyme and mixed well. Centrifuge for 10 minutes at 2500 rpm . Prepare smears from sediment and used used for staining [10] .

Technique for toluidine blue wet-film preparation

The wet –film preparation of sediment from the centrifuged specimen allows rapid assessment of its cellularity, as well as its cell type, that is differentiating between benign and malignant. This examination guides cytotechnologists to follow a suitable method for processing the specimens and helps prevent cross- contamination.

To make Toluidine Blue Wet-Film Preparation place a drop of centrifuged sediment on the slide and place one drop of toluidne blue solution on the slide and mix. Coverslip and examine under microscope.[10]

RAPID ON SITE EVALUATION OF SMEARS

The advent of rapid Romanowsky-type stains, such as the Diff-Quik stain, is a marked improvement in the efficiency of fine needle aspiration and core biopsy procedures. Pathologists, radiologists and clinicians frequently use various types of imaging guidance, such as computerized tomography and ultrasound, to obtain fine needle aspirate and core biopsy specimens from deep lesions. Most of such specimens were sent to the pathology laboratory for time-consuming cytological processing and evaluation. Now these specimens can be evaluated on site using Romanowsky-type stains that take 30 seconds or less to perform. Other rapid stains occasionally are used including the ultrafast Papanicolaou stain [33,34] and the toluidine blue stain , but none has achieved the widespread acceptance of the Diff-Quik stain. Rapid on site evaluation of specimens has reduced significantly the number of unsatisfactory procedures, which has decreased the need for the patient to undergo a repeat biopsy. Further, because of the pathologist ' s ability to carry out all of the duties involved in this process, from performing the procedure, to evaluating its adequacy, to triaging material for ancillary studies such

as cultures and flow cytometry, a new specialist, the “ interventional cytopathologist, ” has been forged .

ANCILLARY STUDIES :

Ancillary testing is not required for the majority of thyroid aspirates and is not recommended as part of routine practice. When appropriate, an adequate specimen should be available before proceeding to ancillary studies. Cell blocks are the most suitable for histochemistry and immunohistochemistry (IHC). Molecular testing can be performed on aspiration fluid, cyst fluid, cell blocks and liquid based preparations (LBP).[35]

The practice of using ancillary tests is essentially based on availability of resources and expertise as well as the clinical demand.

1. Characterisation of cells in the aspirate Cysts

A panel of cytokeratin stains may be useful to confirm the presence of epithelial cells. This exercise may be valuable when assessing specimens that may be deemed non-diagnostic due to paucity of cells.[36, 37]

TTF-1 and thyroglobulin are useful to establish thyroid origin. TTF1 is considered a sensitive marker for thyroid and lung carcinomas. However TTF1 does not have perfect specificity and rare neoplasms from other primary sites, such as breast, colon and prostate have been reported to

express TTF-1.[38, 39] Thyroglobulin is more specific but interpretation of cytoplasmic positivity may be difficult due to background staining.

-PAX8 is another marker that shows consistent positivity in thyroid epithelial cells but is also expressed in renal, ovarian and pancreatic epithelium.[40]

-CDX2 is known to be positive in columnar cell variant of PTC.[41]

-Mucin production is not a feature of primary thyroid neoplasms with the exception of rare tumours such as mucoepidermoid carcinomas.

Confirmation and classification of a specific malignancies like Papillary, medullary and anaplastic carcinomas can be done with a very high degree of accuracy on cytomorphology alone. There are times when the cells are cytologically malignant but the subtyping is uncertain. In other situations the full complement of cytological criteria may be lacking leading to a suspicious rather than a definitive report. Selective use of IHC may be valuable to arrive at a conclusive diagnosis.

- CK19, HBME1 and Galactin combinations have been shown to be reliable in the diagnosis of classical PTC[42,43] .
- The V600E mutation of the BRAF kinase (BRAF) gene is a common event in PTC and the majority of classic (45-77%) and tall

cell variants (80%).[44,71] Detection of this mutation in aspirates should virtually confirm the diagnosis of PTC therefore can be used to confirm a diagnosis in suspicious cases.

- RET / PTC and PAX8 / PPARc gene abnormalities have been described in several thyroid neoplasms. However their use in thyroid cytology is not currently established.

- IHC for calcitonin, CEA and neuroendocrine markers in cell block preparations may confirm medullary thyroid carcinoma.

-Generally ATC does not show any specific IHC pattern. However metastatic malignancies would be the primary differential diagnosis and a negative reaction to site specific antibodies may be of help.

2. To detect genetic or molecular characteristics and provide prognostic information, a panel of mutations including those in the BRAF V600E and RAS genes and rearrangements involving RET/PTC and PAX8/PPARc have been recommended in the management of thyroid nodules. Of these the BRAF mutation is the most evaluated marker for prognosis in relation to PTC as it appears to play a key role in the development and progression of this disease. PTCs with the BRAF V600E mutation are believed to be associated with advanced stage and aggressive biological behaviour although this notion is being questioned lately.[45,46] The recent introduction of the selective BRAF V600E inhibitor PLX4032 in the management of

melanomas harbouring the T1799A point mutation has renewed interest in the identification of BRAF mutated thyroid carcinomas as possible targets for alternative therapy for otherwise treatment - resistant BRAF T1799A-mutated PTC.

3.Flow cytometry : Cell surface marker analysis by flow cytometry is indicated in suspected lymphoproliferative disorders.

The decision to submit material for flow cytometry may have to be made at the time of aspiration with on site evaluation. This decision may be guided by the clinical circumstances in addition to the presence of suspicious or concerning cytological features. Flow cytometry enables characterisation of the lymphoid population with confirmation of clonality. It should be noted, however, monoclonality may occur in Hashimoto thyroiditis.[47,48] . The results should be interpreted in conjunction with the clinical and cytological findings.

- Thyroglobulin assay in aspirates of suspected metastatic papillary thyroid carcinoma can be helpful in establishing a diagnosis. Aspiration samples, the supernatant of cyst fluid or a needle rinse may be tested for thyroglobulin levels.[49,50]
- Parathyroid hormone assay in lesions suspected of being parathyroid origin. The material can be sent for parathyroid hormone testing (needle washed in 0.1- 0.5 ml of normal saline).

NOMENCLATURE USED IN REPORTING

Reporting of thyroid FNA specimens should follow a standard format which should be clinically relevant and helps to direct management. At the National Cancer Institute sponsored thyroid state of the science conference in Bethesda in October, 2007, consensus was reached regarding indications, pre-FNA requirements, FNA techniques, diagnostic terminology, etc [51,69].

The Bethesda System for reporting thyroid cytopathology includes six categories. Each category has an implied cancer risk, which ranges from 0% to 3% for the "Benign" category to virtually 100% for the "Malignant" category [51,69] . As a function of these risk associations, each category is linked to evidence-based clinical management guidelines.

THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY[51]

The recommended diagnostic categories are as follows:

I. Nondiagnostic 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 (subacute) thyroiditis Other

III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm specify if Hürthle cell (oncocytic) type

V. Suspicious for Malignancy

Suspicious for papillary carcinoma

Suspicious for medullary carcinoma

Suspicious for metastatic carcinoma

Suspicious for lymphoma

Other

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 Other

Category I : Nondiagnostic or Unsatisfactory

A specimen is considered “Nondiagnostic” or “Unsatisfactory” if it fails to meet the following adequacy criteria.

Criteria for Adequacy

A thyroid FNA sample is considered adequate for evaluation if it contains a minimum of six groups of well-visualized (i.e., well-stained, undistorted, and unobstructed) follicular cells. Each group should contain at least ten cells , usually on a single slide.

Exceptions to this requirement apply to the following special circumstances:

1. Solid nodules with cytologic atypia

   A sample that contains significant cytologic atypia is never considered ND/UNS. It is mandatory to report any significant atypia; a minimum number of follicular cells is not required

2. Solid nodules with inflammation

   Nodules in patients with lymphocytic (Hashimoto) thyroiditis, thyroid abscess, or granulomatous thyroiditis may contain only numerous inflammatory cells. Such cases are interpreted as Benign and not as ND/UNS. A minimum number of follicular cells is not required.

3. Colloid nodules

Specimens that consist of abundant thick colloid are considered Benign and satisfactory for evaluation. A minimum number of follicular cells is not required if easily-identifiable colloid predominates.

Nodules with an initial ND/UNS result should be re-aspirated, after 3 months ; the 3-month interval is recommended to prevent falsepositive interpretations due to reactive/reparative changes.[52]

Ultrasound guidance with immediate, on-site adequacy evaluation is preferred for repeat aspiration after an initial ND/UNS specimen, especially for solid nodules. Repeating the FNA results in a diagnostic interpretation in up to 60% of cases.[53] Most of the nodules which falls under ND/ UNS category are often benign.

Category II : Benign follicular nodule

The term "benign follicular nodule" applies to a cytologic sample that is adequate for evaluation and consists predominantly of colloid and benign-appearing follicular cells in varying proportions. The general term BFN may be utilized in reporting; alternatively, a more specific term like colloid nodule, nodular goiter, hyperplastic/adenomatoid nodule, or Graves' disease may be used, depending on the associated clinical presentation

It is the most commonly encountered entity in thyroid cytopathology and includes a group of benign lesions with similar cytologic features that are classified histologically as nodules in nodular goiter (NG), hyperplastic (adenomatoid) nodules, colloid nodules, nodules in Graves' disease, and a subset of follicular adenomas such as macrofollicular type . The distinction among these lesions is not possible by FNA, and can be managed in a similar, conservative manner. BFNs are characterized by variable amounts of colloid, benign-appearing follicular cells, Hürthle cells, and macrophages.

Patients with benign thyroid cytology are generally followed clinically with periodic physical examination at 6 to 18 months interval and ultrasonography if needed. Repeat FNA is recommended for nodules showing significant growth or developing US abnormalities, such as irregular margins, microcalcifications, intra-nodular hypervascularity, and hypoechogenicity in solid areas.[54]

The risk of cancer associated with cytologically benign thyroid nodules is difficult to calculate because only a few cases (approximately 10 %) undergo surgery.

Category III : Atypia of Undetermined Significance

The term "Atypia of Undetermined Significance" (AUS) is used only for specimens that contain cells (follicular, lymphoid, or other) with architectural and/or nuclear atypia that is not sufficient to be classified as

suspicious for a follicular neoplasm, suspicious for malignancy, or malignant.

AUS is a cytologic diagnosis of last resort and should not be used indiscriminately

The recommended management for an initial AUS interpretation is the clinical correlation and, for most cases, a repeat FNA at an appropriate interval.[54, 55] Usually a repeat FNA results in a more definitive diagnosis The risk of malignancy in this category is closer to 5–15%.

Category IV: Follicular neoplasm" or "Suspicious for a follicular neoplasm (FN/ SFN)

The term "Follicular neoplasm" or "Suspicious for a follicular neoplasm" refers to a cellular aspirate comprised of follicular cells, most of which are arranged in an altered architectural pattern characterized by significant cell crowding and/or microfollicle formation. Cases that demonstrate the nuclear features of papillary carcinoma are excluded from this category.

The recommended management of a patient with a diagnosis of FN/SFN is surgical excision of the lesion, most often a hemithyroidectomy or lobectomy.[54]

Follicular neoplasm, Hurthle cell type" or "Suspicious for a follicular neoplasm, Hürthle cell type

The cytologist diagnosis of “Follicular neoplasm, Hurthle cell type” or “Suspicious for a follicular neoplasm, Hürthle cell type” refers to a cellular aspirate that consists exclusively of Hürthle cells. Oncocytic cells with nuclear features of papillary carcinoma are excluded from this category.

Category V : Suspicious for Malignancy:

The fine needle aspirate from thyroid is categorised as suspicious for malignancy (SFM) when some features of malignancy (mainly PTC in this context) raise a strong suspicion of malignancy, but the findings are not sufficient for a conclusive diagnosis. Specimens that are suspicious for a follicular or Hürthle cell neoplasm are excluded from this category . For the category SFM, the morphologic changes are of such a degree that a malignancy is considered more likely than not.

Suspicious for Medullary Carcinoma :

The sample is sparsely or moderately cellular. There is a monomorphic population of noncohesive small or medium sized cells with a high nuclear/cytoplasmic ratio

Suspicious for Lymphoma :

The cellular sample is composed of numerous monomorphic small- to intermediate-sized lymphoid cells. Or the sample is sparsely cellular and contains atypical lymphoid cells.

Suspicious for Malignancy, Not Otherwise Specified

The diagnosis “SFM, suspicious for papillary thyroid carcinoma” is an indication for surgery.

Ancillary serologic or immunohistochemical studies, which are of little value for patients with an FNA diagnosis of “suspicious for papillary thyroid carcinoma,” can be very helpful for patients with the diagnosis “suspicious for MTC” or “suspicious for lymphoma.” An elevated serum calcitonin level and/or a repeat FNA that shows strong immunoreactivity for chromogranin, synaptophysin, and calcitonin can convert an initial “suspicious for MTC” interpretation into a conclusively malignant interpretation. A repeat FNA to obtain cells for flow cytometric study is also likely to provide a definite diagnosis for patients with an initial “suspicious for lymphoma” interpretation.

Category VI : Malignant

Papillary Thyroid Carcinoma

Definition PTC is a malignant epithelial tumor derived from thyroid follicular epithelium and displays characteristic nuclear alterations. Papillary architecture may be present but is not required for the diagnosis.

Medullary thyroid carcinoma:

It is a malignant neoplasm derived from and/or morphologically recapitulating the parafollicular cells of the thyroid gland.

Surgical treatment of MTC is usually total extracapsular thyroidectomy with lymph node dissection.

Poorly differentiated thyroid carcinoma

PDTC is a thyroid carcinoma of follicular cell origin characterized by an insular, solid, or trabecular growth pattern. In its pure form, PDTC lacks conventional nuclear features of papillary thyroid carcinoma and is distinguished from the latter by the presence of poorly differentiated features: mitoses, necrosis, or small convoluted nuclei. The most classic form of PDTC is the insular type, defined by its “cellular nests” or insular cell groups outlined by a thin fibrovascular border

Since this carcinoma has poor clinical prognosis, PDTCs are usually managed more aggressively than well differentiated thyroid carcinomas. A recent evidencebased review of therapeutic options for PDTCs recommends using ^{131}I therapy postoperatively.(56)

Undifferentiated thyroid carcinoma

UTC is a high grade, pleomorphic, epithelial-derived malignancy with epithelioid and/or spindle cell features.

Tumors have the following immunochemical profile: · Pan-keratin and vimentin – positive, often focally (some tumors are negative for one or the other); · TTF-1 and thyroglobulin – commonly negative.

Complete surgical resection, with or without pre-operative hyperfractionated radiotherapy and/or chemotherapy to enhance resectability through tumor shrinkage, is the optimal treatment strategy

Younger patients (<45 years old) and individuals with smaller tumors without extensive extrathyroidal tissue invasion or metastases have the best outcome.[57]

The revised Papanicolaou system of reporting is simple and easily reproducible with the following six categories that are useful in triaging patients for either clinical follow-up or surgery: •unsatisfactory, • benign, • atypical cellular lesion, • follicular neoplasm, • suspicious for malignancy, • positive for malignancy[2].

ACCURACY AND LIMITATIONS OF CYTODIAGNOSIS

In experienced hands, and in situations where the pathologist performs the needling, cytology can be a very sensitive tool with sensitivity and specificity of up to 94% and 98% for the diagnosis of malignant lesions and nearly 90% accuracy rates for the identification of malignancy if follicular lesions are excluded [2]. Cytologic diagnosis is generally accurate in thyroiditis, usual type of PC, medullary carcinoma (MC), anaplastic carcinoma (AC) and high-grade lymphoma. False negatives generally occur in cystic lesions harboring malignancy, in low-grade or intermediate-grade lymphomas occurring in a background of Hashimoto's thyroiditis (HT), in AC with necrosis, in focal involvement of

the gland by thyroiditis and in cases with dual pathology where the dominant non-neoplastic lesion overlies or obscures a small carcinoma[2]. False negatives have been shown to be minimized by using US-guided FNA. The false positive rate can be reduced further by excluding indeterminate follicular lesions.

Risk factors for thyroid cancer include a family history of thyroid cancer, a history of head and neck irradiation, male sex, age of less than 30 years or more than 60 years, and a previous diagnosis of type 2 multiple endocrine neoplasia [58].

NORMAL STRUCTURES IN THYROID CYTOLOGY :

Follicular epithelial cells and colloid are regular features in normal thyroids and in colloid goiter. Follicular cells show fragile gray-blue or pale-blue cytoplasm with indistinct or fuzzy cell borders. Coarse blue (paravacuolar) cytoplasmic granules may be seen. Bare nuclei, similar in shape and size to normal lymphocytes, are common. Some cells may show small nucleoli.

In non-bloody specimens, thin colloid stains blue, violet or pink and forms a thin membrane-like coating or film, with folds and cracks due to drying of colloid on the slide in Romanowsky stained smears . Colloid may wash off from the slide while staining but the parched-earth or crazy-pavement artifact of colloid remains and follicular cells are often seen at the smear margins[2]. Thick colloid appears as round, dense clumps of deep blue,

violet or magenta-colored acellular material, or as globular masses with superimposed follicular cells, especially in samples from NG. In Papanicolou (PAP)-stained smears, thin colloid stains pale green or orange, with cracking artifacts seen. Thick colloid appears as clumps of dark green or orange material[2]. The blue violet colour and hyaline texture of colloid appear to be an advantage in May Grunwalds Giemsa (MGG)-stained smears and helps in distinguishing it from fibrillary collagen and deep magenta staining amyloid . In bloody smears, colloid resembles other protein-rich fluids, including serum. Thin eosinophilic colloid appears to be associated with functional activity, whereas thick , markedly eosinophilic colloid occurs in inactive follicles and in some malignant lesions.

PRINCIPAL LESIONS OF THE THYROID[9]

The principal lesions of the thyroid gland that maybe identified in aspiration cytology are as follows:

Cysts

Goiters

Colloid goiter

Thyroiditis

Acute Sub acute (deQuervains)

Lymphocytic (Hashimotos disease)

Riedels Struma (fibrosing thyroiditis)

Tumors

A. Follicular tumors

Follicular adenomas

Follicular carcinoma

B. Hurthle cell tumors

Hurthle cell adenoma

Hurthle cell carcinoma

C. Other carcinomas

Papillary and its variants

Medullary

Anaplastic (large- and small-cell types)

D.Malignant lymphomas

E.Rare malignant tumors

F.Metastatic tumors

CLINICAL FINDINGS

Patients referred for fine needle aspiration cytology of thyroid present with diffuse goiter or multinodular goiter or solitary nodule. Most patients are females. Age is not important because malignant lesions may occur in the very young and very old. Duration of swelling is important and note whether its growth was slow, rapid, or sudden, because slow growing multiple nodules or masses are less likely to be malignant than a

more rapidly enlarging solitary nodule. A sudden increase in the size of the nodule suggests a hemorrhage.

FINE NEEDLE ASPIRATION CYTOLOGY OF THYROID LESIONS

SIMPLE COLLOID GOITER

A diffusely enlarged gland with smears showing a normal cytological appearance .

Abundant or very thick colloid will be present in the background. Colloid can be mixed with blood or form a protein film with folds or mosaic-like cracking or as dense spherical clusters.

Dispersed scanty follicular cells or forming monolayered sheets with honeycomb pattern or distended follicles with smooth contour will be present.

NODULAR GOITER (NG)

Smears show abundant thick or thin colloid, follicular cells in monolayered sheets, poorly cohesive groups and as single cells, globular colloid masses with superimposed follicular cells, bare nuclei and pigment-laden histiocytes (foam cells) in varying proportions. Tissue fragments containing three dimensional balls of cells confined by basement membrane may be present. Involutional follicular cells with small round dark nuclei and fragile, feathery cytoplasm as well as larger, hyperplastic cells with abundant vacuolated cytoplasm or with marginal vacuoles (fire-

flares) are seen. Hyperplastic cells may show anisonucleosis. Oxyphilic cells may be seen[2].

Foam cells, often hemosiderin-laden, suggest degeneration, commonly seen in NG Hyalinized stroma presents as irregular pink/red frayed fragments of vaguely fibrillar material, some with adherent epithelial cells

CYSTIC NODULES

Cystic nodules in thyroid are most commonly due to regressive changes in nodular goiter. They may be small, containing a few drops of fluid or larger cysts yielding substantial quantities. FNA yields brownish colloid-like fluid with altered blood. Smears show foam cells that may contain hemosiderin and sparse degenerating follicular epithelium.

ACUTE SUPPURATIVE THYROIDITIS

Patients present with extremely tender thyroid enlargement, fever and high ESR. Smears show neutrophils, necrotic cells and debris. Intracellular bacteria, (usually Gram-positive cocci), may be present. Less commonly, mycobacteria, viruses, aspergillus, actinomycosis, cryptococcosis and pneumocystis may be the causative factor.

GRAVES' DISEASE (PRIMARY HYPERPLASIA)

Graves' disease (GD) is an autoimmune diffuse hyperplastic thyroid disorder, commonly seen in middle-aged women and usually diagnosed clinically due to hyperthyroidism. Most patients have a diffuse rather than nodular enlargement of the thyroid gland and do not require FNA for

diagnosis. Occasionally, however, large and/or cold nodules develop that raise the suspicion of a co-existing malignancy and thus prompt FNA. The cytologic features of GD are non-specific, and clinical correlation is needed for a definitive diagnosis. Aspirates are often cellular and show similar features to non-Graves' BFNs, including abundant colloid and a variable number of follicular cells. Lymphocytes and oncocytes may be seen in the background. Follicular cells are arranged in flat sheets and loosely cohesive groups, with abundant delicate, foamy cytoplasm. Nuclei are often enlarged, vesicular, and show prominent nucleoli. Few microfollicles may be observed. Distinctive flame cells may be prominent, and are represented by marginal cytoplasmic vacuoles with red to pink frayed edges which are best appreciated with Romanowsky-type stains.[59] Flame cells, however, are not specific for GD and may be encountered in other non-neoplastic thyroid conditions, follicular neoplasms, and papillary carcinoma. Occasionally the follicular cells display focal chromatin clearing and rare intranuclear grooves . These changes are not diffuse, however, and other diagnostic nuclear features of papillary carcinoma are commonly absent. Occasionally, treated GD shows prominent microfollicular architecture, significant nuclear overlapping and crowding, and considerable atypia. Care must be taken not to over-interpret these changes as malignant or neoplastic, and inquiry should be sought regarding prior radioactive iodine therapy

AUTOIMMUNE THYROIDITIS

(HASHIMOTO'S THYROIDITIS/LYMPHOCYTIC THYROIDITIS)

Smears are hypercellular. A bloody background with lymphoid cells, degenerative changes in follicular cells and infiltration of follicular cells by lymphoid cells are characteristic features of Hashimoto's thyroiditis [(2)] . Variable features include oxyphilic cells (Hurthle cells), plasma cells, epithelioid cell granulomas and multinucleated giant cells

The smear background shows lymphocytes with a variable number of plasma cells. There is prominent oxyphilic (Hurthle cell/Askanazy cell) change with single and syncytial aggregates of cells showing abundant, dense, finely granular, gray-blue cytoplasm (MGG), and well-defined cell borders [(2)] . Nuclei are 2–4 times the size of normal follicular cell nuclei and may show atypia and prominent nucleoli. Normal-appearing follicular cells may be present, showing features of hyperactivity. A characteristic feature is that of lymphocytes (and occasionally plasma cells) seeming to adhere to or infiltrate follicular cells, supporting the theory of direct epithelial damage by lymphocytes. Multinucleated giant cells and epithelioid cells can be seen in up to 40% of cases. Neutrophils and eosinophils may be seen adhering to or infiltrating follicular cells in early stages.

An interpretation of lymphocytic thyroiditis does not require a minimum number of follicular/Hürthle cells for adequacy.[60] The lymphoid population is polymorphic, including small mature lymphocytes, larger reactive lymphoid cells, and occasional plasma cells.

The lymphoid cells may be present in the background or infiltrate epithelial cell groups. Intact lymphoid follicles and lymphohistiocytic aggregates may be seen. Hürthle cells (oncocytes), when present, are arranged in sheets or as isolated cells. They have abundant granular cytoplasm, large nuclei, and prominent nucleoli

Anisonucleosis of Hürthle cells may be prominent. Sometimes mild nuclear atypia is encountered, including scattered nuclear clearing and grooves

DE QUERVAIN'S THYROIDITIS (SUBACUTE THYROIDITIS; GRANULOMATOUS THYROIDITIS)

In smears of granulomatous thyroiditis large multinucleate giant cells with numerous nuclei, granulomatous aggregates of epithelioid cells, degenerating follicular cells, neutrophils, macrophages and lymphocytes in a dirty smear background consisting debris and colloid.

The cellularity is variable and depends on the stage of disease. Clusters of epithelioid histiocytes, i.e., granulomas, are present, along with many multinucleated giant cells. The early stage demonstrates many neutrophils and eosinophils, similar to acute thyroiditis.[61] In later stages

the smears are hypocellular. They show giant cells surrounding and engulfing colloid , epithelioid cells, lymphocytes, macrophages, and scant degenerated follicular cells.[61]

RIEDEL´S THYROIDITIS/DISEASE

This is the rarest form of thyroiditis and results in progressive fibrosis of the thyroid gland with extension into the soft tissues of the neck. Criteria : The thyroid gland feels very firm on palpation. The preparations are often acellular. Collagen strands and bland spindle cells may be present. There are rare chronic inflammatory cells. Colloid and follicular cells are usually absent [51] .

FOLLICULAR NEOPLASMS (FN):

Cytologically, follicular lesions include follicular adenoma (FA),follicular carcinoma (FC) and cellular NG . Smears in FN are cellular in a bloody background that is usually devoid of colloid. Many uniform-sized follicular cell clusters, microfollicles and rosette formations are present. Syncytial aggregates, nuclear crowding and overlapping are also often seen. The repetitive smear pattern with uniform cell population is in contrast to the variable pattern of different cell types seen in colloid and hyperplastic nodules. Microacinar clusters with a central lumen represent microfollicles. These are characteristic of FN.

Cytologic preparations are moderately or markedly cellular There is a significant alteration in the follicular cell architecture, characterized by cell crowding, microfollicles, and dispersed isolated cells . Follicular cells are normal-sized or enlarged and relatively uniform, with scant or moderate amounts of cytoplasm. Nuclei are round and slightly hyperchromatic, with inconspicuous nucleoli . Some nuclear atypia may be seen, with enlarged, variably sized nuclei and prominent nucleoli . Colloid is scant or absent.

Variants of follicular neoplasms

• Hurthle (oxyphilic) cell tumors

• Atypical adenoma with bizarre cells

• Neoplasms with clear or signet ring cells

HURTHLE (OXYPHILIC) CELL TUMORS (HCT) :

Specimens are moderately to markedly cellular . The sample consists exclusively (or almost exclusively) of Hürthle cells:

- Abundant finely granular cytoplasm (blue or grey-pink with Romanowsky stains, green with Papanicolaou, pink with hematoxylin and eosin) [51] , enlarged, central or eccentrically located, round nucleus and prominent nucleolus
- Small cells with high nuclear/cytoplasmic (N/C) ratio (small cell dysplasia)
- Large cells with at least 2x variability in nuclear size (large cell dysplasia)

The Hürthle cells are dispersed predominantly as isolated cells , but sometimes arranged in crowded, syncytial-like arrangements or in monolayered sheets of variable sizes . There is usually little or no colloid. There are virtually no lymphocytes (excluding blood elements) or plasma cells. Transgressing vessels are present in some cases.

NEOPLASMS WITH CLEAR OR SIGNET RING CELLS

Clear cell change in follicular epithelium may be due to accumulation of glycogen, thyroglobulin, mucin or lipid. While clear cell morphology is well appreciated in tissue sections, cytologic smears show cells with abundant, finely vacuolated, pale but not totally clear cytoplasm. Clear cell change may be a focal or less frequently diffuse phenomenon in FA, FC, HCT and non-neoplastic lesions

PAPILLARY CARCINOMA (PC) :

Smears in PC are cellular with numerous three-dimensional and papillary fragments with or without vascular cores. Often, papillae not removed intact by the needle appear as flat sheets. Sheets of cells show a distinct anatomical border, formed by a row of cuboidal or columnar cells with focal nuclear crowding and overlapping. The tip of a papilla may be seen as a finger-like aggregate of cells with a similar edge. Naked true papillary connective tissue cores are sometimes found and can be diagnostically helpful.

Tumor cells show uniform enlargement with dense cytoplasm and well-defined cell borders. Intranuclear cytoplasmic inclusions (INCIs) are seen in up to 90% of cases and are characteristic of PC.

Irregular nuclear shapes, convolutions and longitudinal nuclear grooves or creases are visible in cytologic smears (in 85–100% cases)

The presence of ≥3 of the following features – papillae, psammoma bodies, nuclear grooves, INCIs and fine granular chromatin – has been reported to facilitate cytological diagnosis of PC, with frequent grooves and INCIs being the most dependable [(2)].

Criteria (for All Types of PTC, Conventional and Variants):

Follicular cells are arranged in papillae and/or syncytial-like monolayers. Swirling sheets ("onion-skin" or "cartwheel" patterns) are sometimes seen. The altered follicular cells exhibit characteristic nuclear features: Enlarged nuclei Oval or irregularly shaped, sometimes molded nuclei Longitudinal nuclear grooves Intranuclear cytoplasmic pseudoinclusions (INCI) Pale nuclei with powdery chromatin ("Orphan Annie" nuclei) Marginally placed micronucleoli, solitary or multiple Psammoma bodies are sometimes present. Multinucleated giant cells are common.

The amount of colloid is variable and may be stringy, ropy, or "bubble-gum"like. Hürthle cell (oncocytic) metaplasia is sometimes seen. Squamous metaplasia is sometimes seen

Variants of papillary carcinoma[51,72]

• Follicular (and macrofollicular encapsulated) variants

• Oncocytic variant

• Warthin tumor-like variant

• Cribriform-morular variant

• Adenoid cystic variant

• Variant with fasciitis-like stroma,

• High-grade variants: tall cell, columnar, diffuse sclerosing and solid/trabecular variants.

Follicular Variant

The follicular variant of PTC (FV-PTC) is a PTC in which the tumor is completely or almost completely composed of small to medium-sized follicles lined by cells with the nuclear features of a PTC.

Samples are usually hypercellular, with syncytial-like fragments containing microfollicles ("rosettes"). Dispersed microfollicular clusters, isolated neoplastic follicles, and some sheets with branched irregular contours may also be present. Some colloid may be present, typically dense-staining, thick, and sometimes within neoplastic follicles. In contrast to conventional PTC, the nuclear changes are often subtle. The following features are usually absent or inconspicuous: papillary and papillary-like fragments, multinucleated giant cells, INCIs, psammoma bodies, marked cystic change.[51,73]

Macrofollicular Variant

The macrofollicular variant is a PTC in which over 50% of the follicles are arranged as macrofollicles.

Criteria

The sample consists of monolayered (two-dimensional) sheets of atypical epithelium and/or variably sized follicles.

Convincing nuclear changes of PTC must be present for a definite interpretation of malignancy. In contrast to conventional PTC, the diagnostic nuclear features are often more subtle (as with FVPTC). Abundant thin colloid or fragments of thick colloid may also be present[51,73]

Cystic Variant

The cystic variant is a PTC that is predominantly cystic, comprised of thin, watery fluid, abundant histiocytes, and hypervacuolated tumor cells.

Criteria : The neoplastic cells are typically arranged in small groups with irregular borders; sheets, papillae, or follicles may also be present.

Tumor cells look “histiocytoid” (hypervacuolated). Macrophages, often containing hemosiderin, are present. There is a variable amount of thin or watery colloid. Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy. In contrast to conventional PTC, the fine powdery chromatin is usually less prominent, presumably due to cellular

degeneration, and cellular swirls/ onion-skin appearance and cart-wheel arrangement of the follicular cells are more frequently seen.[51]

Oncocytic Variant

The oncocytic variant is a thyroid tumor with the nuclear changes characteristic of PTC but composed predominantly of oncocytic cells (polygonal cells with abundant granular cytoplasm).

Criteria : The sample is composed predominantly of oncocytic cells, arranged in papillae, sheets, or as isolated cells.

Convincing diagnostic nuclear changes of PTC must be present for a definite diagnosis of PTC. Lymphocytes are absent or few in number.[51]

Warthin-like Variant

The Warthin-like variant of PTC is a circumscribed thyroid tumor with papillary architecture and lymphoid follicles that mimics a Warthin tumor of the parotid gland. The neoplastic cells have abundant granular cytoplasm and the nuclear features of PTC.

Criteria : The neoplastic cells are oncocytic and arranged in papillae and as dispersed cells. A lymphoplasmacytic background is present. The lymphocytes permeate the fibrovascular stalk and are intimately associated with the tumor cells. Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy.[51]

Tall Cell Variant

The tall cell variant (TCV) is an aggressive form of PTC composed of papillae lined by a single layer of elongated ("tall") tumor cells (their height is at least three times their width) with abundant dense granular cytoplasm and the typical nuclear changes of PTC. Tall cells with abundant cytoplasm should account for at least 50% of the tumor for it to be classified as a TCV.[62,74]

Criteria: The neoplastic cells have an elongated shape, with a height-to-width ratio of at least 3:1. The neoplastic cells have distinct cell borders and are arranged in papillary fragments

Some lymphocytes may be present. Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy. In contrast to conventional PTC: the nuclear chromatin is sometimes less powdery and more granular , psammoma bodies are fewer in number , INCI tend to be more frequent and more often multiple within one nucleus, imparting a "soap bubble" appearance to the nucleus[62,75]

Columnar Cell Variant

The columnar cell variant is a rare aggressive variant of PTC, characterized by columnar cells with hyperchromatic, oval, and stratified nuclei and supranuclear or subnuclear cytoplasmic vacuoles. The cells are typically arranged in papillae, but trabeculae and follicles can also be seen.

Criteria :

Smears are cellular and generally lack colloid. The neoplastic cells are arranged as papillae, clusters, and flat sheets, sometimes with small tubular structures. The nuclei are elongated and stratified.34 Convincing nuclear changes of PTC must be present for a definitive diagnosis of malignancy. In contrast to conventional PTC:

- The nuclear features of PTC (grooves, INCI) are focal and less prominent
- The nuclear chromatin tends to be hyperchromatic rather than pale and powdery
- Colloid and cystic change (macrophages) are typically not seen[63]

Hyalinizing Trabecular Tumor

Hyalinizing trabecular tumor (HTT) is a controversial neoplasm that some consider a variant of PTC.

The hyalinizing trabecular tumor (HTT) is a rare tumor of follicular cell origin characterized by trabecular growth, marked intratrabecular hyalinization, and the nuclear changes of PTC.

Criteria : Cohesive neoplastic cells are radially oriented around amyloid-like hyaline stromal material. INCIs and nuclear grooves are numerous. Occasional psammoma bodies may be present. Cytoplasmic paranuclear yellow bodies may be present[64]

MEDULLARY THYROID CARCINOMA (MTC):

Smears in MC are cellular in a background of blood. The cell pattern is predominantly dissociated with round, oval, polygonal and spindle cells in varying combinations. Plasmacytoid cells or triangular cells with eccentric nuclei are common and they show moderate amounts of cytoplasm with well-defined cell margins . The small cell type shows scanty cytoplasm, high nuclear cytoplasmic ratio and ovoid nuclei, forming dense clusters, often with nuclear molding. The spindle cell pattern shows cells with elongated, relatively pale nuclei and indistinct attenuated cytoplasm, resembling benign or lowgrade spindle cell soft tissue tumors . Nuclear chromatin may be fine and stippled (neuroendocrine-like) or coarsely granular.

MTC is a malignant neoplasm derived from and/or morphologically recapitulating the parafollicular cells of the thyroid gland.

Criteria

Samples show moderate to marked cellularity. Numerous isolated cells alternate with syncytial-like clusters in variable proportions from case to case. Cells are plasmacytoid, polygonal, round, and/or spindle-shaped. Long cell processes are seen in some cases. The neoplastic cells usually show only mild to moderate pleomorphism.

Rare bizarre giant cells may be seen; they can be numerous in the giant cell variant. Nuclei are round and often eccentrically placed, with finely or

coarsely granular (“salt and pepper”) chromatin. Nuclear pseudoinclusions are occasionally noted. Binucleation and multinucleation are common. Nucleoli are usually inconspicuous but can be prominent in some cells. Cytoplasm is granular and variable in quantity. Small red granules are seen with Romanowsky stains in some cases. Rare cases show cytoplasmic melanin pigment. Amyloid is often present and appears as dense, amorphous material that resembles thick colloid. Cells are typically strongly immunoreactive for calcitonin, CEA, chromogranin, synaptophysin, and TTF-1, and are negative for thyroglobulin. Aberrant results occasionally occur.

POORLY DIFFERENTIATED THYROID CARCINOMA(PDTC)

PDTC is a thyroid carcinoma of follicular cell origin characterized by an insular, solid, or trabecular growth pattern. In its pure form, PDTC lacks conventional nuclear features of papillary thyroid carcinoma and is distinguished from the latter by the presence of poorly differentiated features: mitoses, necrosis, or small convoluted nuclei. The most classic form of PDTC is the insular type, defined by its “cellular nests” or insular cell groups outlined by a thin fibrovascular border. In a subset of cases, PDTCs can also be associated with a better differentiated component showing typical microscopic features of papillary or follicular carcinoma variably admixed with poorly differentiated cells.

Criteria

Cellular preparations display an insular, solid, or trabecular cytoarchitecture . There is a uniform population of follicular cells with scant cytoplasm (sometimes plasmacytoid) . The malignant cells have a high nuclear/cytoplasmic (N/C) ratio with variable nuclear atypia . Apoptosis and mitotic activity are present . Necrosis is often present

ANAPLASTIC CARCINOMA /

UNDIFFERENTIATED THYROID CARCINOMA (UTC)

Three major patterns or combinations of these patterns are seen, namely giant cell, spindle cell and squamoid patterns with large intervening areas of necrosis and hemorrhage.

UTC is a high grade, pleomorphic, epithelial-derived malignancy with epithelioid and/or spindle cell features.

Criteria

Samples show variable cellularity, depending on the amount of necrosis and the site of sampling but are usually moderately to markedly cellular. A malignant background diathesis of necrotic material and neutrophils is often present. Cells are extremely variable in shape and a mixture of spindle and giant cells are seen in about half of the cases. Cells show bizarre nuclei with macronucleoli, irregular nuclear membranes and coarsely clumped chromatin.

Neoplastic cells are arranged as isolated cells and/or in variably sized groups. Neoplastic cells are epithelioid (round to polygonal) and/or spindle-shaped and range in size from small to giant-sized. “Plasmacytoid” and “rhabdoid” cell shapes are seen. Nuclei show enlargement, irregularity, pleomorphism, clumping of chromatin with parachromatin clearing, prominent irregular nucleoli, intranuclear inclusions, eccentric nuclear placement, and multinucleation. Necrosis, extensive inflammation (predominantly neutrophils, “abscess-like”) and/or fibrous connective tissue may be present. Osteoclast-like giant cells (non-neoplastic) are conspicuous in some cases. Neutrophilic infiltration of tumor cell cytoplasm can be seen. Mitotic figures are often numerous and abnormal[2,51].

MATERIALS AND METHODS

The study was conducted in the cytopathology Out Patient Department, Department of Pathology, Tirunelveli Medical College and Hospital, Tirunelveli. It was done after obtaining the necessary approval from Institutional Ethical Committee of Tirunelveli Medical College, Tirunelveli.

STUDY DESIGN:

Prospective experimental study.

STUDY LOCATION:

Fine needle aspirate materials obtained from thyroid lesions of patients attending cytology outpatient department of Tirunelveli medical college hospital , Tirunelveli were used in this study.

SAMPLE SIZE:

65 cases with adequate aspirates.

DURATION OF STUDY:

April 2016 to June 2017.

INCLUSION CRITERIA:

Fine needle aspirate materials obtained from thyroid lesions of patients attending cytology outpatient department of Tirunelveli medical college hospital

EXCLUSION CRITERIA:

Non cooperative patient

Inadequate material on FNA

WRIGHT GIEMSA STAIN

REAGENTS[65]

i. Wright stain dry powder 9 g
ii. Giemsa stain dry owder 1 g
iii. Glycerine 90 ml
iv. Absolute anhydrous methyl alcohol 2910 ml

STAIN PREPARATION[65]

Dissolve dry powder of Wright stain and Giemsa stain in absolute anhydrous methyl alcohol . add glycerine and mix well. Keep it in brown bottle. Allow to stand for 1 month before using. Bottle should be shaken thoroughly once in a day for 3 to 4 days.

Filter with doubled filter paper before using.

BUFFER PREPARATION[65]

Solution No .1

Sodium hydroxide 8 g

Distilled water 1000 ml

Solution no 2

Potassium dihydrogen phosphate 27.2 g

Distilled water 1000 ml

Take 23.7 ml of solution no 1 and add it to 50 ml of solution no 2. Take 20 ml of the above mixed buffer solution to 1000 ml of distilled water . check the pH. This should have a pH of 6.8 .

STAINING PROCEDURE[65]

1. Air dry the slides immediately after smearing.

2. Place the slides on two parallel glass rods. The slide should beabsolutely level.

3. Wright Giemsa stain is dropped on the slide until the smear is completely covered.

4. Allow 2 to 3 minutes time for fixation.

5. Add equal amount of buffer solution.

6. Mixing the stain and buffer can be done by drawing up some fluid into a Pasteur pipette and immediately expressing it again , being very careful not to scratch the smear.

7. The diluted stain is allowed to act for 10 to 15 minutes.

8. Surface scum will be formed.

9. Then it is flooded off with excess of buffer water or with tap water.

10. Stain should not be poured off the slide, to prevent stain precipitates getting deposited on smear.

11. Under surface of slide is wiped clean and allowed to air dry.

1. Mount with DPX mountant for longer storage. [65]

PAPANICOLOU STAIN[32]

i. HARRIS'S HEMATOXYLIN

ii. EOSIN AZURE 5O[32] (EA50)

0.04 M light green SF	10 ml
0.3 M eosin Y	20 ml
Phosphotungstic acid	2 g
Alcohol	750 ml
Methanol	250 ml

Glacial acetic acid 20 ml

iii. ORANGE G 6[32] (OG6)

10% aqueous Orange G 50 ml

Alcohol 950 ml

Phosphotungstic acid 0-15 g

All stains should be filtered before use

PAPANICOLOU STAINING PROCEDURE [32]

1. Fix in methanol , 10 to 15 minutes
2. Hydrate in 95 % alcohol , 2 minutes
3. Hydrate in 70 % alcohol , 2 minutes
4. Rinse in water 1 minute
5. Stain in Harris Hematoxylin , 5 minutes
6. Differentiate in 0.5 % aqueous hydrochloric acid , 10 seconds
7. Rinse in water ,2 minutes
8. Blue in tap water , 2 minutes
9. Rinse in water , 2 minutes
10. Dehydrate , 70 % alcohol , 2 minutes

11.Dehydrate, 95 % alcohol , 2 minutes

12.Stain in OG 6 , 2 minutes

13.Rinse in 95 % alcohol , twice , 2 minutes each

14.Stain in EA 50 , 3 minutes

15.Rinse in 95 % alcohol , 1 minute

16.Clear in xylene 1 to 2 dips

17.Mount with DPX mountant

HEMATOXYLIN AND EOSIN STAIN (H&E)

HEMATOXYLIN SOLUTION PREPARATION[32]

i. Hematoxylin 2.5 g
ii. Potassium alum 50 g
iii. Sodium iodate 0.5 g
iv. Absolute ethanol 25 cc
v. Glacial acetic acid - 20 cc
vi. Distilled water - 500cc

First alum should be dissolved in warm distilled water and Hematoxylin is dissolved in absolute alcohol separately. Then add dissolved hematoxylin to alum solution. The mixture is allowed to boil

and then add sodium iodate.The stain is quickly cooled following with addition of acetic acid.The obtained stain is all set for immediate use.

EOSIN PREPARATION[32]

i. Eosin Y - 1 g

ii. 95% ethanol - 80 cc

iii. Glacial acetic acid - 0.2 cc

iv. Distilled water - 20 cc

Eosin Y is first dissolved in distilled water followed by subsequent addition of 95% ethanol and glacial acetic acid.

FIXATION AND STAINING [10]

1. Fix in isopropyl alcohol – 10 minutes
2. Harris hematoxylin – 5 minutes.
3. Wash in clean water.
4. Bluing using 0.5% lithium carbonate or tap water.
5. Rinse in clean water.
6. Eosin – 15 seconds to 2 minutes based on the age of eosin.
7. Wash in clean water.
8. Xylene –2 dips .

9. Mount the slides using DPX mountant.

METHODS

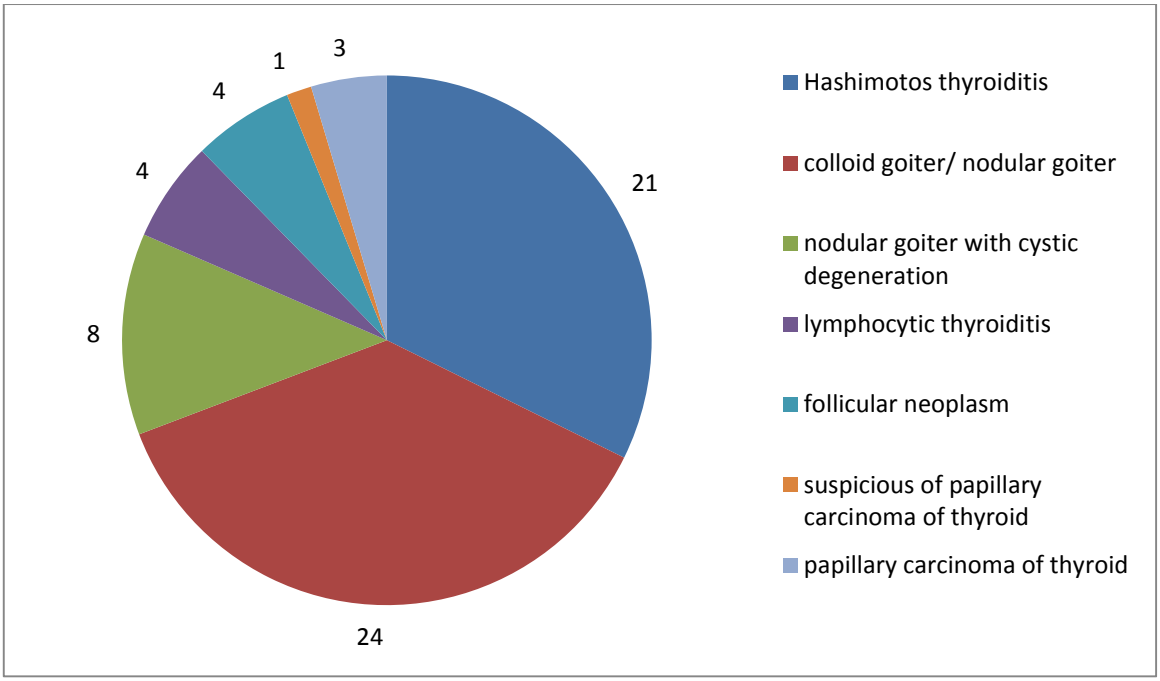
FNAC performed using 23 G needles, after obtaining informed written consent from the patients and multiple smears prepared. Two to three smears were wet fixed and stained with H&E and Pap stain. One to two smears were dry fixed and stained with Wright- Giemsa

Stained slides were observed using light microscope and analysed . The cytomorphologic features of thyroid lesions were analysed using various cytological staining techniques namely Hematoxylin and eosin stain, Papanicolou stain and Wright Giemsa. Cases were followed up and correlated with histopathology if available.

OBSERVATION AND RESULTS

TABLE 1 : DISTRIBUTION OF LESIONS :

S.No	Lesion	Number of cases	Percentage of cases
1	Hashimotos thyroiditis	21	32.3 %
2	Lymphocytic thyroiditis	4	6.15 %
3	Nodular colloid goiter	24	36.92 %
4	Nodular colloid goiter with cystic degeneration	8	12.30 %
5	Follicular neoplasm	4	6.15 %
6	Suspicious of papillary carcinoma of thyroid	1	1.53 %
7	Papillary carcinoma of thyroid	3	4.61 %
	Total	65	19



Distribution of cases:

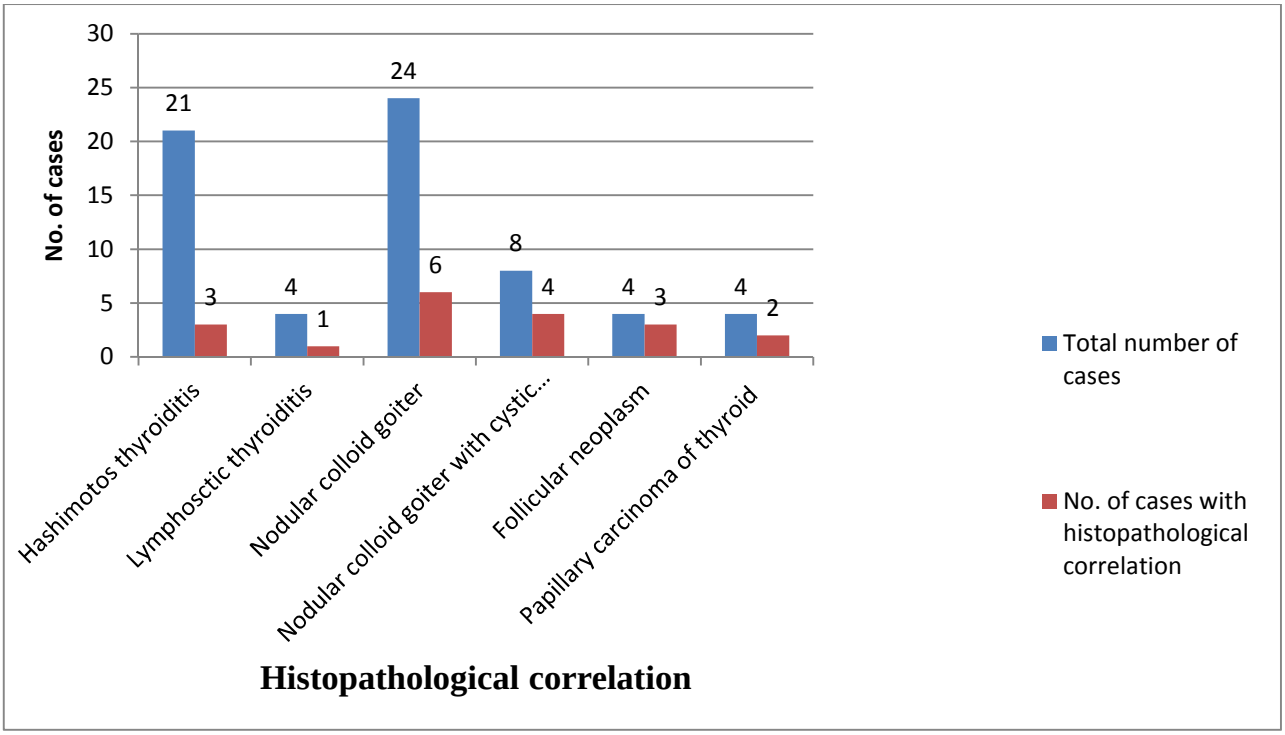
Out of total 65 cases, 49 cases (75.3 %) belonged to Bethesda category II (benign lesions). Hashimotos throiditis 21 cases, lymphocytic thyroiditis 4 cases and nodular colloid goiter 24 cases, 4 cases belonged to category IV (Follicular neoplasm), 1 case belonged to category V (suspicious for malignancy) – suspicious for papillary carcinoma of thyroid, 3 cases were of category VI (Malignant)- all 3 were papillary carcinoma of thyroid , one of them is Hurthle cell variant.

Bethesda category I (Non-diagnostic or Unsatisfactory) cases were excluded from the present study. None of the cases belonged to category III (Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance) in the present study.

HISTOPATHOLOGICAL CORRELATION:

TABLE 2 : HISTOPATHOLOGICAL CORRELATION

S. No	Lesion	Total no. Of cases	Cases with histopathological correlation
1	Hashimotos thyroiditis	21	3
2	Lymphocytic thyroiditis	4	1
3	Nodular colloid goiter	24	6
4	Nodular colloid goiter with cystic degeneration	8	4
5	Follicular neoplasm	4	3
6	Papillary carcinoma of thyroid	4	2



Out of 65 cases in this study, histopathological correlation was available for 19 cases. Histopathological correlation was available for 3 cases of Hashimotos thyroiditis, 1 case of lymphocytic thyroiditis, 6 cases of nodular colloid goiter, 4 cases of nodular colloid goiter with cystic degeneration, 3 cases of follicular neoplasm and 2 case of papillary carcinoma of thyroid. Of those 19 cases, histopathological reports correlated with cytological diagnosis. Three cases reported as follicular neoplasm on cytological basis were reported as follicular carcinoma on histopathology.

The cytomorphologic features of thyroid lesions observed using H&E, Pap and Wright Giemsa stains is as follows.

HASHIMOTO'S THYROIDITIS / LYMPHOCYTIC THYROIDITIS

Wright Giemsa stain

The cells appeared slightly larger. Hurthle cells showed abundant fine granular amphophilic cytoplasm, mild anisokaryosis and centrally or eccentrically placed nucleus with fine chromatin. Follicular cells had pale ill defined cytoplasm and round nucleus with condensed chromatin. Polymorphous population of lymphocytes and plasma cells are very well demonstrated. (Fig 1)

Papanicolou stain:

Pap stained smears showed Hurthle cells with abundant deep eosinophilic cytoplasm, marked anisokaryosis and centrally or eccentrically placed nucleus with fine chromatin (Fig 2). Intra nuclear inclusions were seen in 3 cases stained with Pap stain. Follicular cells had ill defined cytoplasm, round nucleus with fine chromatin and inconspicuous nucleoli. Background showed infiltration of lymphocytes.

Hematoxylin and eosin stain

In H&E stained smears Hurthle cells had abundant eosinophilic cytoplasm, marked anisokaryosis and centrally or eccentrically placed nucleus with condensed chromatin (Fig 3). Follicular cells had ill defined

cytoplasm , round nucleus with condensed chromatin. Background showed infiltration of lymphocytes.

Cytoplasmic granularity of Hurthle cells and polymorphous population of lymphocytes and plasma cells were very well demonstrated using Wright Giemsa stain. Anisokaryosis of Hurthle cells were very well demonstrated using Pap stain. Intra nuclear inclusions were seen in 3 cases stained with Pap stain.

Problems with differential diagnosis arise when stripped follicular cell nuclei which resemble lymphocyte nuclei, were present. Stripped follicular cell nuclei have more homogenous chromatin and denser nuclear rim and lack basophilic rim of cytoplasm seen in lymphocytes. Lymphocytes were better differentiated from naked follicular cell nuclei in Romanowsky stained smears.

Cytological features of Hurthle cells in Hashimotos thyroiditis should be differentiated from Hurthle cell neoplasm. In thyroiditis, oncocytes were large, atypical and pleomorphic. But Hurthle cell neoplasm usually show monotonous cells. Anisokaryosis was well appreciated using alcohol fixed Papanicolou stain.

NODULAR COLLOID GOITER :

Wright Giemsa stain

Follicular cells had pale ill defined cytoplasm and round nucleus with open chromatin. In Wright Giemsa stained smears colloid took bluish violet colour (Fig 4). Thick colloid showed cracking artifacts. And it was easy to identify thin colloid in air dried smears stained with Wright Giemsa stain. One case of nodular colloid goiter in a known hyperthyroid patient showed paravacuolar granules. (Fig 5)

Papanicolou stain :

The thyroid follicular cells had uniform round nuclei that are approximately the size of a red blood cell with scanty delicate cytoplasm. Thick and thin colloid took varying shades of light greenish blue colour , pinkish colour and orangish colour in Pap stained slides (Fig 6). Follicular cells showed anisokaryosis. (Fig 7)

Hematoxylin and eosin stain:

Thyroid follicular cells had scant cytoplasm and round nucleus with condensed chromatin. Colloid appeared as eosinophilic material in H&E stained smears (Fig 8). Anisokaryosis was noted in the follicular cells. Diffuse and nodular goiters had similar cytologic pictures.

When aspirates are overly bloody, serum may be mistaken for colloid, especially on Pap stains. This problem was overcome by using air dried smears stained with Romanowsky stains such as Wright Giemsa / MGG in which thin colloid appears as watery blue. Anisokaryosis was well appreciated in Pap and H & E stained smears.

NODULAR COLLOID GOITER WITH CYSTIC DEGENERATION

Wright Giemsa stain :

In Wright Giemsa stained smears cyst macrophages showed dusky grayish cytoplasm and follicular cells had pale ill defined cytoplasm and round nucleus with condensed chromatin. (Fig 9)

Papanicolou stain :

Benign follicular cells and cyst macrophages were seen in the background of colloid or fluid background. Cyst macrophages showed engulfed colloid within their cytoplasm (Fig 10). Follicular cells showed anisokaryosis.

Hematoxylin and eosin stain:

Follicular cells arranged in microfollicles and macrofollicles admixed with cyst macrophages were in the background of colloid. Anisokaryosis was noted in the follicular cells. The cyst macrophages with vacuolated

cytoplasm were seen in H&E stained smears. One case showed hemosiderin laden macrophages in H&E stained smear. (Fig 11)

Anisokaryosis was well appreciated in Pap and H & E stained smears. Engulfed colloid within the cytoplasm of cyst macrophages were well demonstrated using Pap stain. This can be used to determine whether the cystic degeneration is recent or old.

FOLLICULAR NEOPLASM :

Wright Giemsa stain

In Wright Giemsa stained smears the thyroid follicular cells showed moderately preserved microfollicular architecture with nuclear crowding and overlapping in some foci (Fig 12). Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are enlarged, round to oval and contain uniformly dispersed coarse granular chromatin. (Fig 13)

Papanicolou stain :

Hypercellular aspirate showing predominant microfollicular architecture and three dimensional clusters. The follicular cells were monomorphic with pale ill defined cytoplasm and round nucleus with smooth contour (Fig 14). Background was hemorrhagic and free of colloid.

Hematoxylin and eosin stain:

H&E stained smears showed repetitive microfollicular pattern and three dimensional clusters with uniform cell population in a hemorrhagic background. (Fig 15)

The distinction between follicular neoplasm and nodular goiter with a microfollicular focus may lead to diagnostic difficulties. The amount of colloid in the background is an important feature for differentiating these two entities. Colloid was scant or absent in follicular neoplasm , whereas in case of nodular goiter there was more colloid. The presence of abundant blood in smears helped in the diagnosis of follicular neoplasm, since they are highly vascularised. Another distinguishing feature is the anisokaryosis. Anisokaryosis was observed in cases of non-neoplastic lesions of thyroid such as nodular goiter and thyroiditis. Even mild anisokaryosis was well demonstrated in wet fixed Pap stained smears.

PAPILLARY CARCINOMA OF THYROID:

Wright Giemsa stain

Wright Giemsa stained smears showed papillary clusters and monolayered groups of thyroid follicular cells with enlarged nuclei , irregular nuclear contour , fine granular chromatin and enlarged cytoplasms. Some of the cells showed intranuclear cytoplasmic inclusions. (Fig 16)

Papanicolou stain :

Papillary architecture is very well maintained in Pap stained smears. Nuclear crowding, overlapping and intranuclear cytoplasmic inclusions and nuclear grooves were easily demonstrated (Fig 17). Follicular cells with fine granular chromatin (powdery chromatin) and inconspicuous nucleoli are best appreciated in Pap stained smears. (Fig 18 & Fig 19)

Hematoxylin and eosin stain:

Follicular cells were arranged in papillary clusters with fine granular chromatin. Nuclear crowding, overlapping and intra nuclear cytoplasmic inclusions were seen (Fig 20). One case showed nuclear grooves in H&E stained smear. (Fig 21)

Differential diagnosis include papillary hyperplasia in other lesions of thyroid and Hyalinising trabecular tumour. Difficulty in diagnosis arise in cases of cystic change, lymphocyte infiltrate in papillary carcinoma and follicular variant of PC, which may lead to false positive or false negative diagnosis. In those circumstances, careful inspection of nuclear features such as powdery chromatin, intranuclear cytoplasmic inclusions and nuclear grooves better appreciated using wet fixed Pap stained smears, thus aiding in improvement of diagnostic accuracy.

DISCUSSION

Fine Needle Aspiration Cytology of thyroid lesions is being widely used today and it is a minimally invasive and cost effective outpatient procedure.

The two basic factors that affect the interpretation of the FNAC smears are sampling and the quality of staining. Sampling improves with the experience of the pathologist while staining depends on the nature of stain and staining technique. The choice to select an appropriate stain for FNAC is the basis of obtaining reliable and optimal results . And also to decrease the rates of false negative and false positive reports the choice and quality of stains is important

The present study analyses the cytomorphologic features of individual thyroid lesions using H&E, Pap and Wright Giemsa stains . These three stains are widely used in most cytology laboratories. The present study comprises 21 cases of Hashimotos thyroiditis. Nguyen G-K et al [(1)] observed sheets of follicular epithelial cells with oncocytic change admixed with benign lymphoid cells in Pap stained smear.

Krafts K et al[(11)] states that Romanowsky-type stains enhance cytoplasmic detail, a useful feature for determining differentiation of neoplastic cells, Romanowsky-type stains frequently show excellent granule detail.

Romanowsky-type stains are superior to wet fixed preparations for diagnosing hematolymphoid neoplasms, particularly in body fluid specimens. Certain characteristic features of hematolymphoid cells either are not seen or are more difficult to recognize in Papanicolaou or hematoxylin and eosin stains.

Marluce Bibbo[9] describes that Hurthle cells show marked anisonucleosis and occasionally the nucleus may contain cytoplasmic inclusions in Pap stained smears and irregular –sized Hurthle cells with with polygonal abundant granular cytoplasm , nucleus is slightly eccentric and contain fine granular chromatin and anisokaryosis in Romanowsky stained smears.

In Hashimotos thyroiditis, cellular architecture is very well preserved in wet fixed smears stained with both H&E and Pap stains. When compared to H&E, Pap stained smears showed Hurthle cells with abundant deep eosinophilic cytoplasm and centrally placed nucleus. Anisokaryosis of Hurthle cells are very well demonstrated using Pap stain. Intra nuclear inclusions were seen in 3 cases stained with Pap stain.

In air dried and Wright Giemsa stained slides the cells appeared slightly larger. Fine cytoplasmic granules of Hurthle cells are seen. Hurthle cell cytoplasm took amphophilic colour. Polymorphous population of lymphocytes and plasma cells are very well demonstrated using Wright

Giemsa stain. Our findings were similar to that of Nguyen G-K et al[1] and Krafts K et al[11].

The present study comprised of 24 cases of nodular colloid goiter. Literature described that colloid appear as fine film of varying colour from gray-green to rose [9], Colloid is cyanophilic to eosinophilic and orangish when mixed with blood. [10] In PAP-stained smears, thin colloid stains pale green or orange, with cracking artifacts seen. Thick colloid appears as clumps of dark green or orange material[2]

In Romanowsky stains, thin colloid is recognized easily owing to its characteristic folding and cracking patterns, which imparts a "mosaic like crackling " described by Krafts, Kp et al[27] " crazy pavement" appearance or " cracked glass " and red-violet colour [9]. Orell et al[2] describes blue violet colour and hyaline texture of colloid appear to be an advantage in May Grunwalds Giemsa (MGG)-stained smears and helps in distinguishing it from fibrillary collagen and deep magenta staining amyloid.

In present study colloid appeared as eosinophilic in H&E stained smears.Colloid took light greenish blue colour, pinkish colour and orangish colour in Pap stained slides. In Wright Giemsa stained smears colloid took bluish violet colloid. Thick colloid showed cracking artifacts. And it was easy to identify thin colloid in air dried smears stained with Wright

Giemsa stain. Sidawy and Costa et al describes that a common finding in fine needle aspirates of the thyroid is the presence of paravacuolar granules, which represent lysosomes containing hemosiderin or lipofuscin pigments [18] In our present study one case of nodular colloid goiter in a known hyperthyroid patient showed paravacuolar granules.

The present study comprised of 4 cases of follicular neoplasm. E.A Sinna et al[68] described a case of follicular neoplasm showing atypical follicular cells with high N/C ratio and nuclear pleomorphism, arranged in three dimensional cluster with focal attempt at acinar arrangement in pap stained smears. Marluce Bibbo et al [9] described smears of follicular neoplasm are cellular with tissue fragments showing marked crowding and overlapping. Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are round and enlarged in size in Romanowsky stained smears.

In the present study wet fixed smears stained with hematoxylin and eosin and Pap stains showed hypercellular aspirates with very well preserved cellular architecture . Predominant microfollicular architecture and three dimensional clusters are seen. The follicular cells showed variable nuclear atypia.

In Wright Giemsa stained smears the thyroid follicular cells showed moderately preserved microfollicular architecture with nuclear crowding

and overlapping in some foci. Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are enlarged, round to oval and contain uniformly dispersed coarse granular chromatin.

The present study comprised of 4 cases of papillary carcinoma of thyroid. E.A Sinna et al[68] described a case of papillary carcinoma showing characteristic papillary configuration. The nuclei show ground glass chromatin, intranuclear cytoplasmic inclusions and characteristic clefts in Pap stained smears.

In the present study papillary architecture is very well maintained in H&E and Pap stained smears. Nuclear crowding, overlapping and intracytoplasmic nuclear inclusions are easily demonstrated using H&E and Pap stained smears. One case showed nuclear grooves in H&E stained smear. Follicular cells with fine granular chromatin (powdery chromatin) and inconspicuous nucleoli are best appreciated in Pap stained smears.

Wright Giemsa stained smears showed papillary clusters and monolayered groups of thyroid follicular cells with enlarged nuclei , irregular nuclear contour, fine granular chromatin and enlarged cytoplasms. Some of the cells showed intranuclear cytoplasmic inclusions.

SUMMARY AND CONCLUSION

Thyroid enlargement, whether diffuse or in the form of nodule, has to be investigated to rule out neoplasm. FNAC is the first line of investigation along with other investigations to avoid unnecessary surgeries. Most studies have reported high accuracy rates of FNAC in the diagnosis of neoplasm and thyroiditis and it depends on stains used.

In the present study, the cytoplasmic features such as cytoplasmic granularity, paravacuolar granules and thin colloid are very well demonstrated using Wright Giemsa stain. Cell borders and crisp nuclear features such as chromatin pattern, intranuclear inclusions are best appreciated using wet fixed smears stained with H&E and Pap stains.

Papanicolaou stain is widely used in the practice of cytopathology because of crisp nuclear features such as demonstration chromatin pattern, intranuclear inclusions and cell size comparable to histopathology. Hematoxylin and eosin stain is used in many loboratories because it also provides better nuclear features and it is simple when compared to Pap stain.

Romanowsky-type stains provide several advantages for evaluation of routine cytological specimens including accentuation of cell and nuclear size differences, accentuation of nuclear chromatin, enhanced visibility of

cytoplasmic detail, enhanced visibility of smear background elements, and superior demonstration of intercellular material. In addition, in certain diagnostic settings in which Papanicolaou stains are of little benefit, the use of Romanowsky-type stains is critical. These settings include detection of microorganisms, diagnosis of hematolymphoid neoplasms, and rapid on site evaluation of diagnostic specimens.

We conclude that use of different methods of fixation and multiple stains will complement each other and aid in improving diagnostic accuracy of thyroid lesions. Hence if sufficient material is available different cytological staining techniques can be used to decrease false positive and false negative reports in fine aspiration cytology of thyroid lesions.

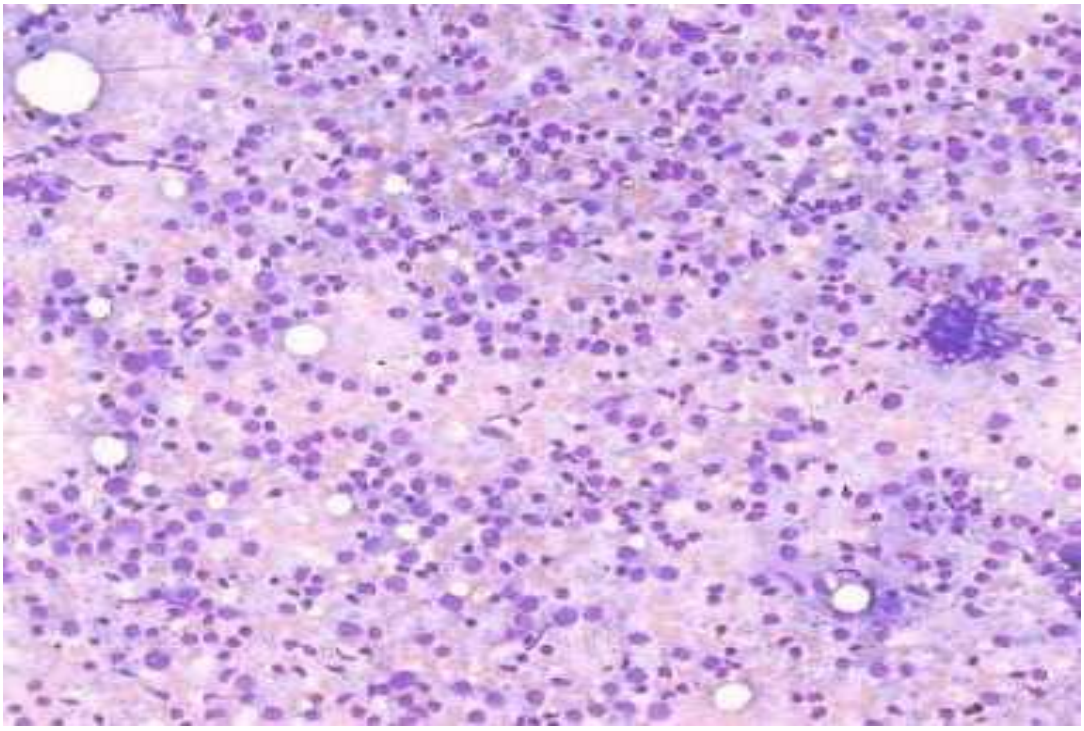


Fig 1: Hashimotos thyroiditis – Wright Giemsa stain, 10 X

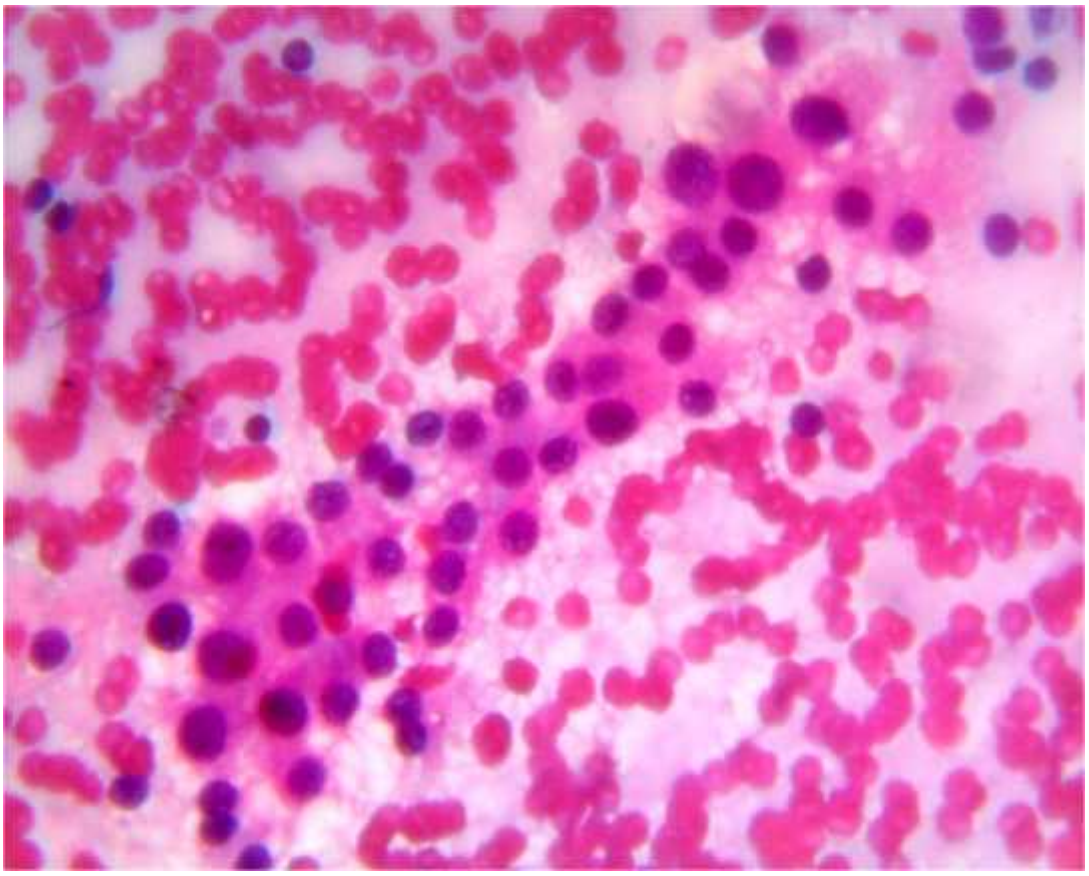


Fig 2: Hurthle cells in Hashimotos thyroiditis – Pap stain, 40 X

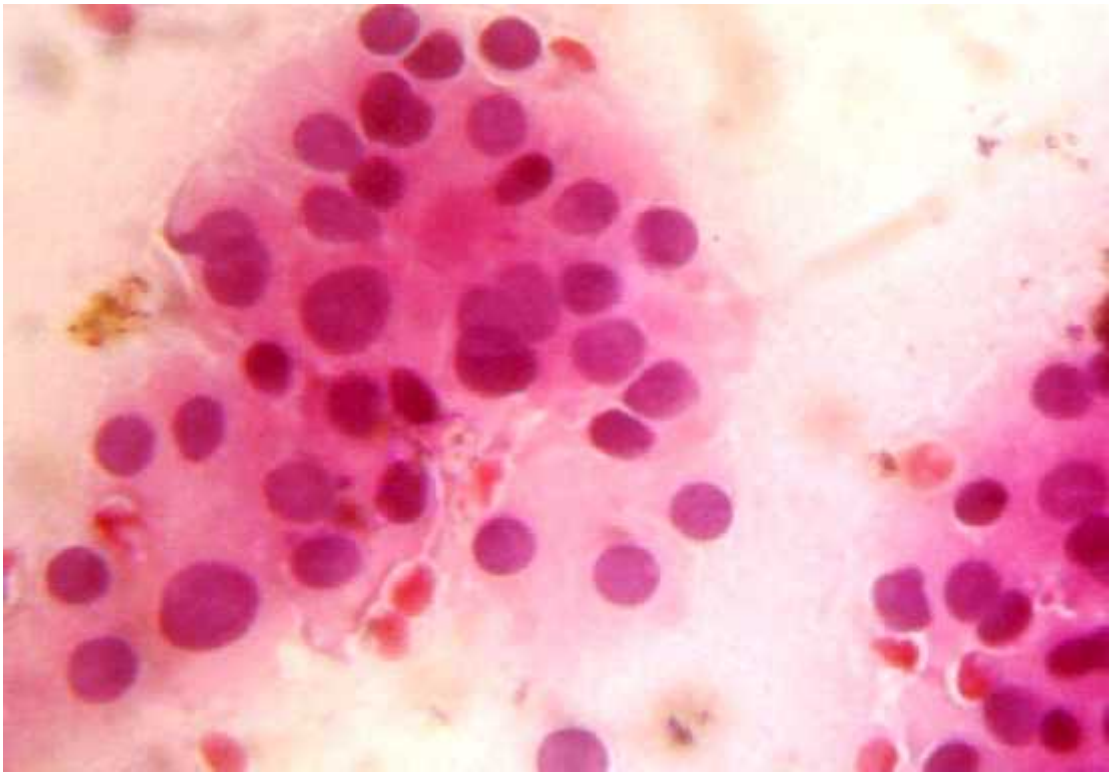


Fig 3: Hurthle cells in Hashimotos thyroiditis – H&E stain, 40 X

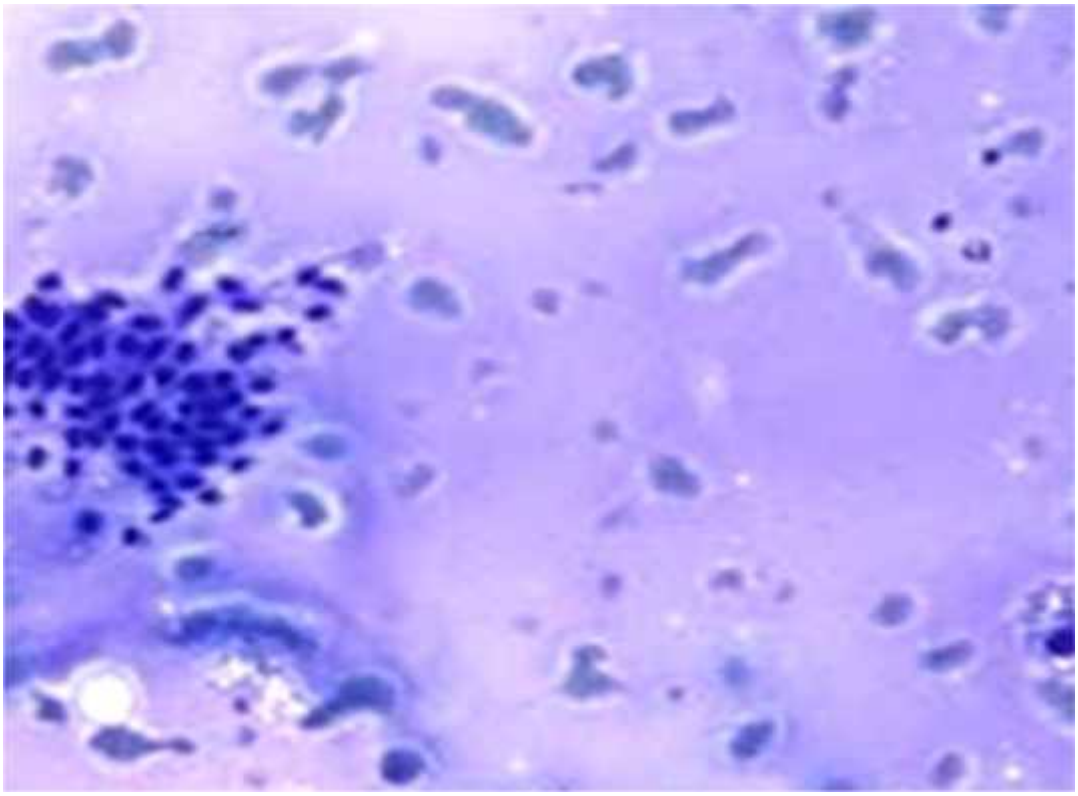


Fig 4:Thin collid with follicular cells in nodular colloid goiter, Wright Giemsa, 40X

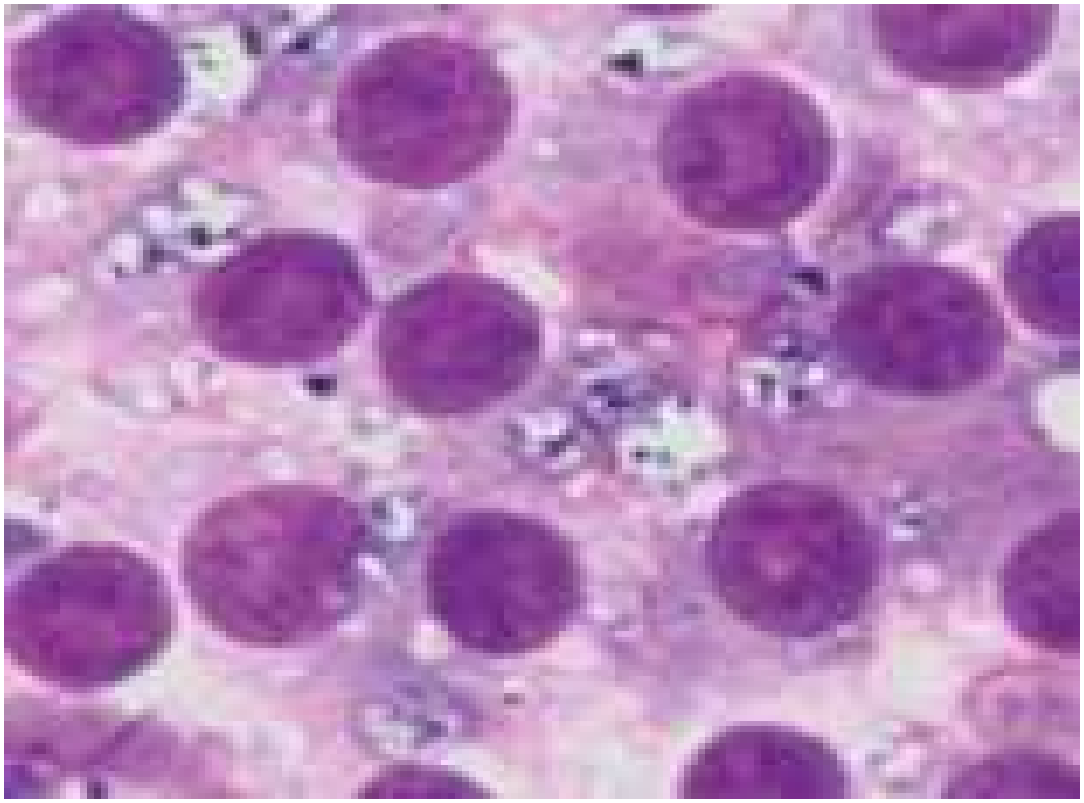


Fig 5: Paravacuolar granules in nodular goiter, Wright Giemsa, 100 X

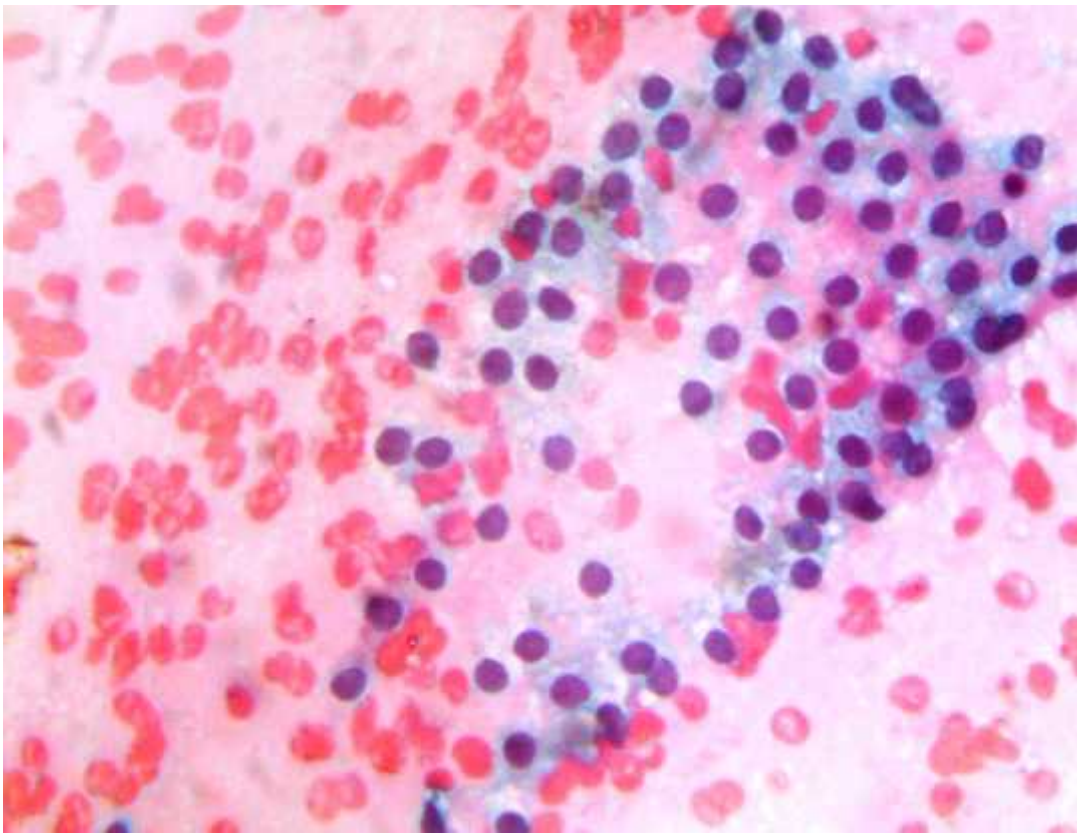


Fig 6: Follicular cells in background of colloid in nodular colloid goiter, Pap stain, 40X

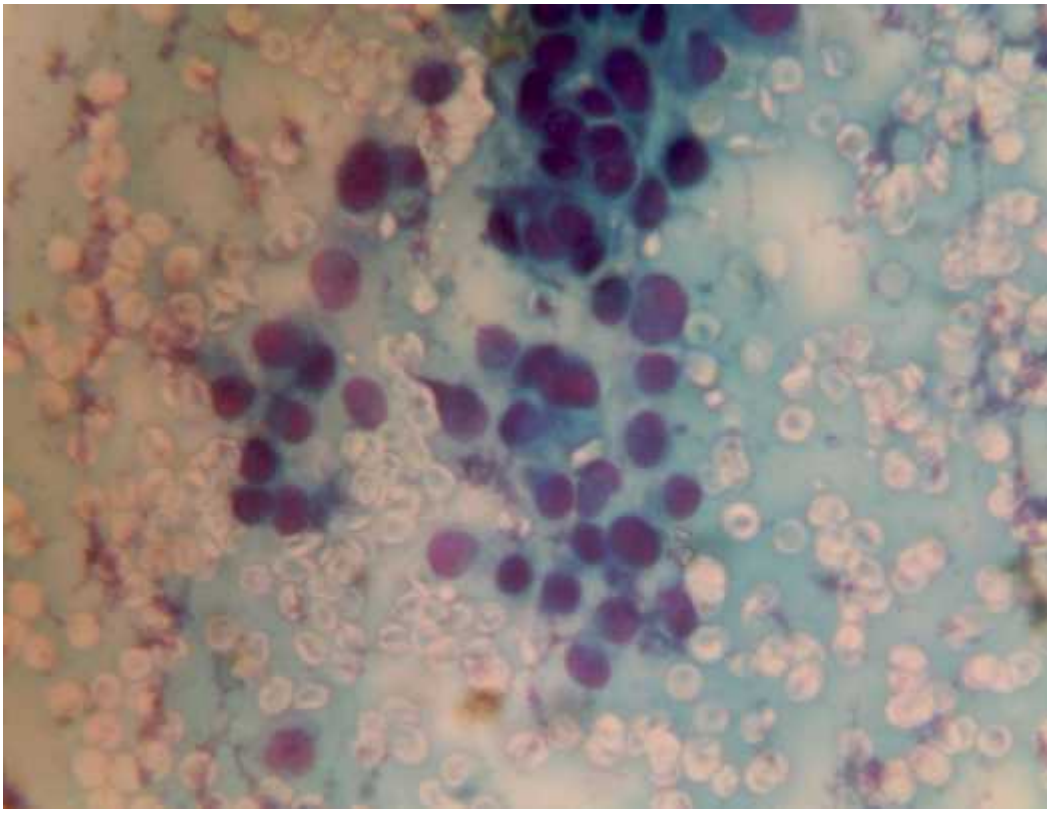


Fig 7: Follicular cells exhibiting anisokaryosis in Nodular Colloid Goiter, Pap stain, 40 X

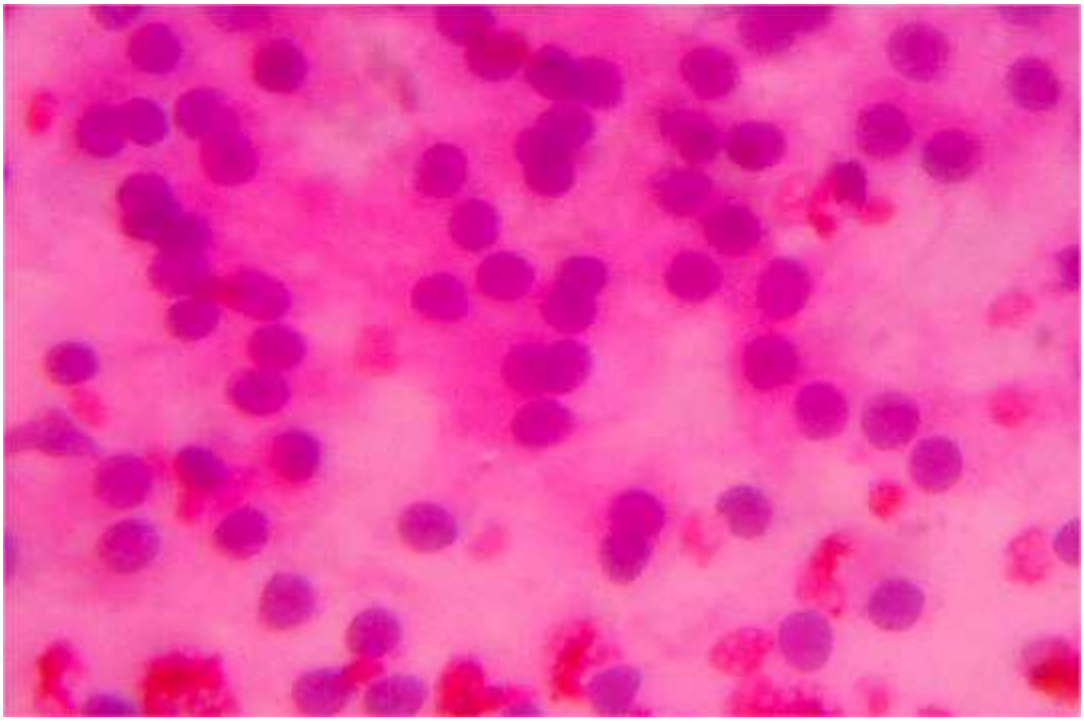


Fig 8: Benign follicular cells in the background of eosinophilic colloid, H&E, 40 X

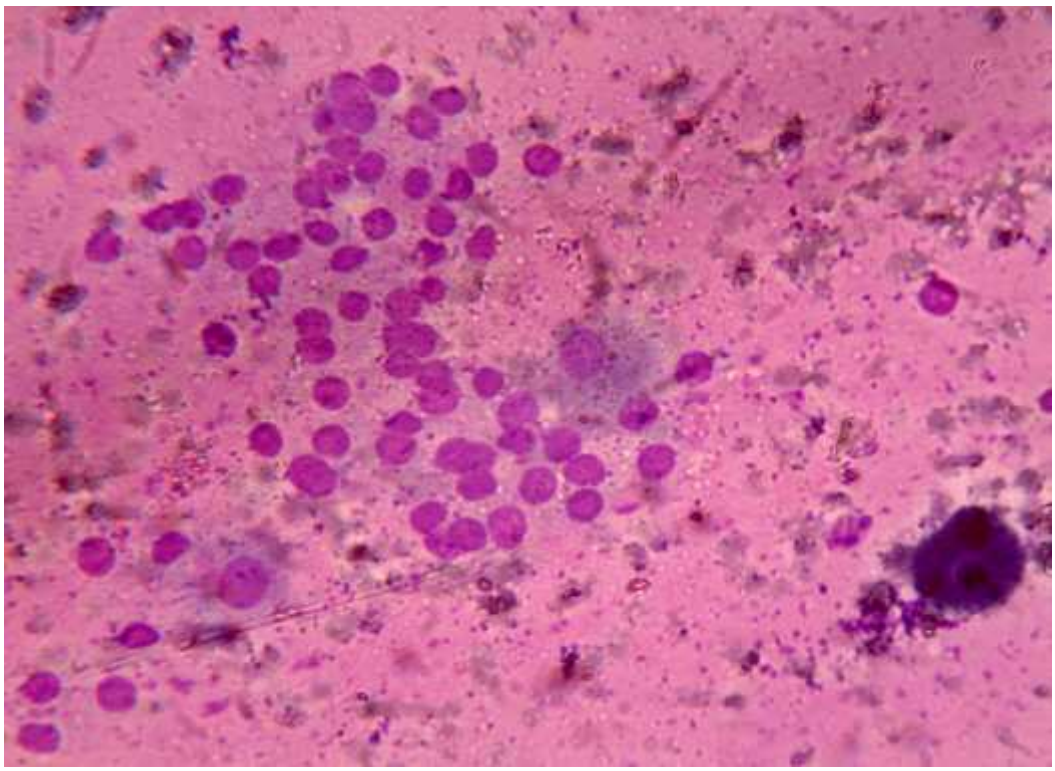


Fig 9: Cyst macrophages , nodular goiter with cystic degeneration, Wright Giemsa, 40X

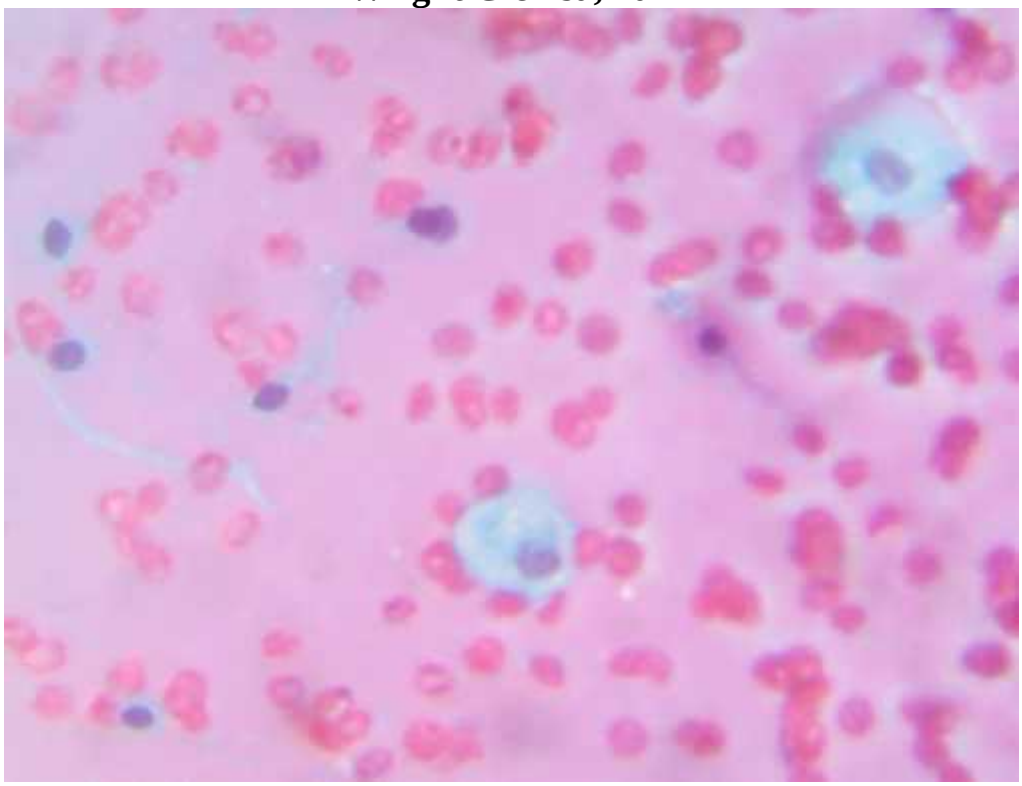


Fig 10: Cyst macrophages with bluish colloid, nodular goiter with cystic degeneration, Pap stain , 40X

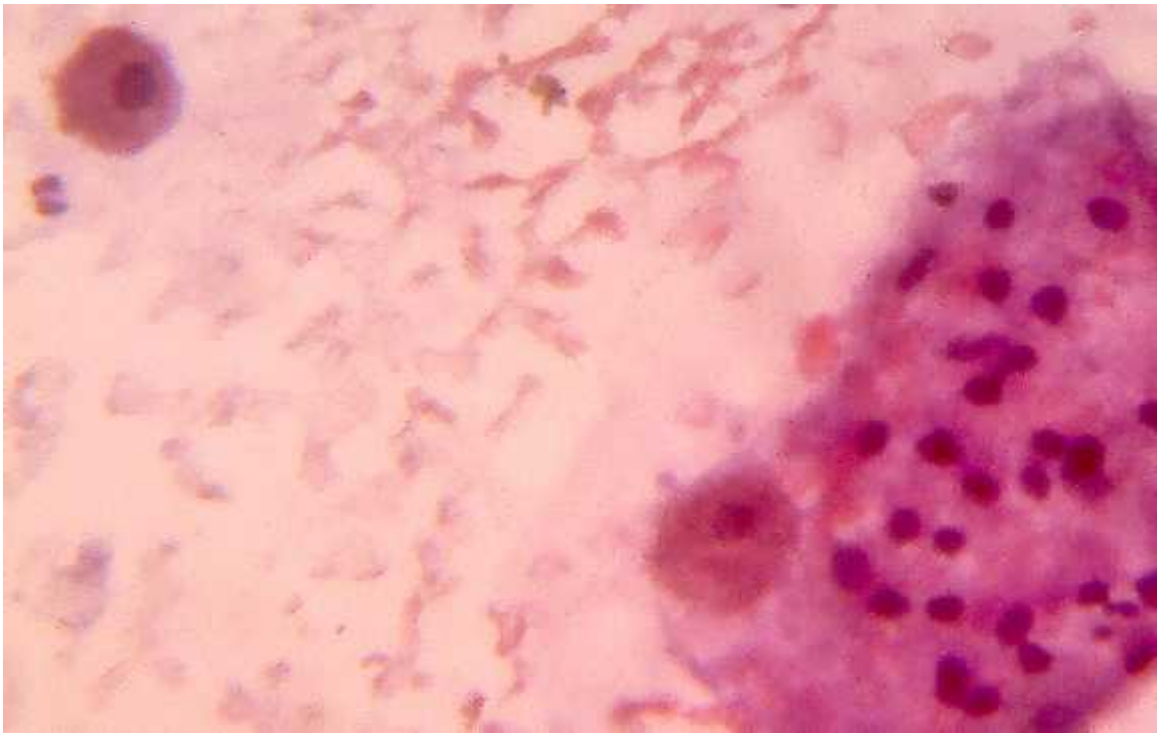


Fig 11: Hemosiderin laden macrophages in nodular goiter with cystic degeneration, H&E , 40X

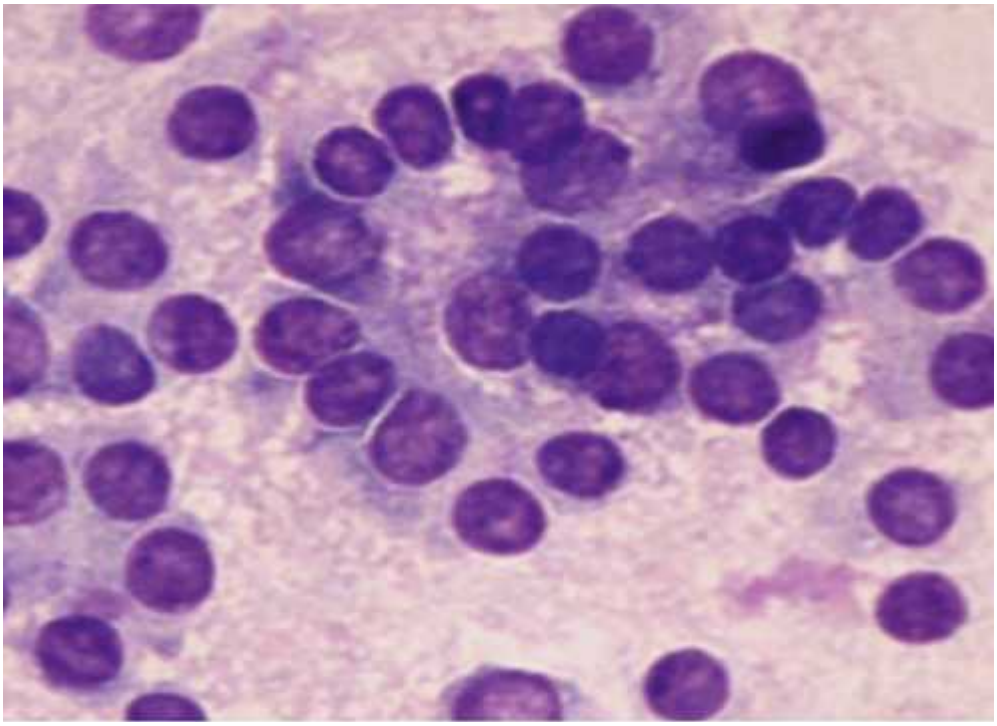


Fig 12: Follicular neoplasm, Wright Giemsa, 40 X

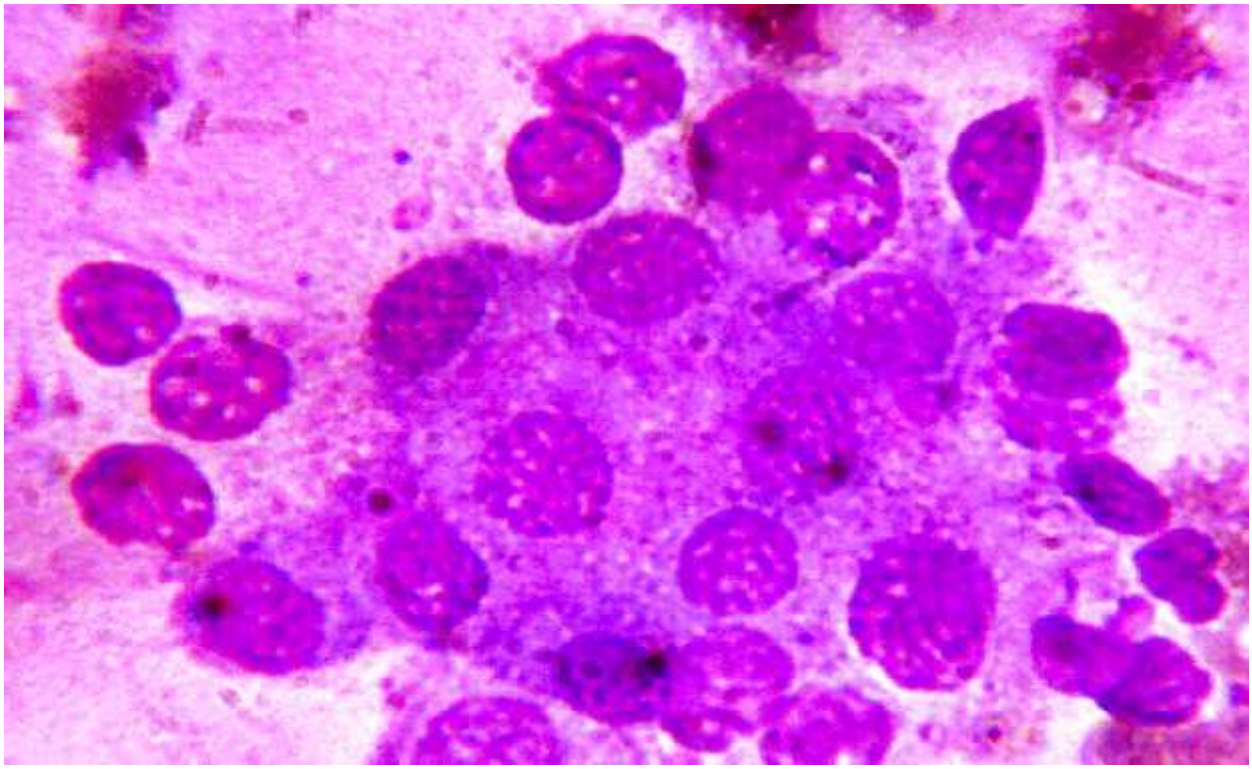


Fig 13: Coarse granular chromatin in follicular neoplasm, Wright Giemsa, 100X

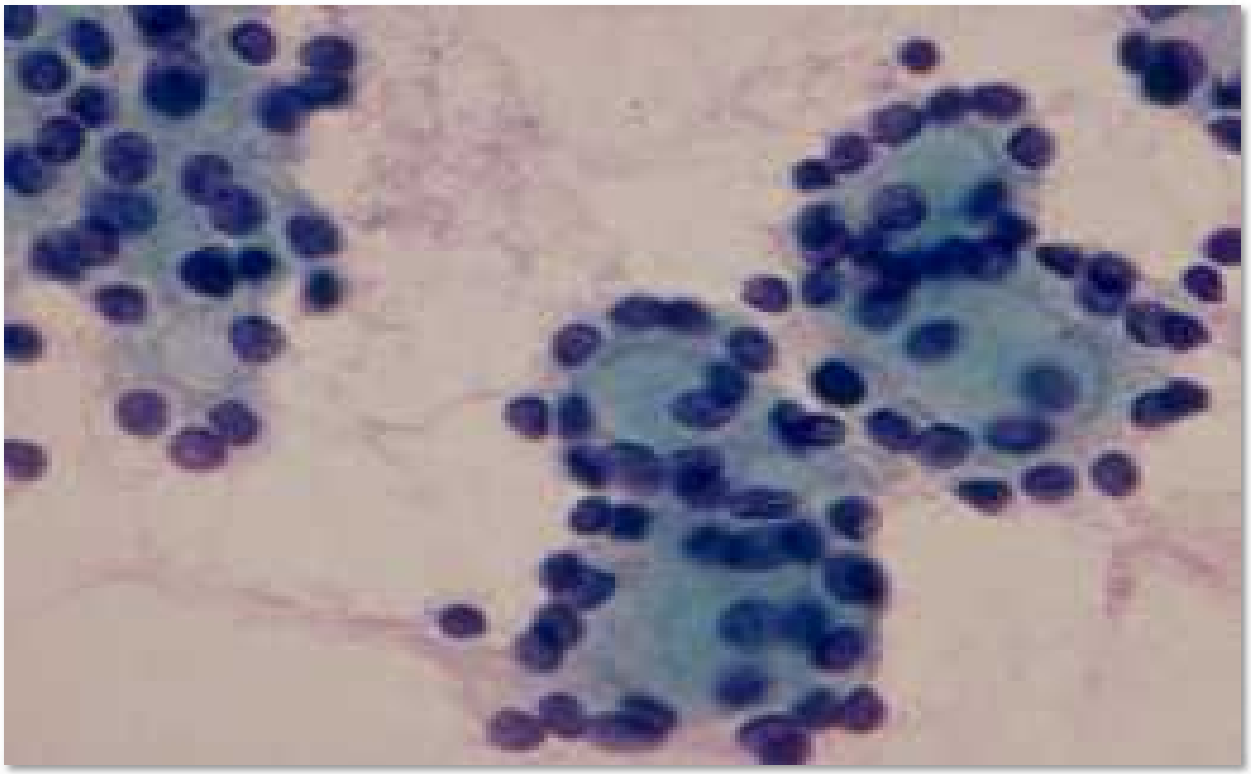


Fig 14: Follicular neoplasm, Pap stain , 40 X

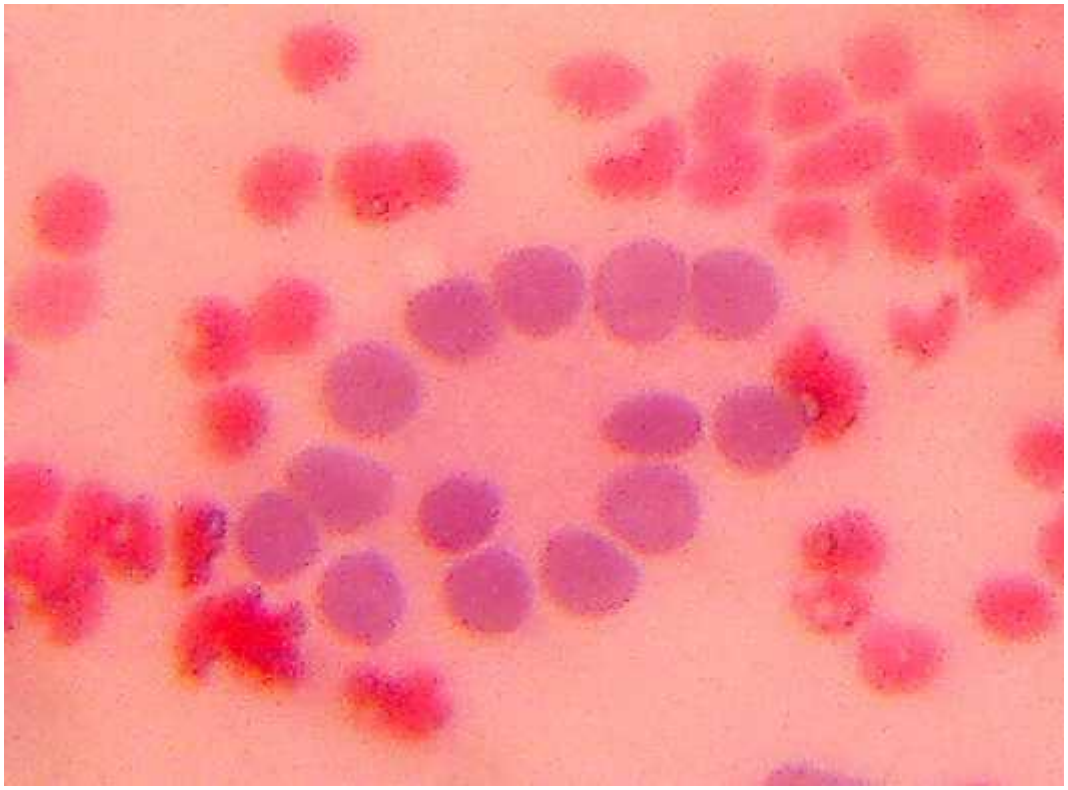


Fig 15: Follicular neoplasm, H&E, 40 X

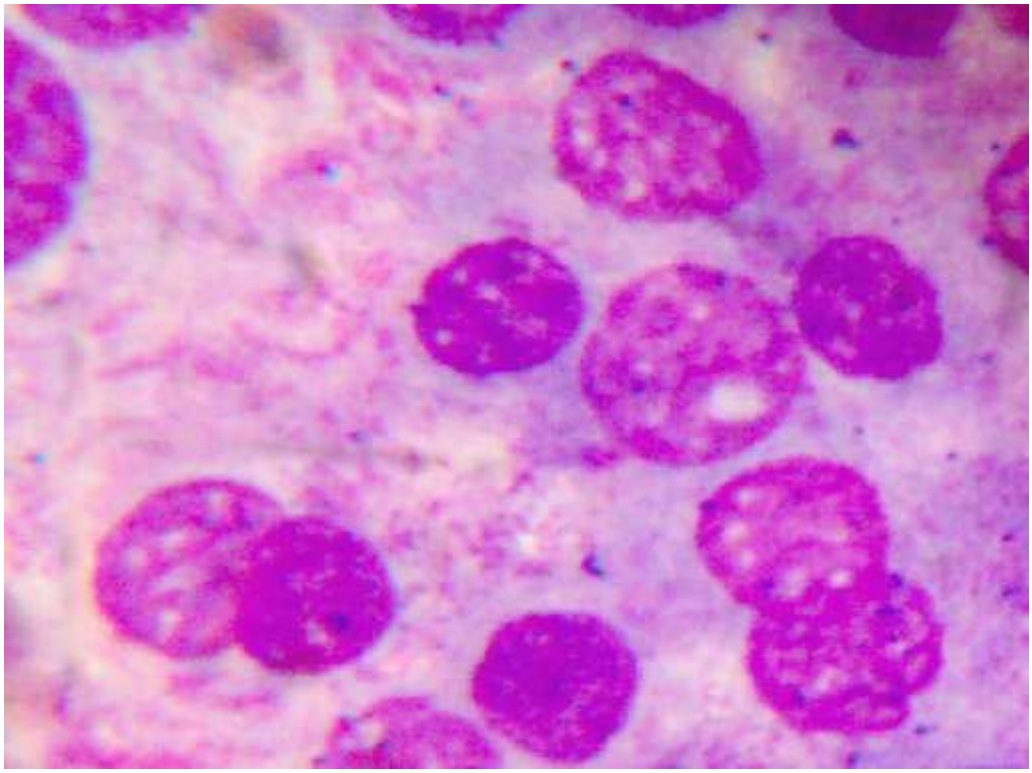


Fig 16: Intranuclear cytoplasmic inclusions, Papillary carcinoma, Wright Giemsa, 100X

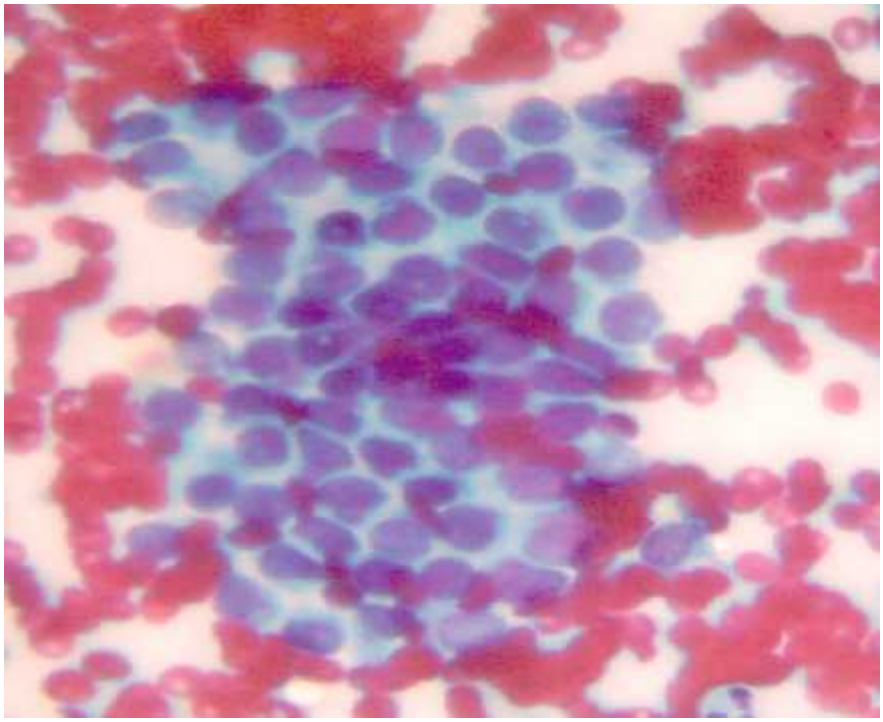


Fig 17: Intranuclear cytoplasmic inclusions, papillary carcinoma, Pap stain, 40X

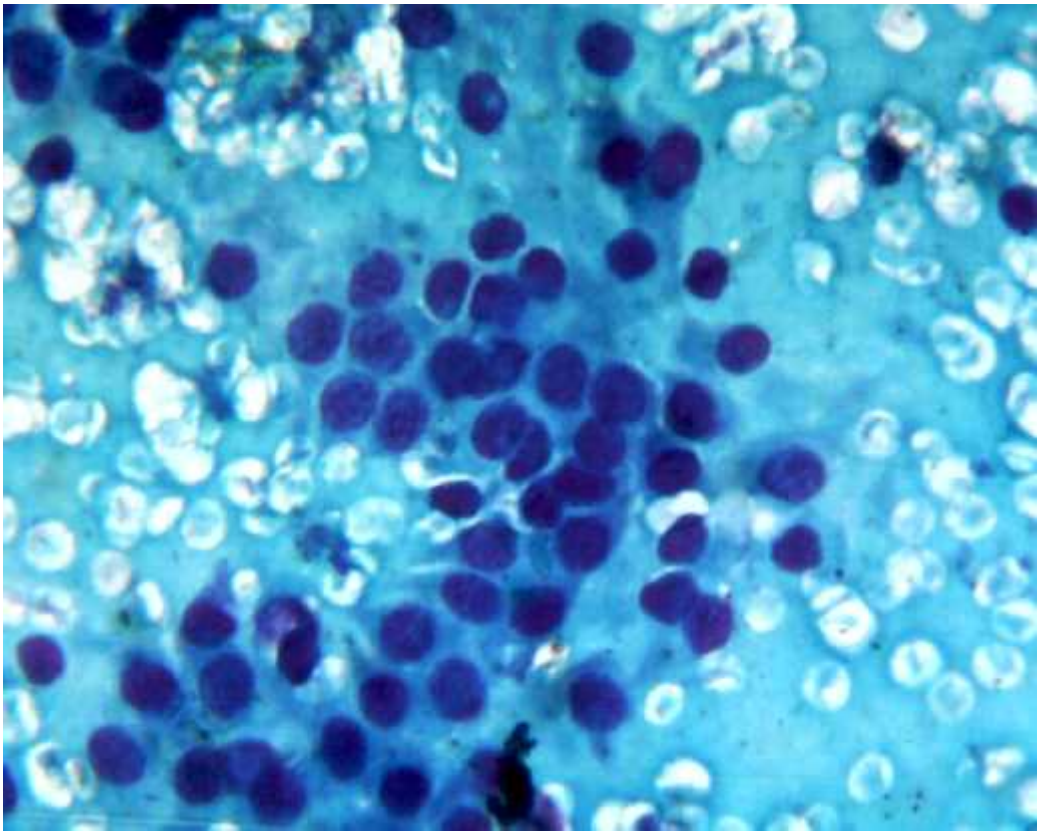


Fig 18:Powdery chromatin in Papillary carcinoma, Pap stain, 40 X

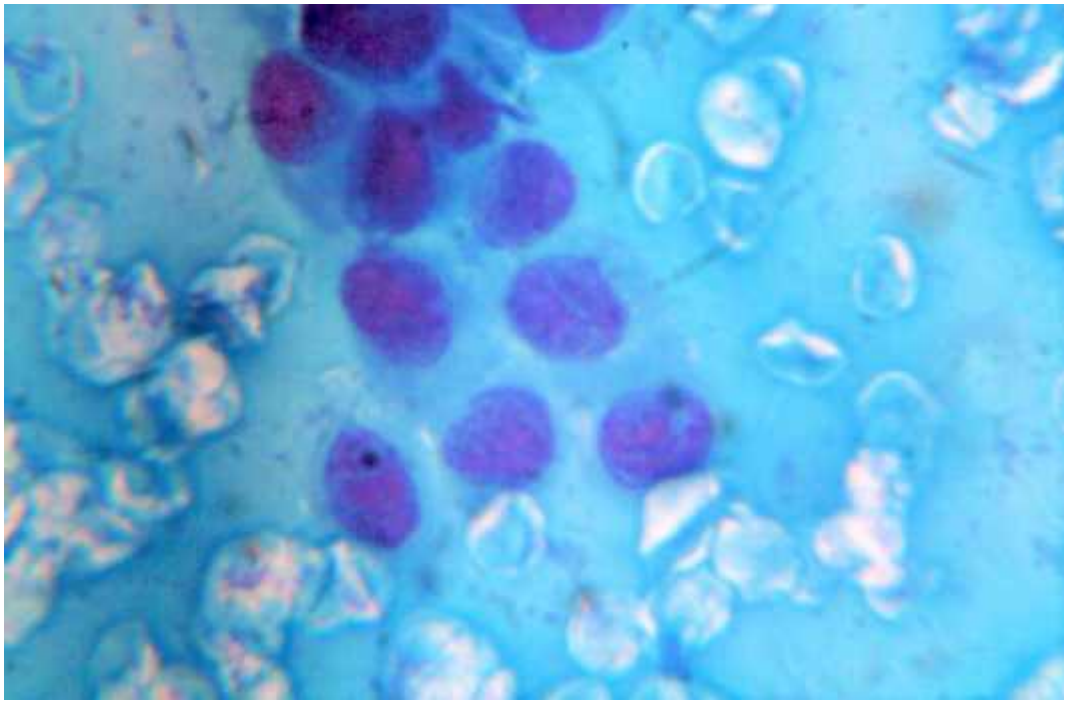


Fig 19: Powdery chromatin and nuclear groove, papillary carcinoma, Pap stain, 100X

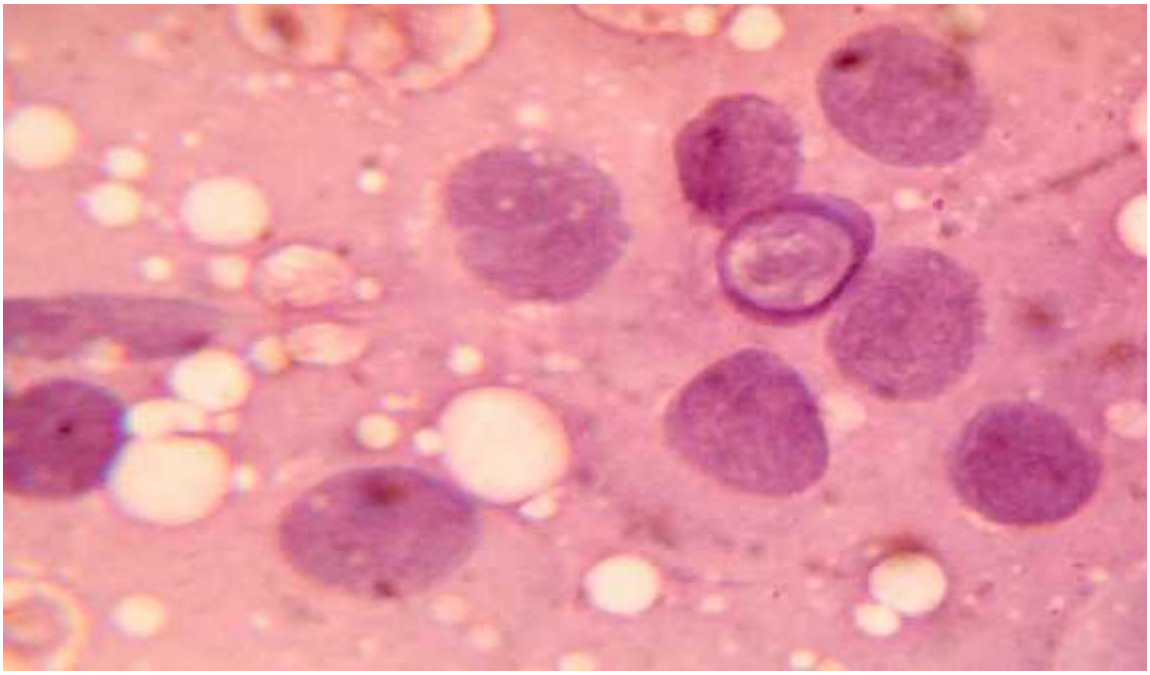


Fig 20: Intranuclear cytoplasmic inclusions in Papillary carcinoma, H&E, 100X

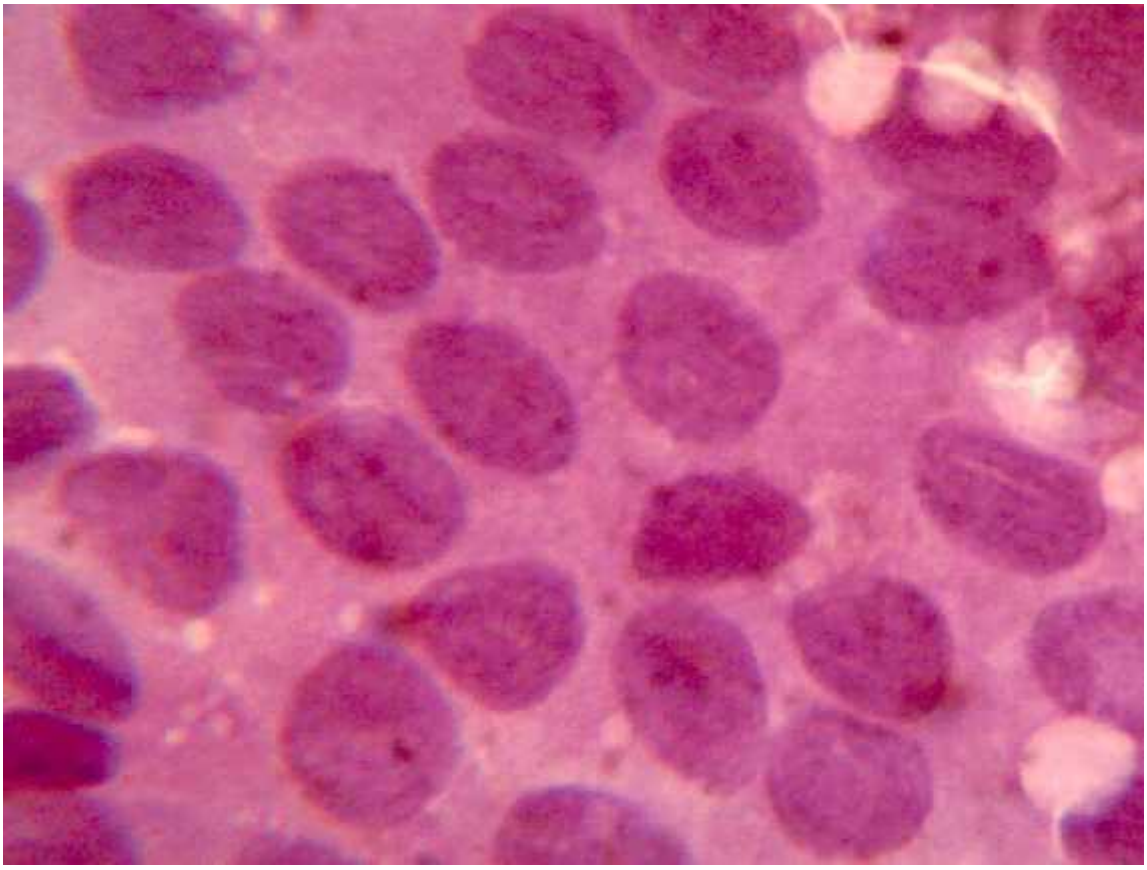


Fig 21 Nuclear groove in papillary carcinoma, H&E, 100X

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Sl. No	Age	Sex	IP/OP no	FNAC no	Diagnosis	HPE correlation
1	47	F	20087	176/16	Hashimotos thyroiditis	NA
2	60	F	3176	212/16	Nodular colloid goiter	yes
3	24	F	34638	213/16	Hashimotos thyroiditis	yes
4	39	F	19228	217/16	Hashimotos thyroiditis	NA
5	35	F	28036	240/16	Adenomatous goiter with cystic degeneration	yes
6	51	F	119087	266/16	Nodular goiter	NA
7	53	F	34521	266/16	Noduar colloid goiter	NA
8	28	F	354763	324/16	Colloid goiter	NA
9	40	F	105913	2148/16	Hashimotos thyroiditis	NA
10	29	F	106969	2159/16	Hashimotos thyroiditis	NA
11	41	F	35421	2423/16	Nodular goiter	YES
12	26	F	117319	2455/16	Colloid goiter	yes
13	69	F	43429	2457/16	Follicular neoplasm	NA
14	42	F	720644	2458/16	Hashimotos thyroiditis	NA
15	49	F	134037	2724/16	Hashimotos thyroiditis	NA
16	52	F	49841	2748/16	Papillary carcinoma	yes
17	22	F	118971	2750/16	Hashimotos thyroiditis	NA
18	26	F	12009	2756/16	Hashimotos thyroiditis	NA
19	73	F	136979	2774/16	Follicular neoplasm	yes
20	38	F	52897	2803/16	Follicular neoplasm	yes
21	34	F	13211	2852/16	Nodular goiter with cystic degeneration	NA
22	24	F	146781	2855/16	Nodular colloid goiter with cystic degeneration	yes
23	30	F	147892	2890/16	Nodular colloid goiter with cystic degeneration	yes
24	44	F	50123	2901/16	Hashimotos thyroiditis	yes
25	33	F	14532	3058/16	Hashimotos thyroiditis	NA
26	70	F	51237	3059/16	Lymphocytic thyroiditis	yes
27	45	F	153898	3076/16	Nodular goiter with cystic degeneration	NA
28	32	F	92582	3126/16	Colloid goiter	yes
29	42	F	97654	3182/16	Papillary carcinoma	NA
30	26	M	93125	3211/16	Hashimotos thyroiditis	NA

31	27	F	109146	3221/16	Hashimotos thyroiditis	yes
32	37	F	160631	3231/16	Nodular colloid goiter with cystic degeneration	NA
33	10	F	56562	2690/17	Nodular colloid goiter	NA
34	29	f	160806	2714/17	Lymphocytic thyroiditis	NA
35	25	F	55718	2765/17	Adenomatous goiter	yes
36	19	F	39669	2766/17	Nodular colloid goiter	NA
37	32	F	478213	2768/17	Nodular colloid goiter	NA
38	33	F	180414	2822/17	Nodular colloid goiter	NA
39	37	F	644591	2823/17	Lymphocytic thyroiditis	NA
40	47	F	48345	2833/17	Nodular colloid goiter with cystic degeneration	NA
41	46	F	58812	2834/17	Nodular colloid goiter	NA
42	46	F	85387	2866/17	Nodular colloid goiter	NA
43	52	F	60784	2912/17	Nodular colloid goiter	yes
44	55	F	488409	2923/17	Suspicious of papillary carcinoma	NA
45	20	F	488437	2925/17	Hashimotos thyroiditis	NA
46	34	F	67531	2934/17	Hashimotos thyroiditis	NA
47	60	F	59402	2935/17	Nodular colloid goiter	yes
48	32	F	188103	2940/17	Nodular goiter	NA
49	50	F	490790	2945/17	Nodular colloid goiter	NA
50	24	F	188967	2951/17	Nodular colloid goiter	NA
51	34	F	189294	2976/17	Follicular neoplasm	yes
52	26	F	492312	2977/17	Hashimotos thyroiditis	NA
53	55	F	191518	3000/17	Hashimotos thyroiditis	NA
54	30	F	136944	3001/17	Hashimotos thyroiditis	NA
55	45	F	80019	3011/17	Nodular colloid goiter	NA
56	30	F	76832	3014/17	Hashimotos thyroiditis	NA
57	58	F	32518	3045/17	Hashimotos thyroiditis	NA
58	19	F	629578	3046/17	Hashimotos thyroiditis	NA
59	42	F	63797	3058/17	Nodular colloid goiter	NA
60	40	F	235538	3060/17	Lymphocytic thyroiditis	NA
61	21	F	166882	3068/17	Nodular colloid goiter with cystic degeneration	NA
62	42	F	492606	3074/17	Nodular colloid goiter	NA
63	40	F	177621	3082/17	Nodular colloid goiter	NA
64	36	F	80123	3083/17	Nodular goiter	NA
65	42	F	167876	3133/17	Papillary carcinoma	yes