

**DIAGNOSTIC UTILITY OF MODIFIED CELL BLOCK FROM
FINE NEEDLE ASPIRATES OF THYROID NODULES AT
KENYATTA NATIONAL HOSPITAL, KENYA.**

By

ALFRED BARTHOLOMEW M'BWINJA

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Declaration

I hereby declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been submitted to the University of Nairobi or any other higher learning institution.

ALFRED BARTHOLOMEW M'BWINJA (BSc. MLT)

MSc Clinical Cytology, University of Nairobi

Signature: _____ **Date** _____

SUPERVISORS:

This dissertation has been submitted for examination with our approval as the supervisors.

DR. W. WAWERU, MBChB, MMed (Path), FC Path ECSA

Senior Lecturer,

Anatomic pathology Unit,

Department of Human Pathology,

School of Medicine,

University of Nairobi.

Signature: _____ **Date** _____

DR. M. MUNGANIA, MBChB, MMed (Path), FC Path ECSA

Consultant Pathologist,

Kenyatta National Hospital.

Nairobi, Kenya.

Signature: _____ **Date:** _____

**JOSEPHINE NYABETA RIOKI, BSc. MLS, MSc. Clinical Cytology, MSc. Molecular
Medicine**

Tutorial Fellow,

Anatomic pathology Unit,

Department of Human Pathology,

School of Medicine,

University of Nairobi

Signature: _____ **Date:** _____

Dedication

This dissertation is dedicated to Mbwinja family for their constant love and support, and also to my wife Christina and my daughter Happiness. I also dedicate this work to my mother and my late father. *“Mom, thanks for all the sacrifices you have made and now it is my turn. I promise to live a life that will do justice to all the sacrifice you have made”*. I love you all and God bless you.

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Abbreviations

AUS:	Atypia of Undetermined Significance
BFN:	Benign Follicular Nodule
CB:	Cell block
CS:	Conventional Smears
FC:	Follicular Carcinoma
FNA:	Fine needle aspiration
FNAC:	Fine Needle Aspiration Cytology
FVPC:	Follicular Variant of Papillary Carcinoma
FN:	Follicular Neoplasm
FLUS:	Follicular Lesion of Undetermined Significance
KNH:	Kenyatta National Hospital
MENs:	Multiple Endocrine Neoplasia syndromes
MTC:	Medullary Thyroid Carcinoma
ND:	Non Diagnostic/Unsatisfactory Specimens
PTC:	Papillary Thyroid Carcinoma
PDTC:	Poorly Differentiated Thyroid Carcinomas
SCC:	Squamous Cell Carcinoma
SFN:	Suspicious for A Follicular Neoplasm
TSH:	Thyroid Stimulating Hormone
TAT:	Turnaround time
TBSRTC:	The Bethesda System for Reporting Thyroid Cytopathology
TCC:	Tissue coagulum clot

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Abstract

Background: Fine needle aspiration cytology (FNAC) is a standard screening tool for the diagnosis and evaluation of thyroid nodules. It is a safe way of evaluating thyroid nodules. However this technique has some limitations such as false negative or positive results, high rate of unsatisfactory results and inability to classify border line lesions which result into indeterminate result.

Objective: To describe the utility of modified cell-block as an adjunct test to FNAC in the diagnosis of thyroid lesions.

Design: This study was a cross-sectional descriptive study.

Subjects: A total of 52 cases suspected of clinically having thyroid lesions at Kenyatta National Hospital (KNH) FNA clinic were recruited.

Setting: This study was done at Kenyatta National Hospital FNA clinic.

Study Period: The study was conducted from February 2016 to April 2016.

Methodology: FNA materials for both conventional smears (CS) and modified cell blocks (CB) were collected simultaneously from 52 patients suspected of clinically having thyroid lesions at KNH. All patients with thyroid lesions were included in this study and patients with a history of thyroidectomy were excluded. Ethical clearance was obtained from KNH/UoN Ethics and Research Committee before carrying out the study. Written informed consent was sought from all participating patients. Cellularity, morphological and architectural preservation, as well as cytologic diagnosis on CS was compared with CB sections. Data was entered on Microsoft excel and analyzed using SPSS software version 20. McNemar's Chi-square statistical tests was performed at 95% confidence level.

Results: Male to Female ratio was 1:8.7, (12%: 88%). Age ranged from 21 to 73 years with mean age of 41 and standard deviation of 13 years. This study showed that modified cell block preparations had high cellularity, minimal obscuring background material with excellent architecture compared to conventional smears. The majority (73.1%) of the thyroid FNA were reported as benign with a high unsatisfactory rate of 25%. The diagnosis of colloid goiter and

thyroiditis were picked up by both methods. Modified cellblock provided additional information for diagnosis of thyroid lesions in 15.4% of the total cases. Unsatisfactory rate of Thyroid FNA cytology was reduced from 25% to 13.4% when both methods were used thereby increasing the diagnostic efficacy to 86.6%. The diagnosis of Suspicious/follicular neoplasm which was missed on conventional smear was picked up by modified cell block preparation. Comparing the diagnosis on McNemar's Chi square test, there was no statistically significant difference in the two methods (p-value >0.05).

Conclusion: This study has not demonstrated statistically significant differences in the diagnostic utility of both methods, but modified cell block preparation provided additional information which was helpful in confirming and establishing new diagnosis.

Recommendations: In resource constrained settings, the cost implications should guide in the selection of the method to use. Proper training and monitoring of clinician performing FNA procedure should be provided in order to reduce unsatisfactory results of the thyroid aspirates.

1 INTRODUCTION

Thyroid nodules prevalence ranges from 4% to 10% in the general adult population and from 0.2% to 1.2% in children (1)(2). Majority of these nodules are benign, and only 5% to 30% are malignant. The main goal of evaluating these nodules is to distinguish benign nodules from malignant ones. Ultrasound and nuclear scanning can also be used in conjunction with FNAC (2).

FNAC is considered the gold standard screening test in the evaluation of a thyroid nodule (2)(3). FNAC is safe, fast, easily repeated, and cost effective procedure, with excellent patient compliance. However, FNAC has limitations. Some of the limitations include false negative or positive results. In addition, FNAC has inability to define malignant follicular lesions in the absence of papillary carcinoma nuclear features. These limitations are as a result of compromised specimens which are poorly preserved, and can be due to sparsely cellular or excessive clotting.. Like other conventional FNA smear, thyroid FNAC also offers limited material for ancillary tests (4).

Cell block (CB) preparation is known to increase cellular yield and improves diagnostic accuracy (5). Cell blocks are micro biopsies embedded in paraffin wax, suitable for sectioning, staining, and microscopic study. This technique has been in use for over a century. There are different methods of cellblock preparation such as bacterial agar method, plasma method, thrombin clot method, tissue coagulum clot (TCC) method, and automated CB preparation system. CB enables the recovery of small tissue fragments to be processed as histological tissue biopsy (5). It also offers extra material for other ancillary tests. Just like paraffin tissue sections in histology, cell block section yield histologic tissue architecture which are useful when making diagnosis. Architectural pattern may not be present on conventional smear. However, CB techniques has some limitation such as increased cost and lengthened turnaround time (TAT).

Modified cell block preparation is an improved technique. This technique offers excellent cytomorphologic features and uses routine safe laboratory chemicals.

Despite the above mentioned diagnostic utility of the cell block in FNAC, it appears to be underutilized in the aspiration of thyroid lesions performed at Kenyatta National Hospital (KNH). This study aims at describing the importance of modified cell-block in combination with FNAC in the diagnosis of different thyroid lesions.

2 LITERATURE REVIEW

Thyroid nodules are found in 4% to 10% of the whole adult population. In children the prevalence of thyroid nodule is very low, approximately 0.2% to 1.2% (1)(2). They are common in elderly persons, especially women and in those with iodine deficiency. The risk of developing thyroid nodules also increases with exposure to radiation. Dietary goitrogens such as calcium and fluoride in water and vegetables such as cabbage also increase the risk of developing thyroid lesions. Thyroid nodules can be noted by the patient or as an incidental finding during scanning or physical examination. Currently, the detection rate of thyroid nodules has increased because of imaging procedures, such as computed tomography scan.

2.1 Anatomy of the Thyroid Gland

Thyroid gland is a highly vascular endocrine organ located at the anterior neck. Thyroid gland is made up of two lobes, right and left lobe. The lobes are connected by isthmus. Each lobe is divided by fibrous septa into lobules, each containing 30 to 40 follicles. Follicles are formed by cuboidal or columnar epithelial cells called follicular cells. Parafollicular cells which produce calcitonin are also found in the thyroid gland. Located at the posterior poles of the thyroid are four, or sometimes more, small parathyroid glands measuring about 6 mm in diameter. The parathyroid glands produce parathormone that regulates the metabolism of calcium(6).

2.2 Indication for Thyroid FNA

Patients with palpable thyroid gland enlargement are candidates for FNA, but further evaluation is required to determine if an FNA is warranted. Thyroid nodules which are 10 mm or greater in diameter, detected on palpation are clinically significant and FNA should be done. To evaluate palpable thyroid nodules, a complete clinical history is needed. Physical examination and hormonal level assessment especially thyroid stimulating hormone (TSH) should also be done (7).

Ultrasound scanning should be considered on all patients with normal or elevated thyroid stimulating hormone to see if an FNA is warranted. Patients with low levels of thyroid stimulating hormone should have a radionucleotide thyroid scanning and correlate the findings with sonographic findings (8).

2.3 Clinical History

Patient clinical history such as family history of thyroid enlargement should be documented during physical examination (9). Patients with history of previous disease and radiation treatment on the head and neck region are also at increased risk of developing thyroid malignancies. Thyroid malignancy rate is four times higher in children than adults (10), as such age should be documented during clinical examination. Older patients, especially men have a high risk of thyroid cancer than women of the same age (11). Overall, thyroid nodules are four times common in women than in men (1).

Thyroid FNA should be considered when there is incidental findings of hot circumscribed nodules during scanning. The risk of malignancy in these nodules is high and ranges from 22% to 66% (11).

2.4 Diseases of the Thyroid Gland Amenable to FNAC

Thyroid disease can be divided into thyroiditis, follicular lesions which include goiters, benign neoplasms, and malignancies.

2.4.1 Thyroiditis

Thyroid inflammatory processes are classified into acute, chronic and sub-acute thyroiditis (12).

2.4.1.1 Acute thyroiditis

Acute thyroiditis begins with fever, swelling and pain in the anterior neck region. There is diffuse gland enlargement with abscesses. Aspiration cytology is rarely done on acute thyroiditis because they are usually detected clinically. Most cases of acute thyroiditis are bacterial in origin and are caused by gram-positive cocci, usually staphylococci particularly *Staphylococcus aureus* and streptococci particularly *Streptococcus pyogenes* or *pneumonia* (13).

2.4.1.2 Subacute Thyroiditis

Subacute thyroiditis is of unknown cause, but is thought to be a post viral syndrome. Patients usually present with subacute thyroiditis following a recent viral illness, such as a cold or influenza, and may follow a seasonal pattern. Other virus such as Mumps, adenovirus, Coxsackie, influenza, Epstein-Barr, have also been associated with painful thyroiditis. Patients are typically young women usually between thirty and forty years of ages. About 25% of cases occur in men, but the disease is rare in children. Patients usually have fever, frequently with chills, and fatigue out of proportion to the apparent systemic illness (12).

2.4.1.3 Chronic Thyroiditis

This includes Hashimoto's thyroiditis; Graves' disease; Riedel's thyroiditis (Riedel's struma) and, nonspecific chronic thyroiditis.

2.4.1.3.1 Hashimoto's Thyroiditis

The commonest type of chronic thyroiditis is Hashimoto's thyroiditis. It is an example of an autoimmune disease which is closely related to Graves' disease. Hashimoto's thyroiditis are associated with defects in antigen-specific suppressor T cells.

Hashimoto's thyroiditis usually affects women of middle-age, who usually present with diffuse goiter and signs of hypothyroidism. It is the most common cause of hypothyroid goiter in places with adequate dietary iodine. Hashimoto's thyroiditis causes goiter in children and is also more common in adolescent girls. Hashimoto thyroiditis is nine times more common in women than men (1). Chronic nonspecific thyroiditis is common in older patients. Approximately, 50% of women and 25% of men have lymphocytic infiltrates in the thyroid gland at autopsy (1).

2.4.1.3.2 Riedel's Thyroiditis

Riedel's thyroiditis (Riedel's struma) also called chronic sclerosing thyroiditis, is extremely rare. Riedel's thyroiditis is four times common in women than men, and usually occurs in middle age. Most patients present with recent enlargement of a long-standing goiter, which may result in dyspnea.(6)

2.4.1.3.3 Painless Lymphocytic Thyroiditis

This subtype of chronic thyroiditis is relatively common. It usually affects women after delivery. It occurs in about 5% to 7% of all pregnancies, but can occur sporadically. The patients have a non-tender goiter. A third of patients develop hyperthyroidism between one to two months after delivery. Another third of patients develop hypothyroidism 4 to 6 months postpartum, which usually lasts about 2 to 3 months, sometimes as long as a year, and occasionally is permanent. The final third of patients develop hyperthyroidism, followed by hypothyroidism (1).

2.4.2 Follicular Hyperplasia

Any thyroid gland enlargement is clinically referred to as goiter. However in pathology, goiter refers to hyperplastic thyroid enlargement. These hyperplastic thyroid enlargements can be nodular or diffuse. Examples of follicular hyperplasia include the following (13).

2.4.2.1 Toxic Diffuse Hyperplasia (Graves' Disease)

Toxic diffuse hyperplasia is an autoimmune disorder, commonly seen in women aged 20 to 50 years. Patients with this disease present typically with diffuse goiter, hyperthyroidism, and exophthalmos, and the gland is usually enlarged(1).

2.4.2.2 Diffuse Colloidal Hyperplasia

The commonest form of goiter in adolescents and young women. This thyroid gland enlargement is a response to deficit of peripheral thyroxine. In this case the gland is enlarged due to distention of follicles and the follicles contains pale colloid (6).

2.4.2.3 Nodular Hyperplasia

Nodular thyroid gland hyperplasia is the commonest thyroid gland disorder which can be sporadic or endemic. The gland enlarges progressively and the nodules are usually multiple, but they can also be solitary. Majority of these nodules are called cold nodules because they are nonfunctional (13).

Hot nodules which are functioning nodules, and hyperthyroidism is seen in only 20% of all cases. The incidence of thyroid cancer in patients with nodular goiters is high and ranges from 5% to 25% (14).

2.4.3 Cystic Nodules of the Thyroid

Approximately 25% of all thyroid enlargement are cystic nodules. Majority of cystic nodules are seen as a result of ischemia in goiters or neoplasia. Half of all cystic nodules may be neoplastic(14). However the incidence of thyroid cancer varies depending on criteria used to select patients for surgery. About 20 to 65% of patients with thyroid enlargement are cured as a result of evacuating their cystic contents. After evacuating the cystic contents, it is important to re-aspirate any palpable nodule because some thyroid malignancies can be cystic (8).

2.4.4 Follicular Neoplasm

Follicular neoplasm include follicular adenoma, Hürthle cell neoplasia, follicular carcinoma, or Follicular Variant of Papillary Carcinoma (FVPC). (13).

2.4.4.1 Follicular Adenoma

Follicular adenoma lacks invasive behavior or markers of PTC. The lesion is considered to be benign, and it shows evidence of follicular differentiation. Cytologically, it is indistinguishable from follicular carcinoma(6)

2.4.4.2 Follicular Carcinoma

This is second common malignancy of thyroid comprises 10% to 15% of all thyroid malignancies.

The tumor shows follicular differentiation and lacks the diagnostic signs of papillary carcinoma.

(1) (12).

2.4.5 Malignancies of the Thyroid Gland Amendable to FNA

Primary thyroid cancers and secondary cancers comprises 5 to 10% of all FNA cases (15)(16). There are different types of malignancies that affect thyroid gland.

2.4.5.1 Papillary Thyroid Carcinoma (PTC)

Eighty percent of all thyroid cancer is Papillary Thyroid Carcinoma (PTC) (4). PTC is common in those aged between 20 to 50 years, but it can occur at any age. The ratio of PTC in male to female is 1:4. There are different ways patients present clinically. Some patients present with solitary nodule while others presents with cervical lymphadenopathy as a sign of metastasis. Cytologically there are several variants of PTC (17).

2.4.5.2 Poorly Differentiated Carcinoma

Poorly differentiated carcinomas are seen in 7% of all thyroid cancers. These are carcinomas which fall in between well differentiated and anaplastic carcinoma with intermediate architectural and nuclear atypia (18).

2.4.5.3 Undifferentiated (Anaplastic) Carcinoma

Anaplastic carcinomas of the thyroid are common in older patients, over 60 years, and is seen in less than 5% of all thyroid malignancies (18). It is fatal, causing 50% of deaths in all patient with thyroid malignancy.

Clinically patients have a history of rapidly growing anterior neck mass with a history of hoarseness of voice. At the time of diagnosis, usually the mass has already spread to adjacent structures (12).

2.4.5.4 Medullary Thyroid Carcinoma (MTC)

MTC are seen in 5% to 10% in all patients with thyroid cancer (18). This malignancy arises from parafollicular cells. Majority of medullary thyroid carcinomas are sporadic. About 10 to 20% occur in children who have a genetic disorder such as multiple endocrine neoplasia syndromes.

2.4.6 Other Malignancies of Thyroid Gland

2.4.6.1 Lymphoma

Malignant lymphoma account for five percent of all thyroid neoplasms. Malignant lymphomas can be primary or involve the gland as a secondary malignancies. The most common primary thyroid malignant lymphoma is B cell lymphoma (3)(6) .

2.4.6.2 Metastatic tumors

Approximately 0.2% of all thyroid aspirates are metastatic tumor to the thyroid (19). The most common primary sites include the esophagus, lungs, kidney and the breast. Metastasis is considered when the cytomorphology is different from the thyroid neoplasm and the patient has a history malignancy elsewhere (14)(19).

2.4.6.3 Squamous Cell Carcinoma (SCC)

One percent or less of all thyroid cancers is SCC. SCC has a similar prognosis as anaplastic carcinoma and occurs more commonly in elderly people. On histology squamous cell carcinoma is defined as squamous differentiated tumor and majority are poorly differentiated tumors. Pleomorphic keratinized squamous cells are seen on cytologic preparations (12).

2.5 Mode of Diagnosis

2.5.1 FNA Cytology

FNA cytology is a standard screening tool for evaluation of thyroid enlargement. Malignant thyroid nodules require surgical treatment while benign thyroid nodules require medical treatment or clinical follow up. Thyroid FNA procedure is fully accepted as a diagnostic workup for patients with thyroid nodules in conjunction with other methods. Several studies on precision and efficiency, in relation to other diagnostic methods have been done regarding this test (13).

2.5.2 Cell Blocks

Cell blocks are micro biopsies prepared from residual tissue fluids and FNA, embedded in paraffin, suitable for sectioning, staining, and microscopic study. The cell block technique has some advantages over conventional smear (5)(20). It increases cellular yield and improves diagnostic accuracy. In Addition, numerous sections can be obtained which can be used for special stains such as immunocytochemistry. Furthermore cell block section yield histologic tissue architecture which may not present on smear (20).

Despite the mentioned advantages of the cell block in FNA, it appears to be underutilized in the aspiration of lesions performed at Kenyatta National Hospital (KNH). This study aims at describing the importance of modified cellblock as an adjunct test to FNAC in evaluation of thyroid lesions.

2.6 Cell Block Preparation Methods

Simple sedimentation technique was is earliest method. The problem with simple sedimentation technique is insufficient cellularity. The first step in cellblock preparation is fixation followed by centrifugation, then transferring of a cell button for tissue processing and finally embedding with molten paraffin wax. Several technical modifications on CB have been reported and are still being improved (21).

2.6.1 Normal Saline Needle Rinse Method

This is the most common method used in many laboratories. In this method of cell block preparation, normal saline is used to rinse the aspiration needle. The material is centrifuged immediately and collected for tissue processing. Alternatively, cellblock can be prepared by rinsing the aspiration needle directly in formalin or 50% ethanol (22).

2.6.2 Modified Cell Block Preparation

Modified cellblock is a simple technique which uses safe chemicals that are readily available in the laboratory (23). It is an alternative method of normal saline needle rinse method. Cell block preparation in modified technique uses Acetic acid Alcohol Formalin (AAF) fixative which is prepared by mixing 34 ml of 95% ethyl alcohol, 4 ml of formalin and 2 ml of Glacial acetic acid. Modified cellblock preparation ensures optimal cell preservation, offers excellent cellular details and maintain architectural pattern (23).

2.6.3 Tissue Coagulum Clot (TCC) Method

TCC method is used to augment cellularity in CB sections, without the material being diluted by normal saline. It allows the aspirated material to form a tissue clot together with the blood in the needle. Tissue coagulum is fixed in formalin container and processed as a histological tissue specimen. TCC is superior compared to normal saline needle rinse methods because it recovers more cells unlike needle rinse method. However, there is no difference in preservation of cells for diagnosis or for immunocytochemistry studies in these two methods (21).

2.6.4 Plasma Thrombin Method

Plasma and thrombin is added to pellet of centrifuged cell suspension to form a clot. Reagents for this method are commercially available. These reagents are prepared from rabbit lung tissue or brain tissue. These reagents contain epithelial cells, hence caution should be taken when reporting to avoid erroneous interpretation (22).

2.6.5 Agar Embedding, Collodion Bag and Other Methods

This method of cellblock preparation uses cell adjuvants such as agar, HistoGel™ and gelatin albumin to support and concentrate cell. The use of cell adjuvants have been developed to overcome the difficulties in cell recovery when processing small fragments of tissue (21).

2.6.6 Shandon™ Cytoblock™ Method

The Shandon™ Cytoblock™ method uses Thermo Shandon Cytocentrifuge to concentrates cells for cell block preparation (24).

2.6.7 Automated CB Preparation System

The first automated method of cellblock preparation is Cellient™ system. Tissue fragments are recovered and processed automatically from a specimen container. This method has high cellular yield compared to other methods of cell block preparation. This method is expensive and requires training (25).

2.7 Diagnostic Terminology for Thyroid Cytology

There are various reporting formats of thyroid cytology which have been suggested by different authors and authorities (26). The most commonly used is the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) which include the following six main categories: (17).

2.7.1 Non Diagnostic or Unsatisfactory specimens

About 10 to 20% of thyroid FNA cases are reported as unsatisfactory. The rate of unsatisfactory specimens depend on the physician's experience performing the FNA procedure. When the FNA procedure is done by a physician with limited experience, the non-diagnostic rate increases (13). For a sample to be adequate, it has to be a representative of the lesion and be adequate in amount.

Satisfactory smear for evaluation should have a minimum of six groups of well-preserved follicular cell which are well-visualized. Each group should have a minimum of ten cells. However, smears which shows atypia are satisfactory even when the atypical cells are fewer than the minimum cells required. In addition, when smears are showing inflammatory processes such as features of abscess, a benign report should be given even when follicular cell are few. Furthermore, smears comprised of abundant thick colloid are reported as benign (17).

2.7.1.1 Management of ND/UNS

The recommended management of non-diagnostic or unsatisfactory aspirate is to repeat FNA procedure. Whenever possible, ultrasound guided FNA should be performed. About 5 to 50% of all ND/UNS result are reported to have cancer on repeat thyroid FNA (27)(28).

2.7.2 Benign Lesions

The majority (50% to 70%) of thyroid FNA samples are reported as benign, in thyroid FNA cytology (13). Whenever possible, benign lesions should be sub-classified to their specific entities such as thyroiditis or benign follicular nodule (1)

Cytologically, the term benign follicular nodule is used when cytologic smears are satisfactory for evaluation. Cytomorphological, the smears are comprised of benign follicular cells in macro-follicular pattern with abundant colloid in the background (6)(17).

2.7.2.1 Management of Benign Lesions

Patients with a benign report have a low risk of malignancy, 0 to 3% (1, 5), as such they are usually on follow up (12).

2.7.3 Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) lesions

This category accounts for 3 to 18% of all thyroid FNAs cases. The category “indeterminate” lesions indicate that the smear has features which are between benign and malignant. This category causes confusion for pathologists and clinicians in thyroid cytopathology.

This diagnostic category is used when specimens contain cells with architectural or nuclear atypia that cannot be classified as suspicious for a follicular neoplasm or malignant. However, the atypia is more marked that cannot be classified confidently as benign. The main reasons to this uncertainty is poor preserved specimen and when the specimen is obscured by blood cellular elements (6).

2.7.3.1 Management of AUS/FLUS Lesions

AUS/FLUS category has 5% to 15% risk of malignancy hence repeating thyroid FNA should be considered (12).

2.7.4 Follicular Neoplasm/Suspicious for a Follicular Neoplasm (FN/SFN) Lesions

All cellular smears comprised of crowded follicular cells arranged in micro follicular pattern with altered architectural arrangement should be reported as FN/SFN. Cases with obvious PTC features such as papillary structure, pale chromatin and nuclear groove should be excluded from FN/SFN category (17).

2.7.4.1 Management of FN/SFN Lesions

Patients with FN/SFN cytology results should undergo hemi thyroidectomy or lobectomy (17).

2.7.5 Suspicious for Malignancy Lesions

About 10 to 30% of all thyroid FNA cases falls in the suspicious for malignancy category (13). This category is used when a specimen has some features of atypia suggestive of malignancy, but the atypia is not marked for conclusive malignant diagnosis. Hürthle cell neoplasia is excluded from this category. The risk of malignancy is 60% in this category (1).

2.7.5.1 Management Suspicious for Malignancy Lesions

Surgery should be done in all patients with suspicious for malignancy result. Papillary thyroid carcinomas are report in 50% to 75% of all patient who had initial report of suspicious for malignancy. A total thyroidectomy should be considered (7).

2.7.6 Malignant Lesions

Primary or secondary thyroid cancers comprise 5 to 10% of all FNA cases (13). There are different types of cancer in thyroid pathology amendable to FNA cytology. In this malignant category, the false positive rate is less than 1% (17).

Cytomorphology of PTC show follicular cells arranged in papillae. Follicular cells have altered arrangement and the nuclei are enlarged, oval or irregular with grooves. The chromatin is fine and pale. The presences of intranuclear cytoplasmic pseudo inclusions and marginally placed micro nucleoli, are also characteristic features of PTC (12).

In Medullary Thyroid carcinoma (MTC), smears are moderate to markedly cellular. The malignant cells are polygonal or plasmacytoid and they originate from parafollicular cells. Sometime the malignant cell can be round, or spindle shaped. The pleomorphism can be mild to moderate (1).

Poorly differentiated thyroid carcinomas (PDTC) originates from follicular cells. They have features which are between well differentiated carcinomas and undifferentiated (anaplastic) thyroid carcinoma (1).

Metastasis is considered when the cytomorphology of smear is different from the cytomorphology of thyroid neoplasm and the patient has a history malignancy elsewhere. Other studies such as flow cytometry or immunocytochemistry can be done to establish the primary site (13).

2.7.6.1 Management of Malignant lesions

Patients with primary thyroid malignancy should undergo surgery. Total thyroidectomy is recommended in all patients with papillary thyroid carcinoma (7).

Poorly differentiated thyroid carcinoma (PDTC) should be managed aggressively because they have a poor clinical prognosis than well differentiated carcinomas (WDTC). In addition to surgery, treatment with ¹³¹I therapy postoperatively is recommend for PDTC (29).

Tracheostomy is required in majority of patients with anaplastic carcinoma due to air way obstruction. Complete surgical resection should be done for both anaplastic carcinomas thyroid SCC (30).

2.8 Study Justification

Even though conventional thyroid FNA smear is a gold standard, it has some limitation such as false positive and negative results. In addition, FNAC has inability to define malignant follicular lesions in the absence of nuclear features of papillary carcinoma. These limitations are as a result of compromised specimens which are poorly preserved, and can be due to sparsely cellular or excessive clotting. Like other conventional FNA smear, thyroid FNAC also offers limited material for ancillary tests. Studies have shown that cell block can increase cellular yield and improves diagnostic accuracy and interpretation but there is little knowledge on its utility on thyroid lesion especially at Kenyatta National Hospital.

2.9 Research Question

Does the use of modified cell block technique as an adjunct to FNAC increase the accuracy in diagnosis of different thyroid lesions?

2.10 Broad objective

To determine the diagnostic utility of modified cell block in combination with FNAC in the diagnosis of thyroid lesions.

2.10.1 Specific objectives

1. To describe cytological patterns of thyroid FNA cytology at Kenyatta National Hospital
2. To describe the cytomorphological features in Cell Block & conventional smear using a scoring point system
3. To compare the cytodagnosis of modified cell block preparation to conventional FNA smears

3 MATERIALS AND METHODS

3.1 Study Design

This was a Laboratory based cross sectional descriptive study.

3.2 Study Setting

The study was conducted in Kenya at Kenyatta National Hospital FNA clinic. Samples were processed at KNH Cytology and UoN histology laboratories.

3.3 Study Population

Patients who were referred to FNA clinic presenting with thyroid nodules at Kenyatta National Hospital.

3.4 Study Period

The study was conducted from February 2016 to April 2016.

3.5 Sampling Method

Convenient sampling method was used.

3.6 Selection Criteria

3.6.1 Inclusion Criteria

This study included all patients aged 18 years and above referred to KNH FNA Clinic with thyroid lesions who gave informed consent.

3.6.2 Exclusion Criteria

All patients below 18 years old and patients who did not give informed consent.

3.7 Sample Size Determination

The sample size in this study was calculated using the R software version 3.2.2, using the McNemar's two-tailed formula for paired proportion as given below.

$$n = \left\{ \frac{Z_{\alpha} * \sqrt{Pdisc} + Z_{\beta} * \sqrt{Pdisc - Pdiff^2}}{Pdiff} \right\}^2$$

Where

n =required sample size.

Z_{α} = Critical value.

Z_{β} = Power.

$P disc$ = Proportion of discordant.

$P diff$ = Proportion difference.

Based on a study by Khan S. et al. in South Africa, the discordant rate was 0.1 and 0.4 (24). At 95% confidence level and a power of 80%, a minimum of 42 samples was calculated as shown below:

n = required sample size.

$Z_{\alpha} = 1.96$.

$Z_{\beta} = 0.84$

$P disc = 0.5$

$P diff = 0.3$

$$n = \left\{ \frac{1.96 * \sqrt{0.5} + 0.84 * \sqrt{0.5 - 0.3^2}}{0.3} \right\}^2$$

$$n = 42$$

A minimum of 42 samples was calculated, however 52 participants were recruited in this study.

3.8 Enrollment of Participants

Participants were identified based on inclusion and exclusion criteria mentioned above. The Principal investigator explained the study risks and benefits before the participant were enrolled. A written informed consent (appendix 1) was obtained from every participant.

3.9 Data Collection

A pre-design data collection form was used to collect demographic information and clinical history (appendix 2) from patient files and requisition forms before sample collection.

3.10 Sample Collection and Processing

Thyroid FNA procedure (appendix 3) was done on all participants by consultant pathologist/pathology registrars. Two clean labelled slides were used to prepare smears. Slides were fixed immediately in 95% alcohol for a minimum of 15 minutes.

Needle rinses were done in clean tubes with acetic acid alcohol formalin (AAF) fixative for preparation of cell block (appendix 4) by the principle investigator. The tube was assigned a unique study identification number.

Both samples were processed at KNH Cytology and UoN histology laboratories. One fixed smear was stained using Papanicolaou stain procedure (appendix 5), and the other slide using Hematoxylin & Eosin staining procedure (appendix 6). Cell blocks were processed in tissue processor (appendix 7). Two cell block sections between 3-5µm were prepared from each specimen. Each slide was stained with pap stain (appendix 5) and Hematoxylin & Eosin stain (appendix 6). Cell block sectioning and staining was done by principal investigator with the help of medical laboratory technologist as a research assistant.

3.11 Format of Reporting

A modified point scoring system developed by Mair et al, cited by Song. H et al (31) was used to score the quality of cytological aspirates (Appendix 2).

3.11.1 Categories of Modified Mair et al Scoring System; cited by Song. H et al, 2015 (31)

1. Background obscuring material

Background obscuring material includes blood, staining artifacts and pus. Qualitative evaluation was done and graded into either:

- Marked when obscuring material covers over 75% of the slide
- Moderate when obscuring material covers 50% to 75% of the slide
- Minimal when obscuring material covers less than 50% of the slide

2. Degree of cellular degradation was graded as follows:

- Marked when cellular degradation affects more than 75% of the slide
- Moderate when cellular degradation affects 50% to 75% of the slide
- Minimal when cellular degradation affects less than 50% of the slide

3. Cellularity was graded as follows:

- Marked when cell cover more than 75% of the slide
- Moderate when cell cover 50% to 75% of the slide
- Minimal when cell cover less than 50% of the slide

4. Architectural and cellular arrangement was done for patterns such as acini, follicles, papillae and graded as follows:

- Excellent/Marked when pattern cover more than 75% of the slide
- Moderate when pattern cover 50% to 75% of the slide
- Minimal when pattern cover less than 50% of the slide

A cumulative score between 0 and 8 was obtained from each specimen and categorized into one of the following two categories:

- a. Category 1 (score 0): diagnostically inadequate
- b. Category 2 (score 1-8): diagnostically adequate

Both the cell blocks and conventional smears were reported using the Bethesda System of Reporting Thyroid Cytopathology (BSRTC) (17).

3.11.2 The 2010 Bethesda System of Reporting Thyroid Cytopathology (17)

The BSRTC aims at helping cytopathologist/cytologist to communicate thyroid FNA results to clinician clearly and helpful clinically. There are six main categories used in this reporting system:

I. Non diagnostic or Unsatisfactory

A thyroid FNA sample is satisfactory for evaluation if it contains a minimum of six groups of well-visualized follicular cells, with at least ten cells per group. An interpretation of Non diagnostic/Unsatisfactory is given when the smears have fewer cells than the minimum requirement. However, there are few exceptions. Smears are considered satisfactory when the smears are showing cytologic atypia even when follicular cells are fewer than the minimum requirement. Similarly, thyroiditis cases may contain only inflammatory cells and are reported as benign.

II. Benign

This category is used when sample is adequate for evaluation and consists predominantly of colloid and benign-appearing follicular cells.

III. Atypia of Undetermined Significance (AUS)

AUS category is used for specimens that contain cells with architectural and/or nuclear atypia that is not sufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignant. On the other hand, the atypia is more marked than benign changes.

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm (FN/SFN)

FN/SFN is used when thyroid aspirate is comprised of follicular cells arranged in an altered architectural pattern with cell crowding and/or microfollicle formation. Cases with the nuclear features of PTC are excluded from this category. It is important to specify Hürthle cell type.

V. Suspicious for Malignancy

This category is used when a specimen has some features of atypia suggestive of malignancy, but the atypia is not marked for conclusive malignant diagnosis.

VI. Malignant

This category is used when thyroid aspirate have unequivocal features of malignancy.

3.12 Materials

3.12.1 Equipment

The following equipment from KNH/UON cytology/histology laboratory were used: Olympus microscope, microtome machine, automatic tissue processor and centrifuge.

3.12.2 Consumables

The following consumables were used: 25cc needles, 10ml syringe, examination gloves, cotton roll, methylated spirit, microscope slides, cover slips, slide boxes, 50 ml conical tubes, glacial acetic acid, cooler box, pasture pippete, buffered formalin, absolute ethanol, slide jars, hydrochloric acid, Scott's tap water, eosin azure 50, eosin, hematoxylin, Orange g 6, paraffin wax pellet(paraplast), May Grunwald- Giemsa, centrifugation tubes, tissue cassette, xylene, lens paper, D.P.X Mountant, Whiteman paper, gauze roll and microtome blade.

3.13 Biosafety and Biosecurity Measures

During sample collection and processing, standard operating procedures were followed. Personal protective equipment such as laboratory coats and gloves were worn during sample collection, processing and analysis. All procedures were done according to the KNH laboratory biosafety and biohazard waste disposal guidelines.

3.14 Variables

Independent Variables

In this study, independent variables included cell block preparation and conventional smear preparation.

Dependent Variables

Cellularity, quality of the background, degree of cellular degeneration, architectural or cellular arrangement and cytodiagnosis were the dependent variables in this study.

3.15 Data Management and Statistical Analysis

Collected data was stored in both a hardcover register and a soft copy in Microsoft excel and analyzed using SPSS software version 20. Data collection form and hard cover register were kept in lockable cabinets where only the researcher had access to ensure that confidentiality. Information stored in soft copy was protected from access from unauthorized persons by a

password. Study identification numbers were assigned to every data collection form to ensure confidentiality. McNemar's Chi-square statistical tests was performed at 95% confidence levels. The descriptive statistics are presented as proportions and in form of table's charts and graphs.

3.16 Quality Assurance

Thyroid FNA procedure were done by consultant pathologist and pathology registrar. Smears were prepared and immediately fixed in 95% alcohol to avoid air drying effects. Syringe needles were rinsed in Acetic Acid Formalin (AAF) fixative for cellblock preparation (23). The specimens were processed at Kenyatta National Hospital and University of Nairobi cytology laboratories using approved standard operating procedures. The stains and reagents were prepared according to the standard operating procedures recommended by the Kenyatta National Hospital and University of Nairobi laboratories. Every time the stains were filtered before each use to ensure good quality staining. Both conventional smears and cellblock slides were reported using (TBSRTC). The principal investigator examined all the slides and were reviewed by consultant pathologist. To ensure quality control in this study, 10% of slide were examined by an independent consultant pathologist who was blinded to the previous results.

3.17 Ethical Considerations

The study was done after obtaining ethical approval from KNH/UON Ethics Review Committee (Appendix 8). Written informed consent was sought from prospective study participants. No participant was coerced in any way to take part in this study and no client was penalized in any way for not taking part in this study. The results were communicated to the attending physician within two weeks for patient management.

4 RESULTS

4.1 Demographic Information

A total of 52 patients with thyroid gland enlargement were enrolled in this study. The study was conducted at Kenyatta national hospital FNA clinic between February and April 2016. The patient age ranged from 21 to 73 years with age mean of 41.4 years and standard deviation of 13.2 years (Table 1).

Table 1: Frequency of Age Group among Patients.

Age Group (years)	Frequency	Percentage (%)
20 – 29	8	15.4
30 – 39	19	36.5
40 – 49	12	23.1
50 – 59	6	11.5
60 – 69	5	9.6
≥ 70	2	3.8
Total	52	100

Majority of patients (37%) were in their third decade of life followed by 23% of patients in their fourth decade (Figure 1).

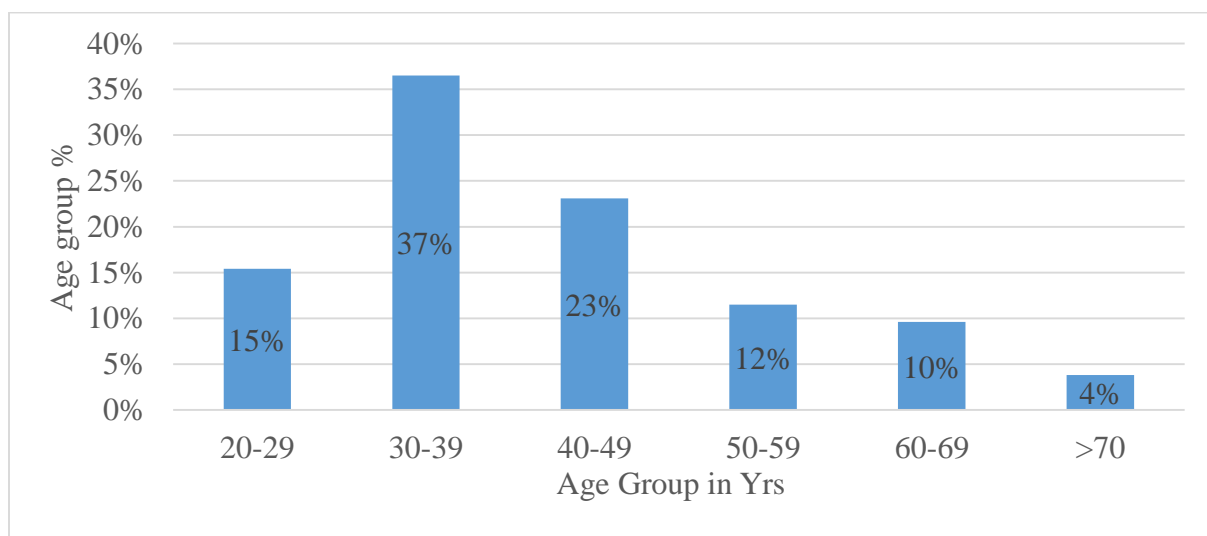


Figure 1: Histogram on age group distribution among patients

The largest number of patients were females accounting for 88% (46/52) of total participants (Figure 2).

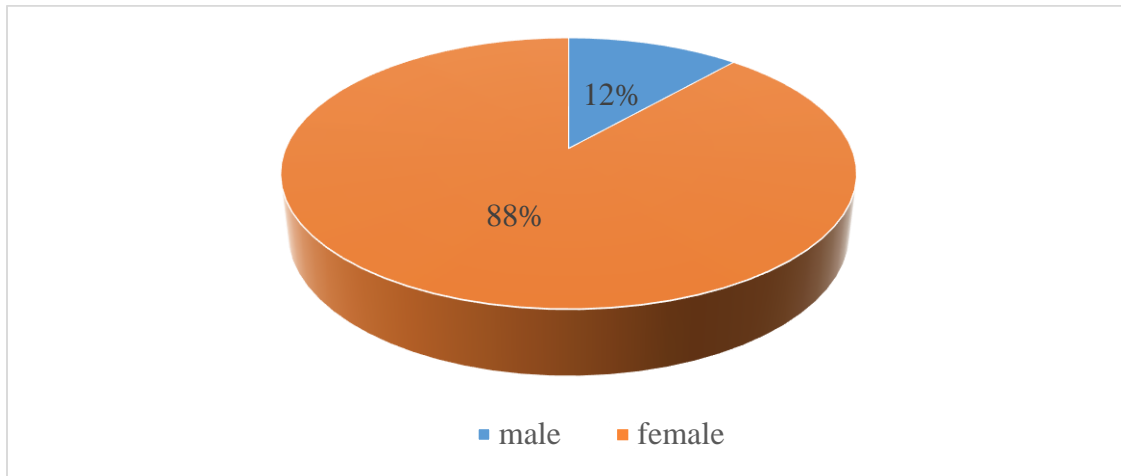


Figure 2: Sex distribution among patients

4.2 Duration of the Thyroid Swelling

The majority of patients (40.4%) had thyroid gland enlargement for the period of one to twelve months. 15.4% of the participants were not able to recall its duration (Figure 3).

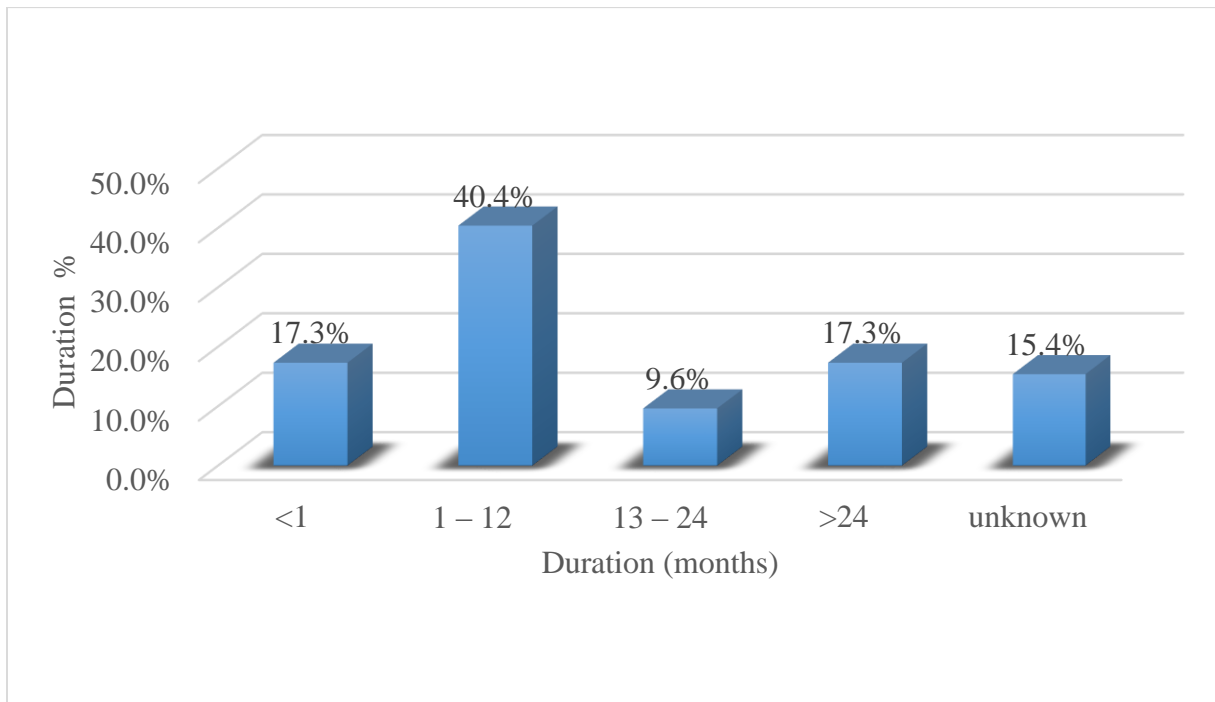


Figure 3: Duration of thyroid lesions among participants.

4.3 Location of Thyroid Lesion

In this study a majority of patient (64%) had centrally placed thyroid enlargement while the left and right sided lesions accounted for 21% and 15 % respectively (Figure 4).

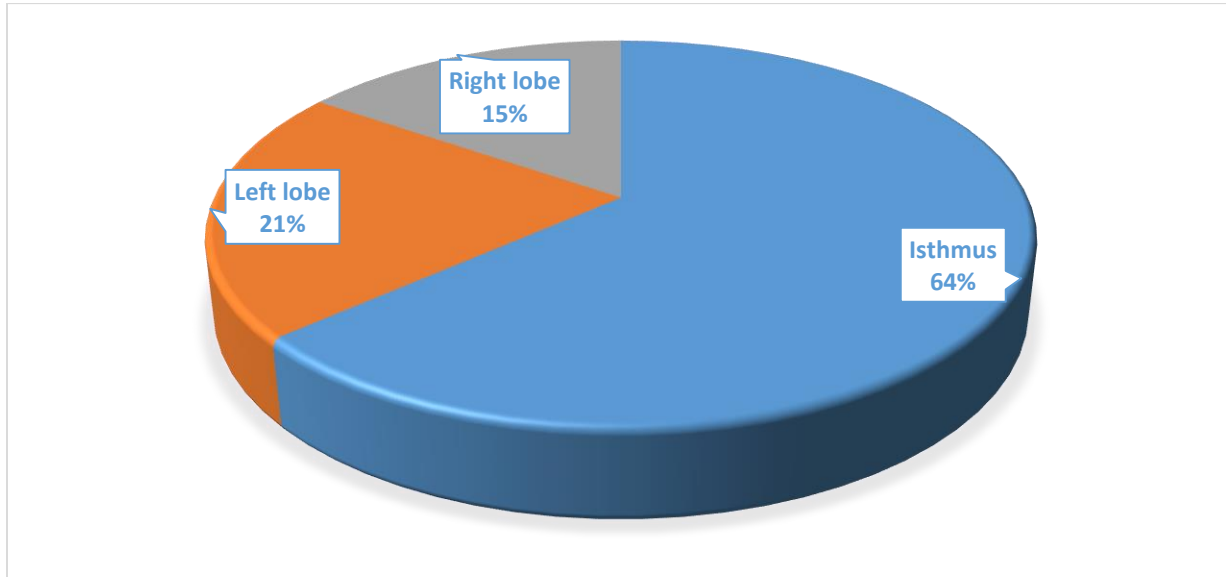


Figure 4: Location of thyroid lesions

4.4 Cytomorphologic Features

Cytomorphology of the two processing techniques (conventional smear and modified cellblock) were scored using Mair et al scoring system for assessing the quality of the slides into four categories: background obscuring material, degree of cellular degradation, cellularity and architectural and cellular arrangement as summarized (table 2). The cytodiagnosis were also compared.

Table 2: Cytomorphology for the two Methods

Mair et al Category	Conventional Smears (%)	Modified Cellblock (%)
BACKGROUND		
Marked	53.8	28.8
Moderate	30.8	36.5
Minimal	15.4	34.6
CELLULAR DEGENERATION		
Marked	25	28.8
Moderate	7.7	5.8
Minimal	67.3	65.4
CELLULARITY		
Minimal	38.5	28.9
Moderate	40.4	26.9
Marked	21.1	44.2
ARCHITECTURE ARRANGEMENT		
Minimal	48.1	34.6
Moderate	32.7	25
Excellent	19.2	40.4

4.4.1 Background Observations

The slides from the two methods were examined for obscuring material which was mostly blood and staining artifacts. This was categorized into marked, moderate and minimal. 35% of cases on modified cellblock had minimal background obscuring material as opposed to 15% on conventional smears. Conventional smears had marked background obscuring in more than half of the cases (53.8%) unlike 29% in modified cellblock cases (Photomicrograph a and b). Both conventional smears and modified cellblock were comparable on moderate background obscuring which was 31% and 37% respectively (Figure 5).

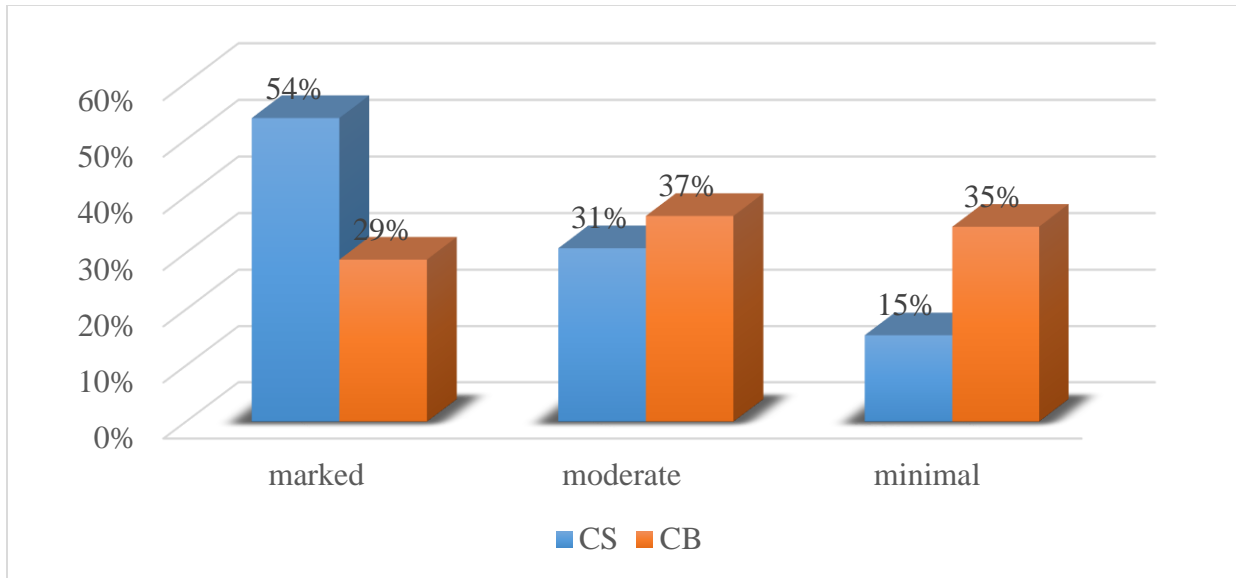


Figure 5: Obscurity on Modified cellblock and Conventional smears

4.4.2 Cellularity

Modified cellblock cases were rated to have marked cellularity (Photomicrograph c) on Mair et al scoring system in 44.2% as compared to 21.1% in conventional smears. Moderate cellularity were scored in 40.4% of conventional smear. Minimal cellularity was seen in 38.5% and 28.9% in conventional and cellblock cases respectively (Figure 6).

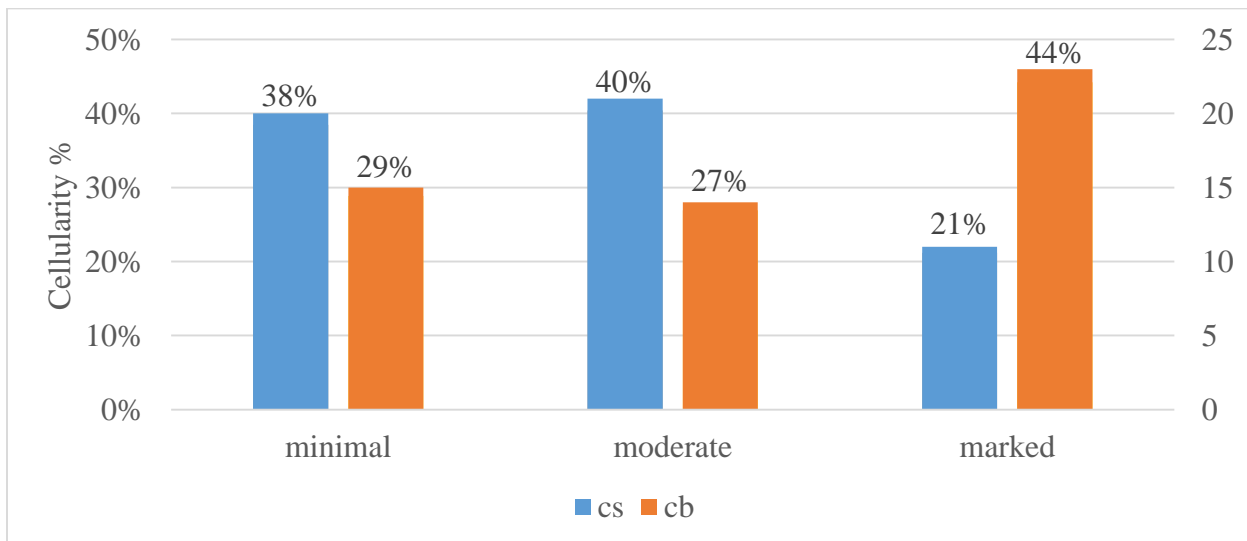


Figure 6: Cellularity on Modified cell block and Conventional smears

4.4.3 Degree of Cellular Degeneration.

In addition to cellularity and background, degree of cellular degeneration was also evaluated using Mair et al scoring system. However, the scores were comparable across all categories on both modified cell block and conventional smears as shown (Figure 7).

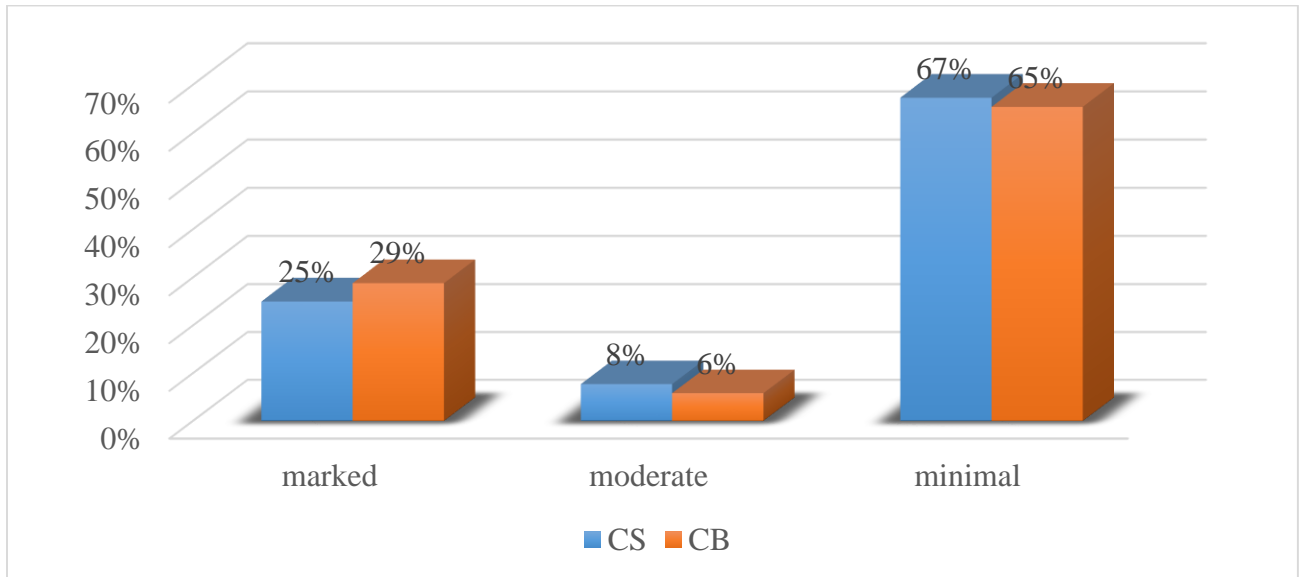


Figure 7: Degree of cellular degradation according to method

4.4.4 Architectural and Cellular Arrangement

Architectural pattern and cellular arrangement was evaluated on both methods. Forty percent of modified cellblock cases were categorized to have marked architectural (Photomicrograph b and c) and cellular arrangement as compared to 19% on conventional smears. Conventional smears were scored high (48%) on minimal architectural arrangement as compared to 35% cases on modified cell block. On the other hand, moderate architecture and cellular arrangement were 33% and 25% on conventional smears and modified cellblock respectively (Figure 8).

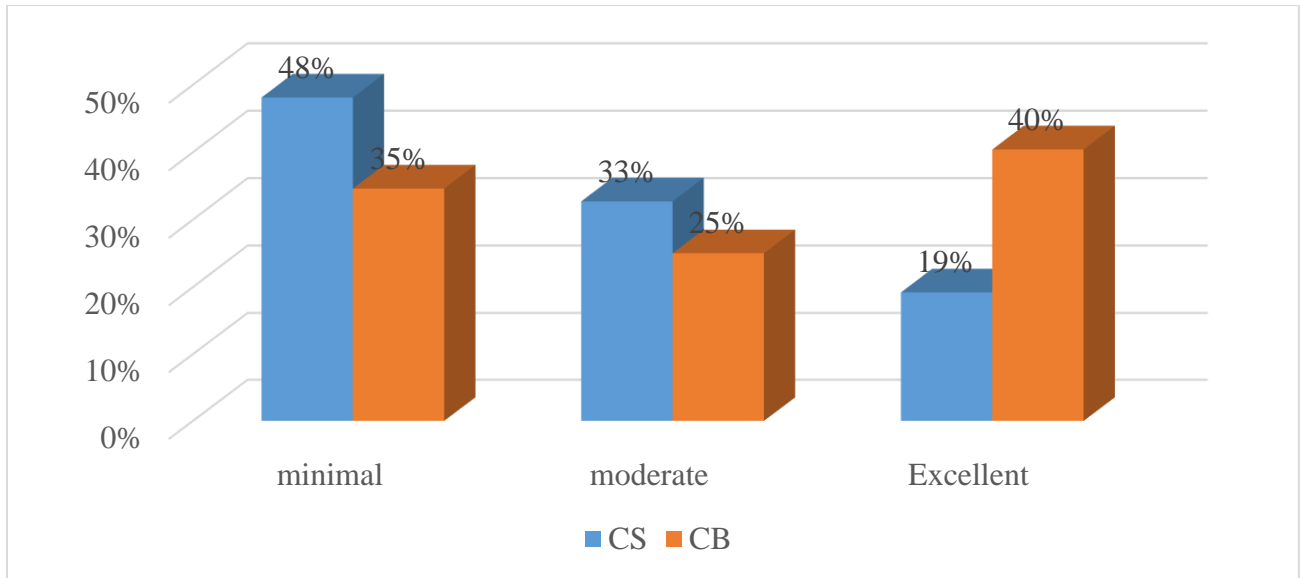


Figure 8: Architecture arrangement on both methods

4.5 Cytodiagnosis Results

Both modified cellblock and conventional smears were reported using the Bethesda System of Reporting Thyroid cytopathology (Table 3).

Table 3: Frequency Table of Cytodiagnosis according to Method

Results	Conventional Smear (%)	Modified Cell Block (%)
Unsatisfactory	13 (25%)	15 (28.8%)
Thyroiditis	2 (3.2%)	1 (1.9)
Colloid Goiter	36 (69.2)	35 (67.3)
Hürthle Cell Adenoma	0 (0.0%)	1 (1.9%)
ACUS	1 (1.9%)	0 (0.0%)
SFN	0 (0.0%)	0 (0.0%)
Suspicious for Malignant	0 (0.0%)	0 (0.0%)
Malignant	0 (0.0%)	0 (0.0%)
Total	52 (100%)	52 (100%)

Unsatisfactory results were reported in 25% cases of conventional smear and slightly higher (28.8%) in modified cellblock section. Among the 13 samples that were unsatisfactory on conventional smears, six samples were satisfactory on modified cellblock method preparation. Two cases of lymphocytic thyroiditis were reported on conventional smears while modified cellblock reported one case as Hashimoto's thyroiditis and another one was unsatisfactory. Colloid goiters were reported in 69.2% and 67.3% in conventional smears and modified cellblock cases respectively. One case that was reported as atypical cell of undetermined significant on conventional smear was unsatisfactory on modified cellblock. One case of colloid goiter on conventional smear was reported as Hürthle cell adenoma on modified cellblock (Photomicrograph d). There was no case of malignant/ suspicious for malignant on both conventional and modified cellblock (Figure 9).

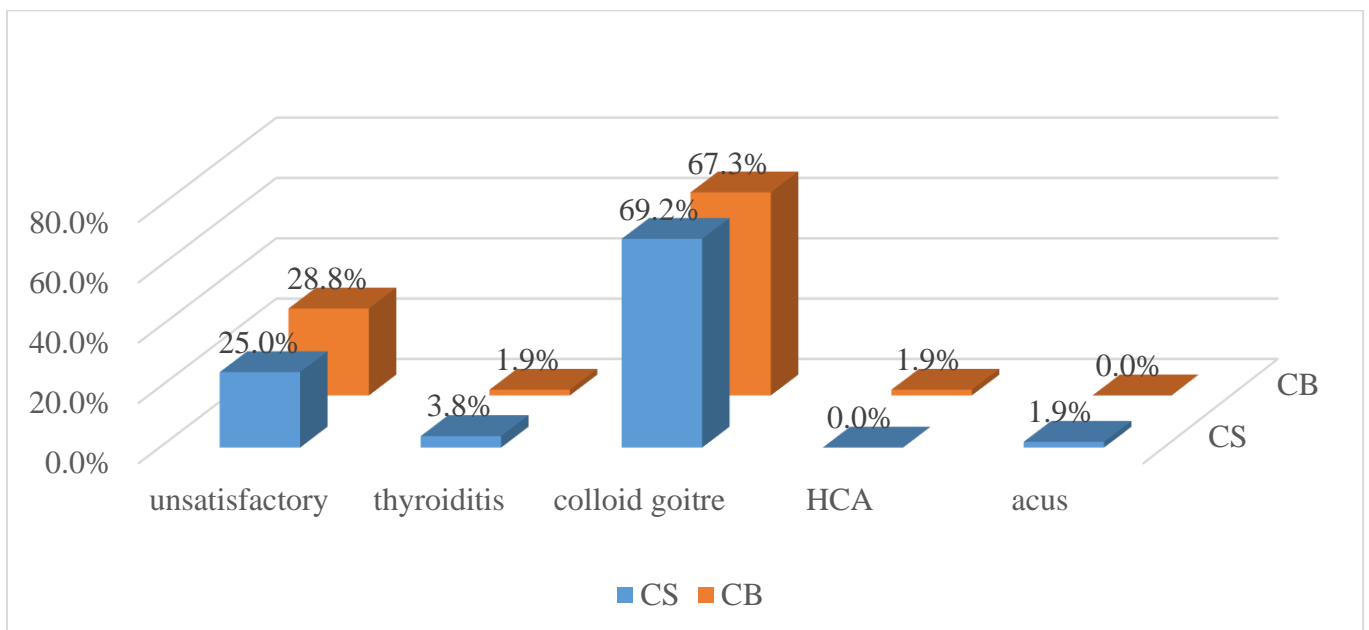


Figure 9: Cytodiagnosis according to method

Comparing the two methods on McNemar's Chi Square test, there was no statistically significant difference in the two methods at *p-value* of 0.791.

Photomicrograph of Conventional smears and Modified cell block section

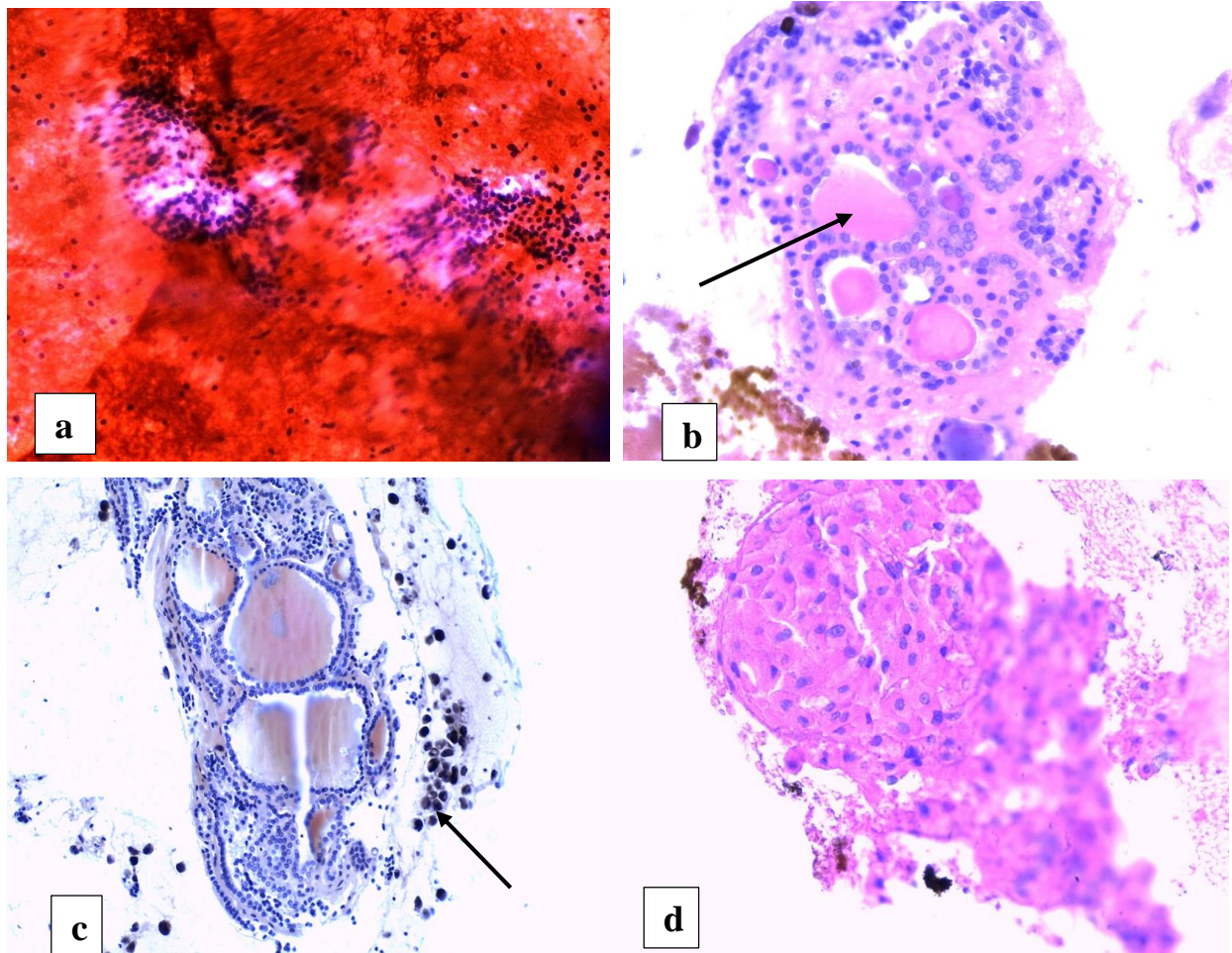


Figure 10: Photomicrographs

(a) Conventional smear showing benign follicular cells obscured by blood cellular elements x10, H/E; (b) Modified cellblock section showing benign follicular cell forming macrofollicles with colloid (arrow) x40, H/E; (c) Modified cellblock section showing benign follicular cell forming macrofollicles with colloid and hemosiderin laden macrophages (arrow) x10 PAP and (d) Modified cellblock section showing Hurthle cells x20, H/E.

5 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

There are variety of cell block methods that have been in use for over a century. The use of cell blocks have been widely advocated in the diagnostic work-up of patients with masses amenable to FNA since they provide diagnostic architectural information which complement FNA smears.

5.1.1 Social Demography

In this study a total of 52 patients were recruited at Kenyatta National Hospital FNA Clinic. The age of patients ranged from 21 to 73 years with mean of 41.4 years. This age range and mean incidence is slightly higher as compared with the study done by Manoj G et al (2) who found that the age range was from 22 to 58 years with mean age of 38.7 years. However, in another study done by Raafat A et al (32), age range was from 15 to 65 years, with a median age of 40 years. This shows that thyroid nodules are found within large age range. In this study we found that majority of patients (37%) were in their third decade of life followed by 23% of patients in their fourth decade. This is in comparable to Manoj G et al study results who showed that majority of patients were in their third decade of life (2).

The majority (46/52) of participants in this study were female accounting for 88% of total participants with a male to female ratio of 1:8.7. This finding agrees with Dorairjan N and Jayashere N, who found that thyroid nodules were 4–9 times more common in females as compared to males (33).

5.1.2 Cytomorphology Features

More than half (53.8%) of conventional smears samples had marked obscuring background on Mair' et al scoring system while only 28.8% were reported to have marked obscuring background on modified cellblock sections. The main background obscuring material was blood cellular elements because thyroid gland is a highly vascular organ. However, 34.6% of modified cellblock cases had a clear background (minimal obscuring background) as compared to 15.6% of conventional cases. This difference can be accounted for because modified cell block fixative fluid had acetic acid which lyses red blood cell. Nithyananda. A et al (5) also found that cell block

sections showed clearly recognizable normal and abnormal cells with minimal shrinkage after using AAF fixative.

Marked cellularity was scored in 44% (23/52) and 21% (11/52) cellularity in modified cellblock cases and conventional smear cases respectively. Marked cellularity in cellblock could be due to the clots in the needle hub which trap cell and these clots were removed for cellblock preparation. This agrees with Basnet et al (34) who showed that Cell block method allows the recovery and processing of minute amounts of cells hence high cellularity.

In this current study, the degree of degradation was comparable on both techniques with minimal degradation rate of 67.3% and 65.4% on conventional and modified cellblock cases respectively. This minimal cellular degradation which was more than half of all cases in both methods was achieved because samples were fixed immediately after collection. This result agrees with Nithyananda. A et al (5). Khan. S et al (24) however, showed a contrary result of marked cellular degradation on Cell block than conventional smears. In their study material for cell block was aspirated after 3 to 4 times of the aspirations for the conventional FNA and this may have contributed to a more traumatized and poorly preserved specimen.

Modified cellblock showed 40.4% marked architecture and cellular arrangement compared to 19.2% in conventional smears. Forty eight percent (25/52) had a minimal architectural arrangement on conventional smears as compared to 34.6% in modified cellblock. This agrees with Brown K et al (35) and Kulkarni et al (36) who concluded that cellblock preserve architecture pattern with excellent nuclear and cytoplasmic details.

5.1.3 Cytodiagnosis

Twenty five percent of conventional smears cases were reported as unsatisfactory. This was a high unsatisfactory rate. The majority of studies have shown that unsatisfactory rate ranges from 2 to 20% (12). The high unsatisfactory rate in this study could be because almost all FNA procedures were done by pathology residents who had variable experience. However six cases out of 13 unsatisfactory cases on conventional smears were satisfactory on modified cellblock reducing the unsatisfactory rate of thyroid FNA cytology from 25% to 13%. During the FNA procedure, aspirated material could clot in the needle hub which was difficult to flush onto the slide for

conventional smears. This clotted material in the hub was removed and used for cellblock preparation. This could explain why six samples which were unsatisfactory on conventional were satisfactory on modified cell block. Out of the two cases which were reported as chronic thyroiditis on conventional smear, one case was Hashimoto thyroiditis on modified cellblock, while the other case was unsatisfactory.

The majority (73.1%) of the cases were reported as benign. This compares very well with Cibas E et al (12) who documented that 70% of all thyroid FNA are reported as benign. Overall modified cell block aid in diagnosis of 8 cases (15.4%) which is in concordance with Brown K et al and Thapar M. et al (35) who showed in their studies that cellblock aided in diagnosis of 14% and 13% of cases respectively.

5.1.4 Study Limitations

1. To obtain material for evaluation from thyroid nodules largely depends on the experience and skill of the physician performing the technique. Many of the insufficient samples were obtained by training pathology residents.
2. In addition, no dedicated passes were done for modified cellblock preparation, limiting the effectiveness of the method.

5.2 Conclusion

This study has not demonstrated statistically significant differences in the diagnostic utility of both methods, but the modified cell block preparations provided additional information helpful in conforming and establishing new diagnosis.

5.3 Recommendation

1. Modified cellblock should be considered as an adjunct test to the conventional smear on thyroid FNA cytology especially in suspected thyroiditis and neoplasms.
2. In resource constrained settings, the cost implications should guide in the selection of the method to use.
3. Proper training and monitoring of the clinicians performing FNA procedure should be provided in order to reduce the unsatisfactory results in thyroid aspirates.