MODIFIED ULTRA FAST PAP STAIN IN CYTOLOGY IN COMPARISION WITH REGULAR PAP STAIN AND MGG

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Partial fulfillment of the regulations required for the award of

M.D.Degree

in PATHOLOGY-BRANCH III

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

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DECLARATION

I solemnly declare that this dissertation entitled **"MODIFIED ULTRA FAST PAP STAIN IN CYTOLOGY IN COMPARISION WITH REGULAR PAP STAIN AND MGG"** was done by me in the Department of Pathology, Coimbatore Medical College, Coimbatore during the period of July 2015 to June 2016 under the guidance and supervision of DR.V.PRABA, MD., Associate professor, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology.

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CERTIFICATE

This is to certify that the dissertation entitled **"MODIFIED ULTRA FAST PAP STAIN IN CYTOLOGY IN COMPARISION WITH REGULAR PAP STAIN AND MGG STAIN"** is a record of bonafide work done by **Dr.P.SELVI.,** Post Graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the guidance and supervision of Dr. V.PRABA, M.D., Associate Professor, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore in partial fulfillment of the regulations of Tamilnadu Dr.M.G.R. Medical University, Chennai towards the award of M.D.Degree (Branch III) in Pathology.

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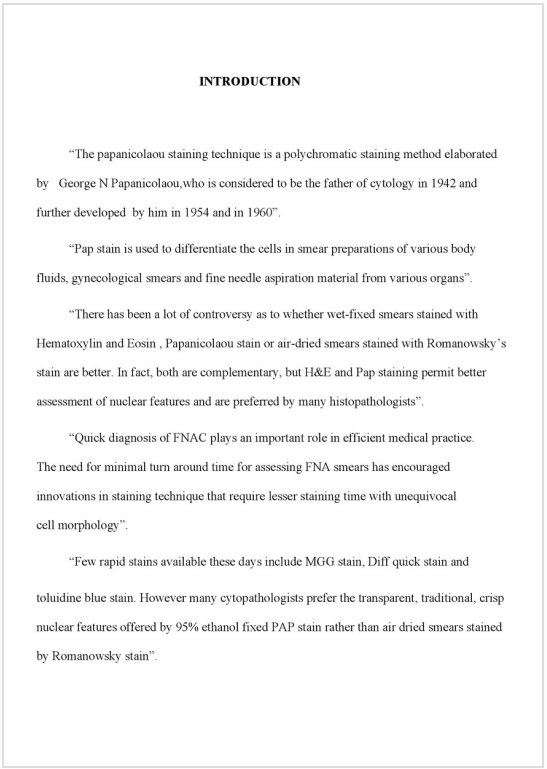


INTRODUCTION

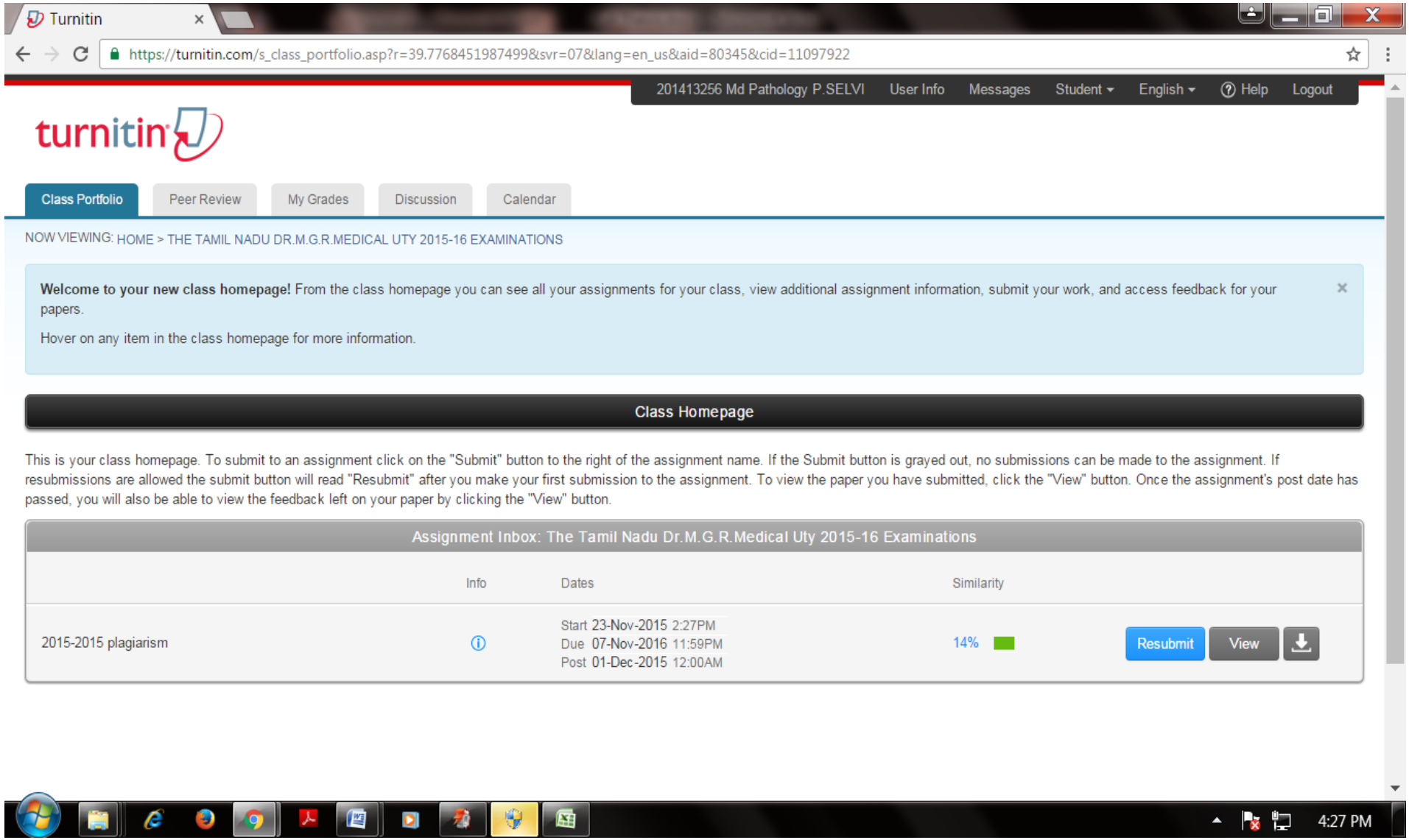
"The papanicolaou staining technique is a polychromatic staining method elaborated by George N Papanicolaou,who is considered to be the father of cytology in 1942 and further developed by him in 1954 and in 1960".

"Pap stain is used to differentiate the cells in smear preparations of various body fluids, gynecological smears and fine needle aspiration material from various organs".

"There has been a lot of controversy as to whether wet-fixed smears stained with Hematoxylin and Eosin , Papanicolaou stain or air-dried smears stained with Romanowsky's stain are better. In fact, both are complementary, but H&E and Pap staining permit better assessment of nuclear features and are preferred by many histopathologists".

"Quick diagnosis of FNAC plays an important role in efficient medical practice. The need for minimal turn around time for assessing FNA smears has encouraged innovations in staining technique that require lesser staining time with unequivocal cell morphology".

"Few rapid stains available these days include MGG stain, Diff quick stain and toluidine blue stain. However many cytopathologists prefer the transparent, traditional, crisp nuclear features offered by 95% ethanol fixed PAP stain rather than air dried smears stained by Romanowsky stain".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Pap stain is used to differentiate the cells in smear preparations of various body fluids, gynecological smears and fine needle aspiration material from various organs.

There has been a lot of controversy as to whether wet-fixed smears stained with Papanicolaou stain or air-dried smears stained with Romanowsky's stain are better. In fact, both are complementary, but Pap staining permit better assessment of nuclear features and are preferred by many histopathologists.

Quick diagnosis of FNAC plays an important role in efficient medical practice. The need for minimal turnaround time for assessing FNA smears has encouraged innovations in staining technique that require lesser staining time with unequivocal cell morphology.

Few rapid stains available these days include MGG stain, Diff quick stain and toluidine blue stain. However many cytopathologists prefer the transparent, traditional, crisp nuclear features offered by 95%

ethanol fixed PAP stain rather than air dried smears stained by Romanowsky stain.

To overcome this, ultrafast papanicolaou stain was introduced by yang and Alvarez in 1994 which is a hybrid of romanowsky stain and pap stain.

It not only reduces the time for pap stain to 90 seconds, but also enhances the quality. Pap stain requires ethanol for fixation. Ethanol is expensive and laboratory needs a license for acquiring ethanol in bulk quantity.

AIM & OBJECTIVES

AIM AND OBJECTIVES

AIM:

Aim of our study is to assess the use of modified ultra-fast pap stain in fine needle aspiration cytology of various organs in comparison with the standard conventional pap stain and MGG stain.

OBJECTIVES:

To compare the results of MUFP, Routine pap and May Grunwald Giesma (MGG) stain".

- To assess the quality of MUFP stain and to find the advantages of the same over routine stains used in cytology.
- To find a cost-effective method which can be adopted in our laboratory.
- To compare the quality of staining procedures used on air dried smears (MGG and MUFP) over the wet fixed smears (Pap stain)
- To assess the utility and applicability of these stains in cytomorphological study in FNAC of thyroid, breast, and lymph nodes.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cytology started as a revolutionary idea of looking at imprints of cut surface of tumors at postmortem. It has evolved through many new methods of procuring, fixing and staining cells. Its main attribute lies in its ability to allow prompt, accurate assessment of cell changes on material taken with minimally invasive procedure and processing.[1]

The evolution of cytopathology occurred through four overlapping eras;

a) 1860-1940: Early history.

b) 1860-1940: Development and expansion of exfoliative cytology in the USA and elsewhere.

c) 1955-1985: Consolidation of cytopathology as a discipline and the parallel developments of population screening and FNA cytology.

d) 1955-1985: Maturation of cytopathology as a discipline and its integration with new technology (1985 to the present day).[1]

In the early historical era microscopic observations of normal and abnormal human cells either in exfoliated or in imprints or scrapes were steadily and independently recorded throughout the nineteenth century.[2,3]

By first decade of twentieth century, exfoliative cancer cells had been described in all types of specimens.[4]

In 1922, professor LS Dudgeon used cytology at St. Thomas Hospital for the diagnosis of a wide variety of neoplastic and inflammatory diseases from imprints of surgical specimens.[5] Dudgeon considered that the stained films were much nicer to examine than paraffin sections.[6] At the same time in USA, FNA cytology was developing and the first series on aspiration of neoplasms was published from memorial Hospital for cancer and allied diseases in New York City.[7]

A second era of cytopathology began in 1941 with the publication of "The diagnostic value of vaginal smears in carcinoma of uterus" by George N. Papanicolaou, an anatomist, and Herbert F. Traut, a gynaecologist.[8] Papanicolaou' contribution to this field was two-fold; he recognized the importance of wet fixation of cytological specimens and he systematically began to accumulate examples of cancer cells in vaginal smears, culminating in his paper New Cancer Diagnosis.[9]

Concurrently with the development of cervical screening the cytological method of cancer diagnosis began to be more widely applied to the respiratory, alimentary and urinary tracts as well as to the serous cavities and the central nervous system.[1]

The era of consolidation was heralded by two publications: the first issue of Acta cytologica in 1957 - the oldest journal devoted exclusively to cytopathology; and in 1961 - by the publication of "Diagnostic cytology and its Histopathologic bases by Leopold G.Koss in association with Grace R. Durfe".[10]

In the last 60 years there is an explosion in the literature of cytopathology, with thousands of articles and scores of books written on the subject.[1]

Population based cervical screening is now practiced to a greater or lesser extent in almost all countries of the developed world.[1] The imperial cancer Research Fund Coordinating Committee on cervical screening made the statement in 1984 that 'with the exception of non smoking, cervical cytology screening offers the only major proved public health measure for significantly reducing the burden of disease,[11] its introduction was highly controversial and has remained so at every stage of its development.

By 1986, there was sufficient evidence from an international multicentre analysis to show that 5 yearly and 3-yearly screening reduced the risk of invasive cancer by 84% and 93% respectively, while little additional benefit was achieved by annual screening.[12]

The impetus of the development of cytopathology as we know it today resulted from the painstaking research of papanicolaou in the USA. Thus papanicolaou is justly referred to as the 'Father of cytopathology'.[13]

PAPANICOLAOU STAIN

For the routine diagnostic cytology, the papanicolaou stain is recommended.

The use of the papanicolaou stain results in well-stained nuclear chromatin, differential cytoplasmic counterstaining, and cytoplasmic transparency.[14]Although, originally developed for interpretation for gynaecological specimen, Papanicolaou staining is now commonly used to facilitate the accurate detection and interpretation of abnormal cells from variety of sources.

NUCLEAR STAINS[15]:

Haematoxylin:

Haematoxylin - most widely used nuclear stain. It is extracted from logwood campeachy wood). The Haematoxylin campechianum is a tree that has been scientifically cultivated in Jamaica since 1715.

Freshly cut wood is colorless. It becomes dark reddish-brown when exposed to atmospheric oxygen. Haematoxylin is not a dye, only the

oxidation product of hematoxylin-hematein is a weak anionic dye. Oxidation of hematoxylin achieved naturally by exposing the solutions to atmospheric oxygen or by using oxidizing agents such as sodium iodate, mercuric oxide, and potassium permanganate. Oxidation process is called "ripening." Solutions should always contain some unoxidized hematoxylin, since complete or over oxidation leads to a breakdown of the solution and the loss of good staining.

Oxidized hematoxylin (hematein) has little affinity for tissue but becomes a strong dye with a particular affinity for nuclei when combined with a metallic mordant.

In some solutions of hematoxylin, the oxidizer also serves as the mordant. For (e.g);iron hematoxylin,but it is not stable.

To achieve stability, the mordant should not oxidize the solution. Ammonium or potassium aluminum sulfate, phosphotungstic acid, and phosphomolybdic acid are mordants. "The mordant-dye combination is called lake". most commonly used hematoxylin lakes are combinations of hematein with either aluminum or iron.

The routine nuclear stains should be called aluminum hematoxylins, or more properly aluminum hemateins, since aluminium is a mordant.

More selective nuclear staining achieved by adding either an excess of acid or an excess of aluminum.

Formulas for some of the aluminum hematoxylins follow,

Harris' Hematoxylin

Hematoxylin….. 5.0 g

Absolute ethyl alcohol….. 50.0 ml

Ammonium aluminium sulfate….. 100.0 g

Distilled water….. 1000.0 ml

Mercuric oxide….. 2.5 g

Gill's hematoxylin (Gill et al. 1974 modified)

Preparation of solution

Hematoxylin 2 g

Sodium iodate 0.2 g

Aluminum sulfate 17.6 g

Distilled water 750 ml

Ethylene glycol (ethandiol) 250 ml

Glacial acetic acid 20 ml

Ehrlich's Hematoxylin

Hematoxylin….. 2gm

95% Alcohol….. 100ml

Distilled water….. 100ml

Glycerol….. 100ml

Ammonium or Potassium Aluminium sulphate….. 3gm

Glacial acetic acid….. 10m

It is used as progressive or Regressive staining. In progressive staining the reaction is stopped once the desired staining intensity is achieved. In Regressive staining, longer time is required to over stain the tissue before the stain is selectively removed in acid alcohol (1% hydrochloric acid in 70% ethanol).

CYTOPLASMIC STAINS[15]:

Counterstain is mostly used as eosin. The staining of eosin is best at a pH of 4.6 to 5.0.

Used properly, three shades of pink can be obtained with eosin alone; erythrocytes, collagen, and the cytoplasm of muscle or epithelial cells should stain with different shades or intensities of pink.

Eosin solution

Eosin Y (1% aqueous solution)….. 200.0 ml

95% ethyl alcohol….. 600.0 ml

Acetic acid, glacial….. 4.0ml

2.Eosin-Phloxine B

Eosin Y (1% aqueous solution)…….. 100.0 ml

Phloxine B (1% aqueous solution)……. 10.0 ml

95% alcohol…….. 780.0 ml

Acetic acid, glacial….. 5.0ml

There are multiple acid dyes also used to provide differential counterstaining and Cytoplasmic transparency.

- Orange G6 (OG 6).
- Eosin azure 36 (EA 36 or EA 50) -contains light green, Eosin and bismark brown.

MODIFICATION OF PAPANICOLAOU STAIN[14] :

Modification of the original papanicolaou stain (1942) was published by Dr.Papanicolaou in 1954 and 1960.

Papanicolaou Technique I: Uses Harris hematoxylin regressively.

Papanicolaou Technique II: Described for urinary and gastric preparations,uses hematoxylin progressively . Other modifications include Gill's modification, Miller's modification, Saccomanno's modification for carbowax fixed smears and Durfee's modification for urine sediment smears.

FIXATIVES[14]:

Rapid fixation of smears is needed to preserve cytological details of cells. For many years the fixative of choice for gynecologic and other smear preparations was the one recommended by Papanicolaou, a solution contain equal parts of ether and 95% of ethyl alcohol. Subsequently, it is was abandoned, since the ether present in the pap stain is a fire hazard. Ninety-five percent ethyl alcohol (ethanol) is now used as a fixative by many laboratories, with excellent results. Smears should remain in 95% ethanol fixative for a minimum of 15 minutes prior to staining.

To obtain ethanol without federal taxation, a license is required.

EQUIVALENT CONCENTRATIONS OF SEVERAL ALCOHOLS FOR PURPOSES OF CELL FIXATION

100% Methanol

95% Ethanol

95% Denatured alcohol

80%propanol

80%Isopropanol

WET FIXATION[14,16] :

Wet fixation is traditional method of fixation in which smears are immediately kept in fixative before air-drying of smears. The disadvantages of wet fixed smears are air-drying artifacts, hemorrhagic background and cell loss during fixation.

To overcome these disadvantages, in 1988, Chan and Kung reported that air-dried smears can be rehydrated by immersing the smears in 0.9% Nacl for 30sec which is used in MUFP.

FINE NEEDLE ASPIRATION CYTOLOGY:

FNA was first introduced in Sweden by Franzen, a haematologist & oncologist – tried the Romanowsky staining methods for cytology.[17]

The technique was further developed by Soderstrom, Fox and also by Lopes Cardoso, Von Haam, Crepinko and Hauptmann.[18]

In the UK, FNA was pioneered by a surgeon, John webb, who was given enthusiastic support by some of the renowned cytopathologists of the time.[19] The technique also became popular in the USA after a long interval since its early use in the 1930s.

The present day focus of FNA cytology is on obtaining a satisfactory specimen on which a reliable diagnosis can be made and therefore, that the aspirate sample should provide true reflection of the disease process.[20] FNA is now established as the first line investigation of mass lesions where-ever they occur in the body.[20]

ADVANTAGES OF FNA [20] :

It is a minimally invasive procedure.

The technique is relatively painless, produces a quick result and is economical.

The method is applicable to easily palpable lesions.

New radiological techniques are now available for internal imaging of organs and also for FNA of deep seated impalpable structures.

It can be done as OP procedure, so hospital stay can be avoided. Diagnosis can be obtained within minutes rather than days

Mostly accurate in many situation, hence it is used as essential preoperative /pretreatment investigation

Risk of complication will be less is an additional advantage that allows FNA cytology to be performed as an OP procedure.

Cells obtained by FNA can be manipulated in a variety of ways useful for ultra structural study, Immunocytochemistry, gene rearrangement, morphometry, image analysis and DNA analysis.

In routine practice, success of FNAC depends on

1. Samples from the representative lesion.

2. Adequate number of cells and other tissue components.

3. Smear making and processing of the samples.

4. Accompanied by correct clinical /radiological information.

LIMITATIONS OF FNAC

"FNA cytology has its own limitations. Scanty samples and loss of histological architecture leads to difficulty in interpretation of FNA smears based on morphology.

There is a risk of complications of FNA. The overall morbidity and mortality to FNA has been estimated in several studies and the risk of death is approximately 1 in 1500.

Serious complications have been reported such as major hemorrhage after FNA of lung, liver and kidney; septicemia after prostate aspiration; bile peritonitis following needling of liver; and acute peritonitis resulting from pancreatic aspiration. However, such complications are very rare. Review of literature shows, the risk is increased due to multiple passes, larger needles, and absence of normal parenchyma covering the lesion .[20]

Contraindications of deep seated aspiration- It include anticoagulant therapy and Intrinsic bleeding problems because it produce increase the risk of bruising and hemorrhage.

Intractable cough and poor respiratory function are absolute contraindications to transthoracic FNA.

Aspirates of unsuspected hydatid cyst carry the potential risk of anaphylactic shock resulting from rupture and is best avoided.

FNA of phaeochromocytomas is contraindicated for fear of inducing a hypertensive crisis.[20]

NEED FOR RAPID ASSESSMENT IN CYTOLOGY:

Quick diagnosis of fine-needle aspiration cytology (FNAC) plays an important role in efficient medical practice.[22] Rapid assessment of FNA smears has become increasingly popular due to the global trend in reducing health care costs.

The goal is minimal time for hospitalization and the fastest possible turnaround time for test results.[23]

Immediate examination of the aspirates for adequacy, while the patient remains in the biopsy suite, reduces the number of inadequate samples and decreases the number of needle passes performed.[24]

The need for minimal turnaround time for assessing fine needle aspiration smears has encouraged innovations in staining technique that require lesser staining time with unequivocal cell morphology.[25]

TWO FUNDAMENTALLY DIFFERENT METHODS ARE USED FOR ROUTINE FIXATION AND STAINING OF CYTOLOGIC SPECIMENS.

Romanowsky-type stains (e.g., Wright's, May- Grunwald Giemsa, Diff-Quick) are based on air-drying.[26]

The trichrome papanicolaou and bichrome Hematoxylin and Eosin are based on wet-fixation.

Many cytopathologists prefer the transparent traditional, crisp nuclear features offered by 95%, ethanol fixed papanicolaou stain than nuclear features (opacity of nuclei, nuclear enlargement etc.) offered by air-dried Romanowsky stains.[25]

MODIFIED ULTRAFAST PAPANICOLAOU STAIN (MUFP):

To overcome the disadvantages of both Romanowsky and pap stains, Yang and Alverez in 1995 suggested an ultrafast Papanicolaou (UFP) stain, which is a hybrid of Romonwsky and Pap stains, and requires only 90 seconds.

It involves rehydration of air dried smears, fixation in alcoholic formalin and subsequent pap staining except that the duration of each step is shortened.[25]

ULTRAFAST PAPANICOLAOU STAIN[23]

1. Normal saline - 30 seconds
2. 95% Ethanol (optional), for storage/transport
3. Alcoholic formalin - 10 seconds
4. Water - 6 slow dips
5. Richard –Allan Hematoxylin 2 2 slow dips
6. Water - 6 slow dips
7. 95% Ethanol - 6 slow dips
8. Richard-Allan Cytostain - 4 slow dips
9. 95% Ethanol - 6 slow dips
10. 100% Ethanol - 6 slow dips
11. Xylene - 10 slow dips
12. Mount and coverslip

Earlier rapid papanicolaou stains (Kline's rapid and Tao's rapid) are identical to the routine stain except that the duration of each step is shortened.

The problem with rapid papanicolaou stain is four fold:

1. Both the cytoplasm and nucleus lose much cellular detail from inadequate fixation.

2. Since FNA samples are inherently bloody, the tumor cells are often covered with ubiquitous RBC's, this is particularly annoying with papanicolaou stain as RBC's stain orange.

3. The wet-fixed cells are much smaller than air-dried cells;

4. Loss of wet fixed cells during processing.[23]

To overcome the first problem, fixative is changed from 95% ethanol to 4% formaldehyde in 65% formalin.[23]

Alcoholic formalin differentiates RNA from DNA in subsequent staining because of acidic PH (PH-5). It renders the nucleoli red and colours more vibrant.[25]

The last three problems can be overcome by chan and kung's rehydration of air-dried smears. Air drying allows the cells to stick firmly

to the glass slide and the rehydration in normal saline allows RBCs to hemolyse, unmasking the cells for morphologic analysis.[23]

The chief limitation of ultrafast papanicolaou stain, is that Richard Allan Haematoxylin (RA-H) and Richard Allan cytostain (RA-C), used in the staining procedure are not universally available.[25]

MM Kamal and MM Munshi (2000) made two modifications in the ultrafast pap stain.

First, Instead of Richard Allan Haematoxlyin (RA-H), Gill's Haematoxlyin is used.

Second, modification was instead of Richard Allan cytostain (RA-C) which is an Alcoholic mixture of orange G, Eosin Y, Light Green and Aniline blue, they used EA modified which is an alcoholic mixture of Eosin Y, light green, Phosphotungstic acid and glacial acetic acid.[25]

As orange G was omitted from the staining solution in Modified ultrafast pap stain, orange discoloration was no longer a problem. Modified ultrafast papanicolaou stain can be used for tissues where chances of cytoplasmic keratinization is negligible.

MODIFIED ULTRAFAST PAPANICOLAOU STAIN:

0.9% Normal saline (30sec)

Alcoholic formalin (10 sec)

6 dips in tap water

Gill's Haematoxylin (30 sec)

6 dips in tap water

95% Alcohol (6 dips)

EA Modified (15 sec)

95% Alcohol (6 dips)

100% Alcohol (6 dips)

Xylene (6 dips)

Modified ultrafast papanicolaou (MUFP) is useful in rapid assessment of adequacy of smears and for intra operative FNA consultations.[25,27]

"Priyanka and Sudhamani et al. showed that Harri's hematoxylin gives good staining as much as Gill's hematoxylin in MUFP".[33]

A study was conducted by Junko Maruta and Hironobu Hashimoto et al., in 2002 showed the applicability of modified ultrafast stain for quick diagnosis of thyroid diseases.

Two specimens from each of 251 thyroid aspirations (120 malignant and 131 benign) were prepared using the modified ultrafast stain and the standard papanicolaou stain. The sensitivities of cytologic diagnosis in specimens stained by standard papanicolaou method and the modified ultrafast method were 95.0% and 93.3% respectively, and the specifities were 99.2% and 97.7% respectively.[22]

Another study done by Grace C.H.Yang and Doreen et all in 2001 on ultrasound guided FNA of thyroid showed that MUFP high lighting the "orphan Annie-eyed" clear nuclei, helped to differentiate follicular variant of papillary thyroid Carcinoma from follicular neoplasms.[28]

MM Kamal and MM Munsi et al., done a study on 'Efficacy of modified ultrafast papanicolaou stain for Breast aspirates' in 2000.

In this study smears from FNA from 100 breasts lumps were stained by the MUFP stain. Eighty six breast aspirates are adequate for interpretation. Smears showed transparent cells with crisp nuclear features, equal to and even better than the conventional papanicolaou stain, in a blood free background.[25]

Shinde and Ajita et al., done a study on ‘Application of Modified ultrafast papanicolaou stain in cytology of various organs’ in 2005. In their study, Group-I included 40 FNAC smears of various organs. In each case, three smears were prepared and stained by MUFP, Papanicolaou, and MGG stains.

For assessment of MUFP stain, scores were given on four parameters; background of smears, overall staining pattern, cell morphology and nuclear staining. Quality index was calculated from ratio of score achieved to maximum possible score. Diagnosis made by MUFP stain was compared with standard stains. The diagnosis was correct except in three cases of metastatic squamous cell carcinoma. Hence, it was concluded that MUFP stain is useful for rapid diagnosis by FNAC, but is not useful for squamous cell lesions.[29]This is because Orange G is not being used in this method.

Luciano.B Lemos and Mithra Baliga in 1997 did a one year study in Fine Needle Aspiration and concluded that ultrafast papanicolaou stain is particularly useful in diagnosing squamous carcinoma because of the bright orange staining it imparts to keratinizing squamous carcinoma cells, which is an important consideration in the diagnosis of many head and neck carcinomas as well as metastatic carcinoma.[31]

Kenji Bando and Reiji Haba et al., done a study on "Utility of Immediate cytologic Diagnosis of Lung masses using ultrafast papanicolaou stain" in 2011. In this study out of 503 cases investigated, the results of immediate cytology using ultrafast pap stain were positive in 348 cases and negative in 153 cases. The study concluded that immediate cytology can be implemented fairly easily in any hospital, and is superior technique for obtaining high diagnostic accuracy.[32]

Priyanka Choudhary and Sudhamani S et al., did a study on 'Comparison of MUFP with the standard rapid papanicolaou stain in cytology of various organs' in 2012. In this study a total of 100 FNAC cases were studied by random sampling. Two smears were prepared for each case and stained by both the MUFP and the rapid pap stain. Scores were given and the quality index was calculated, followed by the statistical analysis. The cases included lymph node (43), thyroid (25), breast (23), salivary gland (02), and soft tissues (07)".

Scores were given on four parameters:

Background of smears, Overall staining pattern,

Cell morphology and nuclear staining.

Quality index was calculated from the ratio of score achieved to the maximum score possible. The study concluded that quality index of

MUFP smears was better compared to the rapid pap stain in all the organs, and was statically significant.[33] Another study was conducted by Shuji Bandoh and Jiro Fujita et al., in 2013 on 'Diagnostic Accuracy and safety of Flexible Bronchoscopy with Multiplanar Reconstruction images (MPR) and Ultrafast Papanicolaou Stain (UFP).

This study includes one hundred consecutive patients with solitary pulmonary nodule who underwent bronchoscopy with multiplanar reconstruction and MUFP stain. The total diagnostic accuracy of bronchoscopy in the MPR and UFP group (91%) was significantly higher compared with the historical control group (58%) [P<0.05]. The conclusion of the study was that combined use of MPR image and UFP during flexible bronchoscopy improved diagnostic accuracy and safety in evaluating solitary pulmonary nodules.[34]

"M.Kamal and Madhura M.Kulkarni et al., in 2011 did a study to find out the efficacy of the ultrafast papanicolaou staining technique for immediate cytologic diagnosis and to check specimen adequacy during radiologically guided FNAC procedure. In this study Group I included 238 out patient FNACs, groups II included 59 radiologically guided FNACs and group III included 50 cases of intraoperative cytology".

Overall diagnosis was possible in 297(85.6%) cases. Only 8 (2.3%) cases could not be diagnosed due to staining difficulties. The overall concordance rate was 98%. The conclusion of the study was UFP staining technique is an accurate and reliable method for rapid cytology reporting. It significantly reduces total turnaround time of the test result, thereby it is cost-effective both for the patient and the hospital.[35]

BREAST

Although most countries in Europe continue to perform fine needle aspiration biopsy (FNB) as their first choice in the investigation of breast lesions in both screening and symptomatic populations, the use of core needle biopsy (CNB) is increasing.[73]

A preoperative diagnosis of FNA breast offers several advantages:

1. Saving of time and relieves the patient's anxiety by immediate diagnosis .
2. A definitive treatment can be planned and discussed with the patient in early.
3. If cancer is confirmed, additional imaging studies (bone scan, liver scan, etc.) Can be done preoperatively to determine stage .
4. With the triple test assessment, many benign conditions can be confidently diagnosed by FNB or CNB and surgery avoided.
5. It is cost-effective and allowing one-step definitive surgery including lymph node sampling in malignant cases.
6. The need for frozen section diagnosis is reduced.

The place of FNB and CNB in the investigative sequence

While FNB of a palpable breast lump should generally be preceded by mammographic and/or ultrasonographic examination, as the

radiological findings help to select the most appropriate area to be biopsied, FNB may be performed as the first-line investigation, especially in symptomatic and screening populations. [74,75]

The main purpose of FNB or CNB of breast lumps is to confirm cancer preoperatively and to avoid unnecessary surgery in specific benign conditions.

The role of FNB in the assessment of a breast lump includes:

1. The diagnosis of simple cysts,
2. The investigation of suspected recurrence or metastasis in cases of previously diagnosed cancer,
3. The confirmation of inoperable, locally advanced cancer,
4. The preoperative confirmation of clinically suspected cancer,
5. The investigation of any palpable lump, clinically benign or malignant, as a guide to clinical management,
6. The ability to obtain tumor cells for special analysis and research, (e.g.) hormone receptor studies, DNA analysis, immunohistochemistry, cell kinetics and molecular studies.

Accuracy of diagnosis in FNB and CNB

However, in our experience the presence of malignant cells on FNB in a palpable mass yields invasive carcinoma at excision in

approximately 98% and thus the addition of a CNB only adds additional information in a few cases.[78]

Core biopsy is needed for fibrotic or collagenous lesion and suspected invasive lobular carcinoma, as these lesions can be paucicellular on FNB.[79]

The reported sensitivities, specificities and positive and negative predictive values for FNB vary depending upon, insufficiency of the samples (as positive, negative or excluded) and atypical samples are categorized as (positive or negative). When insufficent samples and atypical and benign findings are presumed to be negative, sensitivities range from 43.8% to 95%, specificities from 89.8% to 100%, positive predicitive values from 76.2% to 100% and negative predicitive values from 46.3% to 98.8%.

If insufficient samples are excluded, sensitivities and specificities improve to a range of 58.3% to 100% and 55% to 100%, respectively, with a slight change in the negative predictive value to between 46.6% and 98.6%.

The aim should be sensitivity is not less than 95% and this can be achieved by increasing experience. Sensitivity is lower for low-grade

carcinomas (invasive and in situ), for lobular carcinoma, and for very small and very large cancers.[80,81]

The unsatisfactory sample

If samples are not obtained by experienced pathologist or if Smear preparation is not done by technical staff and given to the laboratory.

If the lesion is fibrous in nature with low cellularity, sclerosed FA, desmoplastic ca, or hypertrophic fatty tissue smears can be of low cellularity.A smear with low cellularity must be analysed based on clinical findings and the consistency of the lesion felt by biopsy needle passes.

Poorly prepared smears with crush or drying artifacts or with cells trapped in clotted blood should be rejected as unsatisfactory.

Overall 20% of samples will have scanty cell content. It includes microcalcifications.

However, 'unsatisfactory' samples are mainly occur in benign lesions, and malignant microcalcifications yield much more satisfactory sample.

Standardized reporting of FNB and CNB samples and quality assurance

A national conference conducted by the National Institute of Health in the USA collected the views of many pathologists regarding details required on requestion forms, methods of sampling, cytologic subcategories and details to be furnished in the cytology report.

Five categories of FNB reports are recommended:

Benign, atypical/indeterminate, suspicious/ probably malignant, malignant and unsatisfactory. The benign category is again subdivided into ‘benign specific’ and ‘benign NOS’. This includes specific diagnosis such as cyst, fibroadenoma, fat necrosis, etc.In benign NOS, means simply non-neoplastic breast lesion, for that radiologic and clinical follow-up is mandatory.

Complications

- Complications are uncommon.

Hematomas are very rare. Vasovagal reactions may occur. Pneumothorax is rare one but most important complication,occur in thin patients when the FNA done in medial breast or axilla is sampled.

THYROID

For the past five or six decades, fine needle aspiration (FNA) cytology of the thyroid has been increasingly utilized for the investigation of thyroid lesions.[82]

The prevalence of thyroid nodules is 4–8% in Western populations.[83]

The prevalence of malignancy in solitary cold nodules ranges from 10% to 44.7%. Simplicity, diagnostic accuracy and most of all cost effectiveness, have given FNA the status of the first-line diagnostic test in the preoperative evaluation of thyroid lesions.

With increasing experience, FNA has been shown to be able to categorise many benign and malignant lesions and thereby guide therapeutic protocols.

It is also useful in the diagnosis and monitoring of autoimmune thyroid lesions, especially in clinically equivocal cases and cases where biochemical and immunological parameters are normal or marginally abnormal.

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

1. Evaluation of solitary thyroid nodules (with a view to distinguish benign from malignant),
2. Evaluation of diffuse thyroid lesions (with a view to distinguish inflammatory/autoimmune lesions from nodular goiter),
3. Confirmation and categorization of clinically obvious thyroid malignancy (especially anaplastic carcinomas that may require preoperative palliative treatment, and Lymphoma and metastatic malignancy where surgery is usually not indicated),
4. To obtain material for ancillary tests/prognostic parameters,
5. Evaluation of lesions detected initially by imaging, measuring 1–1.5 cm in diameter with features suspicious of malignancy.[86]

FNA has been shown to be the safest and most accurate of diagnostic tools in thyroid lesions,[87] with a sensitivity as high as 93.4%, a positive predictive value of malignancy of 98.6%, and a specificity of 74.9%.

The accuracy of FNA is distinctly higher in centers where not only the interpretation but the needling too is carried out by the pathologist.

Ultrasonography (US), thyroid function tests, antibody profiles and FNA, used in conjunction in selected cases, complement one another.

US-guided FNA of thyroid is useful, especially in cystic and multinodular lesions harboring malignancy.

Several studies have compared the accuracy and complications of core needle biopsy with that of FNA that has increased adequacy rate but reduced sensitivity, especially for papillary carcinoma.

Combination of core needle biopsy with FNA increases diagnostic accuracy but the problem of distinguishing benign and malignant follicular neoplasms remains. In general, safety and ease of use of FNA outweigh the slight increase in accuracy achieved by core needle biopsy.

Nomenclature used in reporting

Reporting of thyroid FNA specimens should follow a standard format that is clinically relevant in order 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.

The Bethesda System reporting terminology includes six categories: non-diagnostic, benign, atypia of undetermined origin, Follicular neoplasm (FN/) suspicious of FN, suspicious for malignancy and malignant.[88] Every category carries with it the implied risk for

malignancy. Each category should be further qualified as to the possible pathological entity.

If an indeterminate diagnosis is being made due to features suspicious but not diagnostic of a neoplasm, we suggest the revised Papanicolaou system of reporting[89] which 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.

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.[90,91]

Cytologic diagnosis is generally accurate in thyroiditis, usual type of Papillary Carcinoma 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.[92,93]

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.

Complications

There are no contraindications to thyroid FNA.

Local hemorrhage may be caused by needling, occasionally causing a hematoma in the anterior neck.[94] that in turn may cause airway compression[95]

Carotid hematoma is an extremely rare complication.[96]

Transient vocal cord paralysis [97]

Acute transient goiter,[98]

acute suppurative thyroiditis,[99]

Needling may convert a hot nodule to a cold one and vice versa, therefore scans (and in general, all noninvasive investigations) should be done before FNA.

Hemorrhage, necrosis or infarction caused by needling may occasionally obscure the histological pattern of thyroid neoplasms.

Cellular and vascular granulation tissue of organizing 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 needling.[100]

LYMPH NODE

"Lymphadenopathy is a commonly encountered clinical problem which has a multitude of causes. The commonest cause of peripheral lymphadenopathy is a non-specific reactive hyperplasia in which the underlying etiology is infrequently found (probably an asymptomatic inflammatory process)".

In general practice, less than 1% of patients with peripheral lymphadenopathy have a malignant process.

In comparison, retroperitoneal or intra-abdominal lymphadenopathy is usually malignant.

In contrast, in young patients, intrathoracic lymphadenopathy is often associated with infectious mononucleosis, sarcoidosis and tuberculosis.

The likelihood of malignant disease as a cause of peripheral lymphadenopathy increases over the age of 40 years, nodes over 2 cm in size, firm or matted nodes and non-tender/non-painful node.[59]

Fine needle biopsy (FNB) offers the alternative of an immediate, preliminary, although not always specific diagnosis with little trauma and cost, thus providing ample information for further management.[60]

The place of FNA in the investigative sequence

As a rule, cytological examination of FNB smears can determine whether lymphadenopathy is due to reactive hyperplasia, infection, metastatic malignancy or malignant lymphoma**.**

In order to make the most rational use of fine needle aspiration cytology (FNAC), clinicians and pathologists alike must understand and accept that the aims and purpose of FNB of peripheral lymph nodes at the 'primary' or 'community' level are different from those at the 'secondary' or 'specialist' level.

At the primary level, FNAC is used as a triage to distinguish between cases of lymphadenopathy with a high or a low level of suspicion of significant disease by the simplest, least invasive and least costly method.

This preliminary assessment is based on routine cytologic smears only.

At the specialist/secondary level, the role of FNB is to provide material for further cytomorphologic analysis and for ancillary studies.

The aim is to arrive at a definitive diagnosis and lymphoma typing making full use of the armamentarium of ancillary laboratory techniques. This also applies to most abnormal lymph nodes in deep sites.

Lymph nodes clinically suspected of metastatic malignancy constitute one of the commonest indications for FNB.

In patients without a previous malignant diagnosis, not only can metastatic malignancy be confirmed by FNB, but clues to the nature and site of the primary can also be given in most cases.

In addition, a specific diagnosis by FNB of disseminated carcinoma of the prostate, breast, ovary and thyroid, germ cell tumors and neuroendocrine carcinoma should be pursued since treatment is available.

The value of FNB in the investigation of suspected lymphoma can be summarized as follows:

1. At the community/general practitioner level, a preliminary cytological diagnosis suggests appropriate management/referral and further investigations without delay.

2. A representative node can be selected for surgical biopsy by FNB sampling of multiple nodes. The biggest or the most easily

accessible node is not always the most suitable and may show only reactive change.

3. If a suspicion of lymphoma is known beforehand, any surgical tissue specimen will be used to ensure a complete immunologic investigation and the preparation of imprint smears to provide additional cytonuclear detail.

4. Other biopsies – bone marrow, liver, spleen – can be coordinated with node excision, saving time and additional anesthesia.

5. In patients with advanced intra-abdominal or mediastinal lymphoma without involvement of superficial nodes, FNB combined with FCM and clinical and radiological assessment of the extent of disease may be a sufficient basis for therapeutic decisions. Alternatively, a CNB, with or without FNB, can be used to obtain more tissue for ancillary studies, a greatly expanded immunopanel and to give some idea of tissue and immunoarchitecture.[61,62] Surgical intervention, with its risk of morbidity, to obtain tissue for histologic examination can be avoided.

6. Suspected recurrent or residual disease in patients with previously confirmed lymphoma can be substantiated by FNB alone.[63] Any

change in the type or grade of lymphoma will also usually be recognized. Since the recurrent tumor may be the only sign of disease by which the response to systemic therapy can be monitored, it is best left intact.

7. At the secondary/specialist level, an accurate lymphoma subtype may be provided when supported by a range of ancillary studies and by appropriate expertise.

Accuracy of diagnosis

"The accuracy of FNAC of lymph nodes in the diagnosis of metastatic malignancy is influenced by many factors such as the size and site of the node, fibrosis, necrosis, previous irradiation and the number of punctures made.

Diagnostic sensitivity is occasionally limited by the fact that small metastatic deposits, metastases confined to the subcapsular sinus and single-cell metastases can be missed.

However, early micrometastases rarely produce significant lymph node enlargement and if a lymph node is palpable it is likely to contain enough tumor tissue to be detectable by FNB. The diagnostic sensitivity of metastatic and recurring malignancy reported in the literature is usually above 95%.[64,65]

Failure to obtain a representative sample is responsible for most false-negative diagnoses.

Thus, although a negative cytological report makes malignancy unlikely, it is not singularly diagnostic, and if the lymphadenopathy does not show signs of regression within a month or two, FNB should be repeated or a node should be excised for histology.

Diagnostic specificity for malignancy, on the other hand, is high. False-positive diagnoses are rare,[66-67] if particular caution is observed in the interpretation of smears from nodes in fields of previous irradiation and in the presence of necrosis.

Diagnostic accuracy not only depends on the aspirate being representative, but also very much on the quality of the cytological preparations.

Diagnostic sensitivity has generally been found to be lower for lymphoma than for metastatic malignancy.

In an extensive review of the literature, two-thirds of the 30 studies reviewed, in which FNB was supplemented by immunophenotyping, diagnostic sensitivity was over 80% and specificity over 90%.

As a rule, FNB samples from malignant lymphoma are very cellular and can be used for ancillary studies.[68]

The FNB provides superior cytomorphology and is an excellent source for flow cytometry cell suspensions. This combined approach can increase diagnostic accuracy, assist in classification and reduce the number of insufficient samples.[69,70]

Complications:

Significant complications do not occur. Post-aspiration hematoma or necrosis is rare. To date, septic complications or tumor implantation in the needle track have not been reported following FNB of lymph nodes.

Technical considerations

Both reactive nodes and nodes involved by metastatic malignancy or lymphoma are highly cellular and moderately vascular tissues. Sufficient material is therefore easily obtained using 23–27-gauge needles, except sometimes in the presence of fibrosis or necrosis.[71,72]

FNB without aspiration has been used routinely for several years in some institutions. It has the advantage over the traditional aspiration of giving the operator a more direct and sensitive feeling of the consistency of tissues through the needle. This is helpful when small or deep nodes

are biopsied. Non-aspiration also results in less admixture with blood. An abundance of blood adversely affects cell fixation and tends to cause shrinkage and distortion of cells.

If aspiration is used, multiple rapid biopsies from different points of entry are preferable to multiple passes in different directions, in order to obtain a representative and adequate sample without too much blood.

It is not easy to make perfect direct smears from samples of lymphoid tissue and this takes considerable practice. An air-dried smear has to dry quickly for optimal fixation and has to be made thin and even.

The smearing pressure must be well balanced to obtain a thin smear and at the same time avoiding crush artifacts. If the aspirate is bloody or thinned by a large amount of lymph fluid, cells need to be concentrated and separated as much as possible from the fluid. A wet-fixed smear must be fixed immediately to minimize drying artifacts.[59]

While air-dried MGG or Diff-Quik-stained smears are essential for the evaluation of cytoplasm and background elements, alcohol fixation and staining with H&E or Pap is helpful in assessing nucleoli and chromatin pattern. Whenever possible, both air-dried and wet-fixed smears should be made of each FNB sample as they may provide complementary information.[68]

MATERIALS & METHODS

MATERIALS AND METHODS

This prospective study was carried out in Department of pathology, Coimbatore medical college, Coimbatore. During July 2015 to June 2016. Study includes fine needle aspiration from lesions of various organs i.e., thyroid, breast, and lymph nodes.

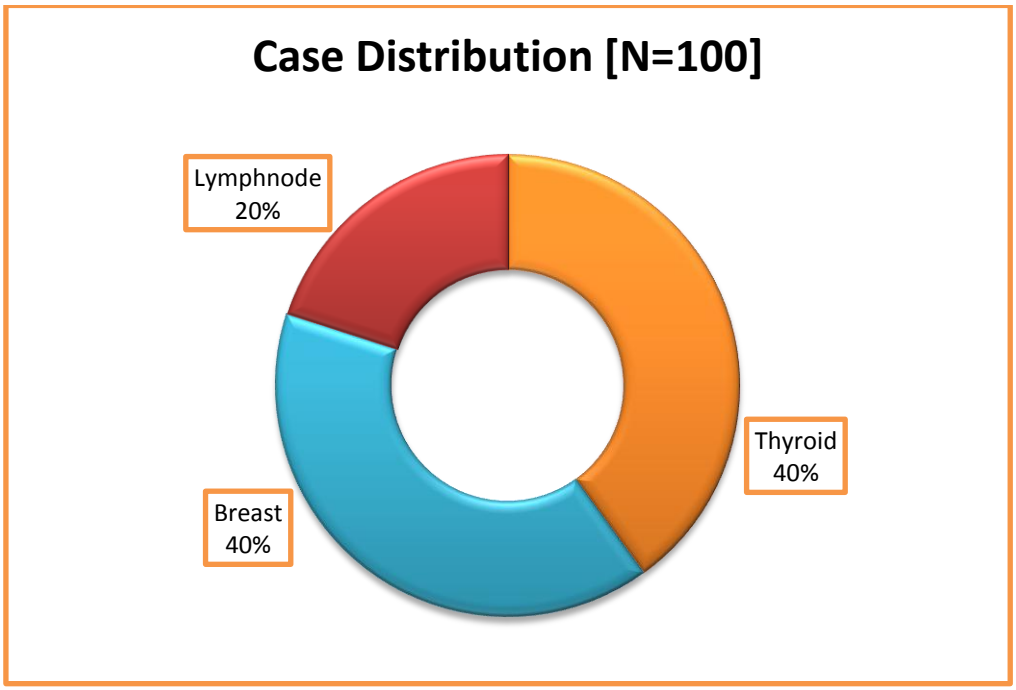
Total number of cases studied - 100

Thyroid - 40

Breast - 40

Lymph node – 20

Graph No: 1 Case distribution



PROCEDURE FOR SMEAR PREPARATION AND FIXATION:

The Fine needle aspiration was done in our hospital by standard technique. A minimum 3 smears were obtained on glass slides. Out of which 1 smear was fixed in 95% ethanol for a minimum of 15 minutes. These smears were stained for pap.

The remaining 2 smears were air dried out of which one was stained by MGG stain and other smear was rehydrated with normal saline and subsequently fixed in alcoholic formalin and stained by MUFP stain.

Inclusion Criteria

Fine needle aspirations from thyroid, breast, and lymph node lesions done in central laboratory, Department of Pathology, Coimbatore medical college, Coimbatore.

Exclusion Criteria:

1. Fine needle aspiration from other organs
2. Cervical cytology
3. Inadequate material on FNAC

STAINING PROCEDURES OF PAP, MGG AND MUFP

PAPANICOLAOU METHOD

REAGENTS REQUIRED:

1. Harri's Haematoxylin

   (Without acetic acid)

2. Orange G 6 (OG 6).

   0.5 Orange G in 95% alcohol 100 ml

   Phosphotungstic acid 0.15g.

3. Eosin azure 36 (EA 36 OR EA 50)

   0.5 Light green SF yellow in 95% alcohol 45ml

   0.5% Bismark brown in 95% alcohol 10 ml

   0.5% Eosin Y in 95% alcohol 45 ml

   Phosphotungstic acid 0.2 g

   Saturated aqueous lithium carbonate 1 drop

TECHNIQUE:

1. Smears are fixed (while still moist) in 95% alcohol – 15 mints.
2. Rinse in distilled water.
3. Stain with Harri's haematoxylin for 4 mints.
4. Wash in tap water for 1-2 mints.
5. Differentiate with acid alcohol (25% HCL in 70% alcohol).
6. Blue in tap water or 1.5% sodium bicarbonate.
7. Rinse the smears in distilled water.
8. Transfer to 70% alcohol and then 95% alcohol for a few seconds.
9. Stain with O G6 for 1-2 minutes.
10. Rinse in 3 changes of 95% alcohol for a few seconds.
11. Stain in EA 50 for 3 – 5 minutes.
12. Rinse in 3 changes of 95% alcohol for a few seconds.

MAY GRUNWALD GIEMSA STAIN (MGG)

Stock Solution of MGG:

0.5 grams of MGG powder dissolved in 100 ml of methanol.

Stock Solution of Giemsa:

0.75 grams of Giemsa Powder dissolved in 100 ml of methanol.

Working Solution of MGG:

Two parts of Stock Solution of MGG and one part of methanol.

Working Solution of Giemsa:

One part of Stock Solution of Giemsa and nine parts of distilled water.

Staining Technique:

Stain with Working Solution of MGG in 1-2 minutes.

Dilution with Working Solution of Giemsa 10 minutes and wash in tap water and dry.

REAGENTS USED IN MUFP STAIN:

1. Normal Saline

2. Alcoholic Formalin- 3 liters of alcoholic formalin was prepared by the following (pH 5)

    40% Formalin-300 ml

    95% Alcohol-2053ml

    Distilled water-647ml

3. Gill's Hematoxylin

4. 95% Alcohol

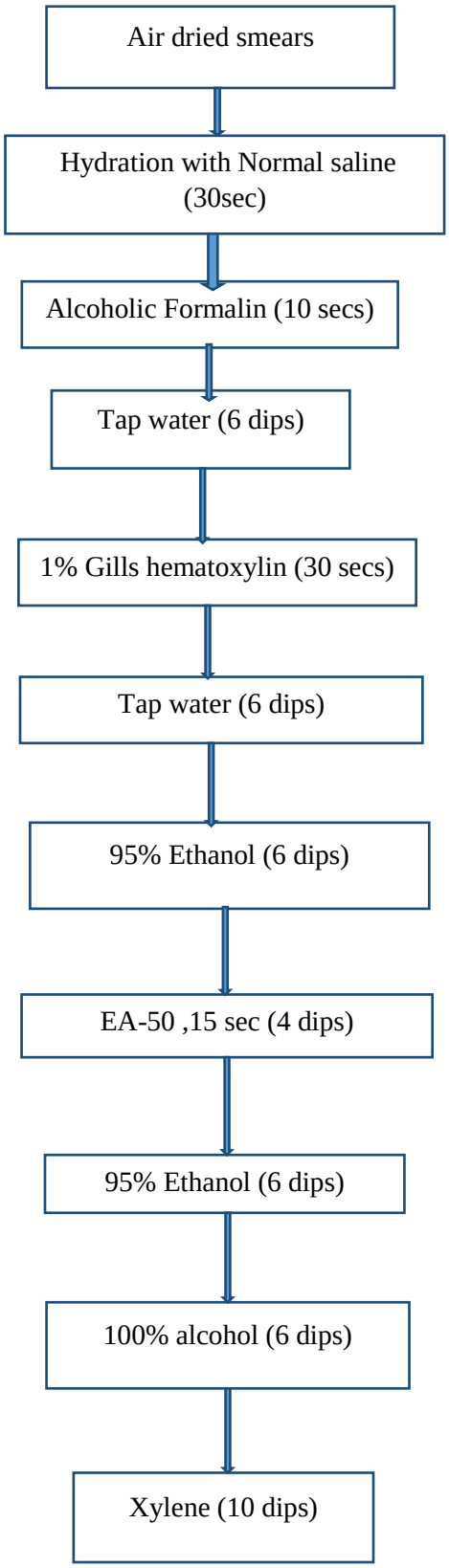
5. 100% Alcohol

6. Tap water

7. EA- 50

8. Xylene

MODIFIED ULTRAFAST PAPANICOLAOU STAIN (MUFP)



SCORING SYSTEM USED IN ASSESSMENT OF STAINING:

PARAMETER	**SCORE=1**	**SCORE=2**	**SCORE=3**
Background	Hemorrhage	Clean	
Overall staining	Poor	Average	Good
Cell morphology	Poorly Preserved	Moderately Preserved	Well Preserved
Nuclear Characteristics	Smudgy Chromatin	Moderately Crisp Chromatin	Crisp Chromatin
Cytoplasmic details	Unsatisfactory	Suboptimal	Optimal
Air drying artifacts	>50%	<50%	0%

The maximum score was 17 for a single case, it was consider into account of all the six parameters,

The "Quality Index" was calculated as the ratio of actual score obtained to the maximum score possible.

Quality Index= actual score obtained /maximum score(17)

Quality Index for each of the three stains of the three organs was compared.

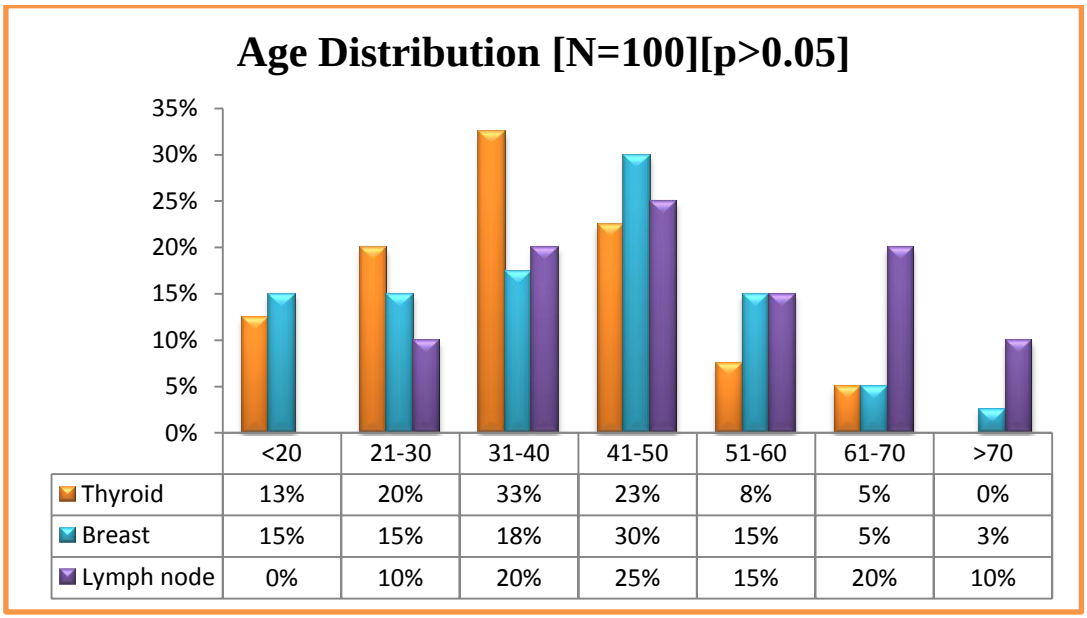
OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Table No: 1 Age distribution of cases studied

Age Distribution				
	Cases			
Age	Thyroid	Breast	Lymph node	Total
<20	5	6	0	11
21-30	8	6	2	16
31-40	13	7	4	24
41-50	9	12	5	26
51-60	3	6	3	12
61-70	2	2	4	8
>70	0	1	2	3
Total	40	40	20	100

Graph No: 2 Age distribution



	<20	21-30	31-40	41-50	51-60	61-70	>70
Thyroid	13%	20%	33%	23%	8%	5%	0%
Breast	15%	15%	18%	30%	15%	5%	3%
Lymph node	0%	10%	20%	25%	15%	20%	10%

Case distribution in thyroid is maximum in 31-40(33%) age group and 41-50 (23%) years.

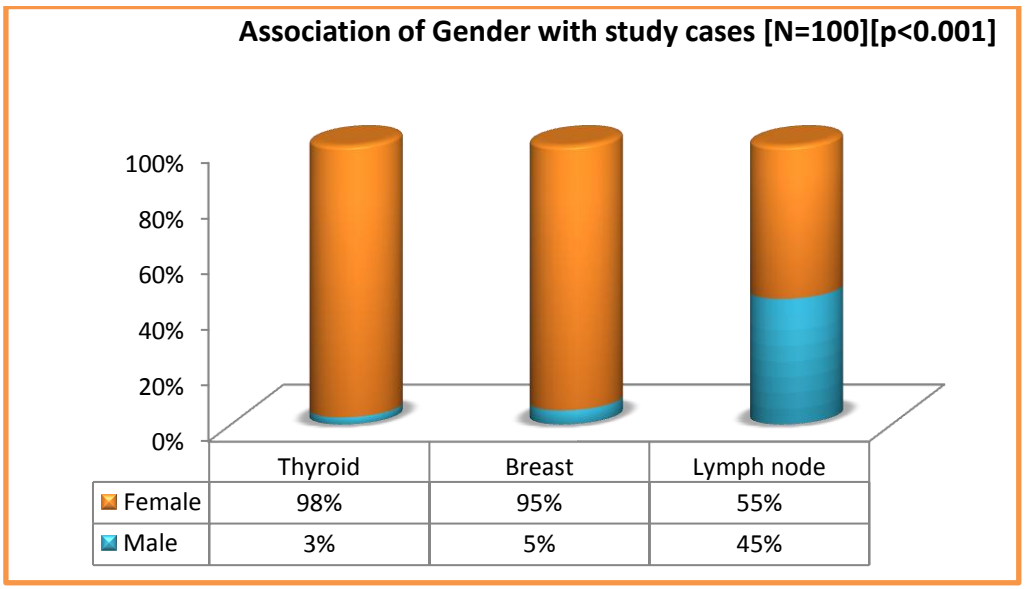
Case distribution in lymph node is maximum in 41-50 (25%) age group and 31-40 (20%) and 61-70(20%) years.

Case distribution in breast is maximum in 41-50 (30%) age group and 31-40(18%) years.

Table No: 2 Association of gender with study cases

Gender	Cases			Total
	Thyroid	Breast	Lymph node	
Male	1	2	9	12
Female	39	38	11	88
Total	40	40	20	100

Graph No.3 Association of gender with study cases



Gender distribution of patients is significant.

Thyroid lesions are more common in females (98%).

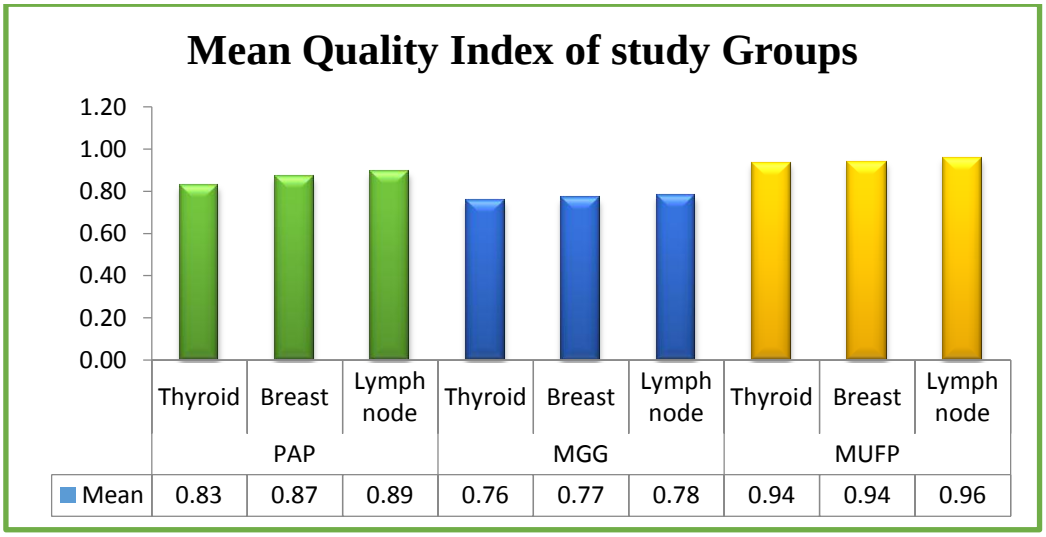
Breast lesions are more common in females (95%).

Lymph node lesions are also common in females (95%).

Table No: 3 Mean Quality index of study Groups

	Clinical Diagnc	Mean	SD	95% CI for Mean Lower	Upper	Minimum	Maximum	Sig
PAP	Thyroid	0.83	0.06	0.81	0.85	0.71	0.94	
	Breast	0.87	0.04	0.86	0.88	0.82	0.94	<0.001
	Lymph noc	0.89	0.03	0.88	0.91	0.82	0.94	
MGG	Thyroid	0.76	0.06	0.74	0.78	0.59	0.94	
	Breast	0.77	0.04	0.76	0.78	0.71	0.88	<0.001
	Lymph noc	0.78	0.04	0.76	0.80	0.71	0.88	
MUFP	Thyroid	0.94	0.04	0.92	0.95	0.88	1.00	
	Breast	0.94	0.03	0.93	0.95	0.88	1.00	>0.05
	Lymph noc	0.96	0.03	0.94	0.97	0.88	1.00	

Graph No: 4 Mean Quality index of study Groups



In thyroid maximum Quality Index score was seen in MUFP stain followed by pap and MGG stain.

In breast maximum Quality Index score was seen in MUFP stain followed by Pap and MGG stain.

In lymph Node maximum Quality Index score was seen in MUFP stain followed by Pap and MGG stain.

Before calculating mean statistics were applied first to specific organ and then inter organ comparision was calculated.

Table No: 4 Associations of Clinical Variables with Study Subjects in Thyroid

	Cases [n=40]						Sig
	PAP		MGG		MUFP		
Background	**n**	**%**	**n**	**%**	**n**	%	
· Hemorrhagic	30	75%	31	78%	0	0%	
· Clean	10	25%	9	23%	40	100%	<0.001
Overall staining							
· Poor	0	0%	1	3%	0	0%	
· Average	21	53%	36	90%	7	18%	<0.001
· Good	19	48%	3	8%	33	83%	
Cell morphology							
· Poorly preserved	0	0%	2	5%	0	0%	
· Moderately Preserved	14	35%	35	88%	5	13%	<0.001
· Well preserved	26	65%	3	8%	35	88%	
Nuclear characteristics							
· Smudgy Chromatin	0	0%	8	20%	0	0%	
· Mod crisp Chromatin	4	10%	29	73%	15	38%	<0.001
· Crisp chromatin	36	90%	3	8%	25	63%	
Cytoplasmic details							
· Unsatisfactory	0	0%	0	0%	0	0%	
· Sub-optimal	20	50%	4	10%	9	23%	<0.001
· Optimal	20	50%	36	90%	31	78%	
Air drying artifacts							
· >50%	0	0%	0	0%	0	0%	
· <50%	27	68%	7	18%	7	18%	
· 0%	13	33%	33	83%	33	83%	<0.001

FNAC OF 40 THYROID LESIONS YIELDED FOLLOWING RESULTS

Clean background was seen in 40(100%) cases of MUFP cases and in 10(25%) cases of Pap. Hemorrhagic background was seen in 31(78%) cases of MGG.

83% of MUFP cases showed good overall staining. 48% of Pap and 8% of MGG cases showing good overall staining. 90% of MGG cases showed average overall staining.

Well preserved cell morphology was seen in in 88% of MUFP, 65% of Pap and 8% of MGG cases.

Nuclear characteristics of crisp chromatin was seen in 63% of MUFP cases 90% of Pap and 8% of MGG cases. 90% of MGG stain showed moderately crisp chromatin.

90% of MGG stain showed optimal cytoplasmic details, 78% of MUFP and 50% of Pap stain showed optimal cytoplasmic details. 50% of Pap cases showed sub-optimal cytoplasmic details.

Least air drying artifacts were seen in MGG (83%) and air dried rehydrated smears like MUFP(83%).

Table No: 5 Association of Clinical Variables with Study Subjects in Breast

	Cases [n=40]						Sig
	PAP		MGG		MUFP		
Background	n	%	n	%	n	%	
· Hemorrhagic	20	50%	32	80%	0	0%	
· Clean	20	50%	8	20%	40	100%	<0.001
Overall staining							
· Poor	0	0%	2	5%	0	0%	
· Average	11	28%	36	90%	6	15%	<0.001
· Good	29	73%	2	5%	34	85%	
Cell morphology							
· Poorly preserved	0	0%	3	8%	0	0%	
· Moderately Preserved	14	35%	31	78%	7	18%	<0.001
· Well preserved	26	65%	6	15%	33	83%	
Nuclear characteristics							
· Smudgy Chromatin	0	0%	3	8%	0	0%	
· Mod crisp Chromatin	5	13%	32	80%	13	33%	<0.001
· Crisp chromatin	35	88%	5	13%	27	68%	
Cytoplasmic details							
· Unsatisfactory	0	0%	0	0%	0	0%	
· Sub-optimal	15	38%	4	10%	7	18%	<0.01
· Optimal	25	63%	36	90%	33	83%	
Air drying artifacts							
· >50%	0	0%	0	0%	0	0%	
· <50%	23	58%	5	13%	7	18%	
· 0%	17	43%	35	88%	33	83%	<0.001

FNAC OF 40 BREAST LESIONS YIELDED FOLLOWING RESULTS:

Clean background was seen in 100% of MUFP and 50% of Pap stain. 80% of MGG stain showed hemorrhagic background.

85%of MUFP and 73% of Pap stain showed good overall staining. 36 cases (90%) of MGG showed average overall staining.

Well preserved cell morphology seen in 83% of MUFP and 65% of pap stained smears. 78% of MGG cases showed moderately preserved cell morphology.

Crisp nuclear chromatin was seen in 88% of Pap and 68 % of MUFP cases. 80% of MGG stain showed moderately crisp nuclear characteristics.

90% of MGG, 83% of MUFP and (63%) of pap stain showed optimal cytoplasmic details .

88% of MGG 83% of MUFP and 43% of Pap cases showed no air drying artifacts.

Table No: 6 Association of Clinical Variables with Study Subjects in Lymph Node

	Cases [n=20]						Sig
	PAP		MGG		MUFP		
Background	n	%	n	%	n	%	
· Hemorrhagic	12	60%	16	80%	0	0%	
· Clean	8	40%	4	20%	20	100%	<0.001
Overall staining							
· Poor	0	0%	0	0%	0	0%	
· Average	7	35%	16	80%	3	15%	<0.001
· Good	13	65%	4	20%	17	85%	
Cell morphology							
· Poorly preserved	0	0%	0	0%	0	0%	
· Moderately Preserved	3	15%	19	95%	2	10%	<0.001
· Well preserved	17	85%	1	5%	18	90%	
Nuclear characteristics							
· Smudgy Chromatin	0	0%	2	10%	0	0%	
· Mod crisp Chromatin	2	10%	17	85%	3	15%	<0.001
· Crisp chromatin	18	90%	1	5%	17	85%	
Cytoplasmic details							
· Unsatisfactory	0	0%	0	0%	0	0%	
· Sub-optimal	6	30%	0	0%	2	10%	<0.05
· Optimal	14	70%	20	100%	18	90%	
Air drying artifacts							
· >50%	0	0%	0	0%	0	0%	
· <50%	6	30%	2	10%	4	20%	
· 0%	14	70%	18	90%	16	80%	>0.05

FNAC OF 20 LYMPH NODE LESIONS YIELDED FOLLOWING RESULTS:

100% of MUFP and 40% of pap stain showed clean background. 80% of MGG cases showed hemorrhagic background.

85% of MUFP and 65% of Pap cases showed good overall staining. 80% of MGG cases showed average overall staining.

95% of MUFP and 85% of Pap cases showed well preserved cell morphology. 95% MGG cases showed moderately preserved cell morphology.

Crisp nuclear chromatin was seen in 90% of Pap and 85% of MUFP stain. 85% of MGG cases showed moderately crisp nuclear characteristics.

100% of MGG, 90% of MUFP and 70% of Pap cases showed optimal cytoplasmic details.

90% of MGG,80% of MUFP and70% of Pap stain showed no air drying artifacts.

RESULTS OF SPECIFIC STAIN IN DIFFERENT ORGANS:

Table No: 7 Association of PAP QL with study cases

PAP QI	Cases			Total
	Thyroid	Breast	Lymph node	
<0.80	10	0	0	10
0.81 - 1.00	30	40	20	90
Total	40	40	20	100

P value of PAP score is <0.001

Mean Quality index score of Pap stain is maximum for lymph node, followed by breast and thyroid.

Graph No: 5 Association of PAP QL with study cases

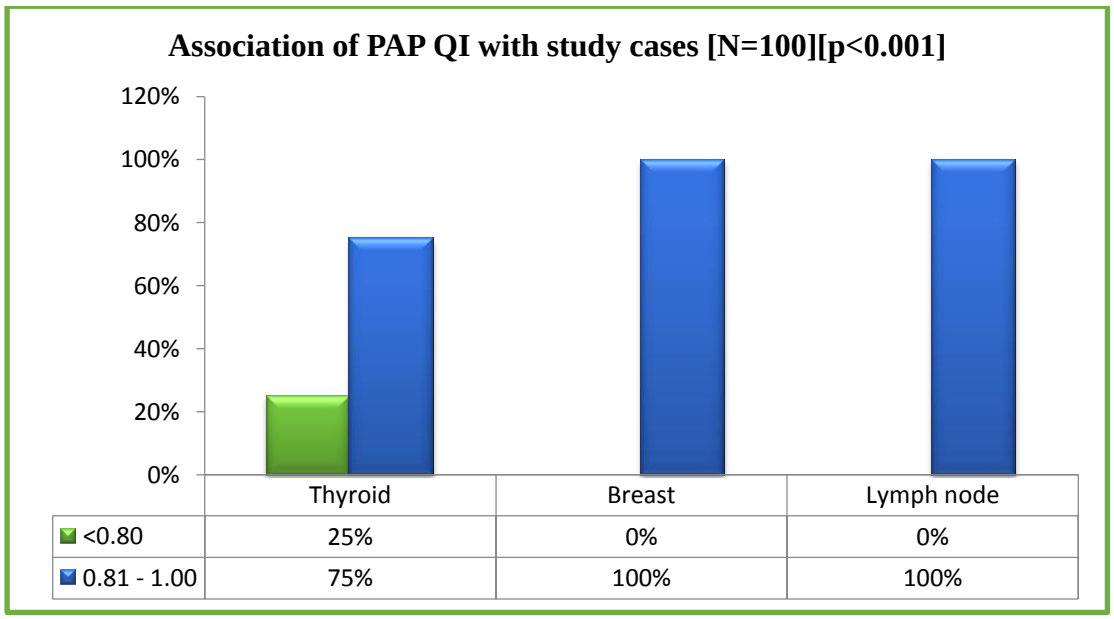


Table No: 8 Association of MGG quality Index with Study Cases

MGG QI	Cases			Total
	Thyroid	Breast	Lymph node	
<0.80	33	31	13	77
0.81 - 1.00	7	9	7	23
Total	40	40	20	100

Mean Quality index score of MGG stain is maximum for lymph node,breast and followed by thyroid.

Graph No: 6 Association of MGG QL with study cases

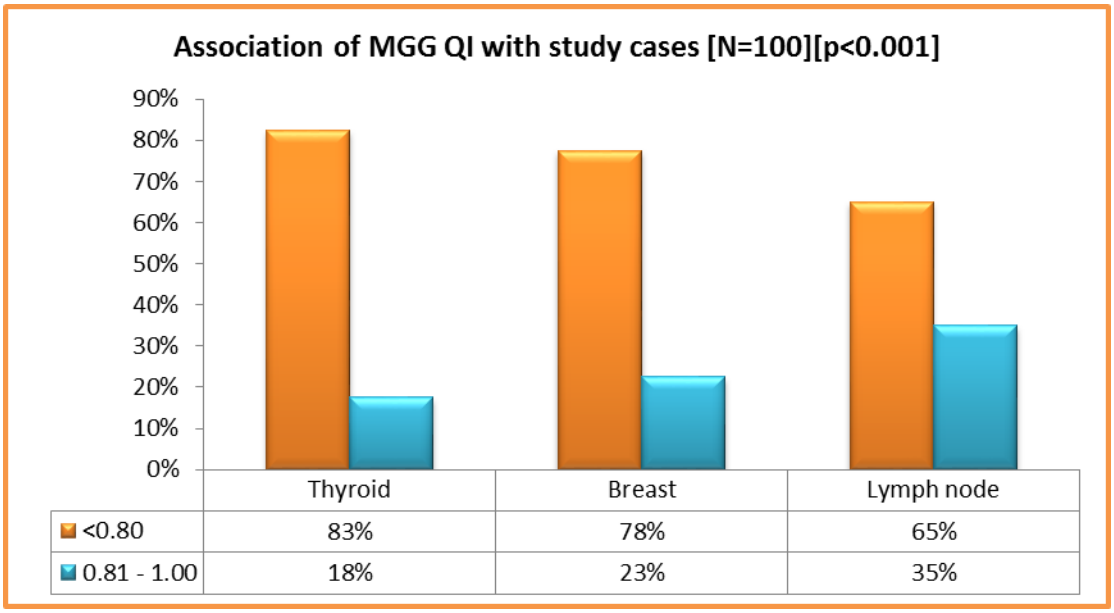


Table No: 9 Association of MUFP quality Index with Study Cases

MUFP QI	Cases			Total
	Thyroid	Breast	Lymph node	
<0.80	0	0	0	0
0.81 - 1.00	40	40	20	100
Total	40	40	20	100

Mean Quality index score of MUFP stain is maximum for lymph node followed by thyroid and breast.

Graph No: 7 Association of MUFP QI with study cases

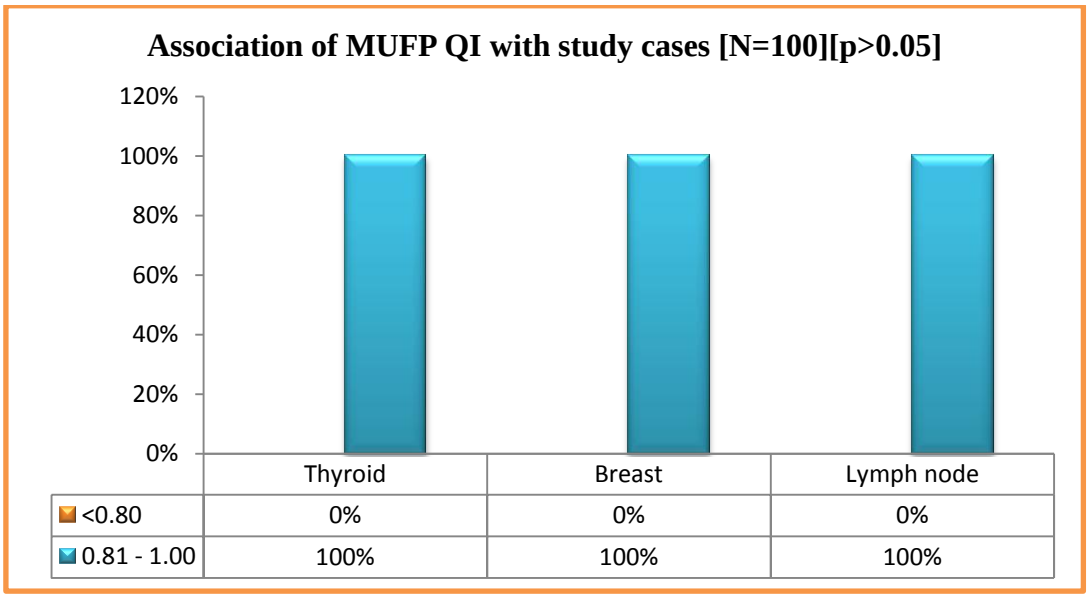


Table No: 10 Association of Background with Study cases

Background	Cases		
	PAP	MGG	MUFP
· Hemorrhagic	62	79	0
· Clean	38	21	100
Total	100	100	100

Graph No: 8 Association of Background with study cases

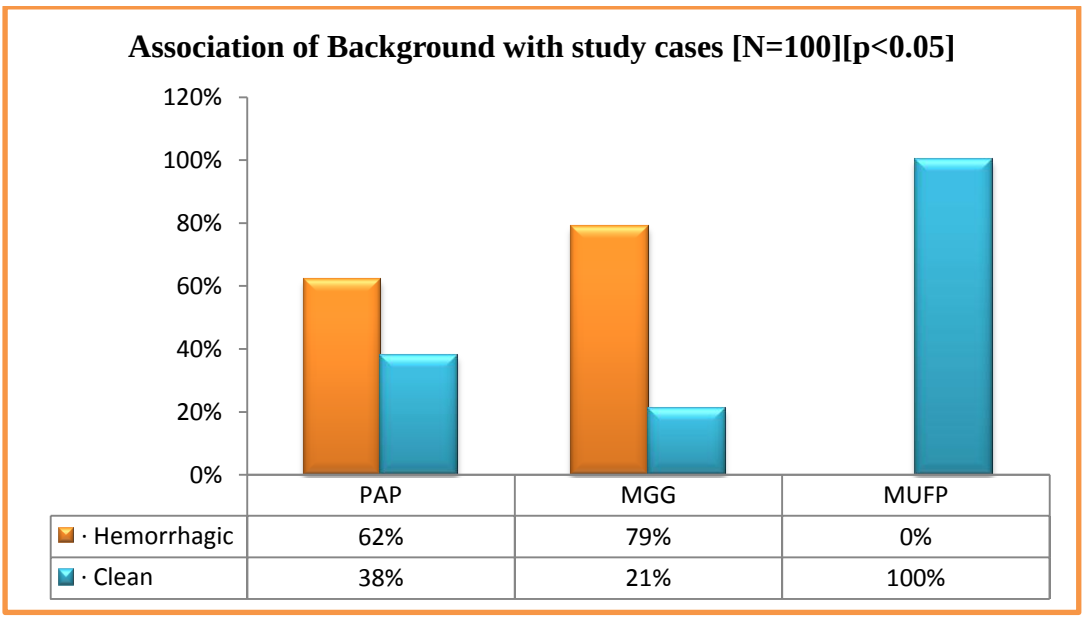


Table No: 11 Association of overall staining with Study cases

Overall staining	Cases		
	PAP	MGG	MUFP
· Poor	0	3	0
· Average	39	88	16
· Good	61	9	84
Total	100	100	100

Graph No: 9 Association of overall staining with study cases

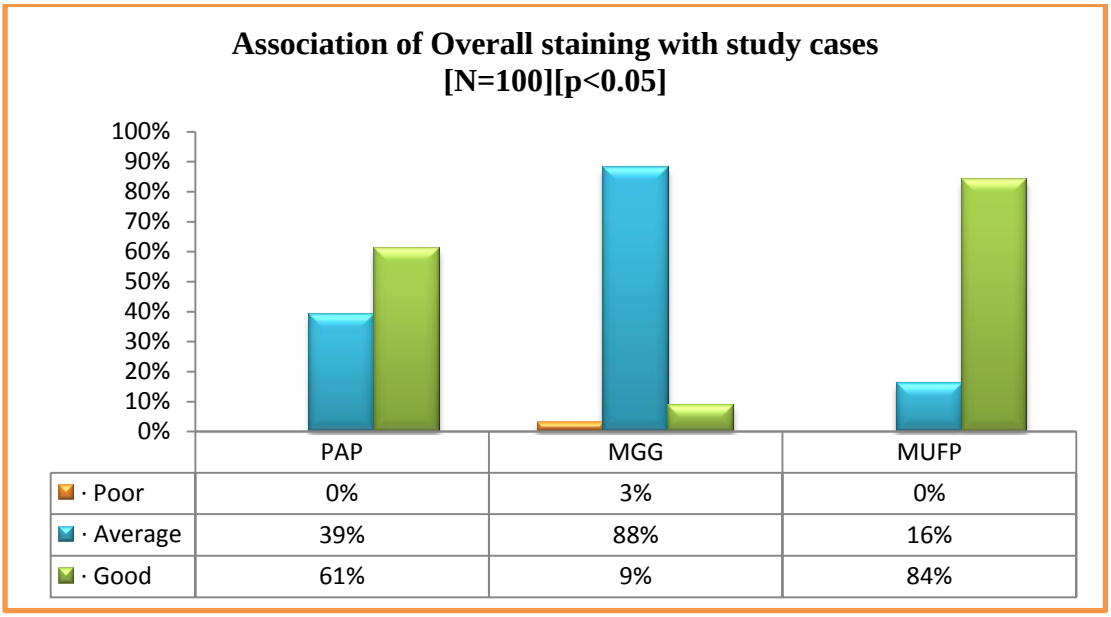


Table No: 12 Association of cell morphology with study cases

Cell morphology	Cases		
	PAP	MGG	MUFP
· Poorly preserved	0	5	0
· Moderately Preserved	31	85	14
· Well preserved	69	10	86
Total	100	100	100

Graph No: 10 Association of Cell Morphology with study cases

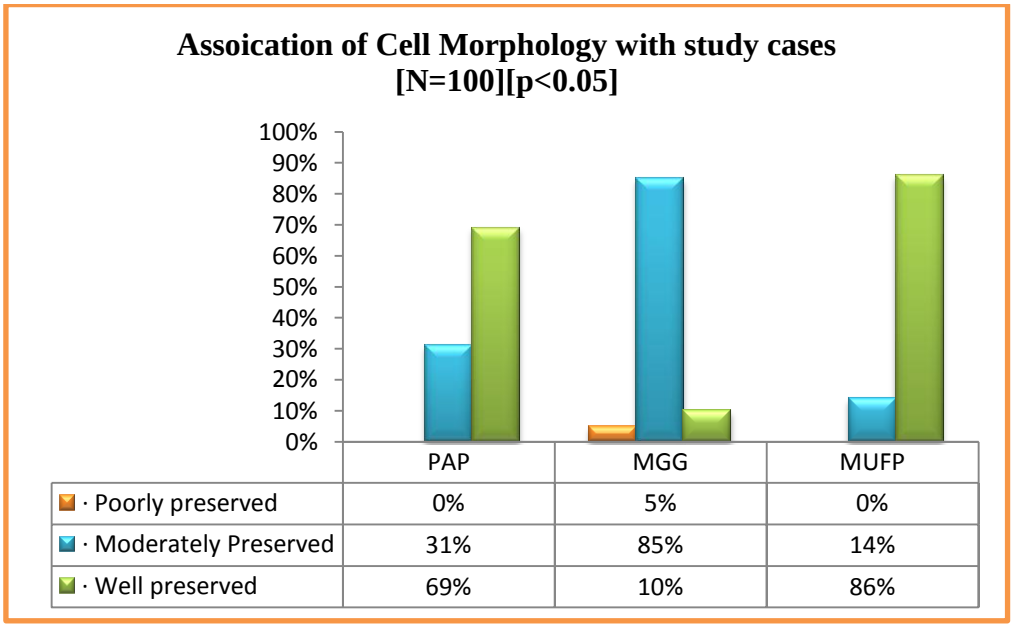


Table No: 13 Association of Nuclear Characteristics with Study cases

Nuclear characteristics	Cases		
	PAP	MGG	MUFP
· Smudgy Chromatin	0	13	0
· Mod crisp Chromatin	11	78	31
· Crisp chromatin	89	9	69
Total	100	100	100

Graph No: 11 Association of Nuclear Characteristics with Study cases

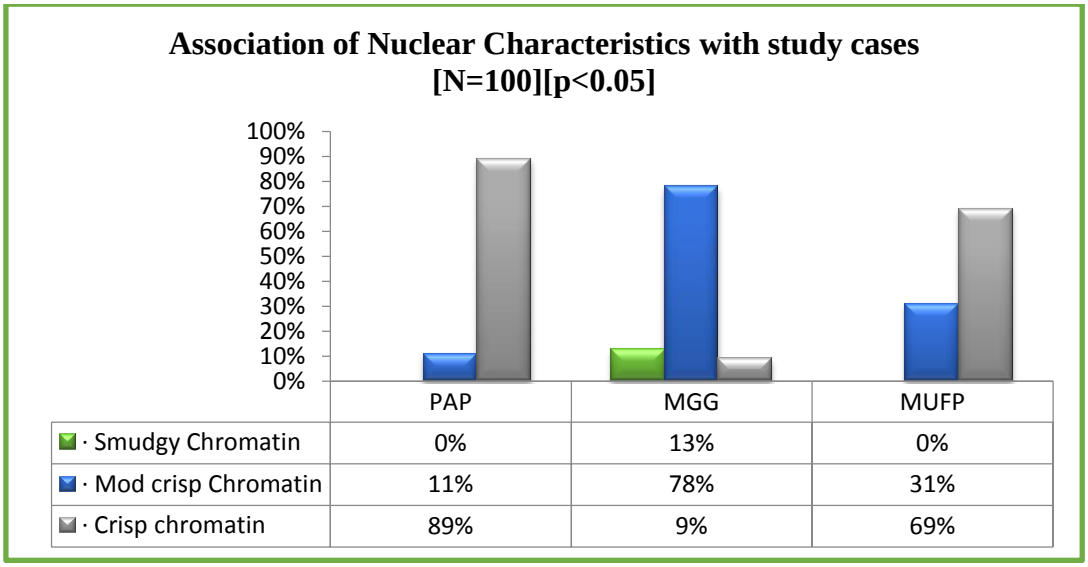


Table No: 14 Association of Cytoplasmic details with Study cases

Cytoplasmic details	Cases		
	PAP	**MGG**	**MUFP**
· Unsatisfactory	0	0	0
· Sub-optimal	41	8	18
· Optimal	59	92	82
Total	100	100	100

Graph No: 12 Association of Cytoplasmic details with Study cases

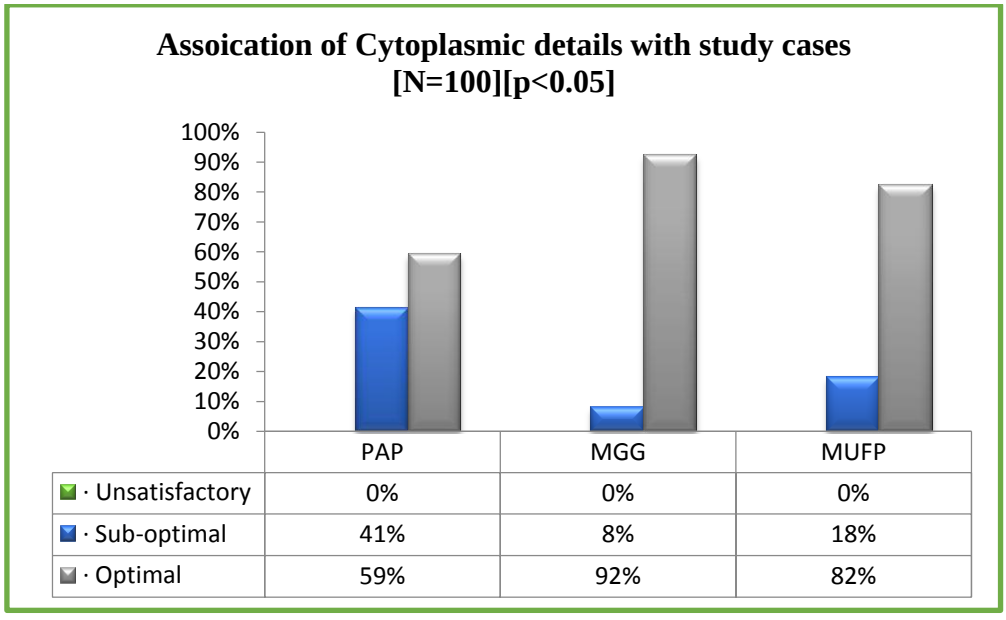
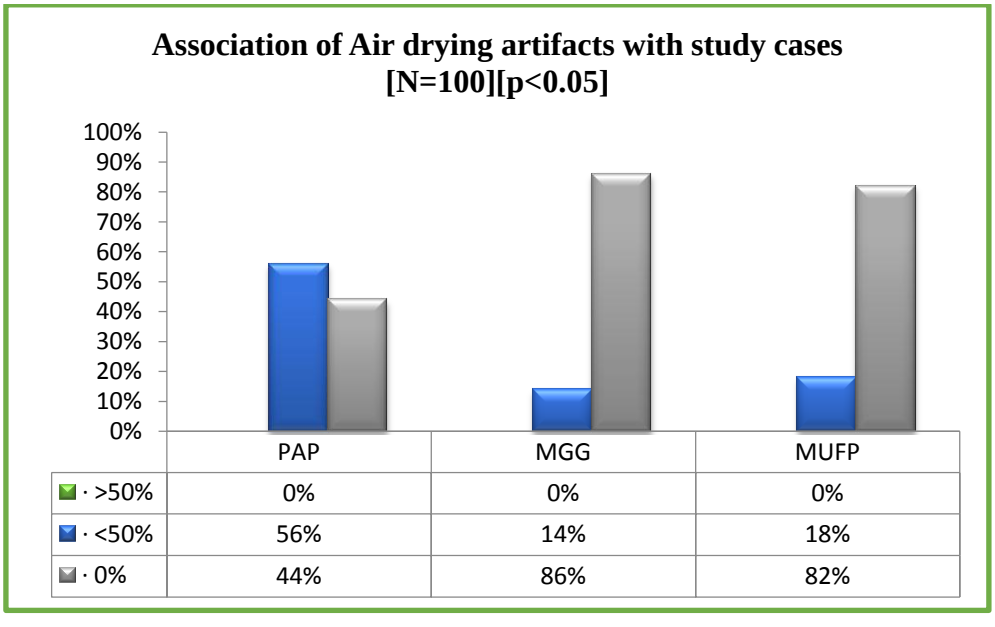


Table No: 15 Association of Air drying artifacts with Study cases

Air drying artifacts	Cases		
	PAP	MGG	MUFP
· >50%	0	0	0
· <50%	56	14	18
· 0%	44	86	82
Total	100	100	100

Graph No: 13 Association of Air drying artifacts with Study cases



STATISTICAL ANALYSIS

STATISTICAL ANALYSIS:

The data are reported as the mean +/- SD or the median, depending on their distribution.

Frequencies are expressed in percentages.

ANOVA was used to assess the quantitative variables.

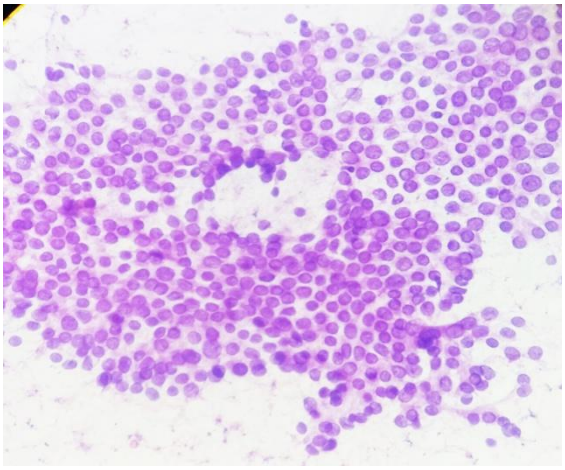
The chi square test & Fisher Exact test were used assess differences in categ oric variables between groups.

A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analysed with a statistical software package. (SPSS, version 16.0 for windows)

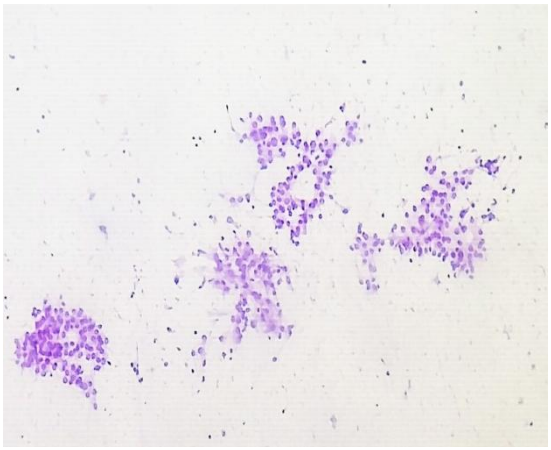
COLOUR PLATES

THYROID

FIGURE: 1

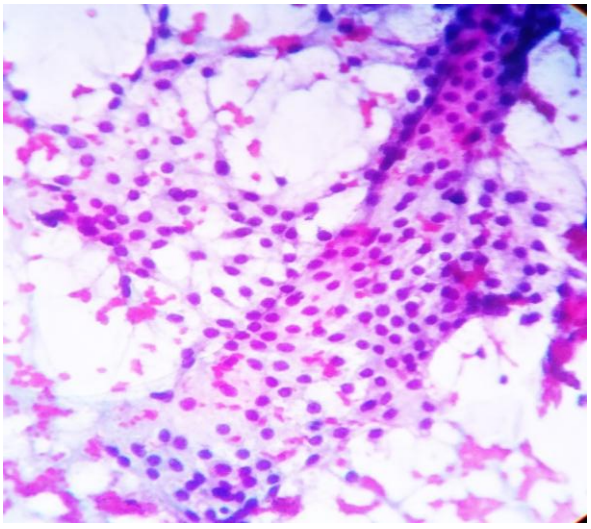


NODULAR GOITER- MUFP STAIN (40X)

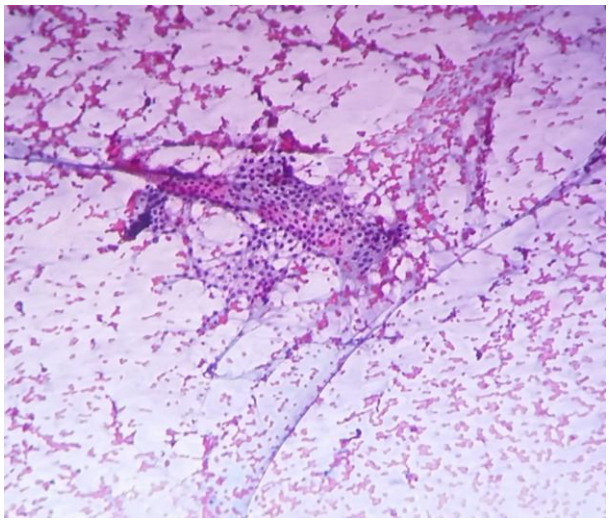


NODULAR GOITER- MUFP STAIN (10X)

FIGUARE: 2

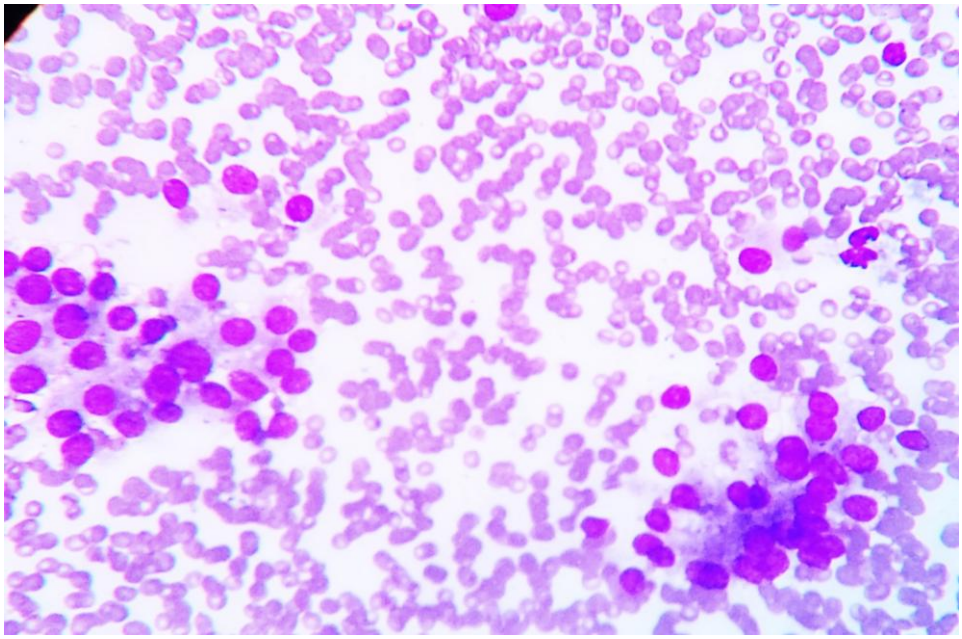


NODULAR GOITER-PAP STAIN (40X)



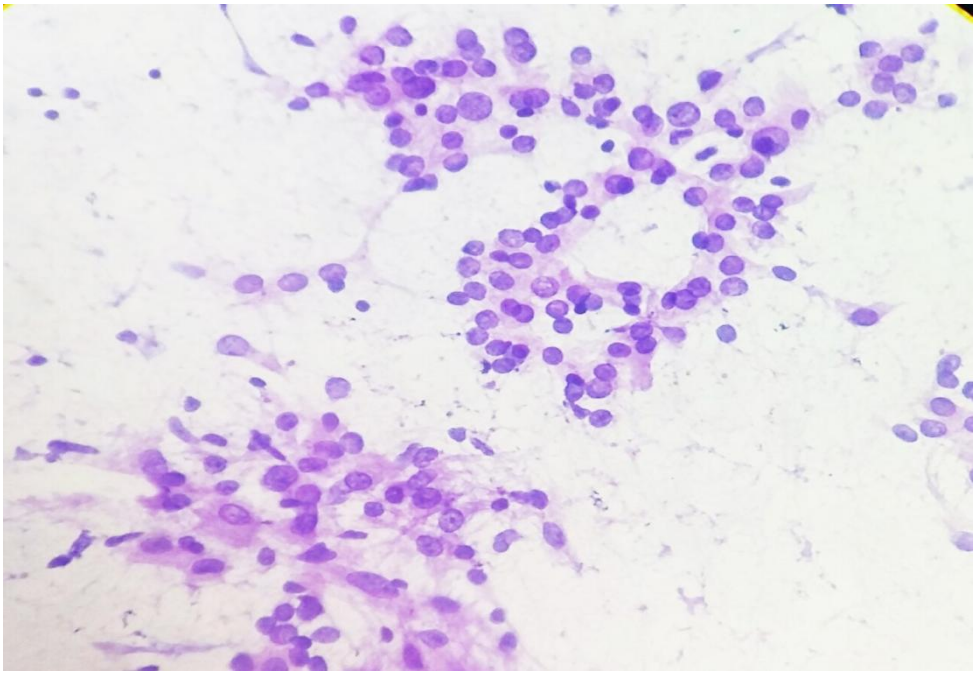
NODULAR GOITER-PAP STAIN (10X)

FIGUARE: 3



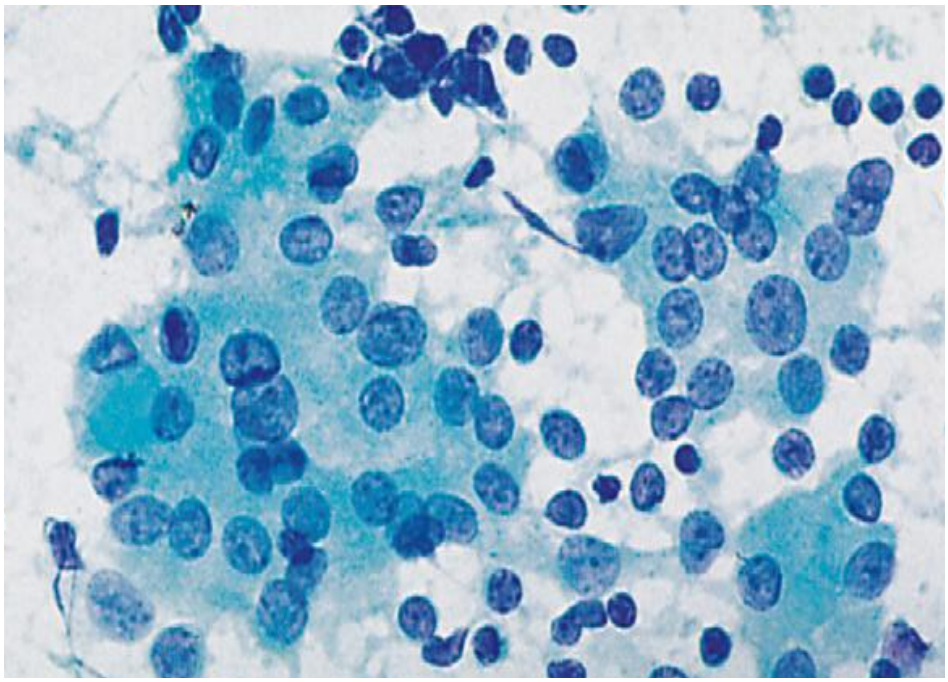
NODULAR GOITER-MGG STAIN (40X)

FIGUARE: 4



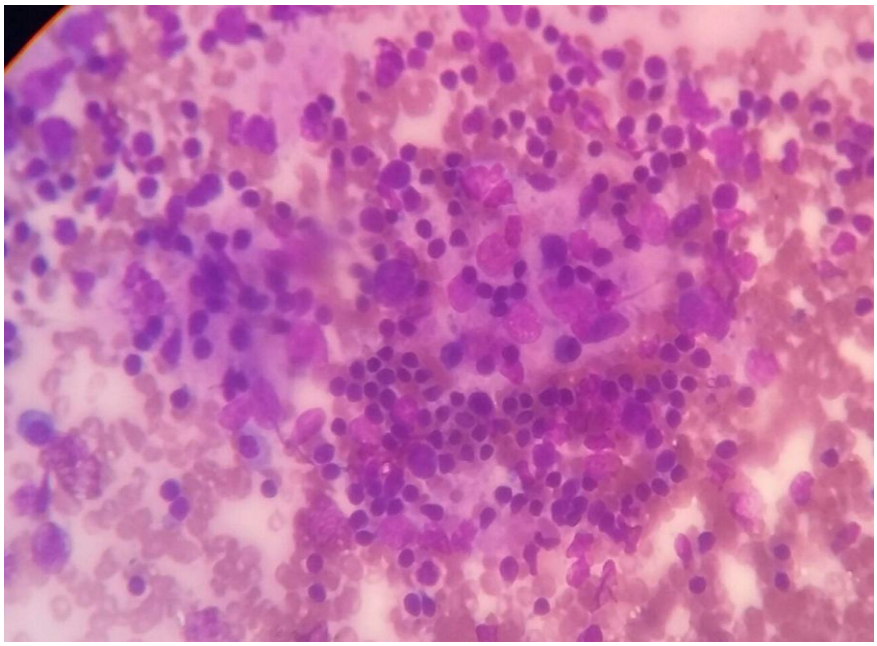
HASHIMOTO'S THYROIDITIS-MUFP STAIN (40X)

FIGUARE: 5



HASHIMOTO'S THYROIDITIS-PAP STAIN (40X)

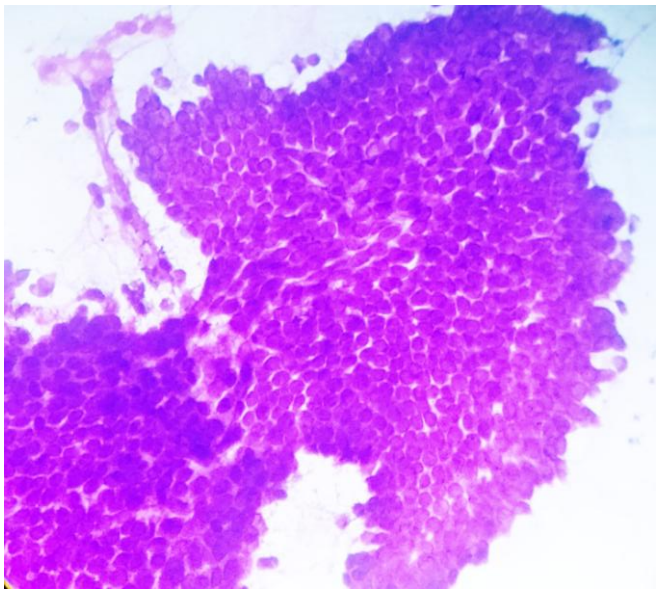
FIGUARE: 6



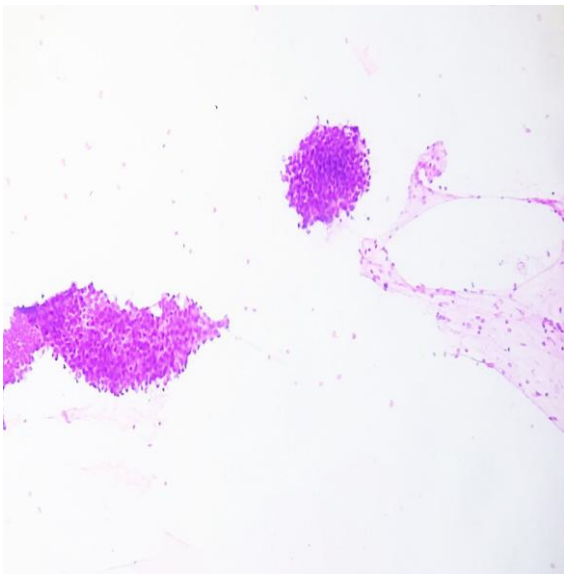
HASHIMOTO'S THYROIDITIS-MGG STAIN (40X)

BREAST

FIGUARE: 7

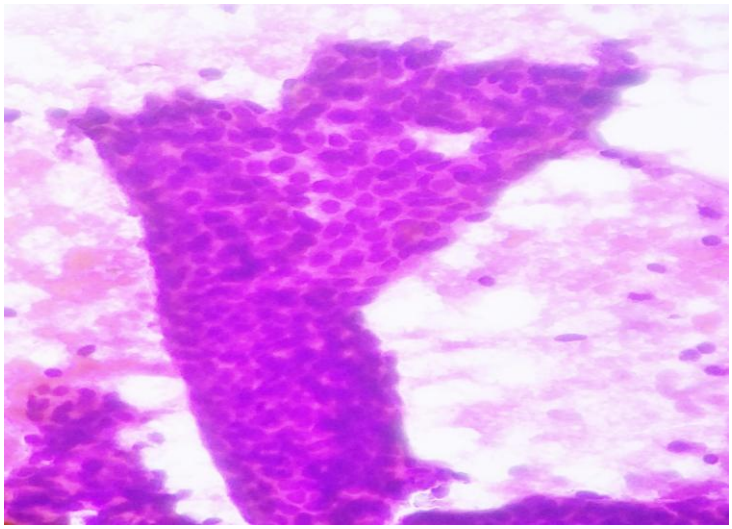


FIBROADENOMA BREAST –MUFP STAIN (40X)

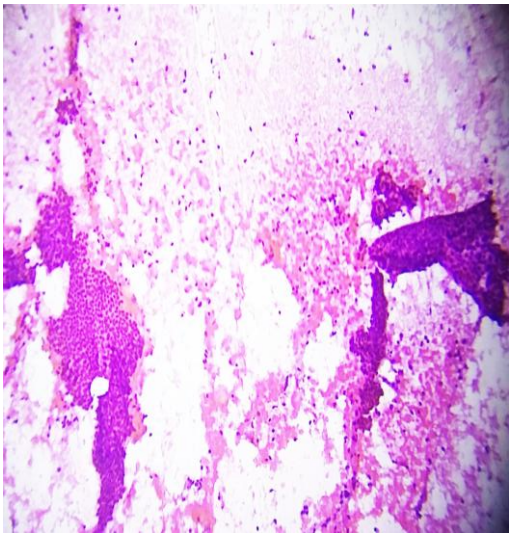


FIBROADENOMA BREAST –MUFP STAIN (10X)

FIGUARE: 8

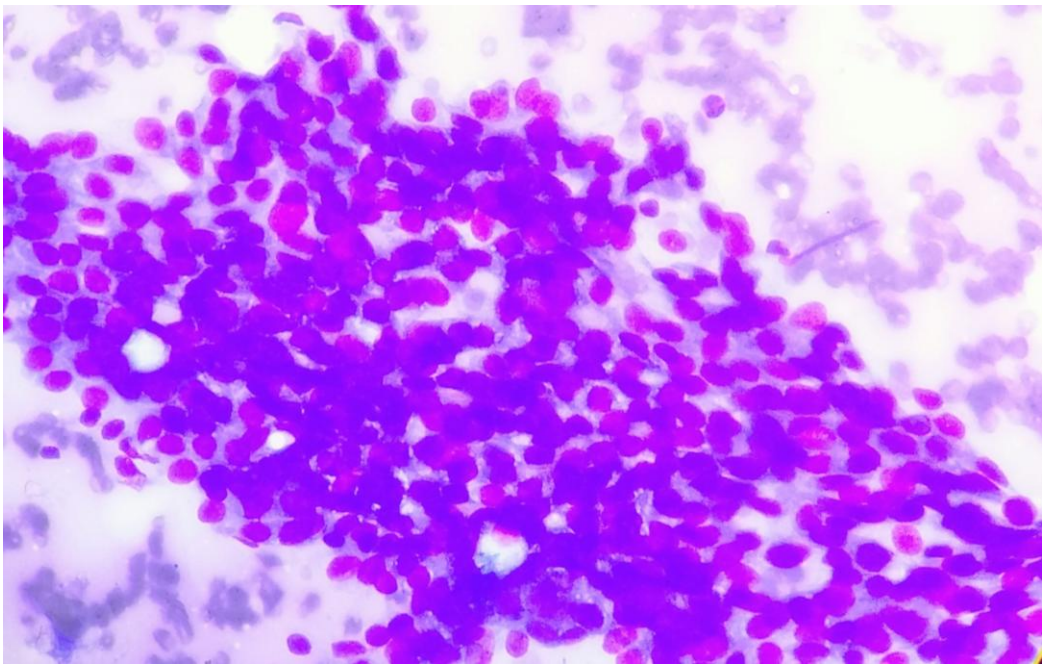


FIBROADENOMA BREAST –PAP STAIN (40X)



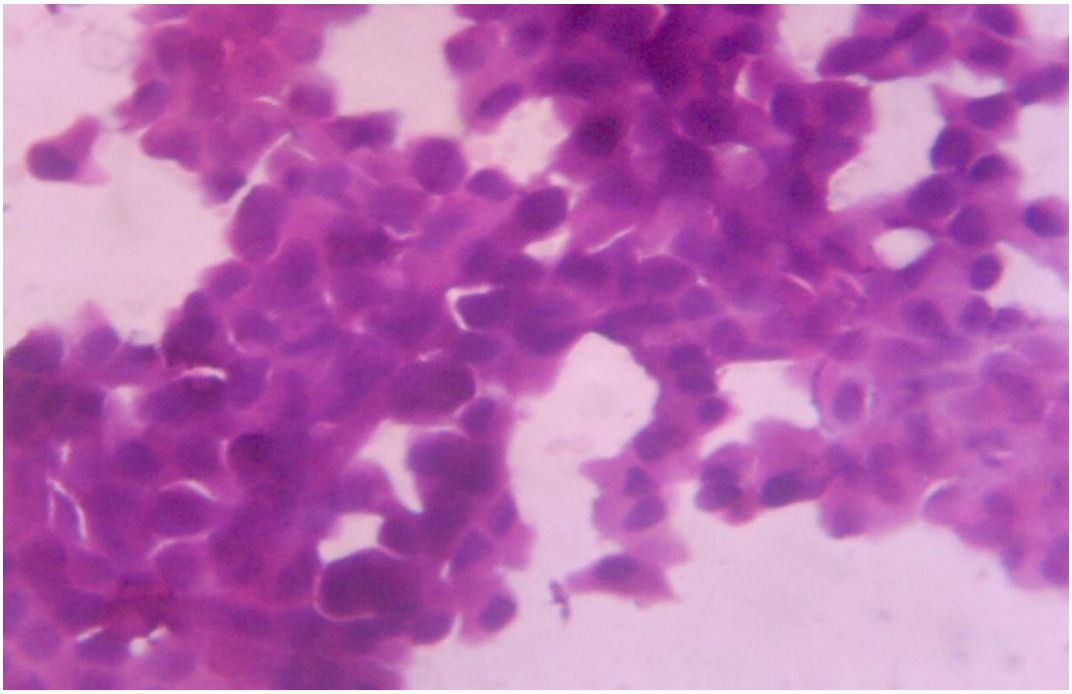
FIBROADENOMA BREAST –PAP STAIN (10X)

FIGUARE: 9



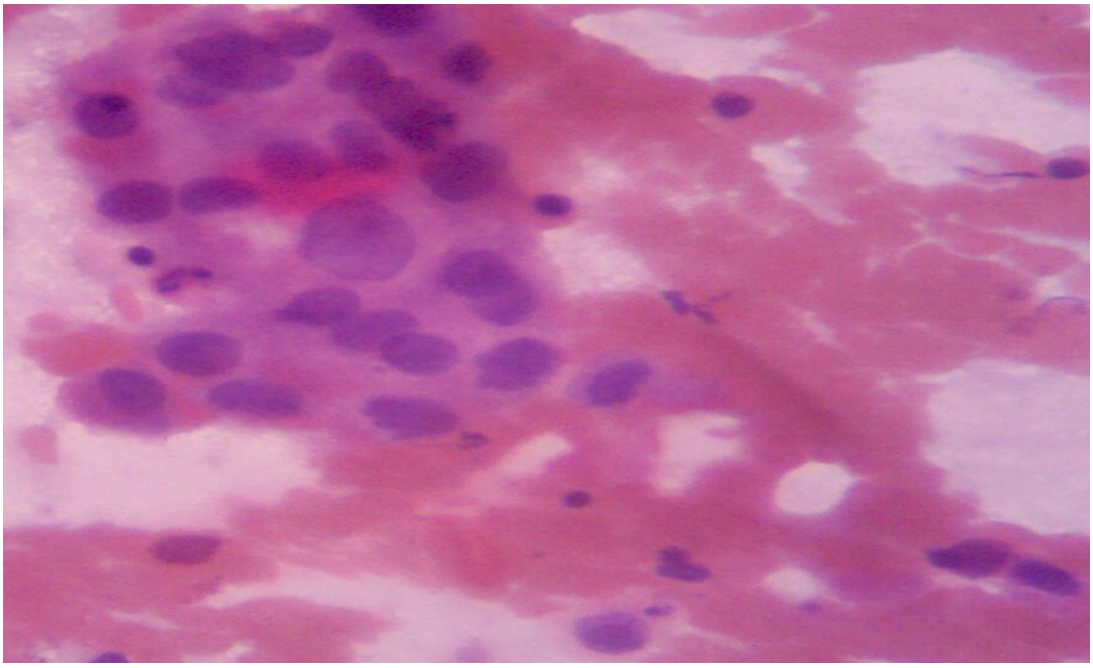
FIBROADENOMA BREAST –MGG STAIN (40X)

FIGUARE: 10



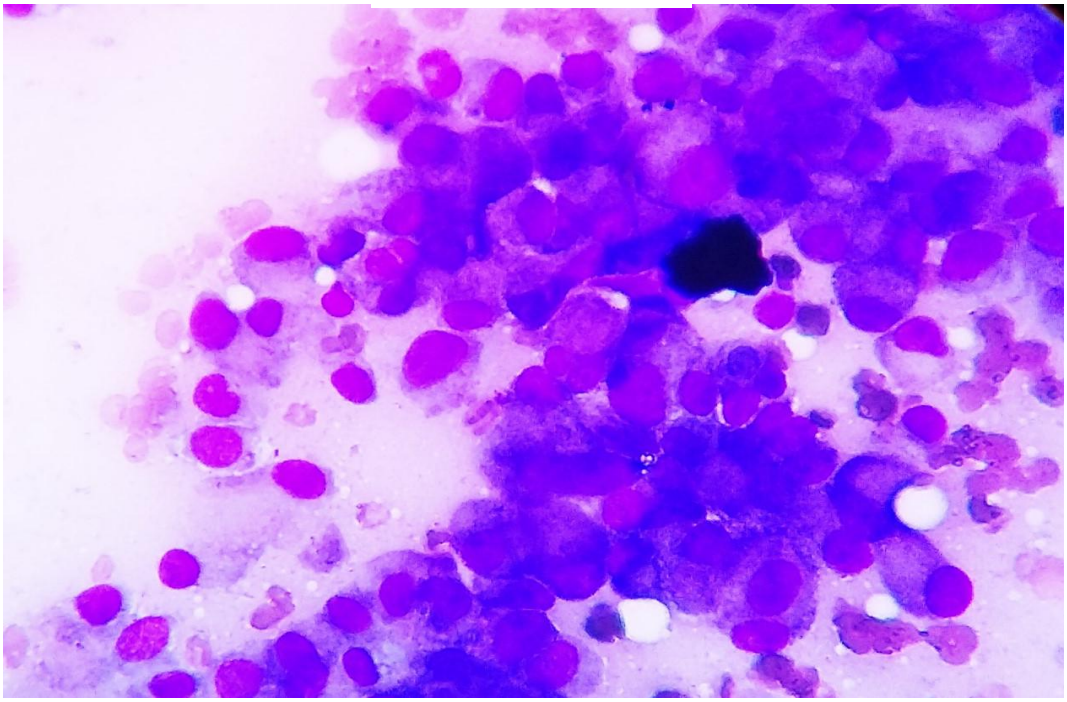
DUCTAL CARCINOMA BREAST -MUFP STAIN (40X)

FIGUARE: 11



DUCTAL CARCINOMA BREAST -MUFP STAIN (40X)

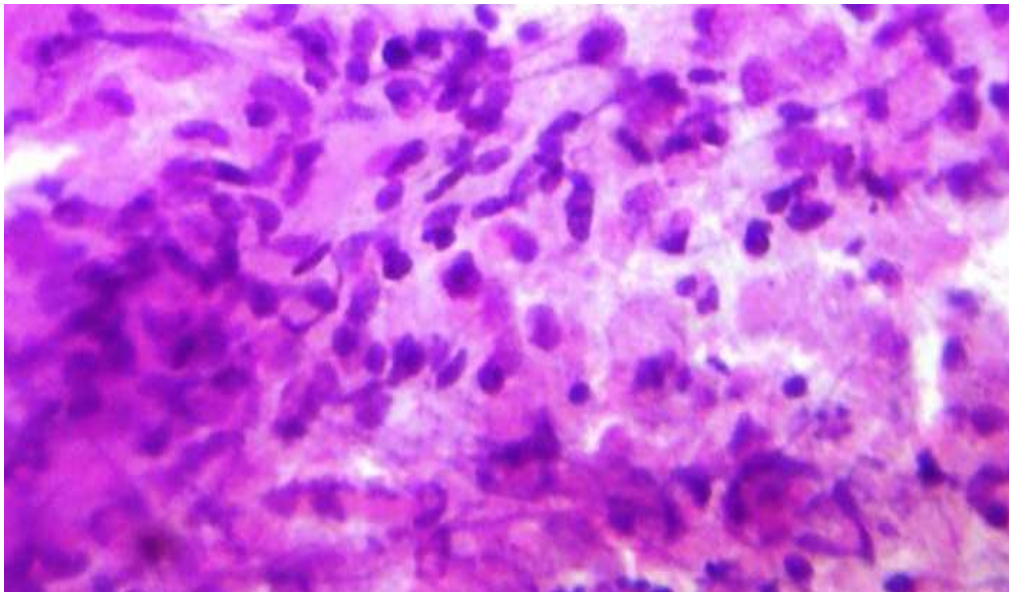
FIGUARE: 12



DUCTAL CARCINOMA-MGG STAIN (40x)

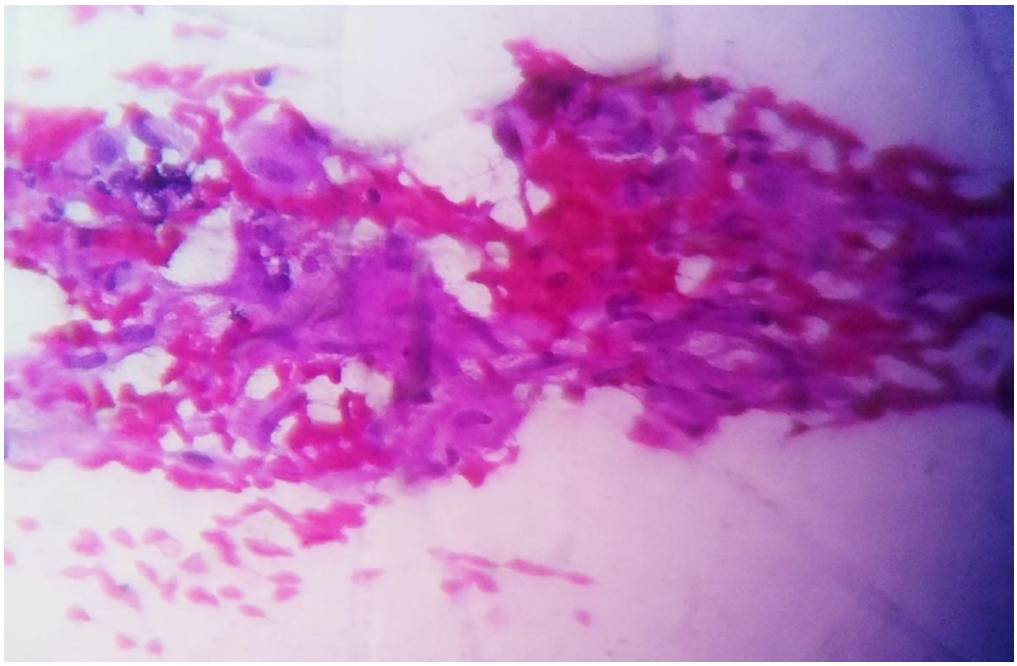
LYMPH NODE

FIGUARE: 13



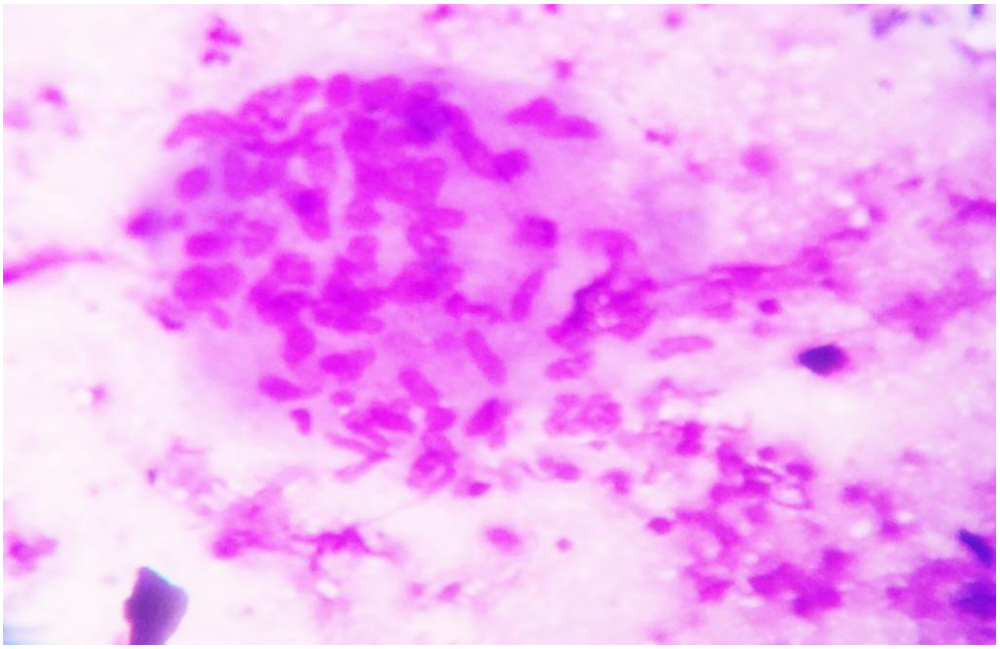
TUBERCULOUS LYMPHADENITIS-MUFP
STAIN (40X)

FIGUARE: 14



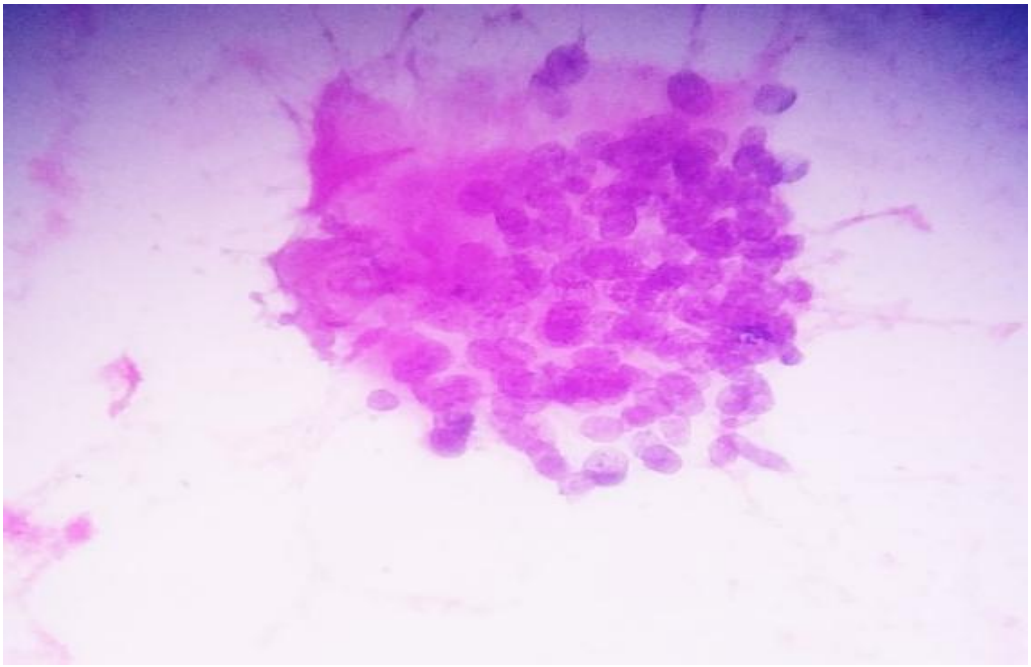
TUBERCULOUS LYMPHADENITIS-PAP
STAIN (40X)

FIGUARE: 15



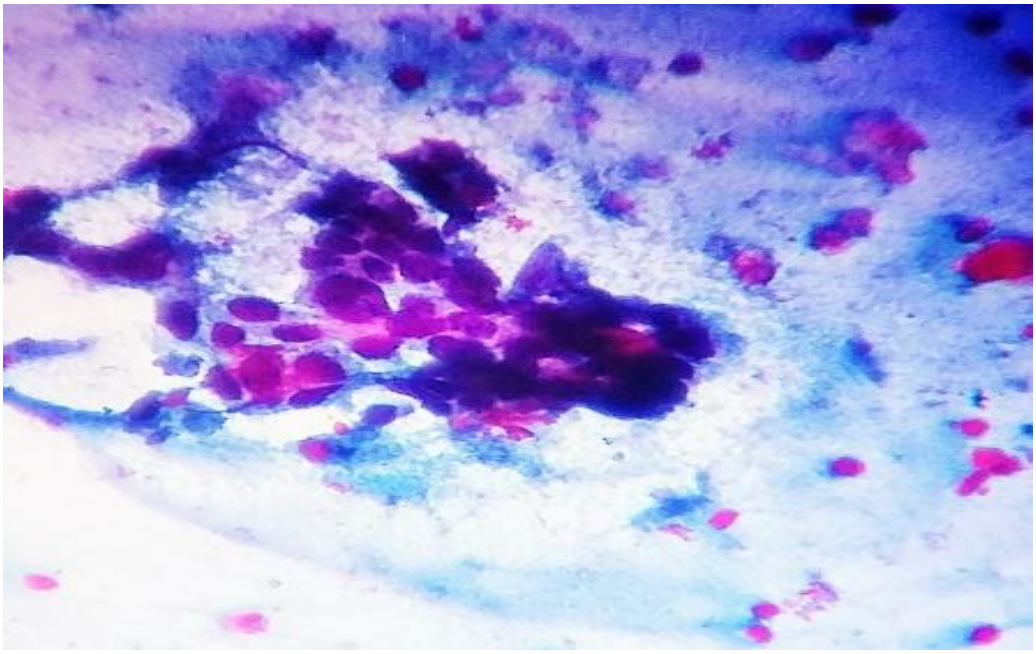
TUBERCULOUS LYMPHADENITIS-MGG STAIN (40X)

FIGUARE: 16



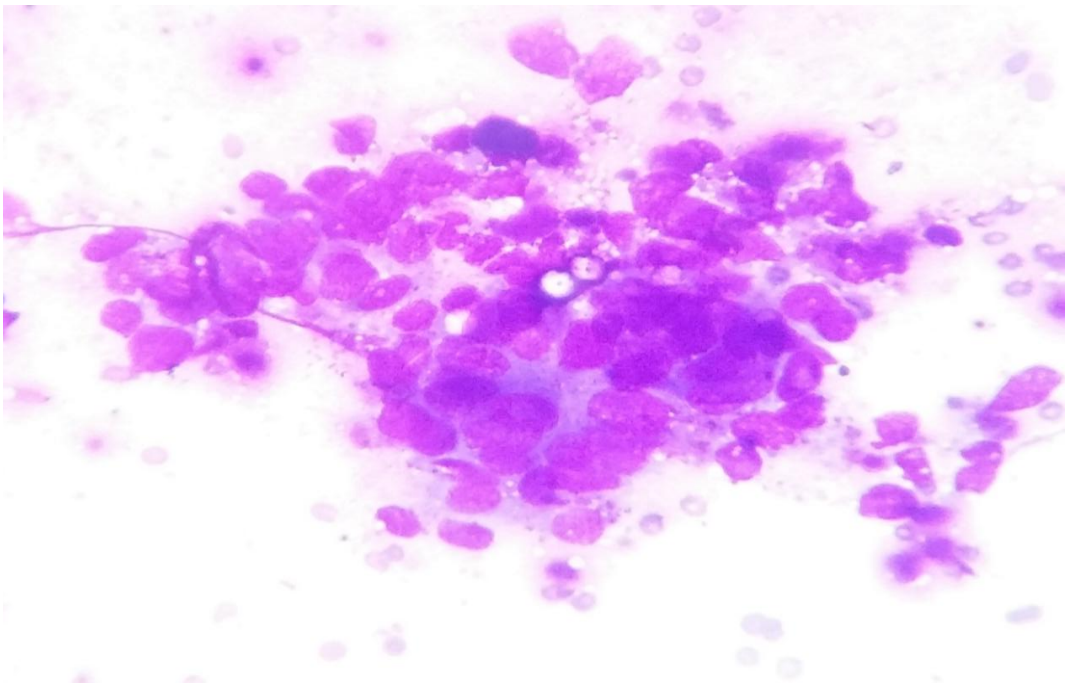
SQUAMOUS CELL CARCINOMA SECONDARIES IN LYMPH NODE, MUFP STAIN, 40X

FIGUARE: 17



SQUAMOUS CELL CARCINOMA SECONDARIES IN LYMPH NODE, MUFP STAIN, 40X

FIGUARE: 18



SQUAMOUS CELL CARCINOMA SECONDARIES IN LYMPH NODE, MGG STAIN, 40X

DISCUSSION

DISCUSSION

Fine needle aspiration cytology (FNAC) is one of the least expensive (most economical), quickest and simplest methods available for early diagnosis of various palpable and deep seated lesions. Since its establisment, PAP stain remains the traditional and most widely used stain, not only for the gynecological cytology, but also for various lesions of other organ.

The different staining methods of air dried smears are MGG, Jenner-Giemsa and Diff-Quick stain, but they will not offer the transparency in the study of subtle nuclear features as seen by the PAP stain.

The conventional pap staining methods proceeds with wet fixation and further staining, together required minimum30 minutes. To cut short the time, kline, Tao and sato developed rapid pap stain which required staining time of 5minutes, 4minutes and followed by 90 seconds. but, the quality of rapid pap stain is usually not good, as the cell morphology is not well satisfactory as seen.

To overcome these problems, ultra-Fast pap (UFP) stain was developed by Yang and Alvarez. It is a hybrid of pap and Romanowsky stains. The staining time is 90 seconds. Further modification of UFP

stain(Modified ultra-Fast pap stain) done by Kamal et al from India, to overcome the shortage of Richard-Allan hematoxylin and Richard-Allan cytostain in Indian set-up. Staining time of this method is 130 seconds and with well appreciated cytomorphology .

In this study, cytomorphology of modified ultrafast papanicolao stain(MUFP) was compared with conventional papanicolaou stain and May Grunwald Giemsa stain.

MUFP stain-rehydrated air dried smear.

PAP stain-ethyl alcohol fixed wet smear.

MGG stain-air dried smear.

All 3 stains quality was evaluated in six parameters, as background, Overall staining, cell morphology, nuclear characteristics, cytoplasmic features and air-drying artifacts.

The quality index of all three stains were calculated and compared with various organs like thyroid, Breast and lymph node.

Ultrafast pap stain was done in thyroid,breast and lymph node lesion by Shinde et al. Quality indices of our study were compared with quality indices of Shinde's study.

In our study, number of cases higher than the Shinde's study.

Table 16. Quality index of various organs in Shinde's study and present study

For Modified ultrafast pap stain:

Organ	Shinde's study		Present study	
	No. of cases	Quality index	No. of cases	Quality index
Thyroid	8	0.98	40	0.94
Breast	16	0.92	40	0.94
Lymph node	15	0.98	20	0.98

Quality index of MUFP stain in the same three organs are compared with rapid pap stain by Priyanka Choudhary et al.

Table 17. Quality index of various organs in Priyanka's study

Organ	No. of cases	Quality index of Mufp
Thyroid	25	1
Breast	23	o.97
Lymphnode	43	0.98

BACKGROUND:

More blood staining background is one of the common problem in conventionally fixed and staining methods, this is overcome by Rehydration of dried smear.

As per table No: 11,

MUFP show 100% clean background,in which air dried smears are rehydrated with normal saline as compared to wet fixed Papanicolaou smears(38%) and air dried MGG smears(21%).

The P value is <0.05. Calculated by applying chi-square test proved the difference to be significant.

These values are compared to various studies. In Shinde's study 95% of the MUFP stained smears showed clean background .Choudhary P. et al. reported MUFP stain has clean, RBC free background, it is very helpful in interpretation of vascular organs like thyroid. Maruta et al. Found that MUFP stain lyses blood cells, making the smear much thinner and clearer. RBC free background provides better cytomorphologic features.

Rehydration solution of MUFP is normal saline, it was introduced by Chang and Kung. It lyses the red blood cells and unmasks the tumour cells. This provide transparent air dried cells and well preserved nuclear details. Cells were appear larger due to air drying with red stained distinct nucleoli.

Our study proves that air-dried smears rehydrated with normal saline provides clean background as compared to wet fixed and air dried smears.

OVERALL STAINING:

According to the table no 12:

84% of MUFP stain showed good overall staining followed by Pap (61%), and MGG (9%). 88% of MGG stain showed average overall staining.

P value is <0.05, thus making significant difference Priyanka Choudhary et al study also showed over all staining score is maximum for MUFP.

CELL MORPHOLOGY:

In the table 13:

Well preserved cell morphology is maximum for MUFP (86%) followed by Pap (69%) and MGG stain (10%).

Value of P is <0.05.showing statistically significant difference. This study is compared to Priyanka Choudhary et al study .this study showed MUFP stain got maximum cell morphology score.

NUCLEAR CHARACTERISTICS:

Crisp nuclear chromatin were assesed.

In the table no14:

P value is <0.05 showing statistically Significant difference.

89% of Pap stain showing crisp nuclear chromatin followed by MUFP stain (69%) and MGG stain (9%). 78% of MGG showing moderately crisp nuclear chromatin.

Follicular variant of papillary carcinoma is easily distinguished from follicular neoplasms of the thyroid by UFP stain as it highlights the Orphan-Annie-eyed nuclei. This is reported by Yang et al.

CYTOPLASMIC DETAILS:

Grading of cytoplasmic features are unsatisfactory, suboptimal and optimal.

In the table no 15:

Optimal cytoplasmic features are seen in 92% of MGG stained smears followed by MUFP (82%) and Pap (59%) stain.

P value is <0.05 thus showing statistically significant difference. Modified ultrafast staining solution has no Orange G. So it is used in tissue with negligible cytoplasmic keratinization.

AIR-DRYING ARTIFACTS:

In the table no 16:

No air drying artifacts are seen in 86% of MGG, 82% of MUFP and followed by 44% of Pap stain.

P value is <0.05 thus showing statistically significant difference.

Kamal et al. found the problem of wet fixation, but the air drying artifacts can be eliminated by rehydration of air dried smears as in MUFP.

Thus our study proved that air drying technique and rehydration of air dried smears was associated with less air drying artifacts as compared to wet fixation.

The amount of air drying artifacts also depends upon skill of person who makes the smear. Thus with rehydration technique even people who are not fully skilled can casually do the procedure without much struggle.

Our study compares the Modified Ultrafast Papanicolaou Stain (MUFP) with conventional Papanicolaou stain and MGG stain. MUFP is quick and has the advantage that

The background is clean and shows very less air drying artifact.

SUMMARY

SUMMARY

This prospective study was carried over a period of 12 months, which included 100 cases (Fine Needle Aspiration Cytology from various organs like Thyroid, Breast and Lymph node).

Three different Stains, Papanicolaou stain and May- Grunwald Giemsa(MGG) and Modified Ultrafast Papanicolaou stain(MUFP) were compared with each other.

Smears were compared with six parameters and significance of Difference was calculated by applying Chi-square/ Fisher Exact test to find the Significance of study parameters on categorical scale between two or more groups.

Statistical Results of specific organ with inter-stain study

- Thyroid cases - MUFP > PAP > MGG
- Breast cases - MUFP > PAP > MGG
- Lymph Node - MUFP > PAP > MGG

> Means better than

PAP, MGG, MUFP stains done for thyroid, breast and lymph node Lesions. Out of which maximum quality index score was obtained by MUFP followed by PAP stain and MGG stain.

Statistical Results of specific stain with inter-organ study

MUFP stain - Lymph Node > Breast/thyroid

PAP stain -Lymph Node > Breast > Thyroid

MGG stain - Lymph Node > Breast > Thyroid

> means better than

MUFP smears stain was best for lymph node, breast and thyroid lesion.

Pap stain showed best for lesion from lymph node ,followed by breast and thyroid .

MGG stain was good for lesion from lymph node followed by breast and thyroid.

CONCLUSION

CONCLUSION

Modified ultrafast pap stain is a excellent staining method for studying FNA material from all three organs like thyroid, breast and lymph node lesions.

MUFP stain showed maximum score for all four parameters and shows optimal cytoplasmic features in 82% of cases and crisp nuclear chromatin in 69% cases.

Fixative of MUFP stain is alcoholic formalin .It provide lesser staining time for fixation and makes nucleoli to appear red and prominent, Compared to conventional Pap stain.

Pap stain is excellent for staining crisp nuclear chromatin and stain bipolar nuclei better than MUFP in fibroadenoma.

MGG stained smears showed less air drying artifacts when compared to wet fixed smears and has better optimal cytoplasmic features.

Lesser time for staining along with excellent morphologic quality is the need of the hour in any cytology laboratory.

MUFP very easily fulfils these parameters either equal to or even better than pap Technique for cytologic staining and organ study.

MUFP staining is quick, reliable and can be done with easily available reagents and is very useful especially in countries like India.

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ANNEXURES

PROFORMA

Name: IP/OP No:

Age : FNAC No:

Sex:

Presenting complaints:

Past history:

Family history:

Personal history:

General Examination :

Pallor, Lymphadenopathy

Icterus, Cyanosis

Clubbing, Oedema

Pulse rate:

Blood pressure:

Respiratory rate:

Systemic examination:RS , CVS , Per abdomen.

Investigations:

Complete blood count

TFT,Ultrasound

Local examination:

Clinical Diagnosis:

Nature of Aspirate:

LIST OF ABBREVIATIONS

FNAC	-	Fine Needle Aspiration Cytology
FNB	-	Fine needle biopsy
CNB	-	Core needle biopsy
PAP	-	Papanicolaou
MUFP	-	Modified Ultrafast Papanicolaou
MGG	-	May Grunwald Giemsa
H&E	-	Hematoxylin and Eosin
OG	-	Orange G
EA	-	Eosin Azure
QI	-	Quality Index
FN	-	Follicular Neoplasm
PC	-	Papillary Carcinoma

ஒப்புதல் படிவம்

பெயர் .

வயது .

பாலினம் .

முகவரி .

எனது மார்பக கட்டியில் / முன் கழுத்து கழலை கட்டியில் / நெறிகட்டியில் இருந்து ஊசி மூலம் திசு / நீர் எடுத்து பரிசோதனை செய்வதற்கு முழு மனதுடன் சம்மதிக்கிறேன்.

இடம் இப்படிக்கு

தேதி கையொப்பம் / ரேகை

KEY TO MASTER CHART

1. Background

   Hemorrhagic-Score 1

   Clean -Score 2

2. OverallStaining

   Poor -Score1

   Average -Score 2

   Good -Score 3

3. Cell Morphology

   Poorly Preserved -Score 1

   Moderately Preserved

   Well Preserved -Score 3

4. Nuclear Characteristics

   Smudgy Chromatin -Score 1

   Moderately Crisp Chromatin-Score 2

   Crisp Chromatin -Score 3

5. Cytoplasmic Details

   Unsatisfactory-Score1

   Suboptimal -Score 2

   Optimal -Score 3

6. Air Drying Artifacts

   >50%-Score 1

   <50%-Score 2

   0% -score 3

MASTER CHART

PAPANICOLAOU STAIN

Sl.No	Cytology no	Age	Sex	organ	Impression	Backround	Overall staining	Cell morphology	Nuclear charecteristics	Cytoplasmic details	Air drying artifact	Quality index
1	893/15	45	female	thyroid	follicular neoplasm	1	3	3	3	3	3	0.94
2	899/15	33	female	thyroid	nodular goitre	1	2	3	3	3	3	0.88
3	906/15	45	female	thyroid	nodular goitre	1	2	3	3	3	3	0.88
4	916/15	27	female	thyroid	nodular goitre	2	3	2	3	2	2	0.82
5	923/15	48	female	thyroid	nodular goitre	1	3	2	3	2	2	0.76
6	925/15	31	female	thyroid	nodular goitre	1	2	2	3	2	2	0.71
7	927/15	42	female	thyroid	nodular goitre	1	2	3	3	2	2	0.76
8	947/15	20	female	thyroid	hashimotos thyroiditis	1	2	3	3	2	2	0.76
9	961/15	20	female	thyroid	hashimotos thyroiditis	1	3	2	2	2	2	0.71
10	1219/15	27	female	thyroid	nodular goitre	1	2	3	3	2	2	0.76
11	1242/15	39	female	thyroid	hashimotos thyroiditis	2	2	2	3	3	2	0.82
12	1244/15	50	female	thyroid	hashimotos thyroiditis	2	2	3	3	2	3	0.88
13	1285/15	24	female	thyroid	nodular goitre	2	2	3	3	3	2	0.88
14	1303/15	20	female	thyroid	hashimotos thyroiditis	1	2	3	3	3	2	0.82
15	1312/15	32	female	thyroid	nodular goitre	1	3	3	3	2	2	0.82
16	490/16	40	female	thyroid	hashimotos thyroiditis	1	3	2	3	3	2	0.82
17	516/16	46	female	thyroid	nodular goitre	1	3	2	3	3	3	0.88
18	518/16	46	female	thyroid	nodular goitre	1	2	3	3	2	2	0.76
19	540/16	32	female	thyroid	hashimotos thyroiditis	2	2	3	2	3	2	0.82

20	541/16	50	female	thyroid	nodular goitre	2	3	3	3	3	2	0.94
21	624/16	36	female	thyroid	nodular goitre	1	3	3	3	2	2	0.82
22	632/16	34	female	thyroid	medullary carcinoma	1	3	2	3	2	3	0.82
23	639/16	62	female	thyroid	papillary carcinoma	2	2	2	3	2	3	0.88
24	648/16	29	female	thyroid	nodular goitre	1	3	3	2	2	3	0.82
25	732/16	68	female	thyroid	papillary carcinoma	1	3	2	3	2	3	0.82
26	782/16	40	female	thyroid	nodular goitre	2	2	3	3	2	3	0.88
27	790/16	39	female	thyroid	hashimotos thyroiditis	1	3	3	3	3	2	0.88
28	792/16	14	female	thyroid	hashimotos thyroiditis	2	2	3	3	3	2	0.88
29	795/16	20	female	thyroid	follicular neoplasm	1	3	3	3	3	2	0.88
30	800/16	38	female	thyroid	hashimotos thyroiditis	1	2	2	3	3	2	0.76
31	802/16	53	female	thyroid	nodular goitre	1	2	3	3	3	2	0.82
32	805/16	28	female	thyroid	follicular neoplasm	2	2	3	2	3	3	0.88
33	809/16	51	female	thyroid	nodular goitre	1	3	3	3	2	3	0.88
34	827/16	21	female	thyroid	hashimotos thyroiditis	1	3	2	3	2	2	0.76
35	834/16	28	female	thyroid	nodular goitre	1	2	3	3	2	2	0.76
36	840/16	37	female	thyroid	nodular goitre	1	3	3	3	2	2	0.82
37	845/16	53	male	thyroid	hashimotos thyroiditis	1	2	2	3	3	3	0.82
38	852/16	29	female	thyroid	nodular goitre	1	2	3	3	3	2	0.82
39	863/16	40	female	thyroid	nodular goitre	1	3	2	3	3	2	0.82
40	873/16	41	female	thyroid	nodular goitre	1	3	3	3	3	2	0.88
41	890/15	50	female	breast	ductal carcinoma	2	3	2	2	3	2	0.82
42	902/15	37	female	breast	fibrocystic disease	1	3	2	3	3	2	0.82

43	903/15	18	female	breast	fibroadenoma	2	3	2	3	2	3	0.88
44	904/15	63	male	breast	gynacomastia	2	2	2	3	3	2	0.82
45	905/15	26	female	breast	fibroadenoma	2	2	2	3	3	3	0.88
46	907/15	33	female	breast	fibroadenoma	2	2	3	3	2	3	0.88
47	912/15	39	female	breast	fibroadenoma	1	3	3	3	2	3	0.88
48	913/15	32	female	breast	fibrocystic disease	1	3	3	3	2	2	0.82
49	914/15	24	female	breast	fibroadenoma	1	3	2	3	3	3	0.88
50	919/15	20	female	breast	fibroadenoma	2	3	3	3	3	2	0.94
51	922/15	38	female	breast	fibrocystic disease	2	2	3	3	3	2	0.88
52	926/15	41	male	breast	gynacomastia	1	3	3	3	3	3	0.94
53	1265/15	33	female	breast	fibroadenoma	1	3	3	3	3	2	0.88
54	1267/15	44	female	breast	fibroadenoma	2	3	3	3	3	2	0.94
55	1271/15	46	female	breast	fibrocystic disease	1	3	2	3	3	2	0.82
56	1278/15	20	female	breast	fibroadenoma	1	3	3	3	2	3	0.88
57	1357/15	62	female	breast	ductal carcinoma	1	2	3	3	2	3	0.88
58	495/16	47	female	breast	ductal carcinoma	2	3	3	3	2	3	0.94
59	520/16	42	female	breast	ductal carcinoma	2	2	2	3	2	3	0.82
60	521/16	55	female	breast	ductal carcinoma	2	2	3	2	3	3	0.88
61	612/16	75	female	breast	ductal carcinoma	2	3	3	2	2	2	0.82
62	620/16	45	female	breast	fibrocystic disease	1	3	3	3	3	2	0.88
63	628/16	58	female	breast	ductal carcinoma	1	3	2	3	3	2	0.82
64	658/16	40	female	breast	ductal carcinoma	1	3	3	3	3	2	0.88
65	784/16	60	female	breast	ductal carcinoma	1	2	3	3	3	2	0.82
66	793/16	45	female	breast	fibrocystic disease	1	3	3	3	2	3	0.88
67	804/16	17	female	breast	fibroadenoma	1	3	3	3	3	2	0.88

68	810/16	29	female	breast	fibrocystic disease	2	2	3	3	3	2	0.88
69	811/16	45	female	breast	ductal carcinoma	2	3	2	3	2	3	0.88
70	813/16	57	female	breast	ductal carcinoma	1	3	3	3	2	3	0.88
71	814/16	19	female	breast	fibroadenoma	2	2	3	3	3	3	0.94
72	833/16	20	female	breast	fibroadenoma	2	3	3	3	2	2	0.88
73	839/16	58	female	breast	ductal carcinoma	2	3	2	2	3	2	0.82
74	844/16	23	female	breast	fibrocystic disease	2	3	2	2	3	2	0.82
75	856/16	47	female	breast	fibrocystic disease	2	3	3	3	3	2	0.94
76	864/16	55	female	breast	ductal carcinoma	2	2	2	3	3	2	0.82
77	880/16	41	female	breast	fibrocystic disease	1	3	3	3	2	3	0.88
78	884/16	26	female	breast	fibroadenoma	1	3	3	3	3	2	0.88
79	885/16	30	female	breast	fibrocystic disease	1	3	3	3	2	3	0.88
80	886/16	50	female	breast	ductal carcinoma	1	3	2	3	3	2	0.82
81	875/15	50	male	lymphnode	metastatic deposits	1	3	3	3	2	3	0.88
82	876/15	38	female	lymphnode	reactive lymphadenitis	1	2	3	3	3	3	0.88
83	883/15	41	female	lymphnode	suppurative lesion	1	3	3	2	3	3	0.88
84	890/15	50	female	lymphnode	metastatic deposits	2	2	3	3	3	2	0.88
85	891/15	34	female	lymphnode	suppurative lesion	2	3	2	3	3	2	0.88
86	894/15	72	male	lymphnode	metastatic deposits	1	3	3	3	2	3	0.88
87	1319/15	29	female	lymphnode	suppurative lesion	1	3	3	3	2	3	0.88
88	1336/15	67	male	lymphnode	metastatic deposits	1	2	3	3	3	3	0.88
89	503/16	60	male	lymphnode	metastatic deposits	2	2	3	2	3	3	0.88
90	534/16	22	male	lymphnode	TB lymphadenitis	2	2	3	3	3	3	0.94
91	551/16	64	female	lymphnode	metastatic deposits	2	3	2	3	3	3	0.94

92	553/16	75	male	lymphnode	metastatic deposits	1	3	3	3	3	2	0.88
93	653/16	68	female	lymphnode	metastatic deposits	1	3	3	3	3	2	0.88
94	659/16	47	male	lymphnode	metastatic deposits	1	2	3	3	3	2	0.82
95	777/16	32	male	lymphnode	reactive lymphadenitis	1	3	3	3	2	3	0.88
96	789/16	56	female	lymphnode	metastatic deposits	2	3	3	3	2	2	0.88
97	799/16	38	female	lymphnode	reactive lymphadenitis	2	3	2	3	3	3	0.94
98	811/16	45	female	lymphnode	metastatic deposits	1	3	3	3	2	3	0.88
99	813/16	57	female	lymphnode	metastatic deposits	1	3	3	3	3	3	0.94
100	816/16	66	male	lymphnode	metastatic deposits	2	2	3	3	3	3	0.94

MAY GRUNWALD GEIMSA STAIN							
S.No	**Backround**	**Overall staining**	**Cell morphology**	**Nuclear charecteristics**	**Cytoplasmic details**	**Air drying artifact**	**Quality index**
1	2	2	2	1	3	3	0.76
2	1	2	2	2	3	3	0.76
3	1	2	2	1	3	3	0.71
4	1	2	2	2	3	3	0.76
5	1	2	2	2	3	3	0.76
6	1	2	3	2	3	2	0.76
7	2	2	2	2	3	3	0.82
8	1	2	2	3	2	3	0.76
9	1	2	2	2	3	3	0.76
10	1	2	2	2	3	3	0.76
11	1	2	2	2	3	3	0.76
12	2	2	1	2	3	2	0.71
13	2	3	2	1	3	3	0.82
14	1	2	2	2	3	3	0.76
15	1	2	2	2	3	3	0.76
16	1	2	2	2	3	3	0.76
17	1	1	2	1	3	2	0.59
18	1	2	2	1	3	3	0.71
19	1	2	2	2	3	3	0.76
20	1	3	3	2	3	3	0.88
21	1	2	2	2	2	3	0.71
22	1	2	2	2	3	3	0.76
23	2	2	2	2	3	3	0.82
24	1	2	2	1	3	2	0.65
25	1	2	2	2	3	3	0.76
26	2	2	3	3	3	3	0.94
27	2	2	2	2	2	2	0.71
28	2	2	2	2	3	3	0.82
29	1	2	2	2	3	3	0.76
30	1	2	2	2	3	2	0.71
31	1	2	2	2	3	3	0.76
32	1	2	2	2	3	3	0.76
33	1	2	2	2	3	3	0.76
34	1	2	2	1	3	3	0.71
35	2	2	1	2	2	3	0.71
36	1	2	2	3	3	3	0.82
37	1	3	2	1	3	3	0.76
38	1	2	2	2	3	3	0.76
39	1	2	2	2	3	3	0.76
40	1	2	2	2	3	2	0.71
41	1	2	2	2	3	3	0.76
42	1	2	2	2	3	2	0.71
43	2	2	2	1	3	3	0.76
44	1	2	2	3	2	3	0.76
45	1	2	1	2	3	3	0.71
46	1	2	2	2	3	3	0.76
47	1	2	2	2	3	3	0.76
48	1	3	1	2	3	3	0.76
49	2	2	2	2	3	2	0.76

50	2	1	3	2	3	3	0.82
51	1	2	2	2	3	3	0.76
52	2	2	2	2	3	3	0.82
53	1	2	2	1	3	3	0.71
54	1	2	2	3	2	3	0.76
55	1	2	2	2	3	3	0.76
56	1	2	2	2	3	3	0.76
54	1	2	3	2	3	3	0.82
58	2	2	2	2	3	2	0.76
59	1	2	2	3	3	3	0.82
60	1	2	2	2	3	3	0.76
61	1	2	2	2	2	3	0.71
62	1	2	2	2	3	3	0.76
63	2	2	2	2	3	3	0.82
64	1	2	3	2	3	3	0.82
65	1	1	3	3	3	2	0.76
66	1	2	2	2	3	3	0.76
67	1	2	2	2	3	3	0.76
68	1	2	2	2	3	3	0.76
69	1	2	2	2	3	3	0.76
70	1	2	2	2	3	3	0.76
71	1	3	3	2	3	3	0.88
72	2	2	2	1	3	3	0.76
73	2	2	2	2	3	3	0.82
74	1	2	2	2	3	3	0.76
75	1	2	2	2	3	3	0.76
76	1	2	2	2	3	2	0.71
77	1	2	2	3	2	3	0.76
78	1	2	3	2	3	3	0.82
79	1	2	1	2	3	3	0.71
80	1	2	2	2	3	3	0.76
81	1	2	2	2	3	3	0.76
82	2	2	2	2	3	3	0.82
83	1	3	2	2	3	3	0.82
84	1	2	2	1	3	3	0.71
85	1	3	2	2	3	3	0.82
86	1	2	2	2	3	3	0.76
87	1	2	2	2	3	3	0.76
88	1	2	2	2	3	3	0.76
89	1	2	2	2	3	2	0.71
90	1	3	2	2	3	3	0.82
91	2	2	2	2	3	3	0.82
92	1	2	2	2	3	3	0.76
93	1	2	3	3	3	3	0.88
94	1	2	2	2	3	3	0.76
95	1	2	2	2	3	3	0.76
96	1	2	2	2	3	3	0.76
97	1	3	2	1	3	3	0.76
98	2	2	2	2	3	2	0.76
99	1	2	2	2	3	3	0.76
100	2	2	2	2	3	3	0.82

MODIFIED ULTRA FAST PAP STAIN							
S.No	**Backround**	**Overall staining**	**Cell morphology**	**Nuclear charecteristics**	**Cytoplasmic details**	**Air drying artifact**	**Quality index**
1	2	3	3	3	3	3	1
2	2	3	3	3	3	3	1
3	2	3	3	3	3	3	1
4	2	3	2	3	3	3	0.94
5	2	3	3	2	3	3	0.94
6	2	2	3	2	3	3	0.88
7	2	3	3	3	2	3	0.94
8	2	3	3	2	3	2	0.88
9	2	3	3	2	3	2	0.88
10	2	3	3	2	3	3	0.94
11	2	3	3	3	3	3	1
12	2	3	2	3	3	3	0.94
13	2	3	3	2	2	3	0.88
14	2	3	3	3	2	2	0.88
15	2	2	3	3	2	3	0.88
16	2	3	3	2	3	3	0.94
17	2	3	3	3	3	3	1
18	2	3	3	2	3	3	0.94
19	2	3	3	3	3	3	1
20	2	2	3	2	3	3	0.88
21	2	3	2	3	3	3	0.94
22	2	3	3	3	2	3	0.94
23	2	2	3	3	3	2	0.88
24	2	3	3	3	3	2	0.94
25	2	3	3	3	2	3	0.94
26	2	3	3	3	2	3	0.94
27	2	3	2	3	3	3	0.94
28	2	3	3	2	3	3	0.94
29	2	2	3	3	3	2	0.88
30	2	3	3	3	3	3	1
31	2	3	3	2	3	3	0.94
32	2	3	3	3	3	3	1
33	2	3	3	2	3	3	0.94
34	2	3	3	2	3	3	0.94
35	2	2	3	3	2	3	0.88
36	2	3	3	3	2	3	0.94
37	2	3	2	3	3	3	0.94
38	2	3	3	2	3	3	0.94
39	2	3	3	3	3	2	0.94
40	2	2	3	2	3	3	0.88
41	2	3	2	3	3	3	0.94
42	2	3	3	2	3	3	0.94
43	2	3	2	3	3	3	0.94
44	2	3	3	3	3	2	0.94
45	2	2	3	3	2	3	0.88
46	2	3	3	3	3	3	1
47	2	3	3	3	3	3	1
48	2	3	3	2	3	2	0.88
49	2	3	2	3	2	3	0.88

50	2	3	3	3	3	3	1
51	2	3	3	3	3	2	0.94
52	2	3	3	2	3	3	0.94
53	2	3	3	3	3	3	1
54	2	3	3	2	3	3	0.94
55	2	3	2	3	3	3	0.94
56	2	3	3	3	2	3	0.94
54	2	3	3	2	3	3	0.94
58	2	3	3	2	3	3	0.94
59	2	3	3	3	3	2	0.94
60	2	3	3	2	3	3	0.94
61	2	3	2	3	3	3	0.94
62	2	3	3	3	3	2	0.94
63	2	2	3	3	3	3	0.94
64	2	3	3	3	2	3	0.94
65	2	3	3	2	3	3	0.94
66	2	3	3	2	3	3	0.94
67	2	3	3	3	3	2	0.94
68	2	2	3	3	3	3	0.94
69	2	3	2	3	3	3	0.94
70	2	3	3	3	2	3	0.94
71	2	3	3	2	3	3	0.94
72	2	2	3	2	3	3	0.88
73	2	3	3	3	3	2	0.94
74	2	3	3	3	3	3	1
75	2	3	3	3	2	3	0.94
76	2	3	2	3	3	3	0.94
77	2	2	3	3	3	3	0.94
78	2	2	3	2	3	3	0.88
79	2	3	3	3	2	3	0.94
80	2	3	3	2	3	3	0.94
81	2	3	3	3	3	2	0.94
82	2	2	3	3	3	3	0.94
83	2	3	3	3	3	3	1
84	2	3	3	3	3	2	0.94
85	2	3	3	2	3	3	0.94
86	2	3	3	3	2	3	0.94
87	2	3	3	2	3	3	0.94
88	2	3	2	3	3	3	0.94
89	2	3	3	3	3	2	0.94
90	2	3	3	3	3	3	1
91	2	2	3	3	3	3	0.94
92	2	3	3	3	2	3	0.94
93	2	3	3	3	3	3	1
94	2	3	3	3	3	3	1
95	2	3	3	3	3	2	0.94
96	2	3	3	3	3	3	1
97	2	2	3	2	3	3	0.88
98	2	3	2	3	3	3	0.94
99	2	3	3	3	3	3	1
100	2	3	3	3	3	3	1