"APPLICATION OF MODIFIED ULTRA-FAST PAPANICOLAOU STAIN IN CYTOLOGICAL DIAGNOSIS"

By

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Dissertation submitted to the

BLDE University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

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ACKNOWLEDGMENT

At first, I pray to the almighty God, thanking him for the bounty of life. I thank my parents who have nurtured me and supported me in all my endeavors, without their love and innumerable sacrifices; I would not be the person I am today.

I would like to take this opportunity to express my sincere and deepest gratitude to my guide **Dr.Surekha U. Arakeri**, Professor Department of Pathology, for her continuous support, patience, motivation, enthusiasm and immense knowledge. Her guidance helped me greatly in the completion of this dissertation.Her meticulous supervision has been a valuable part of my learning process.

I express my sincere gratitude to **Dr. B.R.Yelikar,** Professor and Head, Department of Pathology for his invaluable guidance, constant encouragement and support in any endeavor which I undertook. He is an example to everyone and inculcated a work ethics which will go a long way in developing my career.

I am extremely fortunate to have a supportive and encouraging department who has advised and mentored me and made it conceivable for me to expedite this dissertation. My sincere thanks to **Dr R.M.Potekar** Prof, **Dr.S.B.Hippargi** Prof, **Dr.Mahesh H. Karigoudar** Prof, **Dr Prakash Patil** Assoc Prof, **Dr Girija Patil** Assoc Prof ,**Dr.Vijayalaxmi S Patil** Asst. Prof, **Dr. Anita P Javalgi** Asst prof, **Dr.Savtiri Nerune** Asst prof, **Dr. Mamatha K.** Asst prof and **Dr. Yashaswini S.P**. Asst Prof for their constant inspiration, guidance and encourgament during my study.

My sincere thanks to all my batchmates, seniors and juniors who have helped and encouraged me during my work.

Special thanks to Mr. Ashok Umnabadi, the cytology section in charge for helping me in the collection of my samples and going out of the way to help me with the preparation of solutions and stains.

My heartfelt thanks to Mr. Harshvardhan Maga, the assistant librarian who helped me sort out and collect various articles and research publications.

I am very grateful to all the nonteaching staff of Department of Pathology, who helped me during this work.

I express my gratitude to all patients who contributed in no small way to this dissertation, without whom this entire exercise would have been unimaginable.

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LIST OF ABBREVIATIONS USED

FNAC	Fine Needle Aspiration Cytology
PAP	Papanicolaou
UFP	Ultra Fast Papanicolaou
MUFP	Modified Ultra Fast Papanicolaou
H&E	Haematoxylin and Eosin
MGG	May Grunwald Giemsa
QI	Quality Index
OG	Orange G
EA	Eosin Azure
USG	Ultrasound

ABSTRACT

BACKGROUND:

Need for minimal turnaround time for assessing fine needle aspiration cytology (FNAC) has encouraged innovations in staining techniques that require lesser staining time with unequivocal cell morphology. The standard protocol for conventional Papanicolaou (PAP) stain requires about 40 minutes. To overcome this, ultrafast Papanicolaou (UFP) stain was introduced which reduces staining time to 90 seconds and also enhances the quality. However, reagents required for this were not easily available hence Modified ultra fast Papanicolaou (MUFP) stain was introduced subsequently.

OBJECTIVE:

To assess the efficacy of MUFP staining by comparing the quality of MUFP stain with conventional PAP stain.

METHODS:

FNAC procedure was performed by using 10ml disposable syringe and 22-23G needle. Two smears were prepared & stained by MUFP and conventional PAP stain. Scores were given on four parameters: background of smears, overall staining pattern, cell morphology & nuclear staining. Quality index (QI) was calculated from ratio of total score achieved to maximum score possible.

RESULTS:

Total 131 FNAC cases were studied which were lymph node (30), thyroid (38), breast (22), skin & soft tissue (24), salivary gland (11) & visceral organs (6). The QI of MUFP for thyroid, breast, lymph node, skin & soft tissue, salivary gland

and visceral organs was 0.89, 0.85, 0.89, 0.83, 0.92 and 0.78 respectively. Compared to conventional PAP stain QI of MUFP smears was better in all except visceral organ cases and was statistically significant ($p < 0.001$). MUFP showed clear red blood cell background, transparent cytoplasm and crisp nuclear features.

CONCLUSION:

MUFP is fast, reliable & can be done with locally available reagents with unequivocal morphology which is the need of hour for a cytopathological set-up.

KEYWORDS:

Conventional PAP stain, FNAC, Modified Ultra fast Pap stain.

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INTRODUCTION

Fine needle aspiration cytology (FNAC) has become an important pre-operative and screening test for various lesions. It is also a valuable aid for screening of malignant and potentially malignant lesions. It is easy, economical, non-invasive, quick and feasible method for detection of malignancies.[1-3]

The Papanicolaou (PAP) stain is developed by Dr. George N Papanicolaou, the father of cytopathology. It is a multichromatic staining technique developed in 1942 and subsequently modified in 1954 and 1960.[4]

PAP stain is used to identify and differentiate the cells in smears prepared from various body fluids, gynecological smears and FNAC smears from various organs.[1,2]

Diagnosis by FNAC has a major role in good and efficient medical practice. It is easy to perform and can be done quickly. The need of the hour is minimal turnaround time and quick diagnosis on FNAC. This has encouraged many newer staining techniques with lesser staining time with unequivocal cell morphology.[2,3]

The ever increasing use of FNAC as one of the pivotal diagnostic tools has validated the use of other stains like Romanowsky and haematoxylin and eosin (H&E) stains along with the conventional PAP stain.[4]

Few rapid stains are available now a days which include Diff-Quick stain, toluidine blue stain and May Grunwald Giemsa (MGG) stain. However most of the cytopathologists prefer a multichromatic, transparent stain with crisp nuclear and cytological characteristics which are offered by wet 95% ethanol fixed PAP stain and not by air dried smears stained by Romanowsky stain.[3,5]

The standard protocol for PAP stain requires about 40 minutes.[1] To resolve this issue in 1994, Yang and Alvarez [6] invented the ultrafast Pap (UFP) stain. It is a

hybrid of Romanowsky stain and PAP stain. It reduces the time of staining to 90 seconds as well as enhances the quality.

Kamal *et al* [7] invented the Modified Ultra-Fast Pap (MUFP) stain as reagents required for the UFP stain were not universally available.They used easily available Gills or Harris hematoxylin instead of Richard Allan hematoxylin and modified EA instead of Richard Allan cytostain.

Hence the present study was undertaken to assess the efficacy of MUFP staining in cytological diagnosis of various lesions in the body and comparing these results with conventional PAP stain.

OBJECTIVE OF THE STUDY:

- To assess the efficacy of MUFP staining by comparing the quality of MUFP stain with conventional PAP stain.

REVIEW OF LITERATURE

HISTORICAL PERSPECTIVE:

Cytopathology started off by looking at imprints of cut surface of tumors during postmortem. It then gradually evolved through various methods of procuring, fixing and staining of the cells. Its major attribute was the ability to allow quick and accurate assessment from the material and smears with minimal invasive procedure and processing.[8]

FNAC is minimally invasive and inexpensive method of obtaining quick diagnosis of various lesions. It was first used by Kun in 1847 where he described a ‘‘new instrument for the diagnosis of tumors.’’ Kun used an exploring needle for scooping out tissue from a subcutaneous tumor. For the first time in 15th century syringes and similar instruments were used to aspirate collection of fluids. With the introduction of the achromatic microscope in the 1830s, easy accessibility was provided to observers to examine the aspirated material.[9]

Martin R[9] in in his article titled “Fine needle aspiration biopsy: A Historical Review” mentioned that, Skey in 1851 did breast aspiration for cystic masses. In 1853, Sir James Paget and Erichsen for the first time used aspiration biopsy. Pritchard for the aspiration of breast, used a groove needle with excellent results for the cytology of fat necrosis. In 1883, for the first time Leyden performed transthoracic aspiration biopsy for the diagnosis of pneumonia.

Wied GL [10] in his study faced a major problem of the absence of appropriate stains for tissue microscopy therefore cytologic smears were used for analysis.

In the decade between 1870 to1880 a remarkable change occurred. There was advancement of mechanical microtomes and invention of tissue stains. Thus well stained and thin sections were now possible to be prepared.[10] Aspiration biopsy did

not achieve great acceptance during the first quarter of the 20th century. Controversies over the diagnosis of cancer by means of smears rather than tissue sections were raised.[10,11]

In 1921, Guthrie reported cytologic smears on a various lesions including those of Hodgkins disease. In 1927, Dudgeon and Patrick[12] reported 200 cases with a diagnostic accuracy of 98.6%.

They had proposed needle aspiration of tumors as a means of rapid diagnosis.

In the late 20s and early 30s, two pathologists (Steward and Ewing), a laboratory technologist (Ellis), and one Head and Neck surgeon (Martin) form the Memorial Hospital, New York, started needle aspiration of deep seated palpable tumors.[9,10]

Steward in 1933, described 2500 tumours investigated by aspiration method for 3 years at the Memorial Hospital, New York. He used heat fixed smears stained by rapid H&E stain. In his report, he highlighted and specified points for optimal results:

1. Emphasis on the exact technique of aspiration and preparation of the smears,
2. Importance of clinical correlation before interpretation of the smears,
3. Comparison between cytology and histopathology,
4. Combination of the pattern analysis along with cytologic details should be done for appropriate interpretation, and
5. Awareness about the limitation of the method. Today these points are still the base of knowledge for successful application of needle aspiration. [13-15]

Naylor B[15] in his article mentioned that FNAC continued to be practiced in the late 1930s and into the Second World War. Many articles on aspiration of organs were published between1940 to 1960. He also mentioned in his article that many of clinical doctors were hematologists namely Lopes-Cardozo (Holland), Soderstrom and Franzen (Sweden). They preferred air-dried, methanol fixed, Romanowsky

(Giemsa) smears rather than H&E which was favoured in Memorial Hospital, New York.

The European cytologists introduced the use of thinner needles of 22-gauge or higher. This is the origin of the technique as we use it today. Mannheim in Berlin inspired FNA by using 1.0mm diameter needle in his report of 1931 as quoted in the article by Wied GL.[10]

In 1942, Dr. George Papanicolaou published an article named: ''A New Procedure For Staining Vaginal Smears,'' where he described a new technique for staining, which subsequently came to be known as the PAP stain. Dr. Papanicolaou's work was supported by clinicians but pathologists were reluctant to use the new stain due to differences in fixation and appearance of the cells. Gradually, his work in exfoliative cytology and the clarity of the PAP stain contributed to the resurgence of needle aspiration. During 1950 Dr. Papanicolaou's study became very popular and the interest of the American Society of Cytology was focused on cervico- vaginal cytology. Several years after Dr. Papanicolaou's contribution, interest in needle biopsy again came up at the Karolinska Hospital in Sweden.[16]

During the 60s and 70s there was revival of the FNA technique again due the Swedish experience and the work of many pioneers who published numerous articles on aspiration cytology in many of the international journals .They concluded that FNAC had aided significantly to a timely diagnosis of neoplastic and non-neoplastic lesion. It has replaced or at least complemented tissue diagnosis in many clinical situations. [9,10]

ADVANTAGES OF FNAC:

- This method is simple, painless, accurate, produces speedy results.[13]
- This technique can be performed on superficially palpable lesions. With advancement in radiography internal imaging it is also applicable to lesions that are deep seated and impalpable.[13]
- Reduced hospital stay and faster turnaround time can be obtained as it is done on OPD basis.[13,14]
- There is very little risk of complications with FNAC[14]
- Smears and cell blocks obtained during FNAC can be used in various ways like DNA analysis, ultra structural studies, immunocytochemistry, gene rearrangement, morphometry and image analysis.[13,14]

NEED FOR RAPID ASSESSMENT IN CYTOLOGY: **THE CURRENT SCENARIO:**

Over the years, laboratories have seen many remarkable changes in the type and number of specimens received for cytological evaluation. The use of fibreoptic instruments and other newer imaging techniques has allowed cells to be obtained from almost any anatomic sites by FNAC procedure. Hence many treatment decisions are being made on the basis of the cytological diagnosis. [3,17]

The goal of diagnostic cytopathology is the recognition of cells derived from tissues. The interpretation of smears depends on the skill of the pathologist and can be difficult depending on the site of FNAC. There are two important factors on which the interpretation of these smears depends. One is sampling and site and the second is quality of staining. The quality of smear preparation and staining plays a vital role in the cytopathologist's ability to make a prompt diagnosis.[4]

However, the turn-around time of the cytological diagnosis varies in different institutions and in various clinical situations. The speed of reporting of FNAC can be improvised by rapid evaluation of smears. Quick and efficient diagnosis of FNAC plays a major role in proficient medical practice. On OPD basis, a fast diagnosis of the lesion on aspiration cytology helps the clinician plan management options for the patient and is beneficial to both the clinician and the patient.This need for rapid assessment has encouraged innovations in staining techniques that require less time and also give unequivocal cell morphology.[17]

CYTOLOGICAL STAINS:

Two basic methods of fixation and subsequent staining are used in FNAC. First is complete air-drying of the smear, followed by staining with haematological stains, such as MGG, Jenner-Giemsa, or Diff-Quick.[4]The second method is wet fixation, followed by staining with PAP or H&E stain. The air dried smears followed by staining with MGG stain, toludine blue stain and Diff Quik stain were used traditionally . These Romanowsky stains are air dried rather than wet fixed. The air drying causes cellular swelling and loss of nuclear details. Also it has a greater propensity to precipitate and give high background staining.[4,17]

Most of the cytopathologists even today prefer the crystal clear, crisp nuclear features of wet fixed smears stained by conventional PAP stain, over the opacity of nuclei resulting from air dried smears stained by Romanowsky stains.[3,5,17]

FIXATIVES:

Quick fixation of the smears is essential to preserve the cytological details. For many years the fixative of choice for smear preparations was a solution of equal parts of ether and 95% ethyl alcohol. Subsequently, over years this was abolished because ether presents a fire hazard. Thus 95% ethyl alcohol is employed now a days as a fixative in most laboratories, with good results. Smears should be kept in 95% ethanol for fixation for minimum 15 minutes prior to staining.[4]

WET FIXATION:

Wet fixation is a traditional method wherein the smears are immediately dipped in the fixative before air-drying. The disadvantages are air-drying artifacts if not fixed immediately and also a hemorrhagic background with cellular loss during fixation.[4]

To overcome these limitations, in 1998, Chan and Kung came up with an innovative method. In this method the air-dried smears were placed in normal saline for 30 seconds before fixation with 95% alcohol. This was a simple means of rehydrating the cells. The quality of these rehydrated smears is superior to or similar to wet-fixed smears, keeping in mind that the period of drying does not exceed 30 minutes. This technique of Chang and Kung is now used in MUFP staining procedure.[4,18]

PAPANICOLAOU STAIN:

For the routine cytopathology, the PAP stain is recommended. Its use results in crispy nuclear chromatin, differential cytoplasmic counterstaining, and cytoplasmic transparency. Although, it was originally invented and developed for interpretation for

gynaecological cases, it is now commonly used for detection and interpretation of various cells in a variety of non-gynecological lesions.[4,6]

The PAP stain is a polychromatic staining technique. The advantages include details about the nucleoli and chromatin pattern of the individual tumor cells. This is of utmost importance because nuclear details are used to differentiate between benign and malignant lesions. Thus PAP stain is a gold-standard staining method of choice for diagnosis of neoplastic disease and can also be used in exfoliative cytology or FNAC. However, the conventional PAP stain requires wet fixation and is a multistep lengthy staining procedure.[6,19]

THE CONVENTIONAL PAP STAIN [20]

METHOD

1. Fix slides in alcohol fixative for 15 minutes.
2. Absolute alcohol 2 minutes.
3. 70% alcohol 2 minutes.
4. 50% alcohol 2 minutes.
5. Tap water 2 minutes.
6. Stain with haematoxylin 4 minutes.
7. Rinse in tap water.
8. Differentiate in acid alcohol 5 seconds.
9. Bluing in tap water.
10. Dehydrate in absolute alcohol x 2 times.
11. Stain in Orange G 10 seconds.
12. Rinse in absolute alcohol x2 times.
13. Stain in E.A. 36 2 minutes.
14. Rinse in absolute alcohol x2 times.

15. Clear in xylene x3 times.
16. Mount sections in DPX.

This multi-step process requires around 40 minutes.

MODIFICATIONS IN THE PAPANICOLAOU STAIN:

The original PAP stain of 1942 was modified and published by Dr. Papanicolaou in 1954 and 1960. It uses Harris hematoxylin regressively. PAP Technique II, as used for urinary and gastric preparations, uses hematoxylin progressively. Other changes and variations include Gill’s modification, Miller’s modification, Saccomanno’s modification for carbowax fixed smears and Durfee’s modification for urine sediment smears.[4,20]

Rapid modifications in various stains and their techniques including H&E, PAP stain, have been made to cut down time. Rapid PAP stains were developed by Kline, Tao and Sato, and require 4 min, 5 min, and 90 sec, respectively for staining. However, the quality of rapid PAP staining was not satisfactory or upto the mark, as the cell morphology was not well preserved. The time required for original PAP staining is 30-40mins, H&E staining 20 min, and MGG staining 10 mins. Thus these procedures are not considered ideal for intraoperative cytology. On the other hand, the quality of rapid PAP staining is not satisfactory. Incidentally, Sato’s Rapid PAP staining requires heating haematoxylin upto 60°C to give good nuclear staining.[21,22]

COMPARISON OF RAPID PAPANICOLAOU STAINS[21,22]

Procedure	Kline's Rapid	Tao's Rapid	UFP
Type of smears	Wet smears	Wet smears	Rehydrated air-dired smears
Fixative	95% Ethyl alcohol	95% Ethyl alcohol	4% formalin in 65% Ethyl alcohol
Fixation time	1 minute	2 minutes	10 seconds
Hematoxylin	30-60 seconds	40 seconds	2 seconds (in Hematoxylin II)
Orange G	30 seconds	30 seconds	4 seconds (in cytostain)
EA	30 seconds	15 seconds	-
Total Time	4 minutes	5 minutes	90 seconds

ADVANTAGES OF REHYDRATED AIR-DRIED SMEARS:

In the past years and even today, lot of debate over the superiority of the stains has taken place, as to whether wet-fixed H & E or PAP stains or air-dried Romanowsky's stains give a better quality. As a matter of fact both are complementary to each other .H & E and PAP staining gives better assessment of nuclear features and are preferred .[23]

However, staining becomes highly substandard once air drying takes place. It is very difficult to avoid drying artifacts because it takes atleast few seconds to spread the aspirate and put the slide in the fixative. Various rehydrating agents, that have been used include tap water, normal saline, 50% aqueous glycerin, acetic acid-alcohol solution and hydroxypropyl methyl cellulose ether in water. Of these agents normal saline gave paramount results in many studies. The nuclear and nucleolar features were as crisp and clear as those of wet-fixed smears, and the cytoplasmic details were prominent and distinct. [24]

The technique of rehydration of air dried smears has many advantages over wet fixed smears. Firstly, the issue of air drying in the edges of the smear can be avoided completly. Second, the problem of falling off of larger particles or thicker portion of the smear when placed in 95% ethanol is solved. If the smears are completely air-dried, the cells stick better to the slide and do not fall off. Third, lysis of the red blood cells creates a clean background to allow better interpretation. Fourthly, the cells appear flatter with a more shallower depth of focus on the nuclei.[24,25]

Chan JKC *et al.*[24] in 1988, did a study on 'Rehydration of Air-Dried smears with Normal saline'. In their study air-dried smears were dipped in normal saline for 30 seconds before fixation in 95% alcohol. They found that optimal time for rehydration varies from 5 seconds to 5 minutes and best results were seen if air drying did not exceed 30 minutes.

Ng WF *et al.*[25] did a study in 1994 on ninety fluid specimens (30 cases of urine, 30 of ascitic fluid and 30 cases of pleural fluid) which were studied by preparing three comparative smears. One was air dried for Giemsa stain, one wet fixed in 95% ethanol and the third one dried on a hot plate at 37^0C, rehydrated in normal saline for 30seconds and then fixed in ethanol. The latter two were stained with PAP stain. These smears were studied for retention of red blood cells, retention of epithelial or mesothelial cells and cytologic preparation by comparing the cytomorphological features. They concluded that the third method that is rehydrated smears showed slight cell enlargement, decreased staining chromaticity and more flattened cell clusters. Thus rehydration method was useful for urine and hemorrhagic body fluids.

In a study done by Jones CA [26] on 'Papanicolaou staining of air-dried smears : value in rapid diagnosis' he concluded that rehydration of air-dried smears show superior nuclear features as compared to wet-fixed smears Also the RBC free background improved the staining of epithelial cells.

ULTRA-FAST PAP STAIN:

UFP stain is a combination of air dried Romanowsky stain and wet fixed PAP stain. It includes principles of air drying, followed by rehydration with normal saline and then subsequent fixation in alcoholic formalin. Air drying makes the cell appear larger and therefore increase the resolution for analysis of cellular details. Normal saline rehydrates the cells hence transparency is maintained in addition to removal of hemorrhage in the background blood. Alcoholic formalin at an acidic pH of 5 gives vibrant colors to the cells and the nucleoli, which stain red. The entire procedure is fast and completed in 2minutes quick enough to permit immediate microscopic assessment of the aspirated material . [6,7]

The rapid PAP stains were similar to the routine conventional PAP stain except that the duration of each step was shortened. The problem faced with rapid PAP stain was four fold:

1. Due to inadequate fixation cellular details of both cytoplasm and nuclei were lost
2. Most of the FNA have bloody aspirates most of the smears were hemorrhagic and the RBC's stain orange obscuring the cellular details.
3. The wet-fixed cells appear much smaller than air-dried cells &
4. Cellular loss due to wet fixation.[21]

To overcome the problem of fixation in the same studies, the fixative was changed from 95% ethyl alcohol to alcoholic formalin (4% formaldehyde in 65% formalin).In their studies these authors mentioned that the acidic pH (pH-5) of alcoholic formalin differentiates RNA from DNA because of acidic pH. They also observed that the nucleoli were stained red and more vibrant colour was noted o the smear.[7,21]

The major limitation of Yang and Alvarez's study was that the staining solutions were all commercially prepared. The Richard-Allan hematoxylin and Cytostain which were used by them were manufactured by Richard-Allan, Inc. (Richland, Michigan, USA) and were not available universally.[6]

ULTRAFAST PAPANICOLAOU STAIN: [6]

1. Normal saline 30 seconds
2. 95% Ethanol (optional),
3. Alcoholic formalin 10 seconds
4. Water 6 slow dips
5. Richard –Allan Hematoxylin, 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

Kamal *et al* [7] in the year 2000 for the first time made modifications in the UFP stain. Firstly, instead of Richard Allan Hameatoxlyin, they used Gill's Haematoxlyin. Secondly instead of Richard Allan cytostain which is an alcoholic mixture of orange G, Eosin Y, Light Green and Aniline blue, they used modified EA which is an alcoholic mixture of Eosin Y, light green, phosphotungstic acid and glacial acetic acid.[7,21]

Orange G step was deleted in MUFP. Thus the orange discolouration was no longer a hassel. MUFP stain has rapid assessment. Besides that sharp nuclear features with crisp chromatin with nuclear staining is demonstrated which is of diagnostic accuracy. The smears are preserved for almost 6 months. This is of added advantage for smear review & retrospective studies. The other advantages are, the cells tend to be larger, thus increasing the resolution for analysis of details, clear, hemorrhage free background due to lysis of the RBCs in background and also the vibrant colors in the cells and the nucleoli, which stain red.[22]

Kamal *et al* [7] after these modifications, stained 100 smears of FNAC breast by this method. They observed that the results were encouraging with preservation of cytomorphology. The total staining time was only 130 sec.

Need-based modifications in UFP staining and overall technical innovations have been made by various authors, to get the desired effects. For example, Lemos *et al* [31] and Maruta *et al* [27] had a \study on nuclei of papillary carcinomas of the thyroid. One of the observations in their study was an artifact effect of clear nuclei. This according to Lemos *et al* [27] resembled the ground glass appearance of papillary carcinomas of thyroid. They then reverted to the use of Gill-5 hematoxylin in the procedure and observed that nuclear features were comparable to that of standard PAP stain and clear nuclei were observed only in papillary carcinoma of thyroid.

Yang and Hoda [28] on the contrary, observed 'Orphan Annie' eye nuclei without any modifications in the original UFP stain.

In 2005, Yang CGH and Waisman J [30]did a study on "Distinguishing Adenoid Cystic Carcinoma from Cylindromatous Adenomas in Salivary Fine-Needle Aspirates" They compared 20 cases of adenoid cystic carcinoma with 15 cases of cylindromatous pleomorphic adenoma and 9 cases of basal cell adenoma. The smears were stained with three rapid stains Diff-Quick, H&E, UFP stain. It was concluded that Adenoid cystic carcinoma can be distinguished from cylindromatous pleomorphic adenoma and basal cell adenoma by using UFP stain under oil immersion. UFP stain revealed a difference in the nuclear features and the amount of cytoplasm.

Lemos LB and Baliga M [31]did a study on 'Ultrafast Papanicolaou Stain: One Year's Experience in a Fine Needle Aspiration Service'. They concluded that UFP stain was particularly useful in diagnosis of squamous carcinoma. This is because of the bright orange coloured stain it imparts to keratinizing squamous carcinoma cells, which is important in the diagnosis of most of the head and neck carcinomas as well as metastatic carcinomas.

In 2011, Bando K *et al* [32] did a study on 'Utility of Immediate cytologic diagnosis of Lung masses using ultrafast Papanicolaou stain'. In their study 503 cases were studied, with the use of UFP stain. Positive cytology results were noted in 348 cases and negative in 153 cases. This study inferred that immediate cytology can be executed in any hospital easily, and is a far more superior technique for obtaining diagnostic accuracy.

Another study was conducted by Bandoh S *et al* [33] in 2003 on 'Diagnostic Accuracy and safety of Flexible Bronchoscopy with Multiplanar Reconstruction images (MPR) and Ultrafast Papanicolaou Stain (UFP). Their study included 100

patients with solitary pulmonary nodule who underwent bronchoscopy with multiplanar reconstruction and MUFP stain simultaneously. The total diagnostic accuracy of this group was 91% .This was significantly higher as compared with the control group with an accuracy of only 58% ($p<0.05$). The study thus concluded that combined use of MPR image and UFP during flexible bronchoscopy improved diagnostic accuracy while evaluating cases of solitary pulmonary nodules.

MUFP STAIN [7,22]

Fixation

The smear is first completely air-dried for less than 30minutes. Then it is kept in normal saline for 30 sec, and in alcoholic formalin for 10 sec.

Staining

1. Tap water 6 slow dips
2. Gills haematoxylin 30 sec
3. Tap water 6 slow dips
4. Ethyl alcohol 95% 6 dips
5. EA-36 15 sec
6. Ethyl alcohol 95% 6 dips
7. Ethyl alcohol 100% 6 dips
8. Xylene 10 slow dips
9. DPX and mount with cover slip
10. Mount with cover slip

The total staining time is 130 sec.

Choudhary *et al* [22] in their study titled "Comparision of modified ultrafast Papanicolaou stain with the standard rapid Papanicolaou stain in cytology of various

organs." concluded that Harris hematoxylin gives equal staining properties as that of Gills hematoxylin in MUFP.

In 2002, Maruta J *et al.*[27] conducted a study on "The applicability of modified ultrafast stain for quick diagnosis of thyroid diseases." Two specimens one each of MUFP and standard PAP were made of 251 thyroid cases (122 malignant and 131 benign). The sensitivity of the specimens stained by standard PAP method and MUFP was 95.0% and 93.3%, and specificity was 99.2% and 97.7% respectively.

Yang C.H. *et al* [28] also conducted a study on ultrasound guided FNA of thyroid gland. They observed that "orphan annie-eyed" nuclei were highlighted by the MUFP staining method which helped to differentiate between follicular variant of papillary carcinoma from follicular neoplasms.

In 2000, Kamal MM *et al* [7] in their study on 'Efficacy of modified ultrafast papanicolaou stain for Breast aspirates' studied 100 breast aspirates stained with MUFP. These smears showed a blood free background with transparent cells and crisp nuclear features, equal to or even superior to that of conventional PAP stain.

Shinde *et al* [21] did a study on 'Application of Modified ultrafast Papanicolaou stain in cytology of various organs'. In their study, in group A,40 FNAC smears of various organs were included. In each case, three smears were prepared and stained with MUFP, PAP and MGG stains. Group B, included 10 intraoperative cases for which cytology smears were stained with MUFP and rapid H & E. For assessment, scores were given on four parameters and Quality index was calculated.

Diagnosis made by MUFP stain was compared with the other standard stains. The diagnosis was accurate and precise in MUFP stain except for in three cases of metastatic squamous cell carcinoma. They concluded that MUFP stain is useful for

quick assessment of smears .However it is not beneficial for lesions in squamous cell because of the deletion of Orange G in MUFP stain.

In 2011, Kamal M *et al* [17] did a study to find out the efficacy of the UFP stain for immediate diagnosis and to check specimen adequacy for USG guided FNACs. Group I comprised of 238 outpatient FNACs, groups II included 59 radiologically guided FNACs and group III had 50 cases of intraoperative cytology. Overall diagnosis was possible in 297 cases. The overall concordance rate was 98%. They concluded that 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.

In 2012, Choudhary P *et al* [22] did a study on 'Comparison of MUFP with the standard rapid Papanicolaou stain in cytology of various organs'. In their study 100 FNAC cases were studied. The cases included lymph node, thyroid, breast, salivary gland and soft tissues. Two smears were prepared and stained by MUFP and the rapid PAP stain. Scores were given on four parameters and Quality index was calculated. They concluded that quality index of MUFP smears was better compared to the rapid Pap stain in all the organs, and was statistically significant.

MATERIALS AND METHODS

Source of data:

Patients who were referred for FNAC of various lesions to cytology section, in the Department of Pathology, B.L.D.E.U's. Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapur were included.

Study period – 1st November 2013 to 31st July 2015.

Method of Collection of data:

Patients referred for FNA of various lesions sent to the cytology section of the Department of Pathology for cytological evaluation were included.

FNAC procedure was performed in our laboratory by using Cameco syringe pistol with 10ml disposable syringe and 22-23G needle and multiple smears were prepared.

A total of 2 smears were made on clean glass slides of which one smear was fixed in 95% ethanol for minimum 15 minutes. This smear was submitted for conventional PAP stain and the other smear was air dried and rehydrated with normal saline and was subsequently fixed in alcoholic formalin and stained by MUFP stain.

STAINING PROCEDURES OF CONVENTIONAL PAP AND MUFP

PAPANICOLAOU METHOD[4,20]

REAGENTS REQUIRED :

1. Harris Haematoxylin (Without acetic acid)
2. OG 6

- 0.5 OG in 95% alcohol 100 ml
- Phosphotungstic acid 0.15g.

3. EA 36

- 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. Fix smears (while still moist) in 95% alcohol – 15 minutes
2. Rinse smears in distilled water.
3. Stain in Harris haematoxylin for 4 minutes
4. Wash in tap water for 1-2 minutes
5. Differentiate in acid alcohol (25% HCL in 70% alcohol).
6. Blue in tap water or 1.5% sodium bicarbonate.
7. Rinse in distilled water.
8. Transfer to 70% alcohol, then 95% alcohol for few seconds.
9. Stain in OG 6 for 1-2 minutes
10. Rinse in 3 changes of 95% alcohol for few seconds.
11. Stain in EA 50 for 3 – 5 minutes
12. Rinse in 3 changes of 95% alcohol for few seconds.

MUFP METHOD:[7,22]

REAGENTS REQUIRED IN MUFP STAIN:

1. Normal Saline
2. Alcoholic Formalin- (pH 5)

- 300 ml of 40% Formalin
- 2053 ml of 95% Alcohol
- 647 ml of Distilled water

3. Harris Hematoxylin

4. 95% Alcohol
5. 100% Alcohol
6. Tap water
7. EA- 50
8. Xylene

FIXATION:

1. Air dry smear for less than 30 minutes
2. 0.9% Normal Saline for 30 seconds
3. Alcoholic Formalin for 10 seconds

TECHNIQUE:

1. Tap water (6 slow dips)
2. Harris hematoxylin (30 seconds)
3. Tap water (6 slow dips)
4. Ethyl alcohol 95% (6 dips)
5. EA-36 (15 seconds)
6. Ethyl alcohol 95% (6 dips)
7. Ethyl alcohol 100% (6dips)
8. Xylene (10 slow dips)
9. DPX
10. Mount with cover slip.

SCORING:

Staining quality was assessed for parameters like smear-background, overall staining, nuclear, and cytoplasmic staining by giving the following scores.

Background

- Clean : 2
- Hemorrhagic : 1

Overall Staining

- Good- 3
- Moderate- 2
- Bad- 1

Cell Morphology

- Well preserved and crisp- 3
- Moderately preserved- 2
- Poorly preserved- 1

Nuclear Characteristics:

- Good- 3
- Moderate- 2
- Poor- 1

TOTAL SCORE: 11

The maximum score for each case, taking into account all the four parameters, was 11.

The "Quality Index" for each case with both the stains was then obtained by calculating the ratio of actual score to the maximum score possible.

Quality Index= actual score obtained /maximum score possible

Quality Index for the two stains of the various lesions was compared.

Sample size:

The mean +/- SD index of MUFP obtained in a study conducted by Choudhary P *et al* [22] was 0.97 +/- 0.704 and in PAP staining it was 0.90+/- 0.086.Using mean and standard deviation of MUFP and PAP staining, with 99% confidence interval and 90% power of the study,

The calculated sample size was 130 using the following statistical formula:

$$n = \frac{(Z \quad + Z \;)^2 \times 2SD^2}{d^2}$$

Z - Z value for 99%

Z -Z value for 90%

SD- common standard deviation between two groups =0.15

d- difference between two mean values.

Hence 131 samples were included in the study to compare MUFP stain with conventional PAP stain.

Statistical methods:

1. Students "t'' test and Chi- square test was applied to evaluate the efficacy of MUFP in comparison with conventional PAP stain.
2. Diagramatic representation of the data.

Inclusion Criteria:

All patients referred to the Department of Pathology for cytological evaluation during the study period were included.

Exclusion Criteria:

Cytological samples of cervico-vaginal (gynecological) smears, FNAC smears with inadequate material and only hemorrhagic material were excluded.

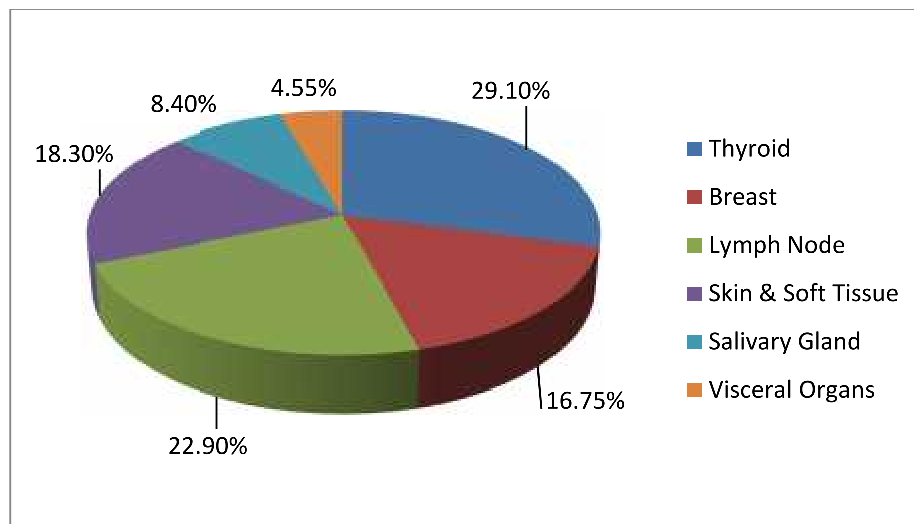
RESULTS

A total of 131 cases were studied in the time period between 1st November 2013- 31th July 2015.These cases were each stained by both MUFP and conventional PAP stain and the cytomorphological characteristics of both stains were then compared.

TABLE 1: Organ Wise Distribution Of Cases Studied

S. No	Organs	Number of cases	Percentage
1	Thyroid	38	29.10%
2	Breast	22	16.75%
3	Lymph Node	30	22.90%
4	Skin & Soft Tissue	24	18.30%
5	Salivary Gland	11	8.40%
6	Visceral Organs	6	4.55%

DIAGRAM 1: Pie Chart Showing Organ Wise Distribution Of Cases Studied

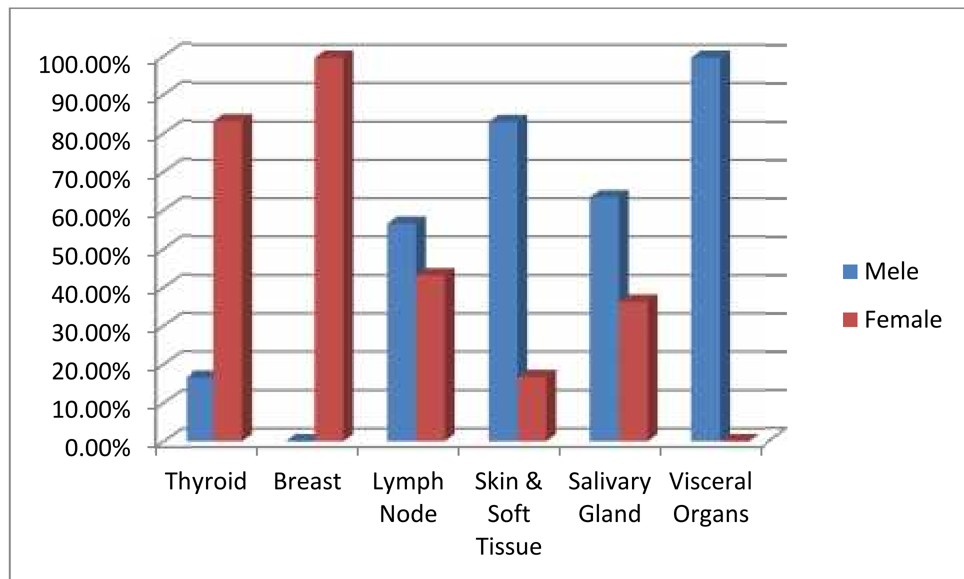


Thyroid cases were the most common lesions accounting for 29.10% of the total cases. This was followed by lymph node 22.9%, skin & soft tissue 18.3% and the least common were the visceral organs accounting for only 4.55% of the cases.

TABLE 2- Gender Wise Distribution Of Cases Studied

Sex	Thyroid	Breast	Lymph Node	Skin & Soft Tissue	Salivary Gland	Visceral Organs
Male	16.50%	0.00%	56.70%	83.30%	63.60%	100%
Female	83.50%	100%	43.30%	16.70%	36.40%	0.00%

DIAGRAM 2: Bar diagram showing gender wise distribution

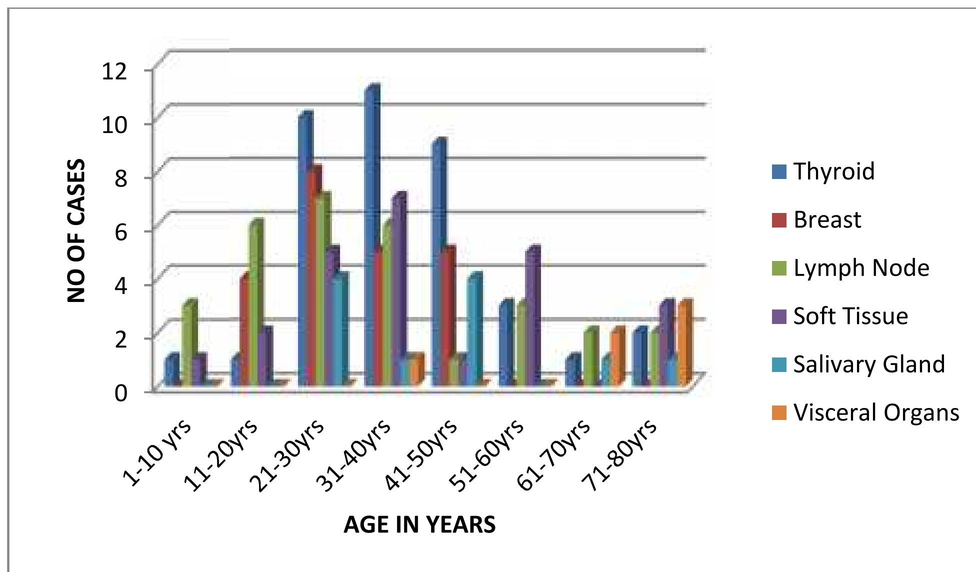


Thyroid lesions were more common in females (83.5%). Lymph node lesions (56.7%), salivary gland (63.6%) and soft tissue lesions (83.3%) were all more common in males.

TABLE 3- Age wise distribution Of Cases Studied

Age in yrs	Thyroid (n=38)	Breast (n=22)	Lymph Node (n=30)	Skin & Soft Tissue (n=24)	Salivary Gland (n=11)	Visceral Organs (n=6)
1-10	1(2.64%)	0 (0%)	3 (10.00%)	1 (4.16%)	0	0
11-20	1 (2.64%)	4 (18.18%)	6 (20.00%)	2 (8.33%)	0	0
21-30	10 (26.32%)	8 (36.36%)	7 (23.33%)	5 (20.85%)	4 (36.37%)	0
31-40	11 (28.94%)	5 (22.73%)	6 (20.00%)	7 (29.15%)	1 (9.09%)	1 (16.67%)
41-50	9 (23.67%)	5 (22.73%)	1 (3.33%)	1 (4.16%)	4 (36.36%)	0
51-60	3 (7.89%)	0	3 (10.00%)	5 (20.85%)	0	0
61-70	1 (2.64%)	0	2 (6.67%)	0	1 (9.09%)	2 (33.33%)
71-80	2 (5.26%)	0	2 (6.67%)	3 (12.50%)	1 (9.09%)	3 (50.00%)

DIAGRAM 3: Bar diagram showing age wise distribution



- Maximum number of cases were in the age group of 21-30 years.
- For thyroid lesions and skin & soft tissue lesions the distribution was seen maximum in the age group of 31-40 years.

- Distribution of cases for breast and lymph node was between the age group of 21-30 years
- For salivary gland lesions a bimodal distribution in the age groups of 21-30 years and 41-50 years was observed.
- For visceral organs elderly age group of 71-80 years was observed.

TABLE 4- Lesion Wise Distribution Of Thyroid Cases (n=38)

Lesions	Number of cases
Nodular Goitre	22
Hashimotos thyroiditis	05
Suspicious for malignancy	04
Papillary carcinoma	04
Follicular neoplasm	03

TABLE 5-Comparison of Staining Quality of MUFP & PAP Stain In Thyroid Cases (n=38)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	36 (94.73%)	4(15.79%)
	Hemorrhagic	2 (5.27%)	32 (84.21%)
Overall Staining	Good	23 (60.53%)	14 (36.84%)
	Moderate	15 (39.47%)	23 (60.53%)
	Bad	0	1 (2.63%)
Cell Morphology	Well preserved	31 (81.58%)	30 (78.95%)
	Moderately preserved	7 (18.42%)	8 (21.05%)
	Poorly preserved	0	0
Nuclear Character	Good	16 (42.11%)	32 (84.21%)
	Moderate	22 (57.89%)	6 (15.79%)
	Poor	0	0

In the overall 38 thyroid cases, hemorrhagic background was seen in only 5.27% cases of MUFP and 84.21% cases of PAP stain. Clean background was observed in 94.73% cases of MUFP and 15.79% in the PAP stain. Nuclear characteristics with crisp chromatin was seen in 42.11% cases of MUFP and 84.21% cases of PAP 57.89% cases of MUFP showed moderately crisp chromatin.

TABLE 6- Lesion Wise Distribution Of Breast Cases (n=22)

Lesion	Number of cases
Mastitis	03
Fibroadenoma	10
Phyllodes tumour	03
Infiltrating Ductal Carcinoma	05
Infiltrating Lobular Carcinoma	01

TABLE 7 - Comparison of Staining Quality of MUFP & PAP Stain In Breast Cases (n=22)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	22(100%)	12 (54.55%)
	Hemorrhagic	0	10 (45.45%)
Overall Staining	Good	8 (36.35%)	9 (40.90%)
	Moderate	13 (59.10%)	12 (54.55%)
	Bad	1 (4.55%)	1 (4.55%)
Cell Morphology	Well preserved	14 (63.64%)	18 (81.81%)
	Moderately preserved	8 (36.36%)	3 (13.64%)
	Poorly preserved	0	1 (4.55%)
Nuclear Character	Good	9 (40.91%)	18 (81.81%)
	Moderate	13 (59.09%)	4 (18.19%)
	Poor	0	0

Out of the 22 cases of breast FNAC, background was hemorrhagic in 45.45% cases of PAP stain whereas clean background was observed in 100% cases of MUFP. Overall staining was good in 36.35% cases of MUFP and 40.90% cases of PAP stain. Nuclear characteristics with crisp chromatin was seen in 40.91% cases of MUFP and 81.81% cases of PAP stain.

TABLE 8- Lesion Wise Distribution of Lymph Node Cases (n=30)

Lesion	Number of cases
Reactive lymphadenitis	18
Granulomatous lymphadenitis	05
Metastatic carcinomas (Squamous cell carcinoma- 2, Adenocarcinoma- 2)	04
Non Hodgkins Lymphoma	02
Hodgkins Disease	01

TABLE 9 - Comparison of Staining Quality of MUFP & PAP Stain In Lymph Node Cases (n=30)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	26 (86.67%)	13 (43.33%)
	Hemorrhagic	4 (13.33%)	17 (56.67%)
Overall Staining	Good	18 (60.00%)	4 (13.32%)
	Moderate	12 (40.00%)	25 (83.35%)
	Bad	0	1 (3.33%)
Cell Morphology	Well preserved	24 (80.00%)	19 (63.27%)
	Moderately preserved	6 (20.00%)	11 (36.73%)
	Poorly preserved	0	0
Nuclear Character	Good	15 (50.00%)	23 (76.59%)
	Moderate	14 (46.67%)	7 (23.41%)
	Poor	1 (3.33%)	0

Clean background was observed in 86.67% cases of MUFP stain and 43.33% cases of PAP stain. Overall staining was good in 60% cases of MUFP and 13.32%cases of PAP stain .Most of the cases stained by PAP stain had moderate staining accounting for 83.35% cases. Cell morphology was well preserved in 80% cases of MUFP stain and 63.27% cases of PAP stain. Nuclear characteristics were crisp and good in 50% of MUFP stain and 76.59% cases of PAP stain.

TABLE 10- Lesion Wise Distribution Of Skin & Soft Tissue Cases(n=24)

Lesions	Number of cases
Lipoma	11
Suppurative lesion	04
Epidermal/sebaceous cyst	06
Positive for malignancy	03

TABLE 11 - Comparison Of Staining Quality Of MUFP & PAP Stain In Skin & Soft Tissue Cases (n=24)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	20 (83.32%)	13 (54.17%)
	Hemorrhagic	4 (16.68%)	11 (45.83%)
Overall Staining	Good	8 (33.33%)	6 (25.00%)
	Moderate	16 (66.67%)	15 (62.50%)
	Bad	0	3 (12.50%)
Cell Morphology	Well preserved	14 (58.33%)	19 (79.17%)
	Moderately preserved	10 (41.67%)	5 (20.83%)
	Poorly preserved	0	0
Nuclear Character	Good	8 (33.33%)	20 (83.83%)
	Moderate	16 (66.67%)	4 (16.17%)
	Poor	0	0

Out of the 24 cases of skin & soft tissue, clean background was observed in 83.32% cases of MUFP stain and 54.17 % cases of PAP stain. Overall staining was good in 33.33 % cases of MUFP with poor staining observed in 12.5 % cases of PAP stain. Cell morphology was well preserved in 58.33% cases of MUFP stain and 79.17% cases of PAP stain. Nuclear characteristics were crisp and good in 33.33% of MUFP stain and 83.83% cases of PAP stain.

TABLE 12: Lesion Wise Distribution Of Salivary Gland (n=11)

Lesion	Number of cases
Sialadenitis	07
Positive for malignancy	02
Pleomorphic adenoma	02

TABLE 13 - Comparison of Staining Quality Of MUFP & PAP Stain In Salivary Glands Cases (n=11)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	11 (100.00%)	5 (45.45%)
	Hemorrhagic	0	6 (54.55%)
Overall Staining	Good	6 (54.55%)	5 (45.45%)
	Moderate	5 (45.45%)	6 (54.55%)
	Bad	0	0
Cell Morphology	Well preserved	9 (81.81%)	9 (81.81%)
	Moderately preserved	2 (18.19%)	2 (18.19%)
	Poorly preserved	0	0
Nuclear Character	Good	8 (72.73%)	8 (72.73%)
	Moderate	3 (27.27%)	3 (27.27%)
	Poor	0	0

Clean background was observed in 100% cases of MUFP stain and 45.45 % cases of PAP stain. Overall staining was good in 54.55 % cases of MUFP and 45.45 % cases of PAP stain .Cell morphology was well preserved in 81.81% cases of both MUFP & PAP stain. Nuclear characteristics were crisp and good in 72.73% of MUFP stain as well as PAP stain.

TABLE 14: Lesion Wise Distribution Of Visceral Organs Cases (n=06)

Lesion	Number of cases
Hepatocellular Carcinoma	04
Poorly differentiated carcinoma- Lung	01
Poorly differentiated adenocarcinoma -Intra Abdominal mass	01

TABLE 15– Comparison Of Staining Quality Of MUFP & PAP Stain In Visceral Organs Cases (n= 06)

CYTOMORPHOLOGICAL PARAMETERS		MUFP	PAP
Background	Clean	5 (83.33%)	1 (16.67%)
	Hemorrhagic	1 (16.67%)	5 (83.33%)
Overall Staining	Good	2 (33.33%)	3 (50.00%)
	Moderate	4 (66.67%)	3 (50.00%)
	Bad	0	0
Cell Morphology	Well preserved	3 (50.00%)	3 (50.00%)
	Moderately preserved	3 (50.00%)	3 (50.00%)
	Poorly preserved	0	0
Nuclear Character	Good	0	5 (83.83%)
	Moderate	6(100%)	1 (16.17%)
	Poor	0	0

USG guided FNAC was done in all the six cases of visceral organs. Four cases were liver masses with a diagnosis of Hepatocellular carcinoma, one lung mass with an impression of poorly differentiated carcinoma and a case of intra abdominal mass was diagnosed as poorly differentiated adenocarcinoma. Clean background was observed in 83.33% cases of MUFP stain and 16.67 % cases of PAP stain. Overall staining was good in 33.33 % cases of MUFP and 50% cases of PAP stain.Nuclear characteristics were crisp 83.83% cases of PAP stain and moderate in 100% cases of MUFP stain.

TABLE 16 - Correlation Of Background Of Both Stains (n=131)

BACKGROUND	MUFP	PAP
Clean	120 (91.60%)	87 (66.41%)
Hemorrhagic	11 (8.40%)	44 (33.59%)

A clean background was seen in 91.6% cases of MUFP stain as compared to only 66.41% cases of PAP stain making the difference statistically significant.
Based on total units (131 of MUFP + 131 of PAP) 262 samples were considered for significance

Test Applied	Value	P value	Difference
Chi square test	25.0609	<0.01	Significant

TABLE 17 - Correlation Of Over All Staining Of Both Stains (n=131)

OVERALL STAINING	MUFP	PAP
Good	65 (49.62%)	41 (31.30%)
Moderate	65 (49.62%)	84 (64.12%)
Bad	1 (0.76%)	6 (4.58%)

Based on total units (131 of MUFP + 131 of PAP) 262 samples were considered for significance

Test Applied	Value	P value	Difference
Chi square test	11.482	<0.001	Significant

TABLE 18 - Correlation of Cell Morphology Of Both Stains (n=131)

CELL MORPHOLOGY	MUFP	PAP
Well preserved	95 (72.52%)	98 (74.81%)
Moderately preserved	36 (27.48%)	32 (24.43%)
Poorly preserved	0	1 (0.76%)

Cell morphology was well preserved in both cases of MUF and PAP stain making the statistical difference insignificant in case of cell morphology.

Based on total units (131 of MUFP + 131 of PAP) 262 samples were considered for significance

Test Applied	Value	P value	Difference
Chi square test	1.281	0.52	Not Significant

TABLE 19- Correlation Of Nuclear Characteristics Of Both Stains (n=131)

NUCLEAR CHARACTER	MUFP	PAP
Good	56 (42.75%)	106 (80.92%)
Moderate	74 (56.49%)	25 (19.08%)
Bad	1 (0.76%)	0

Based on total units (131 of MUFP + 131of PAP) 262 samples were considered for significance

Test Applied	Value	P value	Difference
Chi square test	40.68	<0.001	Significant

TABLE 20 -Mean QI Of All Cases

ORGANS	QI of MUFP	QI of PAP
Thyroid	0.889	0.81
Breast	0.85	0.854
Lymph Node	0.883	0.803
Skin & Soft Tissue	0.825	0.833
Salivary Gland	0.918	0.854
Visceral Organs	0.788	0.811

QI was calculated for all the slides of each organ and then mean of these was further calculated.

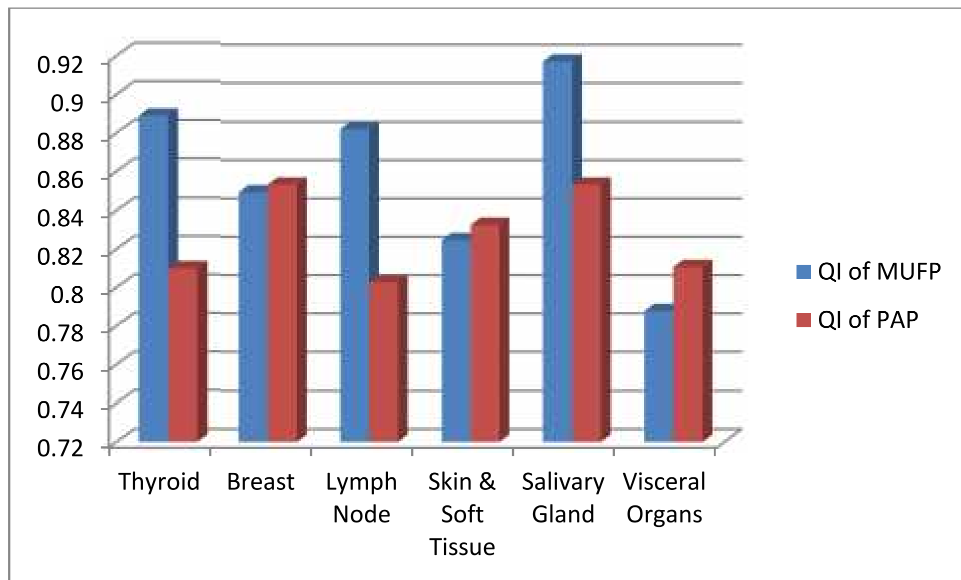
Before calculating the mean, statistics was applied first to specific organs and then inter organ comparison was calculated.

Students't' test was applied to evaluate the two independent Means with a significance level of 0.10 and a two tailed hypothesis.

The results were as follows:

T value	P value	Result
1.458373	0.08771 ($p < 0.10$)	Significant

DIAGRAM 4: Bar Diagram Showing Mean QI Of All Cases



ORGAN WISE QI FOR BOTH STAINS:

Thyroid – MUFP > PAP

Breast– MUFP ~ PAP

Lymph Node – MUFP > PAP

Skin & Soft Tissue– MUFP ~ PAP

Salivary Gland – MUFP > PAP

Visceral Organs- MUFP < PAP

Of both the stains we did, higher QI was seen in MUFP stain as compared to conventional PAP stain except for in cases of visceral organs.

PHOTOMICROGRAPHS

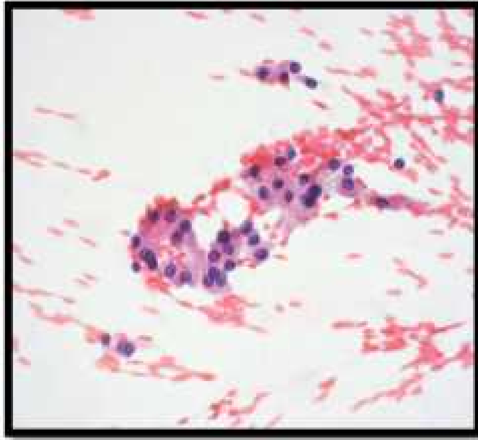


Fig1- Photomicrograph of Hashimotos thyroiditis on hemmorhagic background, PAP stain, 100X

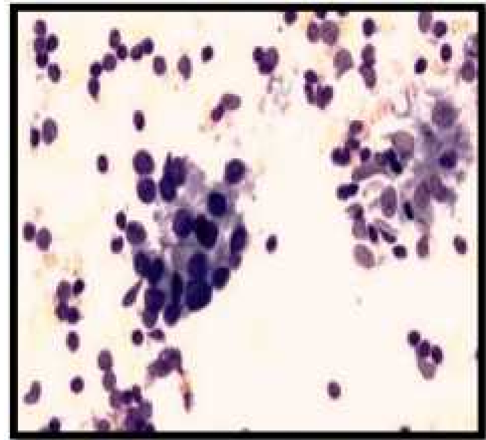


Fig2- Photomicrograph of Hashimotos thyroiditis on clean background, MUFP stain, 100X

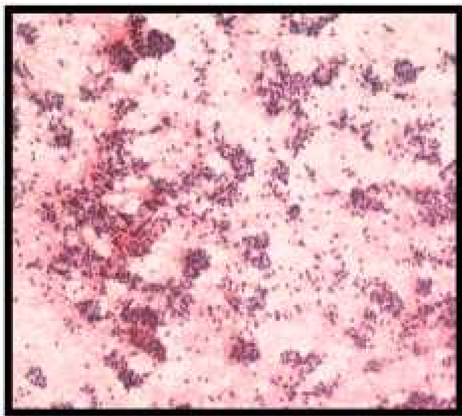


Fig3- Photomicrograph of Follicular Neoplasm, PAP stain, 100X.

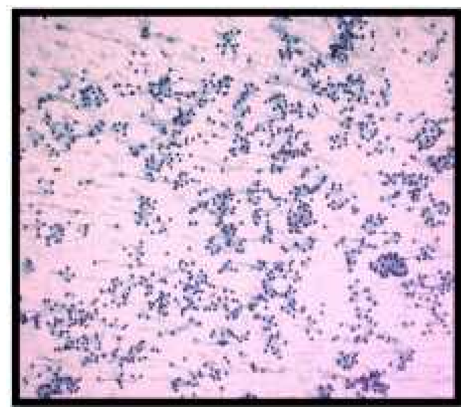
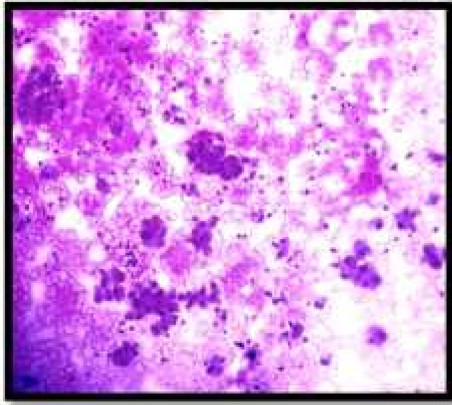


Fig4- Photomicrograph of Follicular Neoplasm on a cleaner background, MUFP stain, 100X.



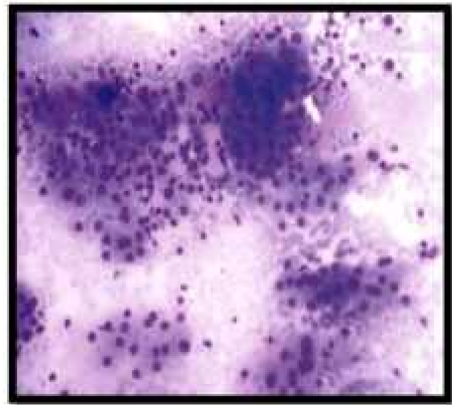
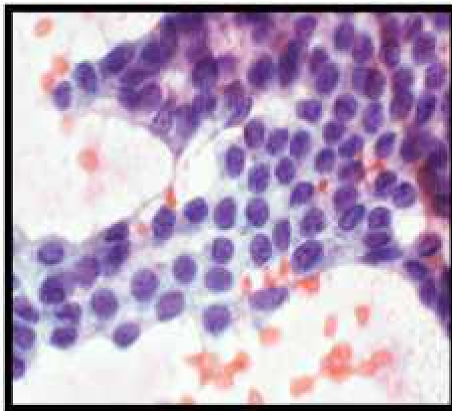


Fig5- Photomicrograph of Follicular Neoplasm on hemorrhagic background, PAP stain, 200X

Fig6 - Photomicrograph of Follicular Neoplasm on a clean background, MUFP stain, 200X



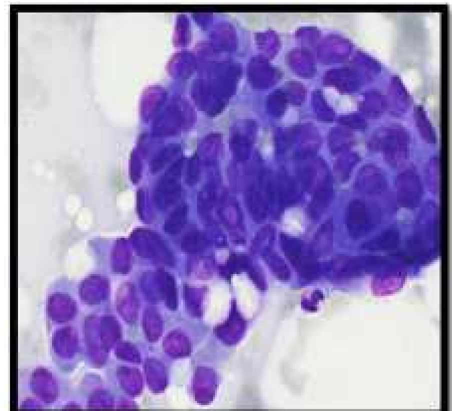


Fig7- Photomicrograph of Papillary carcinoma of thyroid showing prominent nuclear grooves, PAP stain, 600X

Fig8 - Photomicrograph of Papillary carcinoma of thyroid, MUFP stain, 600X

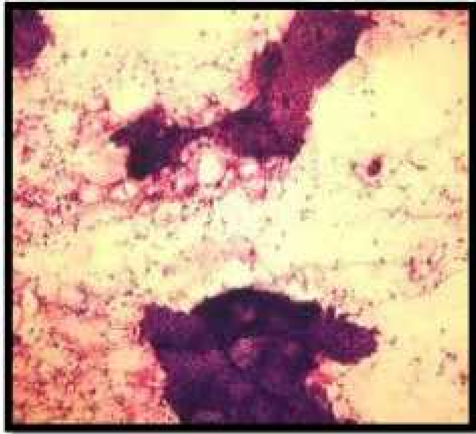


Fig9- Photomicrograph of fibroadenoma of breast, PAP stain, 100X

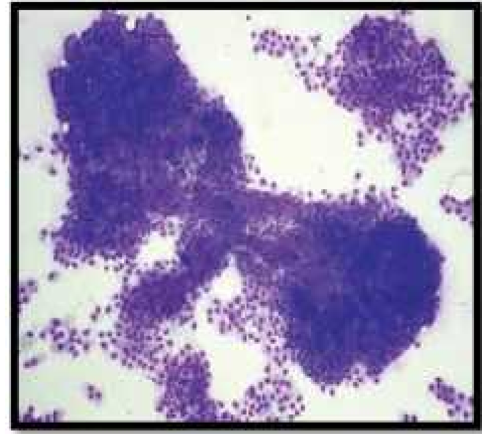


Fig 10 - Photomicrograph of fibroadenoma of breast, MUFP stain, 100 X

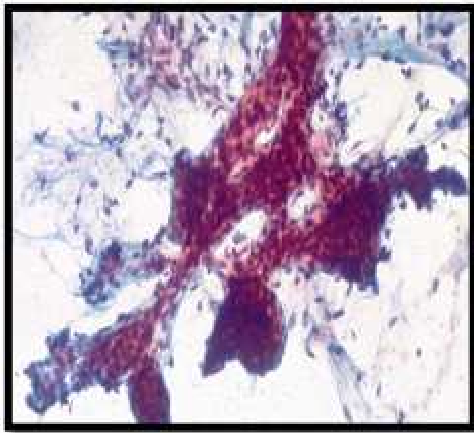


Fig 11- Photomicrograph of fibroadenoma of breast, PAP stain, 200X

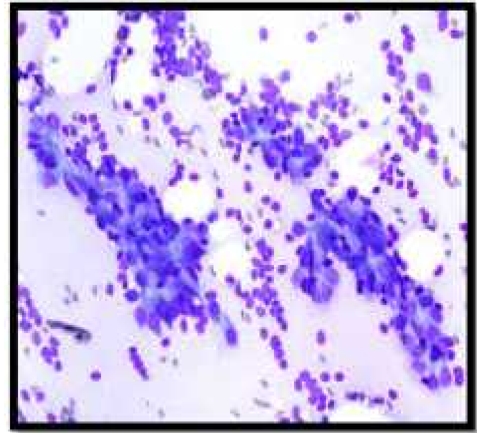
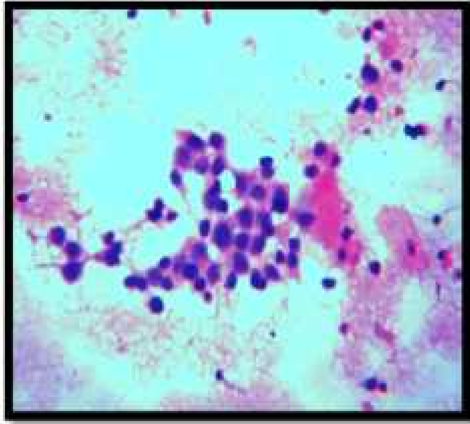


Fig 12 - Photomicrograph of fibroadenoma of breast, MUFP stain, 200 X



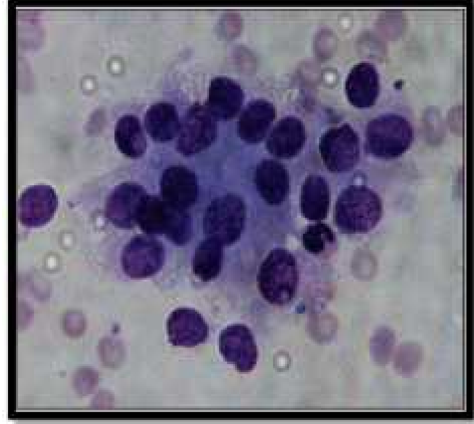
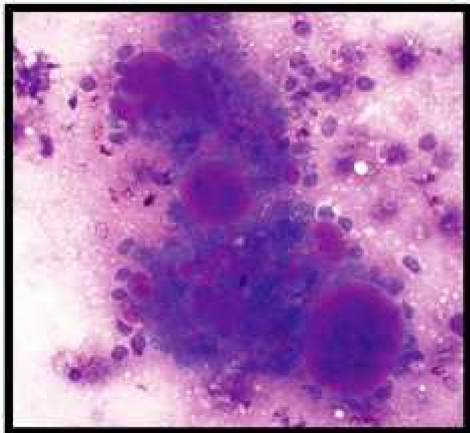


Fig 13- Photomicrograph of IDC of breast, PAP stain, 100x

Fig 14 - Photomicrograph of IDC of breast, MUFP stain, 200 x



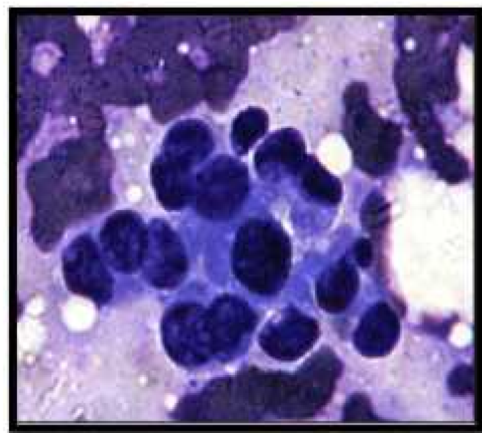
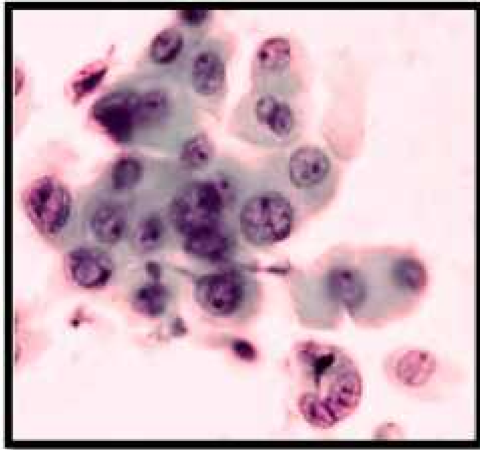


Fig 15- Photomicrograph of IDC of breast, PAP stain, 600X

Fig 16 - Photomicrograph of IDC of breast, MUFP stain, 600X



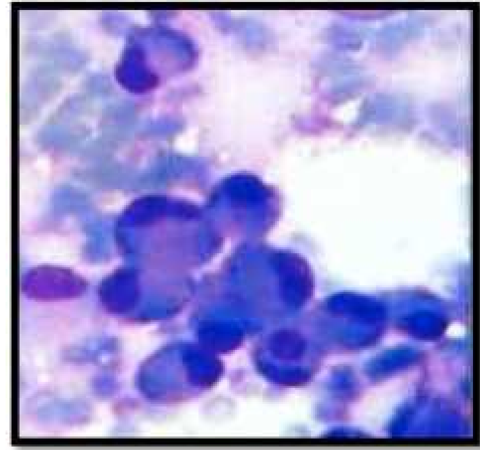
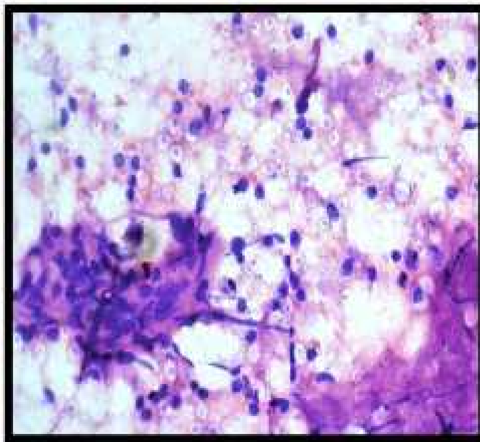


Fig 17- Photomicrograph of invasive lobular carcinoma showing prominent and crisp nuclear features, PAP stain, 400X

Fig 18 - Photomicrograph of invasive lobular carcinoma, nuclear features not well preserved, MUFP stain, 400X



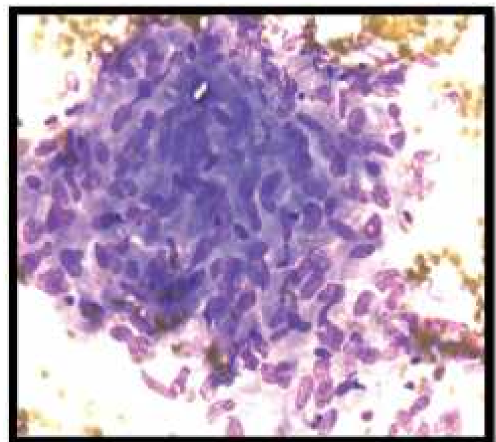
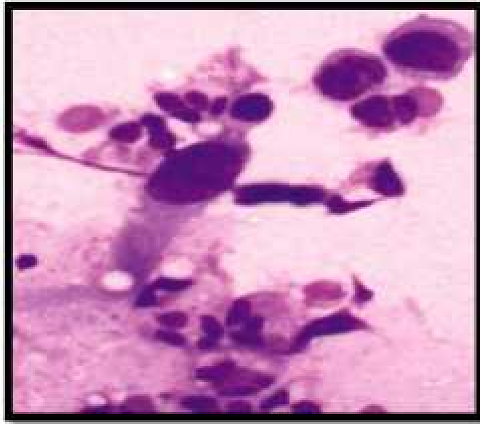


Fig 19- Photomicrograph of tuberculous lymphadenitis, PAP stain, 400X

Fig 20- Photomicrograph of tuberculous lymphadenitis, MUFP stain, 400X



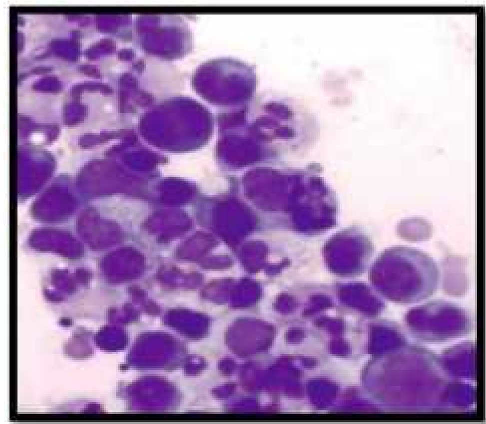
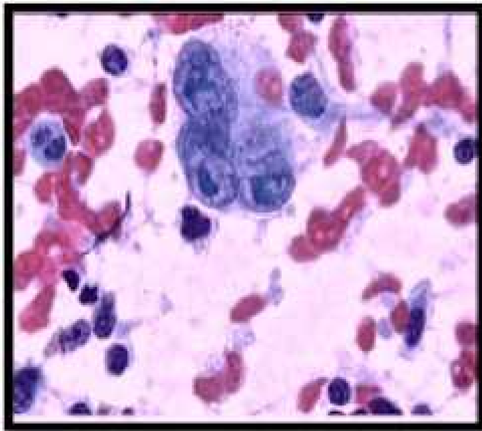


Fig 21- Photomicrograph of NHL of lymph node, PAP stain, 400X

Fig 22 - Photomicrograph of NHL of lymph node, MUFP stain ,400X



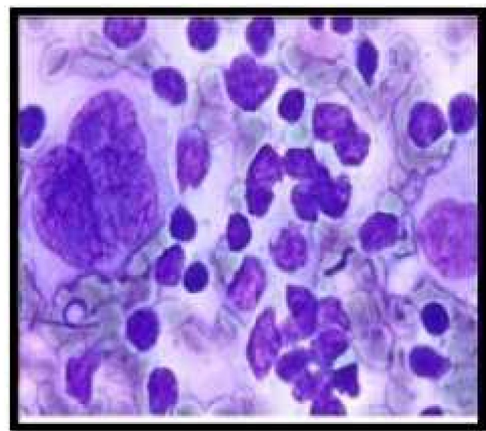
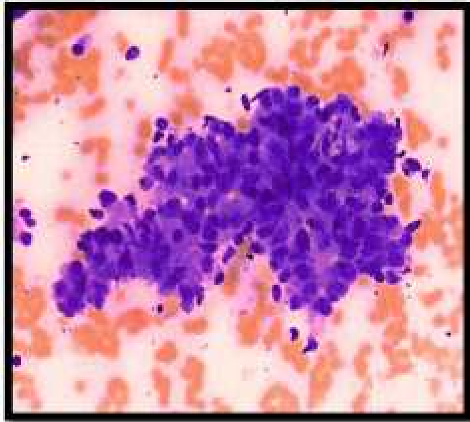


Fig 23- Photomicrograph showing Reed Sternberg cell in Hodgkins disease of lymph node, Nuclear features prominent, PAP stain, 400X

Fig 24 - Photomicrograph showing Reed Sternberg cell in Hodgkins disease of lymph node, with indistinct nuclear features, MUFP stain, 400X



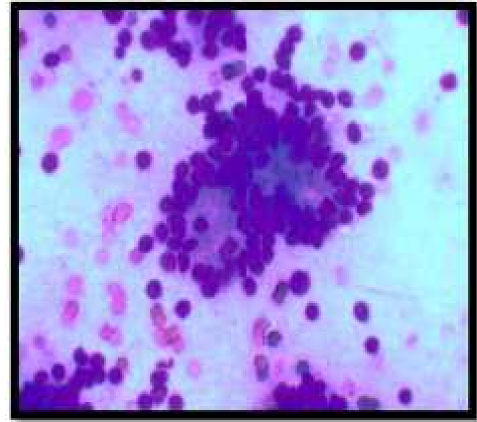
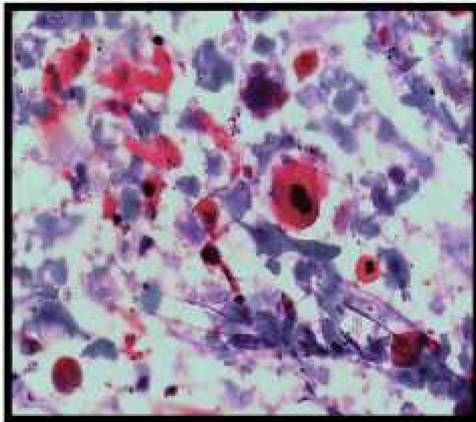


Fig 25- Photomicrograph showing acinar pattern of metastatic adenocarcinoma lymph node on RBC background, PAP stain, 100X

Fig 26 - Photomicrograph showing acinar pattern of metastatic adenocarcinoma lymph node, MUFP stain, 100X



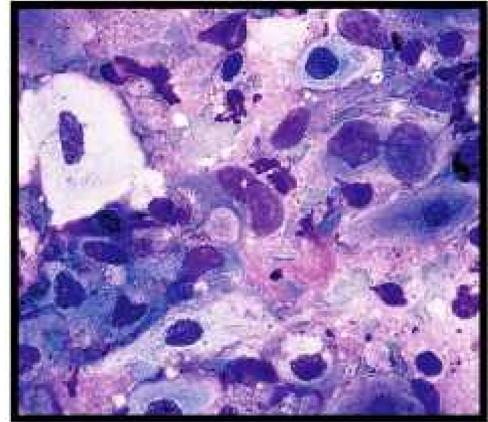
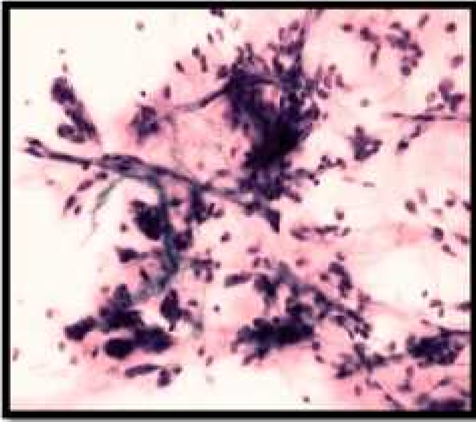


Fig 27- Photomicrograph of metastatic squamous cell carcinoma, lymph node, having distinct cytoplasmic keratinization, PAP stain, 400X

Fig 28 - Photomicrograph showing metastatic squamous cell carcinoma of lymph node, orangiophilia is lost, MUFP stain 400X



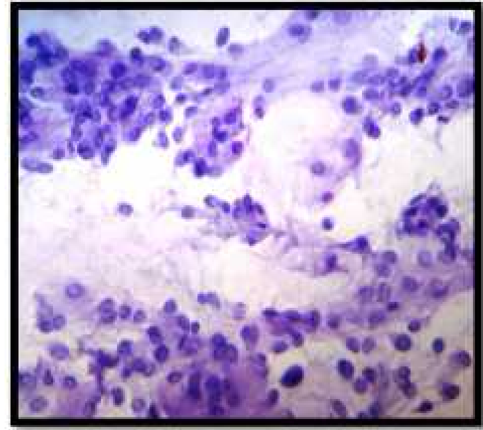
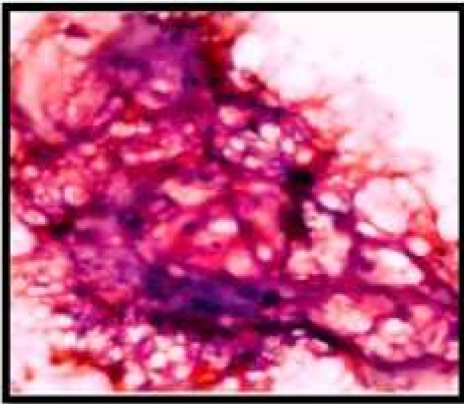


Fig 29- Photomicrograph showing Pleopmorphic adenoma, parotid gland, PAP stain, 100X

Fig 30 - Photomicrograph showing Pleomorphic adenoma, parotid gland, MUFP stain, 100X



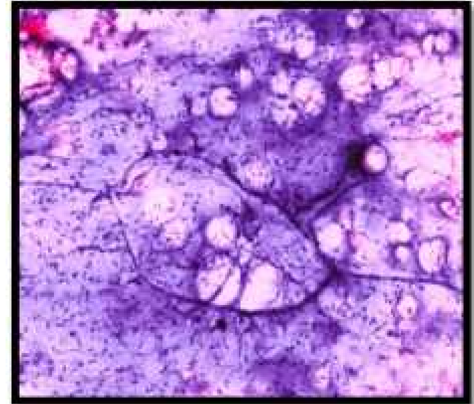
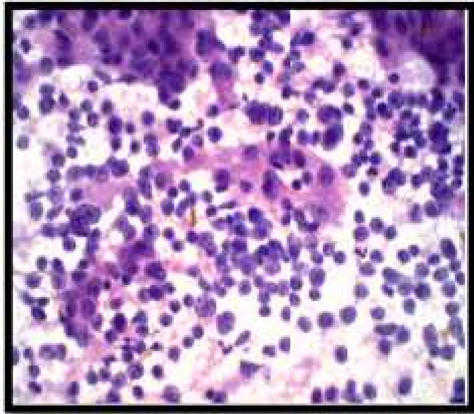


Fig 31- Photomicrograph showing lipoma on a hemorrhagic background with cell loss due to wet fixation, PAP stain, 100X

Fig 32 - Photomicrograph showing lipoma having spindle cells and preservation of cells, MUFP stain, 100X



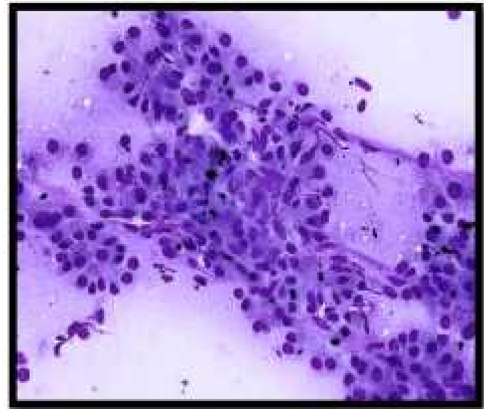
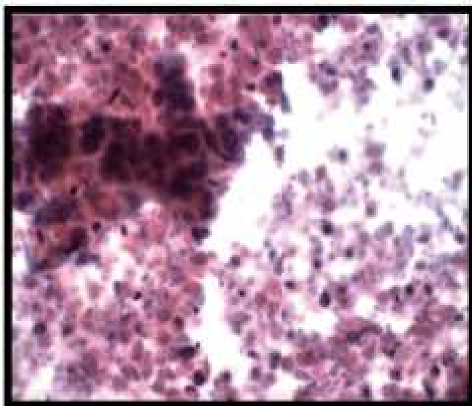


Fig 33- Photomicrograph showing Hepatocellular carcinoma with prominent nuclear featuers, PAP stain, 200X

Fig 34 - Photomicrograph showing Hepatocellular carcinoma, nuclear features not prominent, MUFP stain, 200X



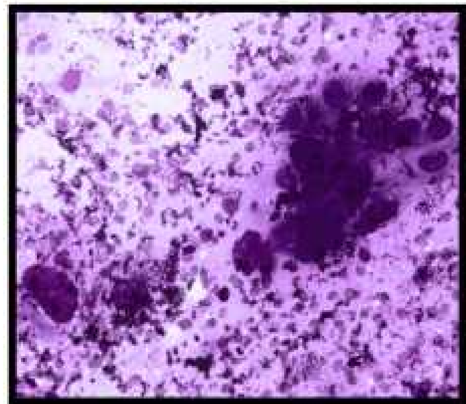


Fig 35- Photomicrograph showing poorly differentiated carcinoma on a dirty necrotic background, PAP stain, 200X

Fig 36- Photomicrograph showing poorly differentiated carcinoma with necrotic debris, MUFP stain, 200X

DISCUSSION

Need for rapid diagnostic assessment has encouraged innovations in staining techniques that require less time and also give unequivocal cell morphology. Since its inception, conventional PAP stain remains the traditional and preferred stain, not only for the gynecological cytology, but also for FNAC studies. The traditional PAP stain involves wet fixation and then subsequent staining, requiring at least 40 minutes. To cut down the time, the rapid PAP stains were developed. However, the quality of rapid PAP stains was not satisfactory, as the cell morphology was not well preserved.[2,3]

To overcome these problems, Yang and Alvarez [6] in 1956, developed UFP stain which was a hybrid of Papanicolaou and Romanowsky stains. The staining time of UFP was 90 seconds. This provided a blood free background that unmasks the cellular material for morphologic evaluation. The cells were larger than wet fixed cells, flatter and more transperant. Air drying makes the cells flat and allows them to stick firmly to the glass slide. The physical hurry for immediate fixation was no longer essential.

Kamal *et al* [7]from India further modified the UFP stain to overcome the problem of shortage of Richard-Allan hematoxylin and Richard-Allan cytostain in Indian set-up. Thus MUFP stain uses locally available reagents with unequivocal cytomorphology and also has a short staining time of 130 seconds. In the present study we compared the cytomorphological characteristics of rehydrated air dried smears stained by MUFP stain with ethyl alcohol fixed smears stained by conventional PAP stain.

Shinde *et al* [21]discussed about the observations they made in connection to MUFP staining. They said that complete air drying revealed a clean, RBC free

background. Kamal *et al* [17] also showed that the problem of air drying artifacts in wet fixed smears, can be reduced by rehydration of air dried smears as in MUFP. Maruta *et al* [27] showed that MUFP stain makes the background hemorrhage free by lysing the RBCs. This also makes the smear thinner and clearer for cytomorphologic observation.

In our study in MUFP stain clean background was seen in 91.6 % cases as compared to 66.4 % cases in wet fixed smears PAP stain. The P value <0.001 was calculated by using chi-square test which proved the difference to be statistically significant. These findings are comparable to findings of studies done by Kamal *et al* [17], Shinde *et al* [21] and Maruta *et al* [27]. Thus our study also showed that air-dried smears rehydrated within 30 minutes with normal saline for about 30 seconds provide cleaner and RBC free background compared to the wet fixed PAP smears.

Also the timing of rehydration after complete air drying was very important and is highlighted in various studies. It was observed that for optimal cell preservation and complete hemolysis, it was best if air dried smears were rehydrated within 30 minutes for 30 seconds. An optimum timing of 30 seconds in normal saline was quoted in studies by Maruta *et al* [27], Shinde *et al* [21]and Kamal *et al*[17]. This timing was strictly observed in our study and we got good results.

The rehydration technique, discovered by Chang and Kung[24] using normal saline restores the transparency of air-dried cells. In addition it hemolyses the RBC's in the smear and unmasks tumor cells. Cells were larger because of air-drying, nucleoli were distinctly seen and stain red. Thus our study shows that air-dried smears rehydrated with normal saline show cleaner, non-hemorrhagic background as compared to only air-dried smears and only wet fixed smears.

Yang and Alvarez[6] advised use of fresh saline for every case in order to avoid incomplete hemolysis of background red blood cells. Kamal *et al*[17]optimized the change of normal saline after every 5 smears. We followed the use of fresh normal saline in every case.

Yang and Alvarez [6] and Kamal *et al* [17] advised daily changing of alcoholic formalin. This solution is storage sensitive and a pH of 5 should be maintained. Prolonged immersion in this fixative affected cytomorphology and caused blurring and wrinkling of nuclei. In our study we followed daily changing of alcoholic formalin and maintained it at a pH of 5.

Shinde *et al* [21]and Kamal *et al*[17] observed that delay in changing hematoxylin caused faint staining of nuclei and thus affected morphology. They advised change of hematoxylin after 60 smears. We changed the solutions after every 30 smears and hence got good morphology and results.

Shinde *et al* [21]and Kamal *et al* [17] advised change of EA after processing of 60 smears.The cytoplasmic stain used in our study was an alcoholic mixture of eosin Y, light green, phosphotungstic acid and glacial acetic acid. This was similar to that used by Kamal *et al* [7,17] Shinde *et al* [21] and Choudhary *et al* [22] in their study. Yang and Alvarez [6] and Yang and Hoda[28] used Richard-Allan cytostain which was an alcoholic mixture of orange-G, eosin Y, light green and aniline blue. As there was lack of orange- G component in the cytostain of our study, it produced difficulties in interpretation of smears of squamous cell carcinoma. We observed that categorization of metastatic squamous cell carcinoma in lymph node cases was not confirmatory due to lack of cytoplasmic keratinization of the squamous cells. A similar problem for diagnosis of squamous cell carcinoma was noted by Shinde *et al* [21]and Kamal *et al* [17] and Choudhary *et al* [22] in their studies.

The quality of the two stains was evaluated on 4 parameters such as background, overall staining, cell morphology, nuclear characteristics in 131 samples collected. The quality index of lesions in the different organs namely; thyroid, breast, lymph node and salivary gland, skin & soft tissue and visceral organs of both the stains were calculated and then compared.

TABLE 21: COMPARISON OF QUALITY INDEX OF MUFP STAIN IN VARIOUS STUDIES

STUDIES	**SHINDE *et al* [21]**		**CHOUDHARY *et al* [22]**		**PRESENT STUDY**	
ORGANS	No. Of Cases	QI	No. Of Cases	QI	No. Of Cases	QI
Thyroid	7	0.98	25	1	38	0.89
Breast	16	0.92	23	0.97	22	0.85
Lymph node	15	0.98	43	0.98	30	0.89
Salivary gland	2	0.95	-	-	11	0.92
Soft tissue	-	-	09	1	24	0.83
Visceral organs	-	-	-	-	06	0.79

Shinde *et al* [21] calculated QI of four organs that is in lymphnode, thyroid, breast, and salivary gland. QI in their study was 0.98 for thyroid and lymph node, 0.92 for breast and 0.95 for salivary gland. However in our study it was 0.89 for thyroid and lymph node. This may be because the number of cases in their study was lesser which led to a high QI.

Choudhary *et al*[22] also calculated QI scores of MUFP in four organs and compared with rapid PAP stain. They found that MUFP smears have RBC free and

clean background. This helped them greatly in interpretation of thyroid lesions giving a QI of 1 for thyroid lesions.

QI of breast lesions was slightly lower in studies done by Shinde *et al* [21] and Choudhary *et al* [22].They explained that the index was lower due to suboptimal staining of single bipolar nuclei in the background in cases of fibroadenoma. However, myoepithelial cells in the clusters were well stained. Hence, it was possible to make a diagnosis of benign breast lesion. They were unable to explain the precise reason as to why the bipolar nuclei in the background were stained suboptimally. The stromal nature of these cells may be responsible for inadequate staining. Similar explanation may be the reason for low QI in breast lesions in our study. In our study out of the 22 cases of breast lesions, 10 cases were that of fibroadenoma, hence QI may be even lower.

Inability to obtain optimum staining even in the pleomorphic adenoma of salivary gland has been observed but well documented studies are not available. Kamal *et al* [17]claim that this could be a result of poor penetration of the cytostain and these modifications needs to be addressed in further studies. In our study out of the 11 cases of salivary gland lesions, only 2 cases were of pleomorphic adenoma and hence we obtained a comparatively better QI for this group.

In our study we compared lesions of visceral organs done by USG guided FNAC. We had six such cases. Four of these were diagnosed as Hepatocellular carcinomas, showing characteristic features on conventional PAP stain with prominent nuclear and nucleolar features. These as compared to MUFP stain had better nuclear morphology thus a better QI was obtained on PAP stain. One case of left lower lung mass in 70/M smoker was diagnosed as poorly differentiated squamous cell carcinoma with highly pleomorphic cells on a dirty and necrotic

background on both stains. Cytoplasmic keratisinsation even though was minimal, was observed on PAP stain making it eaiser to categorise it as squamous cell carcninoma. The case of abdominal mass in a 75/M where the origin could not be made out on imaging studies. It was diagnosed by us as poorly differentiated adenocarcinoma. Even in this case the nuclear features were better appreciated on conventional PAP stain. Thus in all cases of visceral organs PAP stain had a better QI than MUFP stain.

Most studies using MUFP stain have been done for malignant lesions particularly those of breast and thyroid. Very few studies address soft tissue or visceral organ lesions.[21]

MUFP stain gives a clean RBC-free background because of rehydration of air-dried smears by normal saline.[24] A better interpretation is possible, as the epithelial cells are not obscured by RBCs. Also, MUFP stain heightens the quality of nuclear characteristics, essential for cytological diagnosis. Hence, it is useful for rapid diagnosis except in squamous-cell lesions. [17,21,22].

CONCLUSION

- FNAC is a rapid, safe and cost effective technique for on-site cytological diagnosis.
- MUFP is a rapid stain, with staining time of 130 seconds and easily available reagents in Indian set up.
- MUFP smears showed cleaner background.
- Air drying artifacts are less in rehydrated MUFP smears as compared to wet fixed PAP smears.
- Since OG gives a dirty orange background, it is omitted from MUFP stain. As a result, the interpretation of cytoplasmic keratinisation is not possible.
- Nuclear crispness and prominent nucleolar features are better appreciated on conventional PAP stain as compared to MUFP stain.

LIMITATION OF MUFP:

- MUFP stain is technique sensitive. Complete air drying needs to be strictly observed. Inadequate drying gives suboptimal results.
- Alcoholic formalin is storage sensitive and the pH of alcoholic formalin should be maintained at 5.0 or it can lead to poor staining.
- Normal saline, Harris hematoxylin and EA-36 need to be changed regularly.

SUMMARY

The present study included 131 cases of FNAC which was carried over a period of 21 months in the Department of Pathology, BLDE University, Shri B.M.Patil Medical College, Vijayapur.

Patients referred for FNA of various lesions sent to the cytology section of the Department of Pathology for cytological evaluation were included. FNA procedure was performed in our laboratory by using Cameco syringe pistol with 10ml disposable syringe and 22-23G needle and multiple smears were prepared. A total of 2 smears were made on clean glass slides of which one smear was fixed in 95% ethanol for minimum 15 minutes. This smear was submitted for conventional PAP stain and the other smear was air dried and rehydrated with normal saline and was subsequently fixed in alcoholic formalin and stained by MUFP stain. Staining quality was assessed for parameters like smear-background, overall staining, nuclear, and cytoplasmic staining by giving scores. The maximum score for each case, taking into account all the four parameters, was 11.The "Quality Index" for each case with both the stains was then obtained by calculating the ratio of actual score to the maximum score possible.

Quality Index= actual score obtained /maximum score possible

Out of the 131 cases studied maximum cases in our cytology section were those of thyroid accounting for 29% of cases. Benign lesions were much more common than malignant ones and metastasis were rarely encountered. The most common lesion of thyroid was colloid goiter, that of breast was fibroadenoma and in lymph node it was reactive lymphadenitis.

Maximum age distribution was seen in the age group of 31-40 years. Slight female pre ponderance with female to male ratio of 1.5:1 was noted.

After analyzing each parameter separately for each stain and each organ the Quality Index for the two stains of the various lesions was conclusively compared.

The smears of these stains were compared and significance of difference was calculated by applying statistical tests to find the significance of study.

It was concluded on the basis of mean QI that:

- ❖ In thyroid MUFP stain had a much better quality index than PAP stain.
- ❖ In breast both stains were equally good in respect to their quality index.
- ❖ In lymph node and salivary gland MUFP stain had a better quality index than PAP stain.
- ❖ In cases of visceral organs PAP stain had a better quality index than that of MUFP stain.

Thus MUFP is fast, reliable & can be done with locally available reagents with unequivocal morphology which is the need of hour for a cytopathological set-up concluding that MUFP stain can be used as routine cytological stain.

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ANNEXURE-I

ETHICAL CLEARANCE

B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Application of Modified ultra-fast papanicolaou stain in cytological diagnosis" —x—x—

Name of P.G. student Dr. Prachi Sinkar, Department of pathology

Name of Guide/Co-investigator Dr Surekha Arakeri Professor of pathology.

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization

1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: APPLICATION OF MODIFIED ULTRA FAST PAPANICOAOU STAIN IN CYTOLOGICAL DIAGNOSIS.

PRINCIPAL INVESTIGATOR : DR.PRACHI SINKAR
P.G. DEPARTMENT OF PATHOLOGY

P.G.GUIDE : Dr. SUREKHA U. ARAKERI M.D
PROFESSOR,
DEPARTMENT OF PATHOLOGY

PURPOSE OF RESEARCH:

I have been informed that this study is done to know the efficacy of modified ultra fast Pap Stain over conventional Pap Stain in Cytological Diagnosis.

PROCEDURE: I understand that I will undergo detailed clinical history, thorough clinical examination and after which fine needle aspiration cytology will be performed and subjected to cytological examination.

RISK AND DISCOMFORTS:

I understand that, I may experience some pain and discomfort during the examination of the lesion or during FNAC. This is mainly the result of my condition and procedures of this study are not expected to exaggerate these feelings which are associated with usual course of treatment.

BENEFITS: I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the treatment.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed.

REQUST FOR MORE INFORMATION:

I understand that I may be asked more questions about the study at any time.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore I agree to participate in the present study.

______________________ ________________

Participant / Guardian Date:

______________________ ________________

Signature of Witness Date:

I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____________________ ________________

Dr. Prachi Sinkar Date:

_____________________ ________________

Witness to Signature Date

ANNEXURE-III

CASE PROFORMA

Name:	Age:
Sex:	IP/OP no:
Unit:	Cytology no:

Clinical presentation:

Past history:

Family history:

Personal history:

General physical examination:

Pallor	Lymphadenopathy
Icterus	Cyanosis
Clubbing	Oedema

Pulse rate:

Blood pressure:

Respiratory rate:

Systemic examination:

RS

CVS

Per abdomen

Clinical Diagnosis:

Cytomorphological features:

Cytology: Lesion: Site

Size

Number:

Nature of Aspirate:

Adequacy:

	Background	Overall staining	Cell morphology	Nuclear characteristics	Total Score
PAP					
MUFP					

Impression on cytology:

ANNEXURE-IV

KEY TO MASTER CHART

M-Male

F- Female

IDC-Infiltrating Ductal Carcinoma

ILC-Invasive Lobular Carcinoma

Chr Non Sp- Chronic Non Specific

LN-Lymph Node

LAD-Lymphadenitis

NHL-Non-Hodgkins Lymphoma

HD-Hodgkins Disease

Met- Metastatic

S/0 –Suggestive of

Ca-Carcinoma

Diff Ca-Differentiated Carcinoma

SCC-Squamous Cell Carcinoma

PTC-Papillary Carcinoma of Thyroid

CG-Colloid Goitre

HT-Hashitmotos thyroiditis

USG-Ultrasound

ANNEXURE-V

MASTER CHART

Sr No.	Name	Age/Sex	Site	FNAC no.	Cytological Dignosis	Modified Ultra Fast Pap Stain						Conventional PAP Stain					
						Back ground	Overall Staining	Cell Morphology	Nuclear character	Total Score	QI	Back ground	Overall Staining	Cell Morphology	Nuclear character	Total Score	QI
1.	Annapurna	50/F	Breast	2115/13	Acute Mastits	2	1	3	2	8	0.7273	1	3	1	2	7	0.63
2.	Ambika	34/F	Breast	2017/14	Fibroadenoma	2	3	2	2	9	0.8182	2	3	2	2	9	0.81
3.	Kalavati	42/F	Breast	58/14	Fibroadenoma	2	2	3	2	9	0.8182	2	2	3	2	9	0.81
4.	Renuka	35/F	Breast	134/14	IDC- High Grade	2	3	3	2	10	0.9091	1	2	2	3	8	0.72
5.	Mahananda	25/F	Breast	546/14	Phyllodes Tumour-Benign	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
6.	Devibai	30/F	Breast	511/14	Fibroadenoma	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
7.	Jyoti	26/F	Breast	322/14	Fibroadenoma	2	2	3	3	10	0.9091	1	1	3	3	8	0.72
8.	Rajashree	24/F	Breast	677/14	Benign Breast Lesion	2	3	3	2	10	0.9091	1	2	3	2	8	0.72
9.	Rubina	28/F	Breast	1075/14	Chronic Mastitis	2	3	2	3	10	0.9091	1	2	3	3	9	0.81
10.	Sridevi	50/F	Breast	1084/14	IDC	2	3	3	3	11	1	2	3	3	3	11	1
11.	Sakkamma	19/F	Breast	52/15	Fibroadenoma	2	2	3	3	10	0.9091	1	2	3	3	9	0.82
12.	Jariamma	19/F	Breast	80/15	Chr Non-Sp Mastitis	2	2	3	2	9	0.8182	1	2	3	3	9	0.82
13.	Laxmi	30/F	Breast	81/f	Fibroadenoma	2	2	3	2	9	0.8182	2	3	3	3	10	0.9
14.	Shreedevi	40/F	Breast	228/15	ILC	2	2	2	2	8	0.7273	1	3	3	3	10	0.9
15.	Guaridevi	47/F	Breast	184/15`	IDC	2	2	2	3	9	0.8182	2	2	3	3	10	0.9
16.	Sulochana	18/F	Breast	212/15	Fibroadenoma	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
17.	Neeta	26/F	Breast	344/15	Fibroadenoma	2	3	2	2	9	0.8182	2	3	3	3	11	1
18.	Aprana	38/F	Breast	56/15	Fibroadenoma	2	2	3	2	9	0.8182	2	3	2	3	10	0.9
19.	Shilpa	16/F	Breast	321/15	Fibroadenoma	2	3	2	2	9	0.8182	2	3	3	3	11	1
20.	Mamta	38/F	Breast	50/15	Positive For Malignancy	2	2	2	2	8	0.7273	1	3	3	3	10	0.9
21.	Anita	28/F	Breast	150/15	Fibroadenoma	2	2	2	2	8	0.7273	2	2	3	3	10	0.9
22.	Gurubai	47/F	Breast	184/15	IDC	2	3	3	3	11	1	1	2	3	3	9	0.81
23.	Alka	38/F	LN	2228/13	Reactive LAD	1	3	3	2	9	0.8182	2	2	2	2	8	0.72
24.	Hmiythom	19/F	LN	2077/13	Necrotising LAD	2	3	3	2	10	0.9091	2	2	2	2	8	0.72
25.	Vijaylaxmi	58/F	LN	1040/14	Supraclavicular LN Mets-AdenoCa	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
26.	Chandrabala	9/F	LN	1059/14	Reactive LAD	1	3	2	2	8	0.7273	1	2	3	2	8	0.72
27.	Abdul	24/M	LN	79/14	Reactive LAD	2	3	3	2	10	0.9091	2	2	2	3	9	0.81
28.	Ashok	27/M	LN	325/14	Reactive LAD	2	3	3	3	11	1	1	2	3	3	9	0.81
29.	Appanna	76/F	LN	2226/13	Met in LN from Ca Penis-SCC	2	3	3	3	11	1	1	2	3	3	9	0.81
30.	Aliya	28/F	LN	2102/13	Reactive LAD	2	2	3	2	9	0.8182	2	2	2	2	8	0.72

31.	Shankarappa	30/M	LN	569/14	Reactive LAD	2	3	3	3	11	1	1	3	3	3	10	0.9
32.	Bapagouda	10/M	LN	42/14	Reactive LAD	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
33.	Satawwa	75/F	LN	720/14	Tubercular LN	2	2	3	3	10	0.9091	1	2	3	3	9	0.81
34.	Saikumar	14/M	LN	1084/14	Reactive LAD	2	2	2	2	8	0.7273	2	1	3	3	9	0.81
35.	Lakawwa	60/F	LN	1523/14	Reactive LAD	2	2	3	2	9	0.8182	2	2	3	3	10	0.9
36.	Kavita	18/F	LN	1570/14	Reactive LAD	2	3	3	3	11	1	1	3	3	3	10	0.9
37.	Riyana	28/F	LN	1157/14	Tubercular LN	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
38.	Chidanand	13/M	LN	169/15	Granulomatous LAD S/0 Tuberculosis	2	3	3	2	10	0.9091	1	3	3	3	10	0.9
39.	Parvati	22/F	LN	198/15	Reactive LAD	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
40.	Chidanand	56/M	LN	251/15	NHL	1	2	2	2	7	0.6364	1	3	2	2	8	0.72
41.	Mahesh	32/M	LN	47/15	Reactive LAD	2	3	3	3	11	1	1	2	3	3	9	0.82
42.	Irramma	38/F	LN	312/15	Reactive LAD	2	3	3	3	11	1	1	2	2	3	8	0.72
43.	Mohan	45/M	LN	471/15	Granulomatous LAD	2	2	3	3	10	0.9091	1	2	2	3	8	0.72
44.	Bhavan	23/F	LN	332/15	Reactive LAD	2	3	3	3	11	1	1	2	2	3	8	0.72
45.	Maheashwar	20/M	LN	147/15	Reactive LAD	2	3	3	3	11	1	2	2	3	3	10	0.9
46.	Basvaraj	35/M	LN	445/15	Suppurative Lesion	2	3	3	3	11	1	2	2	3	3	10	0.9
47.	Yadappa	65/M	LN	347/15	S/O Met Poorly Diff Ca	2	2	3	3	10	0.9091	2	2	2	3	9	0.81
48.	Moshin	3/M	LN	322/15	Suppurative Lesion	2	3	3	3	11	1	2	2	3	3	10	0.9
49.	Siddanand	70/M	LN	3393/15	S/O Metastatic SCC	1	3	2	1	7	0.6364	1	2	3	3	9	0.81
50.	Bangaouda	38/M	LN	251/15	HD	2	2	3	2	9	0.8182	2	2	3	3	10	0.9
51.	Laxman	18/M	LN	205/15	Reactive LAD	2	3	2	2	9	0.8182	1	2	2	2	7	0.63
52.	Tipanna	36/M	LN	336/15	Reactive LAD	2	3	2	2	9	0.8182	1	2	2	2	7	0.63
53.	Shallappa	46/M	Salivary Gland	2013/13	Chronic Sialadenitis	2	2	2	2	8	0.7273	1	2	2	2	7	0.63
54.	Maibub	43/M	Salivary Gland	71/14	Chronic Sialadenitis	2	2	3	3	10	0.9091	1	2	3	2	8	0.72
55.	Jeevan	21/M	Salivary Gland	579/14	Chronic Sialadenitis	2	3	3	3	11	1	1	2	2	2	7	0.63
56.	Anita	25/F	Salivary Gland	1039/14	Chronic Sialadenitis	2	3	3	3	11	1	1	3	3	3	10	0.9
57.	Mahanta	42/M	Salivary Gland	1514/14	Pleomorphic Adenoma	2	2	3	2	9	0.8182	2	2	3	3	10	0.9
58.	Rudragouda	50/M	Salivary Gland	1150/14	Positive For Malignancy	2	3	3	2	10	0.9091	2	3	3	3	11	1
59.	Meenakshi	28/F	Salivary Gland	1515/14	S/O Chronic Sialadenitis	2	3	3	3	11	1	2	3	3	3	11	1
60.	Shankarappa	65/M	Salivary Gland	1514/14	Pleomorphic Adenoma	2	2	3	3	10	0.9091	1	3	3	3	10	0.9
61.	Nazeer	36/F	Salivary Gland	1550/14	Acute Sialadenitis	2	2	3	3	10	0.9091	1	2	3	3	9	0.82
62.	Sagar	27/M	Salivary Gland	50/15	Chronic Sialadenitis	2	3	3	3	11	1	2	3	3	3	11	1
63.	Parvati	79/F	Salivary Gland	443/15	Chronic Sialadenitis	2	3	2	3	10	0.9091	2	2	3	3	10	0.9
64.	Visvanath	15/M	Soft Tissue	1046/14	Benign Soft Tissue Tumour	1	2	2	2	7	0.6364	1	2	2	2	7	0.63
65.	Bhimav	21/M	Soft Tissue	1060/14	Lipoma	2	2	3	3	10	0.9091	1	1	3	2	7	0.63
66.	Somanna	72/M	Soft Tissue	228/14	S/O Malignant Round Cell Tumour	2	3	3	2	10	0.9091	1	2	3	3	9	0.81
67.	Hanmanth	55/M	Soft Tissue	372/14	S/O Poorly Diff Ca	2	3	3	3	11	1	1	2	3	3	9	0.81
68.	Raju	12/M	Soft Tissue	380/14	Acute Suppurative Inflammation	1	3	3	2	9	0.8182	1	3	3	2	9	0.81
69.	Parsuram	55/M	Soft Tissue	468/14	Benign Soft Tissue Tumour	1	2	2	2	7	0.6364	1	2	2	2	7	0.63

70.	Renuka	28/F	Soft Tissue	368/14	Epidermal Cyst	2	2	3	3	10	0.9091	1	2	3	3	10	0.9
71.	Anusuyaa	32/F	Soft Tissue	510/14	Lipoma	2	3	3	2	10	0.9091	1	1	3	3	8	0.72
72.	Ranga	40/M	Soft Tissue	571/14	Lipoma	2	3	3	2	10	0.9091	1	1	3	3	8	0.72
73.	Nangouda	45/M	Soft Tissue	582/14	S/O Epidermal Cyst	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
74.	Jumanna	37/M	Soft Tissue	40/14	Lipoma	2	2	2	2	8	0.7273	2	2	2	3	9	0.81
75.	Mallinath	35/M	Soft Tissue	1603/15	Infected Sebaceous Cyst	2	3	2	2	9	0.8182	2	2	3	3	10	0.9
76.	Dhariyappa	80/F	Soft Tissue	1136/14	S/O Necrotic Lesion	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
77.	Sadanand	55/M	Soft Tissue	1219/14	Acute Suppurative Inflammation	1	2	2	2	7	0.6364	1	2	2	3	8	0.72
78.	Aravind	40M	Soft Tissue	1219/15	S/O Epidermal Cyst	2	2	2	3	9	0.8182	2	2	3	3	10	0.9
79.	Kamlabai	60/F	Soft Tissue	1550/14	Poorly Diff Ca	2	3	3	2	10	0.9091	1	2	3	3	9	0.81
80.	Sachin	28/M	Soft Tissue	1543/14	Lipoma	2	3	3	3	11	1	2	2	2	3	9	0.82
81.	Manappa	75/M	Soft Tissue	2135/14	Acute Suppurative Inflammation	2	2	3	3	10	0.9091	2	3	3	3	11	1
82.	Bassangouda	27/M	Soft Tissue	184/15	Lipoma	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
83.	Tipanna	58/M	Soft Tissue	185/15	Lipoma	2	2	2	2	8	0.7273	2	3	3	3	11	1
84.	Anuroop	28/M	Soft Tissue	192/15	Lipoma	2	2	2	2	8	0.7273	2	3	3	3	11	1
85.	Basvaraj	2/M	Soft Tissue	185/15	Lipoma	2	2	2	2	8	0.7273	2	3	3	3	11	1
86.	Rajhans	34/M	Soft Tissue	255/15	Lipoma	2	2	3	3	10	0.9091	2	2	3	3	10	0.9
87.	Shivanand	33/M	Soft Tissue	332/15	Lipoma	2	2	2	2	8	0.7273	2	3	3	3	11	1
88.	Indumali	42/F	Thyroid	2196/13	CG	2	3	3	2	10	0.9091	1	3	2	2	8	0.72
89.	Shantappa	65/M	Thyroid	2013/13	Suspicious For PTC	2	3	3	2	10	0.9091	1	3	2	2	8	0.72
90.	Shridevi	46/F	Thyroid	2078/13	Cystic CG	2	3	3	2	10	0.9091	1	2	3	2	8	0.72
91.	Mallamaa	60/F	Thyroid	1058/14	HT	2	3	3	2	10	0.9091	1	2	2	2	7	0.63
92.	Mohanva	40/F	Thyroid	2012/13	Nodular Goitre	2	3	3	2	10	0.9091	1	3	3	3	10	0.9
93.	Shanbann	26/F	Thyroid	98/14	HT	2	3	3	2	10	0.9091	1	2	2	3	9	0.81
94.	Aarti	25/F	Thyroid	23/14	HT	2	3	3	3	11	1	1	3	3	3	10	0.9
95.	Nurjan	45/F	Thyroid	97/14	HT	2	3	3	3	11	1	1	3	3	3	10	0.9
96.	Lalasingh	35/M	Thyroid	84/14	Nodular CG	2	2	3	2	9	0.8182	1	3	2	3	9	0.81
97.	Kasturi	46/F	Thyroid	86/14	CG	1	3	2	2	8	0.7273	1	2	2	2	7	0.63
98.	Suvarna	18/F	Thyroid	324/14	S/O PTC	1	3	3	2	9	0.8182	1	2	2	3	8	0.72
99.	Ningraj	7/M	Thyroid	381/14	Nodular Goitre	2	3	3	3	11	1	1	2	3	3	9	0.81
100.	Sunanda	42/F	Thyroid	566/14	Lymphocytic Thyroiditis	2	3	3	3	11	1	1	3	3	3	10	0.9
101.	Maadevappa	60/M	Thyroid	565/14	SCC-Thyroid Mets	2	2	2	3	9	0.8182	1	2	3	3	9	0.81
102.	Kashibai	35/F	Thyroid	606/14	CG	2	3	3	2	10	0.9091	1	1	3	3	8	0.72
103.	Parvati	26/F	Thyroid	1056/14	Nodular CG	2	3	3	3	11	1	1	2	3	3	8	0.72
104.	Jainabee	30/F	Thyroid	1053/14	Follicular Neoplasm	2	3	3	2	10	0.9091	1	2	3	3	9	0.81
105.	Jayashree	40/F	Thyroid	1085/14	Benign Thyroid Lesion	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
106.	Gangashri	25/F	Thyroid	1083/14	Nodular Goitre	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
107.	Ganagabai	35/F	Thyroid	1074/14	Simple CG	2	3	3	3	11	1	1	3	3	3	10	0.9
108.	Kasturi	35/F	Thyroid	1622/15	CG	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
109.	Lailta	32/F	Thyroid	1582/14	Granulomatous Thyroiditis	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
110.	Rajashree	50/F	Thyroid	1192/14	HT	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
111.	Lalita	23/F	Thyroid	1185/14	S/O CG	2	2	3	3	10	0.9091	1	2	3	3	9	0.81
112.	Kamlabai	48/F	Thyroid	1216/14	Cystic Nodular Goitre	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
113.	Shakira	33/F	Thyroid	12116/14	Nodular CG	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
114.	Nilangawa	80/F	Thyroid	1240/15	Nodular CG	2	3	3	3	11	1	1	3	3	3	10	0.9
115.	Praveenabai	25/F	Thyroid	1522/14	Nodular Goitre	2	2	2	3	9	0.8182	1	2	3	3	9	0.82
116.	Vijaylaxmi	28/F	Thyroid	162/15	Nodular Goitre	2	3	3	3	11	1	1	3	3	3	10	0.9

117.	Surekha	48/F	Thyroid	1382/14	S/O Nodular Goitre	2	3	3	3	11	1	1	3	3	3	10	0.9
118.	Darshana	37/F	Thyroid	253/14	Suspicious For Malignancy	2	2	3	2	9	0.8182	1	2	3	3	9	0.82
119.	Sudha Ravi	24/F	Thyroid	22/15	Multinodular Goitre	2	2	3	2	9	0.8182	1	2	3	3	9	0.81
120.	Shantabai	60/F	Thyroid	311/15	S/O Folllicular Neoplasm	2	3	2	2	9	0.8182	1	3	3	3	10	0.9
121.	Sunanda	42/F	Thyroid	463/15	Cystic CG	2	2	3	3	10	0.9091	1	2	3	3	9	0.81
122.	Sushilabai	75/F	Thyroid	254/15	Nodular Goitre	2	3	2	3	10	0.9091	1	2	3	3	10	0.9
123.	Darshini	37/F	Thyroid	253/15	Suspicious For Malignancy	2	3	3	2	10	0.9091	1	3	3	3	10	0.9
124.	Pranita	39/F	Thyroid	143/15	Nodular Goitre	2	2	2	3	9	0.8182	1	2	2	3	8	0.72
125.	Annapurna	28/F	Thyroid	221/15	Nodular Goitre	2	3	2	3	10	0.9091	1	3	3	2	9	0.81
126.	Shivaji	40/M	Visceral Organ (USG Liver)	1579/14	Hepatocellular Ca	2	3	2	2	9	0.8182	1	3	3	3	10	0.9
127.	Vithoba	75/M	Visceral Organ (USG Liver)	1377/14	Hepatocellular Ca	2	2	2	2	8	0.7273	1	3	3	3	10	0.9
128.	Danappa	64/M	Visceral Organ (USG Liver)	1559/14	Hepatocellular Ca	2	2	2	2	8	0.7273	1	3	3	3	10	0.9
129.	Sharanappa	80/M	Visceral Organ (USG Liver)	129/15	Hepatocellular Ca	2	2	3	2	9	0.8182	2	2	2	3	9	0.82
130.	Irrapa	70/M	Visceral Organ (USG Lung Mass)	1523/14	S/0 Poorly Diff Ca	1	2	3	2	7	0.7273	1	2	2	3	8	0.72
131.	Dundappa	75/M	Visceral Organ (USG Abdominal Mass)	22/14	Poorly Diff AdenoCa	1	2	2	2	7	0.9091	1	2	2	3	8	0.63