"RAPID CYTODIAGNOSIS BY DIFFERENT STAINING TECHNIQUES IN COMPARISON WITH CONVENTIONAL STAINS IN FINE NEEDLE ASPIRATION CYTOLOGY"

BY

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DISSERTATION SUBMITTED TO

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DOCTOR OF MEDICINE
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UNDER THE GUIDANCE OF

Dr. CSBR PRASAD, *MD*

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR
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Date: *Signature of the Candidate*

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LIST OF ABBREVIATIONS

CG	**-**	**COLLOID GOITRE**
DC	**-**	**DUCTAL CARCINOMA**
DPX	**-**	**DISTYRENE PLASTICIZER XYLENE**
FA	**-**	**FIBROADENOMA**
FCC	**-**	**FIBROCYSTIC CHANGE**
FN	**-**	**FALSE NEGATIVE**
FNT	**-**	**FOLLICULAR NEOPLASM THYROID**
FNAC	**-**	**FINE NEEDLE ASPIRATION CYTOLOGY**
FP	**-**	**FALSE POSITIVE**
GL	**-**	**GRANULOMATOUS LYMPHADENITIS**
H&E	**-**	**HEMATOXYLIN & EOSIN**
HG	**-**	**HYPERPLASTIC GOITRE**
LT	**-**	**LYMPHOCYTIC THYROIDITS**
M/E	**-**	**METHYLENE BLUE/EOSIN**
MGG	**-**	**MAY GRUNWALD AND GIEMSA STAIN**

NG	**-**	**NODULAR GOITRE**
NPV	**-**	**NEGATIVE PREDICTIVE VALUE**
OSAA	**-**	**ONSITE ADEQUACY ASSESSMENT**
PAP	**-**	**PAPANICOLAOU STAIN**
PPV	**-**	**POSITIVE PREDICITVE VALUE**
PT	**-**	**PHYLLODES TUMOR**
PTC	**-**	**PAPILLARY THYROID CARCINOMA**
RL	**-**	**REACTIVE LYMPHADENITIS**
SCC	**-**	**SQUAMOUS CELL CARCINOMA**
SFM	**-**	**SUSPICIOUS FOR MALIGNANCY**
SL	**-**	**SUPPURATIVE LYMPHADENITIS**
SP	**-**	**SPECIFICITY**
SS	**-**	**SENSITIVITY**
TBWM	**-**	**TOLUIDINE BLUE WET MOUNT**

ABSTRACT

TITLE OF THE STUDY: "RAPID CYTODIAGNOSIS BY DIFFERENT STAINING TECHNIQUES IN COMPARISON WITH CONVENTIONAL STAINS IN FINE NEEDLE ASPIRATION CYTOLOGY"

INTRODUCTION

Fine needle aspiration cytology (FNAC) technique has become more common and popular nowadays. FNAC is a very important component of pre-operative / pre-treatment investigation in combination with clinical, radiological and other laboratory data. Rapid staining and immediate interpretation with increased accuracy provide diagnostic information for further management of the patient.

OBJECTIVES OF THE STUDY

1. To find out the diagnostic rapidity of the following staining techniques in FNAC

   Toluidine blue wet mount preparation [TBWM]

   Methylene blue/eosin stain [M/E]

2. To compare the morphological features and results obtained from rapid stains with conventional PAP and H&E techniques in FNAC.

MATERIALS AND METHODS

The study was conducted on 320 fine needle aspirates from patients of R.L Jalappa hospital attached to Sri Devaraj Urs medical college. Consent was taken from all the patients included in the study. All 320 cases were stained with all four stains [PAP, H&E, M/E, TBWM].

RESULTS

The time taken for each stain was assessed to find out the rapidity of the stain. TBWM took 2 minutes for the staining followed by M/E – 5 minutes, H&E – 8 minutes and PAP – 10 minutes.

The diagnostic accuracy of TBWM is 76%, sensitivity was 77% and specificity 94%. This reduced diagnostic accuracy and sensitivity was due to three dimensional clusters of cells due to which the morphology was obscured. Quality index of this stain is 0.68

M/E stain showed sensitivity, specificity and diagnostic accuracy of 98%, 98% and 100%. Quality index of this stain is 0.73

Quality index of PAP and H&E were 0.86 and 0.83

CONCLUSION

Toluidine blue wet mount and Methylene blue/Eosin stain can be used as a rapid diagnostic test. It is also used to assess adequacy of sample especially for deep seated lesions and in USG guided FNAC. It can be used for intra operative cytodiagnosis as an adjunct to frozen section diagnosis. Thus, both stains can be routinely undertaken as a supplementary procedure for conventional stains to improve the cellularity and to reduce the time taken for re-sampling.

KEY WORDS: Rapid stains, Quality Index, Toluidine blue wet mount, Methylene blue/Eosin

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INTRODUCTION

Fine needle aspiration cytology (FNAC) technique has become more common and popular nowadays. Though it is not a substitute for conventional histopathology, it should be regarded as a very important component of pre-operative / pre-treatment investigation in combination with clinical, radiological and other laboratory data.[1]

Conventional FNAC has its own advantages and limitations, which are well known to any cytopathologist. In spite of its advances and advantages, conventional FNAC fails to achieve a 100% accuracy.

This is partly because of,

(i) A lack of sufficient cellularity in desmoplastic lesions.

(ii) Wastage of aspirated cells when they stick to the hub and lumen of the needles.

(iii) Morphological distortion produced when the cells are trapped in fibrin mesh.

(iv) Distortion of fragile cells during smearing.

(v) Loss of cell to cell and cell to stromal architecture.[1,2]

Hence in an attempt to improve its accuracy, a supravital stained Toluidine blue wet mount (TBWM) preparation of the aspirate and Methylene blue/Eosin (M/E) stained smears are studied which will give additional information regarding the lesion on which FNAC is done. These supravital stains attains good definition of cell outline, cytoplasmic contents and nuclear details.

Here for wet mount preparation, toluidine blue is aspirated in the same needle and syringe which is used to aspirate for conventional fixed smear - Papanicolaou (PAP) and Hematoxylin & Eosin (H&E) stains. The mixed material is poured on a clean slide and cover slip is placed over it. Then this wet mount is examined under microscope for morphological details.

Methylene blue/Eosin is an smearing technique where the slide is air dried and then fixed in methanol followed by staining.

Toluidine blue and methylene blue are rapid supravital stains that produce good nuclear detail. Toluidine blue gives a three dimensional view of cell in a wet mount.[1,2] Both stains are easily available and inexpensive.[3,4] Addition of eosin stain gives a pale pink cytoplasm to the cell and gives better contrast to appreciate cytomorphology.[2] Supravital stained wet mount of fine needle aspirate and methylene blue/eosin smears are becoming popular as a supplementary procedure to conventional FNAC smears.[5,6]

NEED FOR THE STUDY

Fine needle aspiration cytology (FNAC) is a simple, reliable, cost effective and low risk tool for diagnosing palpable or non-palpable mass lesions.[1] FNAC is important in pre-operative and pre-treatment investigation of a patient as an adjuvant to clinical, radiological and other laboratory data. Rapid diagnostic methods are very useful in managing the patients.[1,2] Since the majority of lumps are benign, FNAC plays a major role in making a diagnosis and planning appropriate management.[3,4]

The diagnostic accuracy of FNAC depends on adequacy and representativeness of sample and good cytomorphological detail without much artifactual distortion. Rapid staining and immediate interpretation with increased accuracy provide diagnostic information for further management of the patient.[5,6] Diff quick stain is used for rapid cytodiagnosis in the west, which is not available in India and is expensive.[3,6,7]

Most of the time for rapid assessment of cellularity in FNACs, Methylene blue is used and diagnosis cannot be rendered as it stains everything blue. Alternatively smear stained with Methylene blue/Eosin (M/E) is another rapid technique which can be used in Indian conditions. Addition of eosin stains the cytoplasm and the morphology can be well appreciated.[2,7]

Another technique for rapid cytodiagnosis is Toluidine blue wet mount, a supra vital stain that accentuates a good nuclear detail and enables a three dimensional view of cells in a wet mount. Eosin stain can be added to wet mount to enhance the contrast.[2] Supravital stain is not only inexpensive but is also easily available.[3]

Rapid diagnosis also facilitate preoperative decision making and to avoid unnecessary invasive procedures for patients with primary or metastatic lesions.[5,6] Toluidine blue wet mount and

Methylene blue/Eosin staining of fine needle aspirates can be used along with the conventional H&E and PAP stained FNAC smears to improve the accuracy of diagnosis.[2]

Therefore this study is aimed at assessing the utility of FNAC samples prepared by Toluidine blue wet mounts and smears stained by Methylene blue/Eosin techniques in rendering a rapid cytodiagnosis when compared with routine PAP and H&E stains.

OBJECTIVES OF THE STUDY

1. To find out the diagnostic rapidity of the following staining techniques in FNAC

   Toluidine blue wet mount preparation

   Methylene blue/Eosin stain

2. To compare the morphological features and results obtained from rapid stains with conventional PAP and H&E techniques in FNAC.

REVIEW OF LITERATURE

For over 100 years, the discipline of anatomical pathology has centered on diagnostic histopathology and this in turn on the surgical biopsy. Diagnostic cytology is the science of interpretation of cells that are either exfoliated from epithelial surfaces or removed from various tissues. For the last 60 years, exfoliated and abraded samples of cells have also been collected from accessible anatomical surfaces especially from uterine cervix and the bronchus. In the United Kingdom, Dudgeon and Patrick in 1927 proposed the needling of tumors as a means of rapid microscopic diagnosis. George N Papanicolaou introduced cytology as a tool to detect cancer and pre-cancer in 1928. Consequently, Martin and Ellis in 1930 used needles of thicker caliber for aspiration biopsy than those commonly in use today. It was in Europe and particularly Scandinavia the fine needles aspiration cytology began to flourish in the 1950s and 1960s. FNAC is a diagnostic procedure used to investigate superficial lumps or masses. In 1981, the first fine needle aspiration biopsy in the United States was done at Maimonides Medical Centre, eliminating the need for surgery and hospitalization.

Fine needle aspiration cytology became more popular and common now. Initially the method was applicable to lesions that are easily palpable-superficial growth of the skin, sub cutis and soft tissues and organs such as thyroid, breast, salivary glands and superficial lymph nodes. Modern imaging techniques mainly ultrasonography and computed tomography applied to organs and lesions in sites not easily accessible to surgical biopsy offer vast opportunities for fine needle aspirations of deeper structures. So sample may be obtained from the lung, mediastinum, abdominal, retroperitoneal and pelvic organs and, deep seated lesions in head and neck and soft tissues.

FINE NEEDLE ASPIRATION CYTOLOGY [FNAC]

NEEDLES

The size of the needles used for aspirating materials are varied according to the site of lesion. Standard disposable 27-22 G, 30-50 mm needles are suitable for superficial palpable lesions, 22 G, 90mm disposable lumbar puncture needles with trocar are convenient for most deep biopsies.[7] There have been some variations in needle design as aspiration biopsy has become more widely used. The Franzen needle used for prostatic FNAC has a notched tip and stylus was used by William J. Frable.[8] The Milex and Inrad needles that were designed to improve sampling in firm fibrous breast masses have a slot on the side, so called side port needles. Radiologists use the Chiba needle of 21-22 G most frequently for transthoracic and trans-abdominal aspirations.[8] In some centers, bone lesions are also aspirated and combined with core biopsy was performed by Layfield et al., 1987 and White et al., 1988.[9,10] There are virtually no complications by employing only the thin needle technique except for pneumothorax with trans-thoracic aspiration and some cases of excessive bleeding with trans-abdominal aspiration was reported by Powers et al., in 1995.[11] [Figure 1: Various sizes of needles used for FNAC]

TECHNIQUE

The skin should be cleansed with an alcohol swab prior to puncture for superficial FNAC. Local anesthesia is used for percutaneous radiologically guided needle biopsies. The sterile needle is inserted in the target area. Staying within the lesion, the needle is moved in a cutting motion, withdrawing cells into the needle hub. The force of the cutting motion needed to obtain an adequate sample must be adjusted for the body site and characteristic of the lesion. These biopsies may be performed with “Suction” (Aspiration) or “Non Suction” (Non aspiration) technique. Once the cellular material is seen in the needle hub, suction is released and needle is

withdrawn. The cellular material is expressed on to one or more slides and is smeared with a second slide. One of the slide is fixed in a fixative and the other is air dried.[7,12] For wet mount preparation, toluidine blue is aspirated in the same needle used for FNA and the mixed material is poured onto slide and coverslip is placed over it.[1,2]

FIXATIVES

For routine wet fixation of smear either 70-90% alcohol in Coplin jar or commercial spray fixatives are used. Carnoy's fixative has the advantage of lysing red blood cells and being a good nuclear fixative.[7,12]

SAMPLING OF FLUIDS

In case of cystic lesions, as much fluid as possible is removed. Then the cyst fluid can be handled as liquid specimen. If there is a residual mass, the procedure for solid lesions as described above should be followed.[7]

Serous or body cavity fluids are usually aspirated with aseptic technique by a needle puncture and fluid is collected in a dry container. Fluid sample is submitted in fresh state to the laboratory. If delay in transportation to the laboratory is unavoidable, fluid may be kept in a refrigerator at 4^{o}C up to 6 hrs. Fluid samples intended for cytological analysis should never be frozen. If longer delay is expected, preservation at the time of collection with 50% ethanol equal to the volume of specimen is suggested. For small fluid accumulations the entire specimen is submitted for laboratory evaluation.[7,12]

For large effusions, 50-200 ml of well mixed fluid should be sent for cytological examination. Fluid received is centrifuged at 1500 rpm for 10 minutes. The supernatant decanted and smears are made from the sediment. If the specimen is very bloody, variable amount of Carnoy's solution depending on the amount of red blood cell present can be added, mixed, left to

stand undisturbed for 10 minutes. The supernatant containing the hemolysed red blood cells is discarded. Saline solution is added and mixed with cells and the solution passed through a membrane filter.[13,14,15] [Figure 2: FNAC Technique]

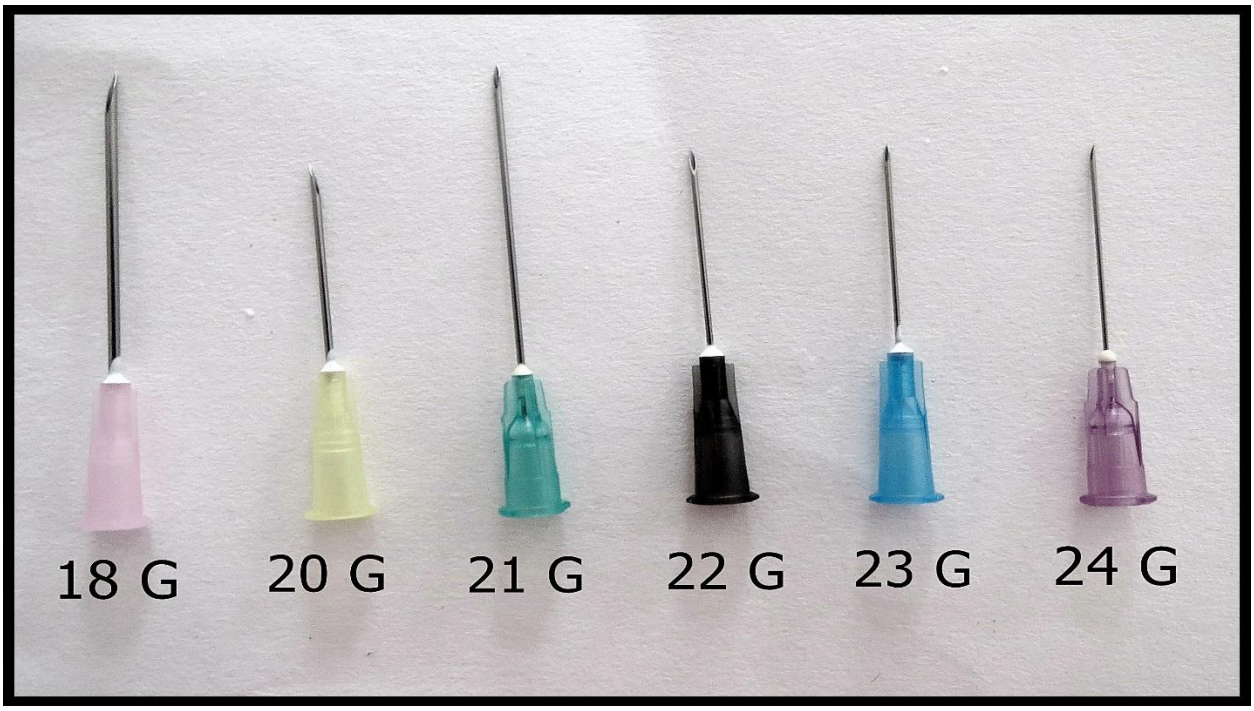


Figure 1: Various sizes of needles used for FNAC procedure

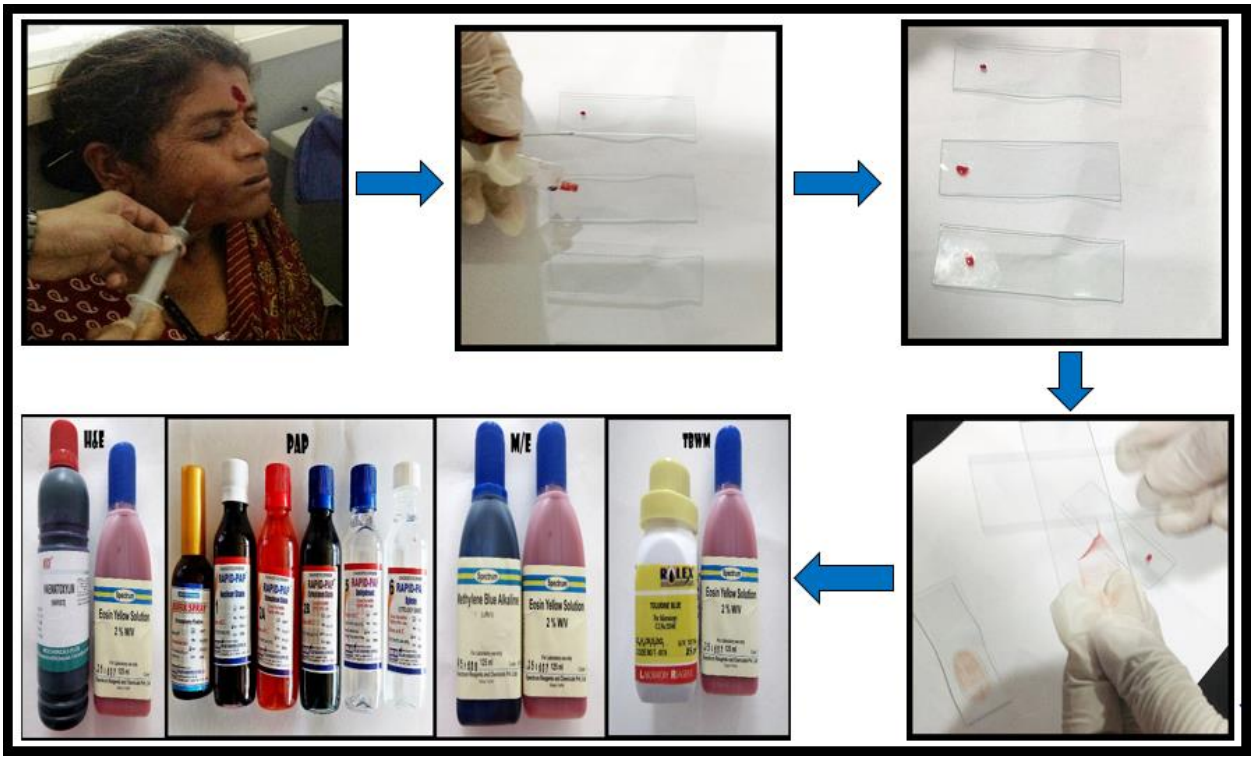


Figure 2: FNAC Technique

STAINS USED IN FNAC

Fundamentally two different methods of fixation and staining are used in FNA cytology. Air drying followed by staining with a hematological stains such as MGG, Wright, Giemsa Stain or Diff quik. Second one is alcohol fixation and staining with PAP or H&E. Both methods have their advantages and disadvantages. Air drying causes the cell to flatten on the glass surface. Therefore it appears larger than the cell fixed in ethanol. But nuclear enlargement and variation in nuclear size are exaggerated in wet fixed Papanicolaou stain and this is helpful in cytological diagnosis.[7,12]

Difficulties can also occur with wet fixation particularly drying artifacts if samples are thick and highly cellular. However with both these methods, cells were lost either in the fixative solution or subsequently during staining.[2]

In the past decade, FNAC has gained tremendous growth with many improvements in the technique. The new advanced techniques which has improved the efficacy of FNAC includes special stain techniques, immuno-cytochemistry, cell block preparation techniques, quantitative techniques like morphometry, object counting, flow cytometry, in situ hybridization technique and polymerase chain reaction.[7]

In spite of development of new advanced ancillary techniques, FNAC has its own limitations. The diagnostic accuracy of FNAC depends on adequacy of sample, representativeness of the sample and good cyto-morphological detail without much artifactual distortion. Several studies had been done to reduce pit falls and improve the diagnostic accuracy of FNAC.[2,3,6]

In 1958 Chandler Foot et al., experimented with the supra vital stains (Neutral red - Janus green) in sediments of effusion for rapid diagnosis. The most frequent and disturbing obstacle to

accurate cytological diagnosis of cancer in sediments of serous effusions is the fact that histiocytes and mesothelial cells may undergo misleading metaplastic change under certain circumstances eg. congestive cardiac failure, cirrhosis and are then readily mistaken for cancerous elements.[16] This Neutral red - Janus green procedure which has been extensively studied by others which offers a simple method for positively identifying histiocytes and leukocytes. Neutral red stains the granules of the histiocytes a brilliant orange red and janus green stains lymphocytes diffusely sky blue and colours the mitochondria. Mesothelial cells do not stain at all by this method. Their study established a diagnostic accuracy of 38.2% (13/34) by supra vital stains.[17,18] This pilot study using supra vital stain wet mounts had its limitations as the authors themselves acknowledge that neoplastic cells could not take up these stains.[16]

Other rapid stains used for wet sediment examination are thionine blue, methylene blue and toluidine blue. Harris and Keebler in 1976 suggested the use of these stains as a,

(i) control when setting up new cytopreparatory procedure

(ii) check on the cellular preservation of the sample

(iii) method of identifying highly positive samples so they may be stained separately

(iv) check on existing cytopreparatory method

(v) Mean of estimating the cellularity of a sample.

Other cytopathologists recommended the use of these stains as a definitive diagnostic procedure for some fluid specimens such as urine, effusions and spinal fluid.[19]

Ferreira et al., in 1959 studied fresh material cytology using toluidine blue. Toluidine blue is a basic dye of thiazine group that selectively stains acidic tissue components like carboxylates, sulfates, phosphate radicals, Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).[20] Herlin et al., and Martin et al., studied that cancer cells contain quantitatively more

DNA and RNA than normal epithelial cells and so toluidine blue stains nuclei of malignant cell well and give satisfactory nuclear details.[21,22] Bennion et al., and Lepage et al., studied and demonstrated the greater affinity of tumor RNA for basic dyes like toluidine blue.[23,24]

Richart et al., in 1962 and Shedd et al., in 1965 studied in vivo staining of oral cancer using toluidine blue. They concluded that neoplastic cells stained intensely with toluidine blue and non dysplastic epithelial cells fail to retain toluidine blue stain. Malignant epithelium may contain intra cellular canals that are wider than normal epithelium and this is a factor that would enhance penetration of dye.[25,26] Later Silverman et al., in 1984 studied the usefulness of toluidine blue in detection of oral precancerous and malignant lesions. Their result yielded the accuracy of toluidine blue uptake 91%, false negatives were 2%. There were 30% false positives in benign lesions.[27]

This in vivo staining technique using toluidine blue were also studied in many tissues other than oral lesions. In 1976, Giler et al., experimented per oral intra gastric staining method using toluidine blue in forty two cases with suspected neoplastic lesions of the stomach. Their study showed fifteen out of eighteen malignant lesions and one out of thirteen benign lesions were stained. The normal mucosa, areas of inflammation and most of the benign lesions appeared unchanged. They concluded that in vivo toluidine blue staining prior to endoscopy might be of help in demonstrating minute malignant lesions of gastric mucosa and in differentiating benign and malignant ulcers of stomach.[28]

Since toluidine blue is a good nuclear stain, combination of toluidine blue with other dye is used to improve cytomorphology by creating excellent contrast. This combination stain study was experimented by Henriques et al., in 1972, where he used toluidine blue and eosin as a stain for rapid cytodiagnosis.[29] Later Vartanian et al., in 1998 tried the efficacy of this combination

stain. He used Leung stain which is a combination of toluidine blue and alcian yellow for demonstration of Helicobacter pylori. Their study on the reliability of the Leung stain in endoscopic mucosal biopsy specimen revealed that it is cheapest, easiest to prepare and good choice as a standard for routine Helicobacter pylori staining.[30]

A comparison study between different stain were experimented by Linda et al., in 1978. Studies had been done to compare the efficacy of toluidine blue stain as a supplementary staining with Grocotts modification of Gomori's stain for demonstration of Pneumocystis carinii. Their study showed positive results in 67/78 (86%) of cases with toluidine blue stain and 60/78 (77%) of cases with Gomori's stain alone and accuracy using both stains was 100%. They concluded that it is a rapid stain, accurate and highly constant.[31]

Humphreys et al., in 1996 did a pilot study comparing toluidine blue and H&E staining of Basal cell carcinoma and Squamous cell carcinoma during Moh's surgery. Their study showed that toluidine blue revealed stromal changes associated with the presence of Squamous cell carcinoma and Basal cell carcinoma. H&E provided more prominent visibility of individual cell keratinization and necrosis, which are common features seen in squamous cell carcinoma.[32,33]

Adequacy of the sample is more important for cytological diagnosis. Inadequate and scanty sample pose a major problem in arriving at inconclusive diagnosis. Several authorities stated that immediate on site smear evaluation by cytopathologist optimizes diagnostic accuracy and minimizes the technique insufficiency rate. This favorable effect on FNA diagnostic accuracy is most pronounced for deep seated lesions where FNA is guided by computed tomography, ultrasound, bronchoscopy and endoscopy. Several studies had been done on immediate assessment of cellularity using rapid stains to improve diagnostic accuracy.[5,6]

Civardi et al., in 1988 studied the value of rapid staining and immediate cellularity assessment of ultrasound guided fine needle aspirate samples. Their result yielded a sensitivity of 95.6%. They concluded that rapid evaluation of aspirated material can reduce the number of punctures needed per case resulting in less discomfort and reduced likelihood of complication for the patient.[34]

Silverman et al., in 1989 studied the accuracy of immediate interpretation of FNAC from various sites. He utilized Diff-quik as a rapid stain for immediate assessment.[35] Diff-quik is a modified Wright stain. It provides good cellular detail and identifies stromal fragments by metachromasia. It includes 2 solutions. Solution I is buffered eosin Y. Solution II is a buffered solution of thiazine dyes methylene blue and azure A. Azure A undergoes slow constant oxidation to azure B which is the actual staining solution of the original Romanowsky method. Here air dried fixed smears were dipped for 5 seconds in solution I and II respectively. Then the slide was rinsed with water. The slide was allowed to dry or examined wet. Their study yielded a sensitivity of 96%. There were 14 false negative or falsely insufficient immediate interpretations and one false positive immediate diagnosis. They concluded that immediate assessment can,

(i) Determine whether an adequate specimen is present.

(ii) Render a specific preliminary diagnosis.

(iii) Guide further clinical investigations or treatment.

(iv) Determine whether ancillary studies are needed to make a more accurate or specific diagnosis from the FNA specimen.[35,36]

Methylene blue/Eosin is a Romanowsky stain, family of polychrome stains used in differentiating cytoplasmic and nuclear components. M/E stain is useful in enhancing pleomorphism and distinguishing extracellular from intracytoplasmic material.[36]

A study done by Fabre et al., in 1999 for immediate assessment of guided FNAC from deep seated masses using Diff-quik stain showed a rapid evaluation increases the diagnostic yield allowing near 100% in sensitivity, specificity and predictive value of positive cases.[37]

Kusum Verma et al., in 1991 studied the diagnostic accuracy of immediate interpretation of fine needle aspirates from various sites. But here they followed rapid May Grunwald stain and Giemsa stain (MGG) staining method as a rapid stain for their study. Air dried fixed smears were stained by applying MGG for one minute each respectively. Then the smears were air dried, examined and morphologic diagnosis was rendered. The entire procedure took 10 - 15 minutes. Later the routine MGG staining was done on the same slide where May Grunwald stain and Giemsa stain was applied for 10 minutes and 15 minutes respectively. They compared this MGG staining and routine Papanicolaou staining and the results of immediate interpretation were compared with the final diagnosis and statistically analyzed. Their result showed a sensitivity of 97%. The diagnostic accuracy of immediate interpretation of FNA smears found by them were comparable with 97.5% to 98.4% accuracy reported for frozen section diagnosis done by Saltzstein et al., 1973 and Lessells et al., 1976, Rogers et al., 1987. Their study confirmed the utility of the test for rapid intra operative diagnosis and this technique proved to be a valuable tool and replaces the frozen section particularly when facilities for the latter are not available.[38,39,40,41]

In 1992, Srivannaboon et al., studied and compared the diagnostic accuracy of toluidine blue with that of Papanicolaou stain in non-gynecologic cytology. In their study, a sensitivity of 95.3% and 96.9% was achieved by using toluidine blue and Papanicolaou stain respectively. They found that the diagnostic accuracy of toluidine blue stain approximates that of the Papanicolaou stain.[42,43]

Later the application and accuracy of intra operative immediate cytological assessment by rapid stains in place of frozen sections were studied. Chang et al., in 1993 studied three hundred and five fresh specimens submitted for intra operative frozen stain for immediate intra operative cytological assessment. They experimented Liu's stain as a rapid stain. Liu's stain is one of the Romanowsky stain that take only 2 minutes. The accuracy of diagnosis was measured by comparison with final histological diagnosis. Sensitivity and specificity of intra operative cytology were 94.9% and 95.6% respectively. In combination of intra operative cytology and frozen section, sensitivity and specificity become 96% and 96.3% respectively. They concluded that Liu's stain is a simple, rapid, reliable staining method in intra operative cytological diagnosis. It is apparent that intra operative cytology is able to provide a useful adjunct to the frozen section diagnosis.[44]

Yang et al., in 1995 studied an alternative procedure for fine needle aspiration cytology. The objective of this study was to develop a Papanicolaou stain as fast as Diff-quik yet the cytomorphology as exquisite as that processed by thin prep for the optimal evaluation of fine needle aspirates.[45]

Satisfactory results were obtained after three modifications were made,

(i) Rehydration of air dried smears with normal saline.

(ii) Use of a 4% formaldehyde or 65% ethanol fixative.

(iii) Use of Richard Allen Hematoxylin 2 and cyto-stain.

The first modification restored the transparency of the cells and hemolysed red blood cells, the second modification reduced the time needed for proper fixation and staining from minutes to seconds and the third modification simplified the procedure. This 90 second protocol yields a transparent polychromatic stain with crisp nuclear and cytoplasmic features. The

cytomorphology processed by this protocol is at least equal to, if not better than the quality of specimens prepared by thin prep and superior to those processed by the standard papanicolaou procedure. This ultra-fast stain can also be adapted for permanent FNA smears.[45,46]

Computed Tomography, endoscopic and ultrasonography guided FNAC (USG guided FNAC) is safe, rapid and cost effective method for securing a sample of abnormal tissue to diagnose and stage a variety of pathologic conditions in deep seated organs. The rate of false negative results is more dependent upon sampling failure and poor preparation of aspirated material than on interpretation error. Lachman et al., in 1995 studied three hundred and forty one cases of image directed fine needle aspirates for onsite adequacy assessment (OSAA). The diagnostic accuracy before and after the implementation of this OSAA were compared. This study yielded a diagnostic sensitivity of 86% before OSAA and of 98% after OSAA.[47]

Studies done by many authors on immediate assessment of FNAC from various sites proved the value of its diagnostic accuracy. Later this was concentrated on FNAC of particular tissue and studied. Stewart et al., in 1996 studied the value of immediate assessment of cytology in FNAC of lung. They utilized Diff-quik as a rapid stain. The diagnostic accuracy was examined by review of clinical and radiological data in all patients. All malignant diagnoses were confirmed on clinical or pathological review and the diagnostic sensitivity was 96.6%. They concluded that immediate cytology assessment reduces the number of unsatisfactory and false negative lung FNAC. The complication rate is also minimized by decreasing the number of pleural punctures.[48]

In 1997, Tsou et al., experimented Riu's stain as a rapid stain for cytologic diagnosis of thyroid and liver tumors. Riu's stain is a Romanowsky type stain and has been in use in Taiwan over the past forty years. Their study showed a sensitivity of 93.5% and specificity of 100% for

the detection of malignancy. They concluded that Riu's stain is a reliable quick stain in the diagnosis of lung and thyroid malignancy.[49, 50]

Later, Baloch et al., in 2000 evaluated the combined impact of ultrasound guidance rapid on site evaluation of FNA specimen and different cytologic preparations (fresh and alcohol fixed smears, Millipore filter) and staining method by Diff-quik and Papanicolaou stain on the diagnostic yield of thyroid FNA. A definite diagnosis could be made solely on the basis of air dried Diff-quik stained preparations in 65% cases, alcohol fixed papanicolaou stained smears in 68% cases and Millipore filter preparation in 91% cases. They concluded that ultra sound guided FNA combined with onsite evaluation and different cytologic preparation on significantly improve the diagnostic accuracy of thyroid FNA specimens.[51]

One of the limitations of fine needle aspiration of thyroid is difficulty in distinguishing the follicular variant of papillary thyroid carcinoma from follicular neoplasms. By highlighting the "orphan-annie eyed" clear nuclei of the former, the ultra-fast Papanicolaou stain easily separates these two entities. In 2001, Yang et al., assessed 1135 ultrasound guided FNAs of thyroid. Of the 1127 satisfactory FNAs with 2-6 years clinical follow up, a false negative rate of 0% and a false positive rate of 1.5% were obtained. Of the 169 surgical follow-ups with satisfactory FNAs, a sensitivity of 100%, specificity of 66.7%, positive predictive value of 87.4%, negative predictive value of 100% and global accuracy of 89.9% were obtained.[52]

Shirley et al., in 2003 again studied the utility of rapid staining of FNAC. Sensitivity and specificity values were similar for rapid and routine stained slides and ranged from 80 100%. Their study concluded that rapid staining of cytological smears is a useful adjunct to the evaluation of aspirated material, improving adequacy rates and overall performance of the FNA service and should also result in significant savings in time and cost to patients.[53]

Now rapid staining of FNAs is an accepted procedure for evaluation of adequacy and rapid diagnosis. Several studies had been conducted on the rapid staining techniques so far using stains such as Diff quik, rapid MGG, Liu's stain, Riu's stain, ultra-fast PAP. However those stains are imported and expensive.[3,6]

Joy MP et al., in 2003 used toluidine blue as an alternative rapid stain. It is an inexpensive stain, and easily available. Although toluidine blue has been used in evaluation of touch imprint, frozen sections and in squash preparation of central nervous system tumors by Dusmez et al., 2001, there were no previous reports using toluidine blue for rapid diagnosis of ultrasound guided fine needle aspirates. They studied the reliability of toluidine blue stain as a rapid stain for quick diagnosis in ultra sound guided aspiration cytology. Here smears were air dried and dipped in a Coplin jar containing freshly filtered staining solution for one minute. Then rinsed in tap water and slides were examined under the microscope. They observed that cytoplasmic, nuclear details were well appreciated in toluidine blue stained smear permitting rapid diagnosis. The sensitivity of their study for malignant/suspicious for malignancy was 98.54%. Sensitivity and specificity for an inflammatory condition was 100%. They concluded that toluidine blue staining is not only a reliable method for rapid staining and diagnosis, it also permits preservation of cytological material by de-staining and re-staining with permanent stains. De-staining was done by putting the smear in 95% alcohol for ten minutes and then it was used for re-staining with MGG.[3,54]

Wet mount studies were done previously in effusion fluids, urine cytology using toluidine blue for rapid diagnosis by Zuher et al., 1985.[55] A study done by Robertson et al., in 1990 used toluidine blue for rapid on-site evalualion of transbronchial aspirates greatly improves the diagnostic yield. A small drop of the aspirated specimen was expressed onto a slide, mixed with

an equal drop of stain, cover slipped to make a stained wet film, and immediately examined microscopically. Cells appear slightly larger in stained wet films. Small nucleoli may be more visible in small cell anaplastic carcinoma. As compared to H&E stain, cytoplasm of keratinized squamous cells does not take up the stain and appears pale in toluidine blue.[56]

A study done by Jan et al., in 1990 used Diff Quik stain for rapid diagnosis and concluded that it gives a good differential staining between cells and stroma, fibrous tissue, cartilage and also mucinous/myxoid material was obtained with this stain.[57]

Selvi et al., in 2001 studied 27 synovial fluids using Diff Quik stain and wet mount. Monosodium urate and calcium pyrophosphate crystals were identified better on wet mount. Thus it was concluded that wet mount methodology is the gold standard for crystal detection, including Diff Quik stained smears might provide a useful tool.[58]

In 2003, Lambah et al., done a study on imprint cytology stained with toluidine blue and concluded the positive and negative predictive value of the test using toluidine blue were very high which suggest that the technique could be applied as a diagnostic tool with the reassurance that a positive result can be wholly relied upon.[59]

Erkilic et al., in 2006 done a wet mount study on 160 effusions concluded that in wet films it is easier to detect cytoplasmic and nuclear features of the well preserved cells. Because of preservation of cytoplasmic vacuoles, it is easier to identify macrophages and distinguish them from mesothelial cells.[4]

Sumathi et al., in 2012 done a study on 197 FNA's and studied cell morphology both in supravital toluidine blue wet film and Hematoxylin and eosin stained wet fixed smear. Cytomorphology was well appreciated in wet film study as it showed three dimensional view of

unfixed cells. Degenerated cells and neoplastic cells are more fragile and distorted easily by smearing. These artifacts are not seen in wet mount preparation with supravital stain which increase the cytomorphology especially for diagnosing deep-seated mass lesions.[1]

Ammanagi et al., in 2012 done a study on 200 cases using toluidine blue and Diff quik rapid stains and concluded that these rapid stains gives a fair idea about the nature of the lesion as it allows for the easy identification of cells.[6]

Another study done by Sumathi et al., in 2012 on 190 aspirates concluded that wet mount toluidine blue improves the diagnostic accuracy by minimizing the smearing and drying artifact, loss of cell sample during fixation and staining. Sensitivity increased with combined H&E and wet mount study, when compared to H&E alone. The study results yielded good diagnostic accuracy of 97.4% by combining rapid stain as a supplementary procedure for conventional H&E.[2]

A study done by Sofi et al., in 2013 employed supravital stain and found nuclear and nucleolar details were as good with conventional stains. Cytological morphology in less crowded areas and cellular differentiation were excellent.[60]

Microfluidics represents a novel approach for the processing of pathological biopsy specimens. Microfluidics requires the dissociation of cells from tissue. The cells were recovered and subjected to wet mount by supravital stain and conventional stains. Cells appear to be cytologically similar in both the methods. Three dimensional clusters seen in wet mount preparation reduce the quality of images.[61,62]

Various studies on supravital stains reveals that, it is a good nuclear stain and a reliable rapid stain. In all above studies smeared samples are studied for immediate assessment of cytomorphology and rapid diagnosis. One problem in FNAC is morphological distortion due to

drying and smearing artifact. This may be avoided in the great extent by using wet mount techniques. Therefore cytodiagnosis by Toluidine blue wet mount and Methylene blue/Eosin stain can be used as a rapid technique as it reduces the time taken for reporting of FNACs. These techniques can be used as a supplementary diagnostic procedure along with conventional stains.

MATERIALS AND METHODS

SOURCE OF DATA: The study was conducted on Fine needle aspirates from patients of R.L Jalappa hospital attached to Sri Devaraj Urs Medical College. Consent was taken from all the patients included in the study.

DURATION OF STUDY: December 2013 to July 2015 (1 year and 8 months).

SAMPLE SIZE: 320 Fine needle aspirates from patients presenting with different swellings.

Sample size was calculated by Glenn D Israel method.

$$n = \frac{2\left[Z_\alpha\sqrt{2\,\bar{\rho}(1-\bar{\rho})} + Z_\beta\sqrt{\rho_1(1-\rho_1)+\rho_2(1-\rho_2)}\right]^2}{d^2}$$

$\rho_1 = 91$, $\rho_2 = 81$, $Z_\alpha = 1.96$, $Z_\beta = 0.84$, $d = \rho_1 - \rho_2 = 10\%$, $\bar{\rho} = \frac{\rho_1 + \rho_2}{2} = 88\%$

$$n = \frac{2[88.2 + 38.44]^2}{(10)^2}$$

$n = 320$ samples.

Sample size was calculated based on sensitivity and specificity of rapid stains used in different studies.[2]

INCLUSION CRITERIA: All routine and guided FNACs from all sites of the body.

EXCLUSION CRITERIA: Aspirates yielding very little material.

MATERIALS REQUIRED FOR FNAC

(i) Needles – 23 – 20 G. 20-50 mm needle for superficial lesion.

(ii) 23-22 G – 90 mm needle for guided FNAC.

(iii) Syringes, spirit, cotton, slides, coverslips, test tubes, centrifuge, xylene, DPX

(iv) Standard H&E and PAP stain. [Figure 3: H&E and PAP staining kit]

(v) Loffler's Methylene blue alkaline. [Figure 4a: M/E staining kit]

(vi) Toluidine blue 0.5% [Figure 4b: TBWM staining kit]

(vii) Eosin 0.5%

(viii) A fixative 70-90% ethanol for H & E

(ix) Gloves and masks are required as a precautionary measure.

The staining solutions were prepared as explained in the Annexure III.

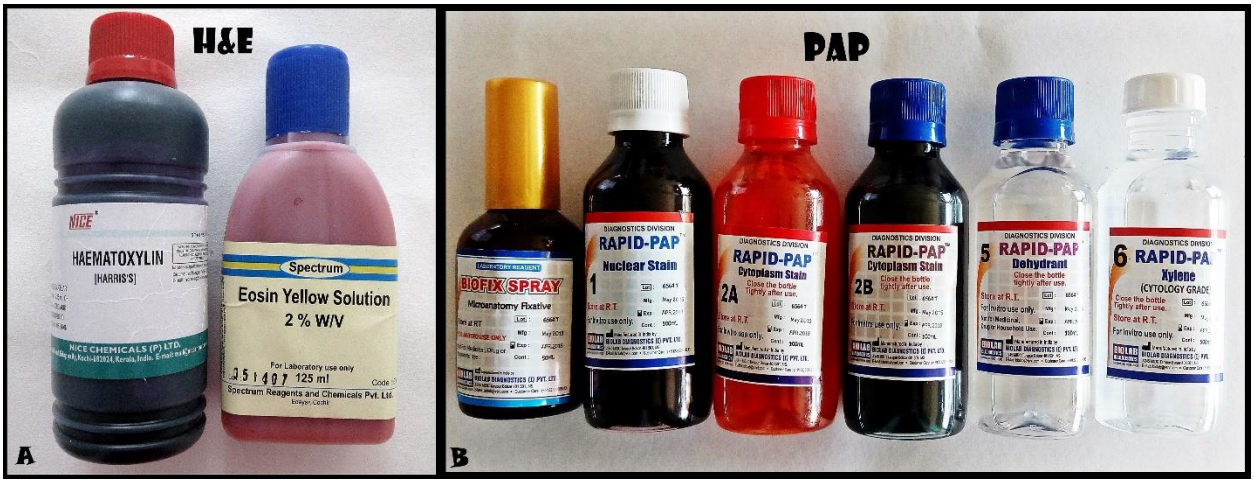


Figure 3A: H&E, 3B: PAP staining kit

Figure 4a: M/E staining kit, 4b: TBWM staining kit

PROCEDURE

1. WET MOUNT TECHNIQUE

TOLUDINE BLUE WET MOUNT STAINING

Step 1: Fine needle aspirate is expressed on centre of slide

Step 2: In case of body cavity fluid cytology drop of fluid was placed in the center of slide if fluid was turbid or the fluid was centrifuged at 1500 rpm/min for 10 minutes. The supernatant fluid was discarded. Then a drop of well mixed sediment was placed in the center of slide.

Step 3: Add drop of 0.5% Toluidine blue stain

Step 4: Mix with needle

Step 5: Add a drop of diluted Eosin stain and mix well (optional)

Step 6: Cover with cover slip

Step 7: Wet mount was examined under microscope and cytomorphology was observed.

ALTERNATIVE METHOD OF PREPARATION OF WET MOUNT

This method was tried whenever aspirates were very scanty and adhered to hub of the needle. Under such condition it was very difficult to express the aspirate over the slides.

Step 1: A few drops of toluidine blue stain was aspirated using the same syringe and needle and rinsed.

Step 2: Then the stain mixed material was expressed in the center of slide.

Step 3: A drop of eosin solution was placed next to cell stain mixture and mixed well (optional)

Step 4: Cover with cover slip

Step 5: Wet mount was examined under microscope and cytomorphology was observed.

Morphology of cells in toluidine blue wet mount staining: Three dimensional clusters and stromal elements can be appreciated with this technique with ease. Cells appear bigger than the conventional stains. Nuclei appear blue and cytoplasm is pale pink.[1,2]

2. SMEARING TECHNIQUES

A. METHYLENE BLUE ALKALINE/EOSIN STAINING

Step 1: Air dry the smear

Step 2: Fix smear in 95% methanol – 1 min

Step 3: Wash in running tap water

Step 4: 1 Dip in 1% eosin

Step 5: Wash in running tap water

Step 6: 1 dip in Loffler's methylene blue alkaline

Step 7: Wash in running tap water

Step 8: Air dry and mount with DPX

Post fixation after air-drying facilitates chromatin staining. Morphology of cells in Methylene blue and eosin stain: As it is done on air dried smears the nuclei appear bigger than the conventional stains. Nuclei stain blue to purple and cytoplasm is pale pink or pale blue.

B. PAP STAINING

Step 1: Fix the smear in 95% Ethanol

Step 2: Rinse in tap water

Step 3: Harris or Gill Hematoxylin 3-5 minutes

Step 4: Rinse in tap water

Step 5: Dip in acid alcohol

Step 6: Wash in running tap water

Step 7: Add equal amounts of 2A and 2B solution (30 seconds)

Step 8: Wash in tap water

Step 9: Air dry the smear

Step 10: Mount with permanent mounting medium (DPX)

Morphology of cells in PAP stain: The nuclei stain blue and cytoplasm is pink or green depending on the cells.[7]

C. HEMATOXYLIN AND EOSIN STAINING (H&E)

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

Step 2: Immerse in hematoxylin solution – 3-5 minutes

Step 3: Wash in running tap water

Step 4: Dip in acid alcohol

Step 5: Wash in running tap water

Step 6: Dip in eosin stain

Step 7: Wash in running tap water

Step 8: Air dry and mount with DPX

Morphology of cells in H&E stain: The nuclei stain blue and cytoplasm is pink.[1,7]

All 320 samples were stained by above four staining techniques. All the cases were reviewed by two pathologists who were blinded for final diagnosis. Time taken for each procedure was noted to access the rapidity. Diagnosis are rendered based on standard diagnostic criteria. Results obtained by Toluidine blue/Eosin wet mount and Methylene blue/Eosin stain are compared with Conventional H&E and PAP techniques to obtain diagnostic accuracy.

ASSESSMENT OF QUALITY INDEX

The morphology of the cells was assessed using the following scoring system for all the four stains on all 320 cases using the following scoring system. [Table: 1]

Table 1: The scoring system to assess Quality Index.[63]

SCORE	1	2	3
SLIDE QUALITY Background	Hemorrhage/Necrosis	Clean	
Overall staining	Bad	Moderately good	Good
Cell morphology	Not preserved	Moderately preserved	Well preserved
Nuclear characteristics	Smudgy chromatin	Moderately crisp chromatin	Crisp chromatin

The maximum score for a single case, taking into account of all the four parameters, was 11. Thus, the maximum possible score in the study was calculated by multiplying the number of cases by 11 for each of four stains. The "Quality index" was obtained by finding out the ratio of actual score obtained to the maximum score possible.

QUALITY INDEX = Actual score obtained / maximum score possible

STATISTICAL ANALYSIS

We used the IBM SPSS Software (v.22) to perform the statistical analysis. Validation of rapid cytodiagnostic tests was done against the gold standard PAP and H&E stains using tests like Sensitivity, Specificity, Positive predictive value and ROC curve. P-value less than or equal to 0.05 was considered significant.

CYTOMORPHOLOGY

THYROID LESIONS

Colloid goitre (CG): Abundant thick and thin colloid with few benign appearing follicular cells in monolayered sheets. [Figure: 5A]

Nodular goitre (NG): Abundant thick or thin colloid. Follicular cells in monolayered sheets, poorly cohesive clusters and single cells. Hyperplastic, involutional and oxyphilic cells seen.

Lymphocytic thyroiditis (LT): Lymphoid cells impinging on follicular cells. Hurthle cell change. Lymphoid and plasma cells in the background. [Figure: 5B]

Hyperplastic goitre (HG): Follicular cells in monolayered sheets, follicular or ring structures. Moderate pale, cobweb like, delicately vacuolated cytoplasm. Marginal vacuoles (fire flares). Colloid free background. [Figure: 6A]

de-Quervains thyroiditis: Multinucleate giant cells with numerous nuclei, phagocytosed colloid. Granulomatous aggregates of epithelioid cells. Degenerating follicular cells, parvacuolar granules. Dirty smear background with cell debris, colloid, neutrophils, lymphocytes and macrophages. [Figure: 6B]

Follicular neoplasm thyroid (FNT): Prominent micro follicular pattern, Rosettes, syncytial groups and equal-sized cell clusters. Nuclear crowding and overlapping. Bloody, usually colloid free background. [Figure: 7A]

Papillary thyroid carinoma (PTC): Papillary sheets of monomorphic cells with moderate pale pink cytoplasm, large round uniform nucleus with nuclear crowding and overlapping, prominent small basophilic nucleolus. Intranuclear cytoplasmic inclusions and nuclear grooves. Scanty, viscous, stringy (chewing gum) colloid. [Figure: 7B]

Anaplastic carcinoma: Necrotic background with dissociated and/or clustered highly pleomorphic malignant cells. Multinucleate, bizarre giant cells and or spindle/squamoid cells showing marked atypia. Frequent, abnormal mitosis. [Figure: 7C]

BREAST LESIONS

Fat necrosis: A dirty background of granular debris, fat droplets and fragments of adipose tissue. Foamy macrophages, multinucleated giant cells and adipocytes with bubbly cytoplasm. Absence of epithelial cells. [Figure: 8A]

Galactocele: Poorly cohesive epithelial cells of acinar type with abundant fragile cytoplasm with secretory vacuoles and frayed borders, rounded vesicular nuclei and central nucleoli. Dirty background due to lipid secretion. [Figure: 8B]

Fibrocystic change (FCC): Epithelial fragments of usual epithelial cells. Scattered single bare bipolar/oval nuclei. Background of variable amounts of cyst fluid, macrophages and apocrine metaplastic cells. [Figure: 9A]

Fibroadenoma (FA): Cellular smears with a bimodal pattern containing epithelial and stromal fragments. Large, branching sheets of bland epithelial cells. Numerous single, bare bipolar/oval nuclei. Fragments of fibromyxoid stroma. [Figure: 9B]

Phyllodes tumor (PT): Cluster of duct epithelial cells round to oval cells with scanty cytoplasm, round uniform nucleus with single small nucleoli, and cluster of stromal cell-oval to spindle cells with indistinct cytoplasmic membrane, scanty pink cytoplasm mild anisokaryotic nucleus with granular chromatin and small nucleolus. [Figure: 9C]

Papillary neoplasm: Complex folded and branching epithelial sheets and finger-like fragments, true papillary fragments with nuclear atypia. Rows of palisaded columnar epithelial cells. Dispersed epithelial cells with nuclear atypia. [Figure: 10A]

Ductal carcinoma (DC): Single epithelial cells with intact cytoplasm. Cells shows moderate to severe nuclear atypia: enlargement, pleomorphism. irregular nuclear membrane and chromatin. Necrotic material in the background. [Figure: 10B]

LYMPH NODE LESIONS

Reactive lymphadenitis (RL): Mixed population of lymphoid cells – Small lymphocytes, centroblasts, centrocytes, immunoblasts and plasma cells. Scattered histiocytes with intracytoplasmic nuclear debris (tangible body macrophages). [Figure: 11A]

Suppurative lymphadenitis (SL): Clusters of neutrophils and pale pink granular necrotic material, karyorrhectic debris. Sometimes bacteria is also seen.

Granulomatous lymphadenitis (GL): Histiocytes of epithelioid type forming cohesive clusters are characteristic. Multinucleated giant cells usually of langhans type. [Figure: 11B]

Metastatic malignancy: Abnormal non-lymphoid cells amongst normal/reactive lymphoid cells. Cytological criteria for malignancy –pleomorphic cells with nuclear atypia

Adenocarcinoma: Pleomorphic cells with scanty blue cytoplasm, large round, oval, irregular hyperchromatic nucleus with prominent nucleoli. Attempted gland formation and cytoplasmic vacuolations. [Figure: 12A]

Squamous cell carcinoma (SCC): Pleomorphic cells with scanty to moderate pink cytoplasm and large irregular hyperchromatic nucleus with inconspicuous nucleoli. [Figure: 12B]

Lymphoproliferative lesions: Monomorphic population of large cells with scanty blue cytoplasm, large round nucleus with granular chromatin and prominent nucleoli

SOFT TISSUE LESIONS

Epithelial inclusion cyst/Keratinous cyst: Polyhedral purple colored anucleated squames. [Figure: 13A]

Cystic lesions: Thick pus like fluid from bronchial cyst lesions showed sqaumous epithelial cells with few neutrophils and lymphocytes in the background.

Lipoma: Polyhedral to large round fat cells in tissue fragments with abundant cytoplasm and peripherally placed nucleus. [Figure: 13B]

Lymph cyst: lesions showed clear fluid aspirate and scattered population of uniform lymphocytes.

Thyroglossal duct cyst: Scattered thyroid follicular cells with purplish pink granular colloid in the background. [Figure: 13C]

Angiomatous lesions: Full of pale pink RBC.

Malignant lesions: Spindle, polyhedral, pleomorphic cell with abundant pale pink cytoplasm, large spindle to round nucleus with hyperchromatism and nucleoli and tumor giant cells

SALIVARY GLAND LESIONS

Pleomorphic adenoma: Variable cellularity of single cells, ovoid, plasmacytoid or spindle with abundant well defined cytoplasm, regular ovoid nuclei with bland finely granular nuclear chromatin and smooth nuclear membrane. Fibrillary chondromyxoid ground substance. [Figure: 14]

Acute suppurative sialadenitis: Variable number of acute inflammatory cells with scanty material

LIVER LESIONS

Diffuse parenchymal liver disease: Decreased cohesion of hepatocytes, degenerative changes in hepatocytes. Hepatocytic regeneration – Cells and nuclei vary in size, uneven chromatin pattern, large nucleoli, cytoplasmic staining is uneven, multinucleation and mitosis. Increased lymphocytes and Kupffer cells, bile duct epithelial cells. [Figure: 15]

Adenocarcinomatous deposits: Pleomorphic cells with scanty blue cytoplasm, large round, oval, irregular hyperchromatic nucleus with inconspicuous nucleoli. Intracytoplasmic neolumina.

THYROID LESIONS

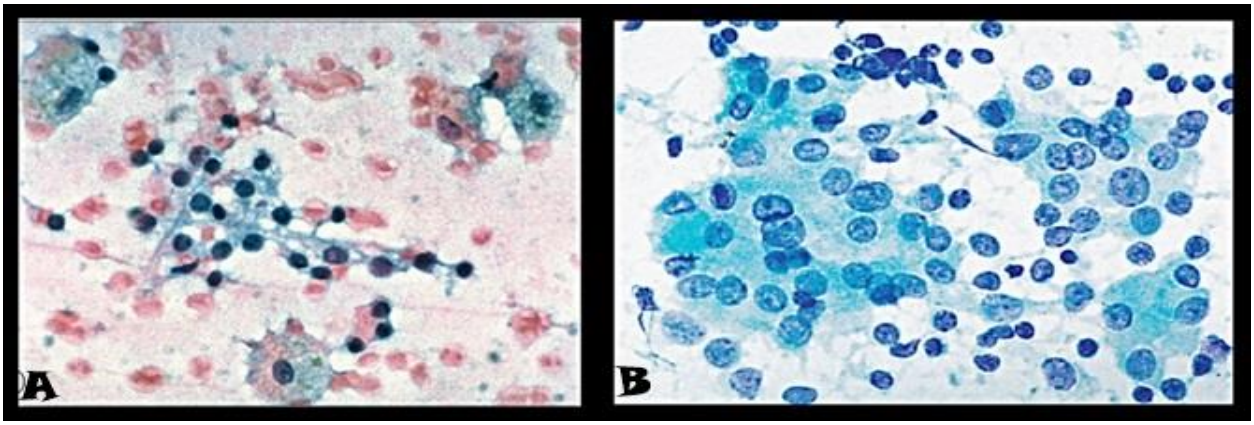


Figure 5: A – Colloid goiter (PAP), B – Lymphocytic thyroiditis (PAP)

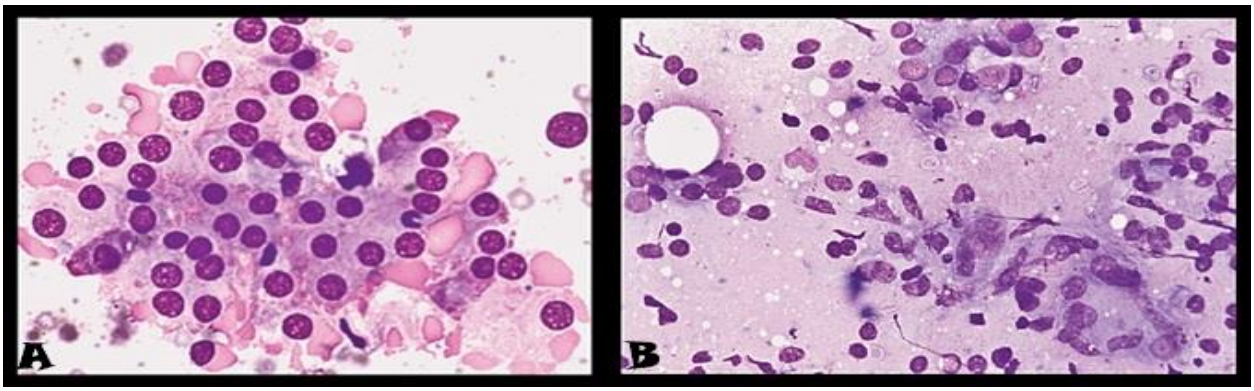


Figure 6: A – Hyperplastic goiter (H&E), B – de Quervains thyroiditis (H&E)

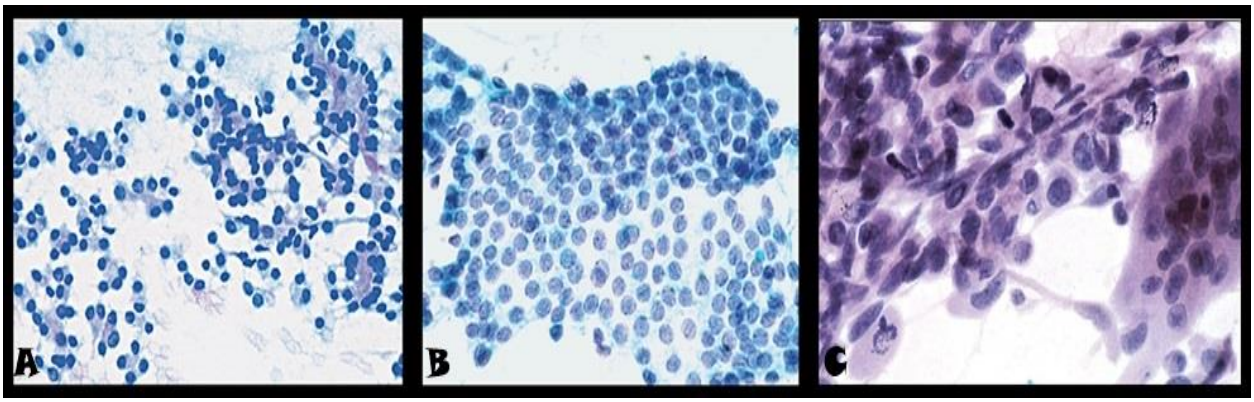


Figure 7: A-Follicular neoplasm (PAP), B-Papillary carcinoma (PAP), C-Anaplastic carcinoma of thyroid (H&E)

BREAST LESIONS

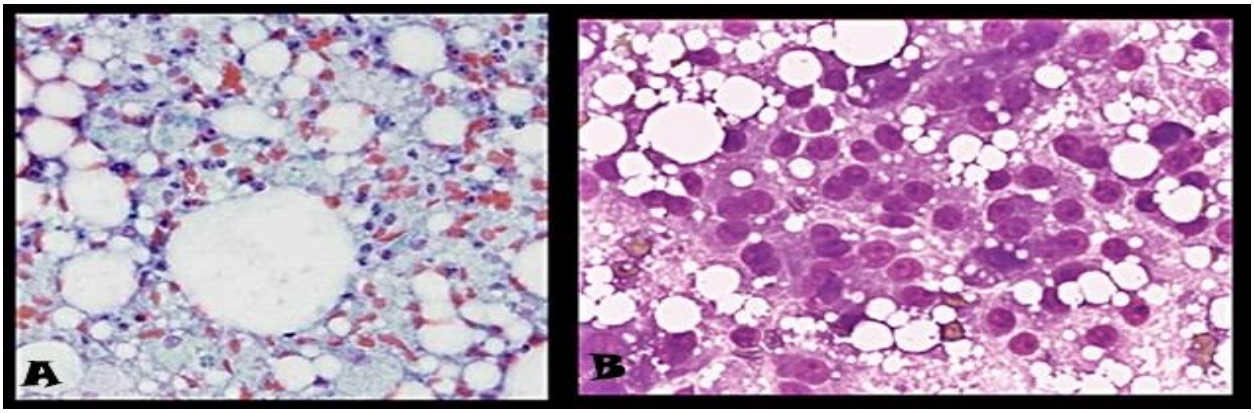


Figure 8: A – Fat necrosis (PAP), B – Galactocele (H&E)

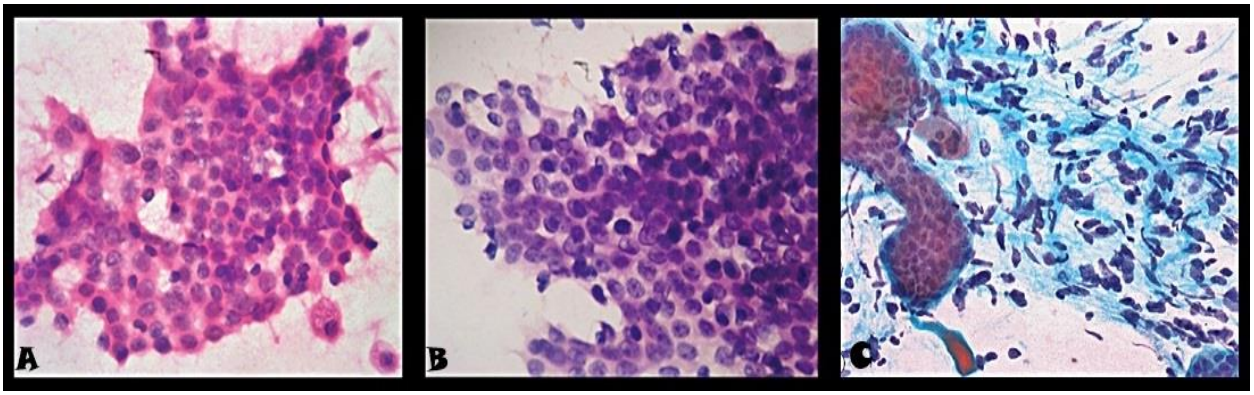


Figure 9: A-Fibrocystic change (H&E), B-Fibroadenoma (H&E), C-Phyllodes Tumor (PAP)

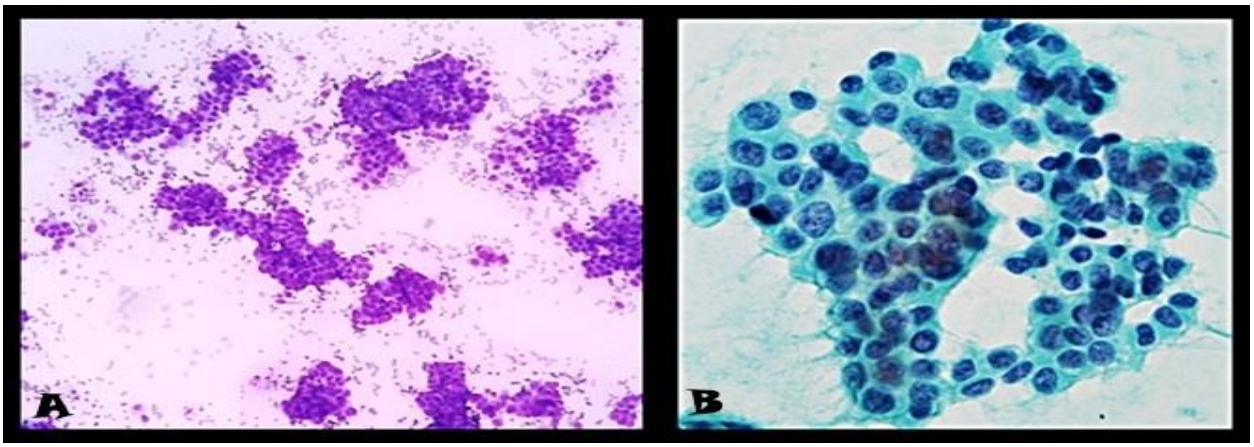


Figure 10: A – Papillary neoplasm of breast (H&E), B – Ductal carcinoma (PAP)

LYMPH NODE LESIONS

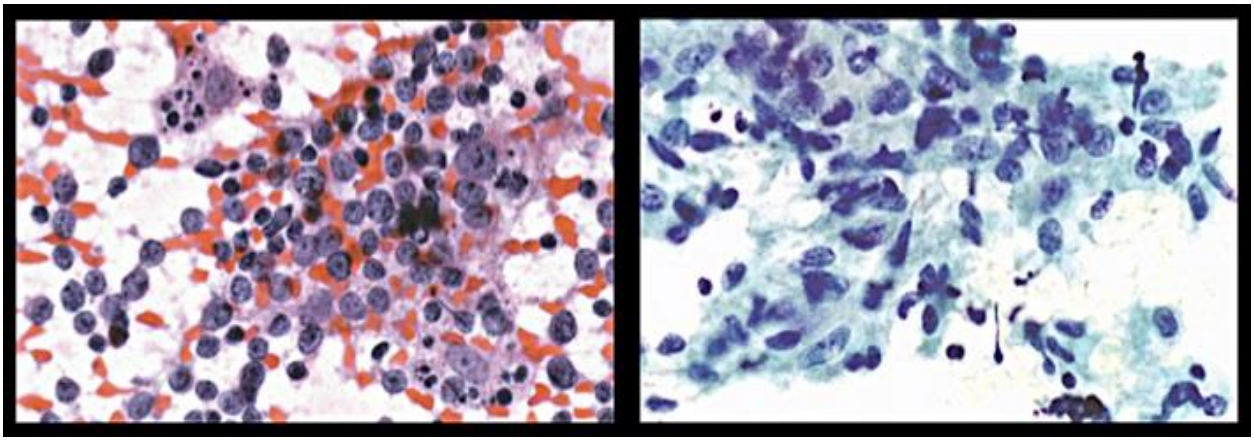


Figure 11: A – Reactive lymphadenitis (H&E), B – Granulomatous lymphadenitis (PAP)

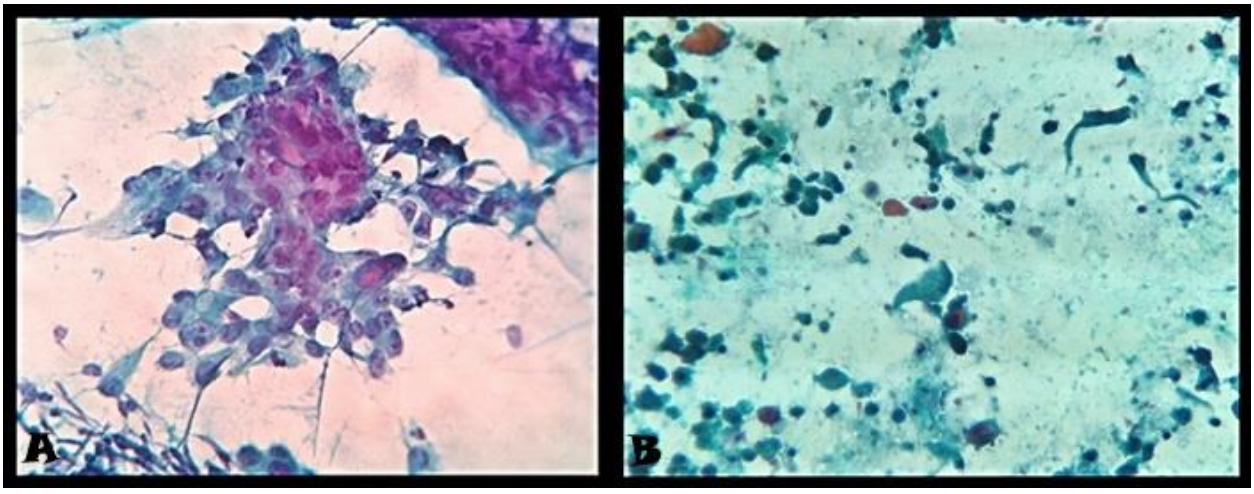


Figure 12: A – Adenocarcinomatous deposits (PAP), B – Squamous cell carcinoma deposits (PAP)

SOFT TISSUE LESIONS

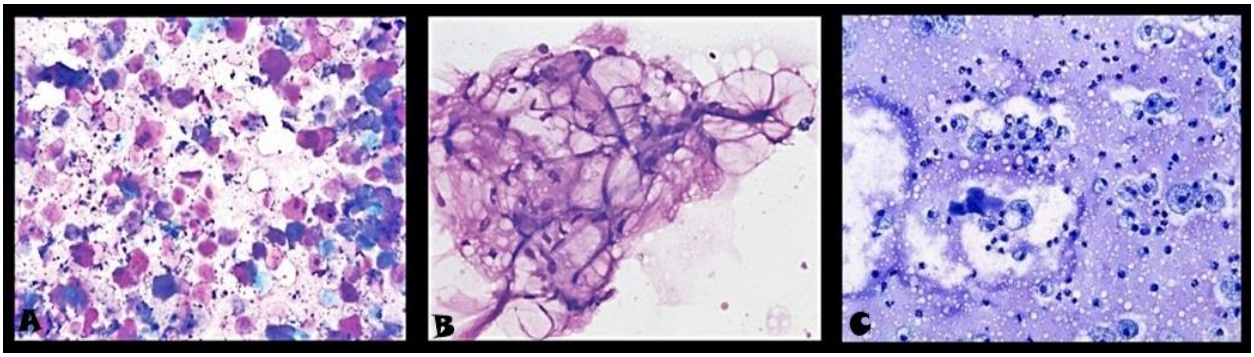


Figure 13: A - Keratinous cyst (PAP), B - Lipoma (H&E), C - Thyroglossal cyst (PAP)

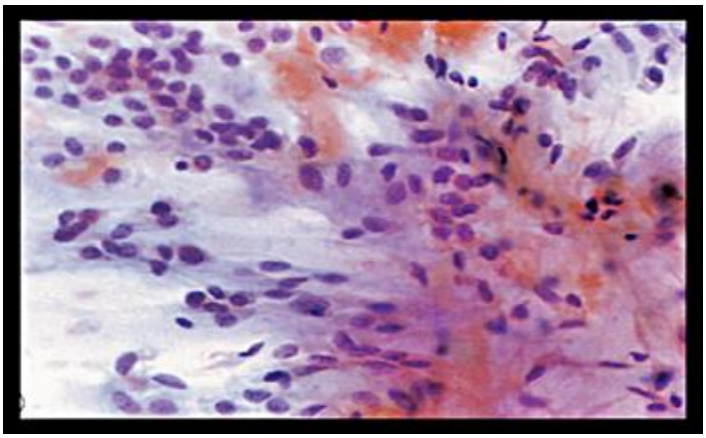


Figure 14: Pleomorphic adenoma (PAP)

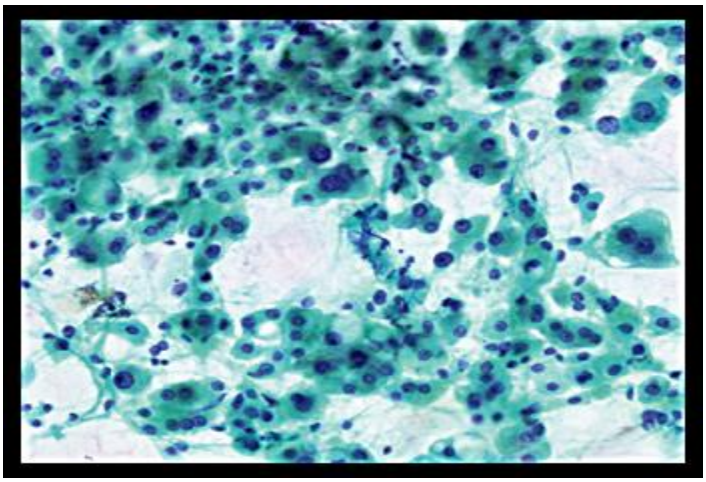
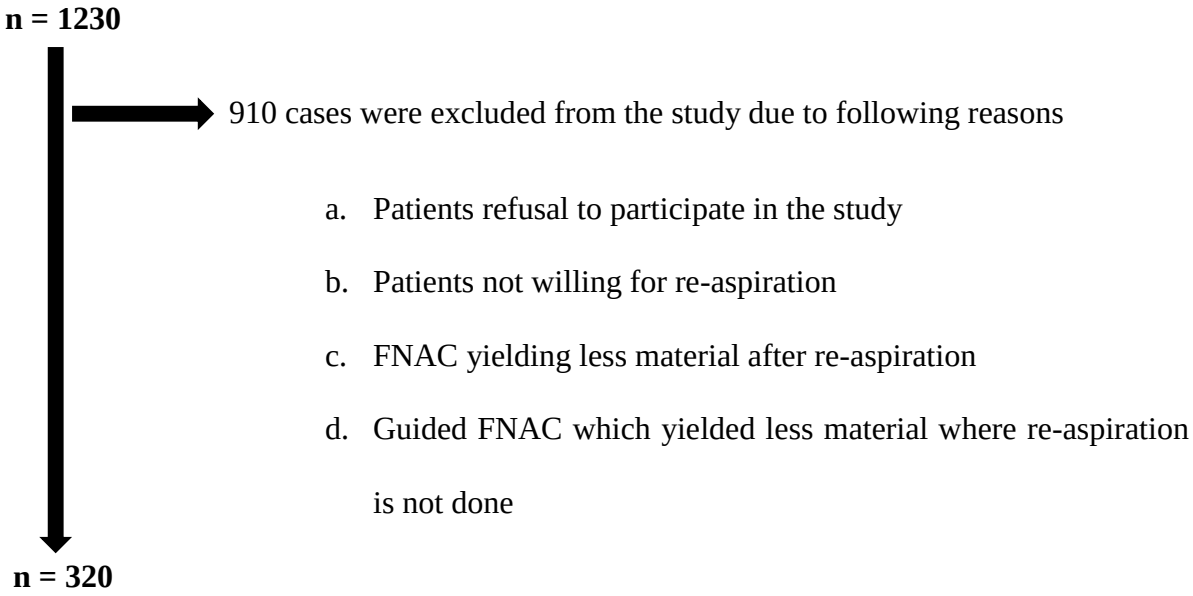


Figure 15: Diffuse parenchymal liver disease (PAP)

RESULTS

Over a period of 1 year and 8 months 1230 FNAC was done in the Department of Cytology in R.L Jalappa Hospital. 320/1230 cases were selected for the study.



320 cases had good cellularity where all stains can be performed and were chosen for the study. For each case four stains have been done and were analyzed by two pathologists for the morphological features and for final diagnosis. Pathologists were blinded for final diagnosis.

RAPIDITY ASSESSMENT

The rapidity of the stains was assessed for all four stains. Toluidine blue wet mount takes only 2 minutes for staining followed by Methylene blue/eosin which takes 5 minutes. [Table: 2]

Table 2: Time taken for each staining technique

S. NO	STAINING TECHNIQUES	TIME TAKE FOR THE PROCEDURE
1	Toluidine blue wet mount stain	2 minutes
2	Methylene blue/Eosin stain	5 minutes
3	H&E stain with artificial drying	8 minutes
4	PAP stain with artificial drying	10 minutes

COMPARISON OF RESULTS OBTAINED FROM RAPID STAINS WITH CONVENTIONAL PAP AND H&E TECHNIQUES IN FNAC

Distribution of cases: In our study, out of 320 cases, thyroid lesions were the commonest followed by breast lesions, lymph node lesions, soft tissue lesions, salivary gland lesions and liver lesions.

Table 3: Distribution of cases based on sites

LESIONS	TOTAL CASES (n=320)	PERCENTAGE
Thyroid	100	31%
Breast	96	30%
Lymph node	86	27%
Soft tissue	28	9%
Salivary gland	7	2%
Liver	3	1%

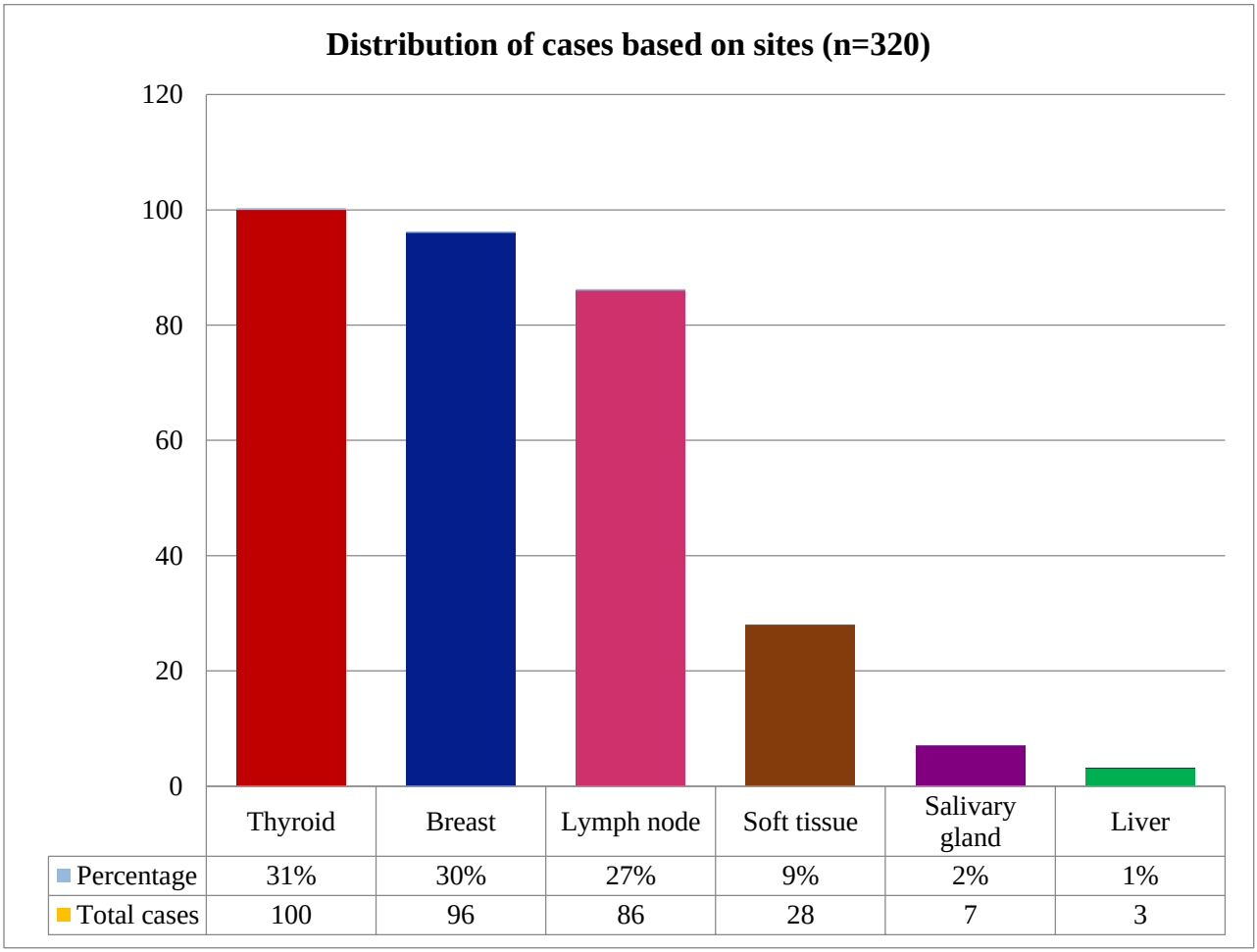


Chart 1: Distribution of cases based on sites

On analyzing the overall benign and malignant lesions, discrepancy was found in TBWM. M/E almost gives the same diagnosis as conventional stains. The overall distribution of cases is given in the following table.

Table 4: Distribution of lesions based on stains

LESIONS	CONVENTIONAL STAINS [PAP and H&E]				METHYLENE BLUE/EOSIN STAIN				WETMOUNT TECHNIQUE			
	B	M	SFM	NO OP	B	M	SFM	NO OP	B	M	SFM	NO OP
Thyroid (100)	86	13	1	-	86	13	1	-	94	5	-	1
Breast (96)	68	27	1	-	68	27	1	-	62	24	8	2
Lymph node (86)	58	28	-	-	58	28	-	-	61	23	1	1
Soft tissue (28)	24	4	-	-	24	4	-	-	20	4	-	4
Salivary gland (7)	7	-	-	-	7	-	-	-	2	-	3	2
Liver (3)	2	1	-	-	2	1	-	-	2	1	-	-

*B – Benign, *M – Malignant, *SFM – Suspicious for malignancy, *NO OP – No opinion

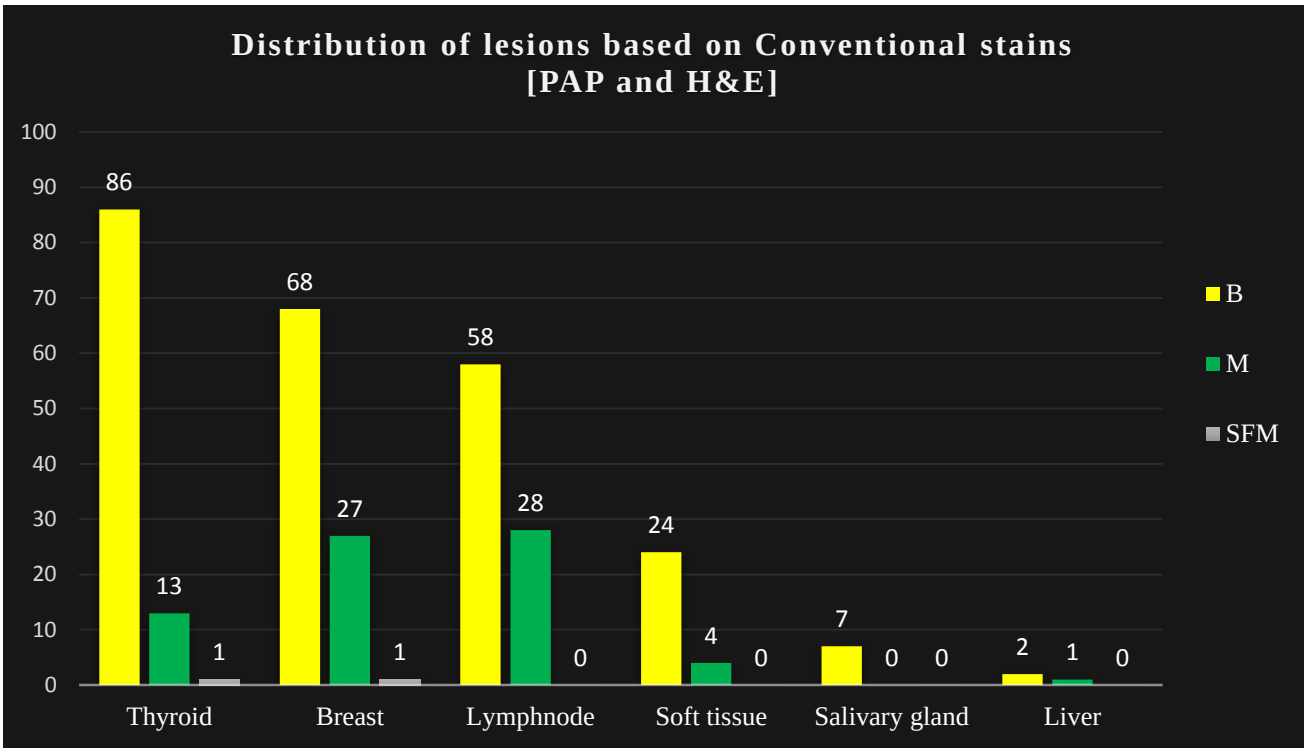


Chart 2: Distribution of lesions based on Conventional stains [PAP and H&E]

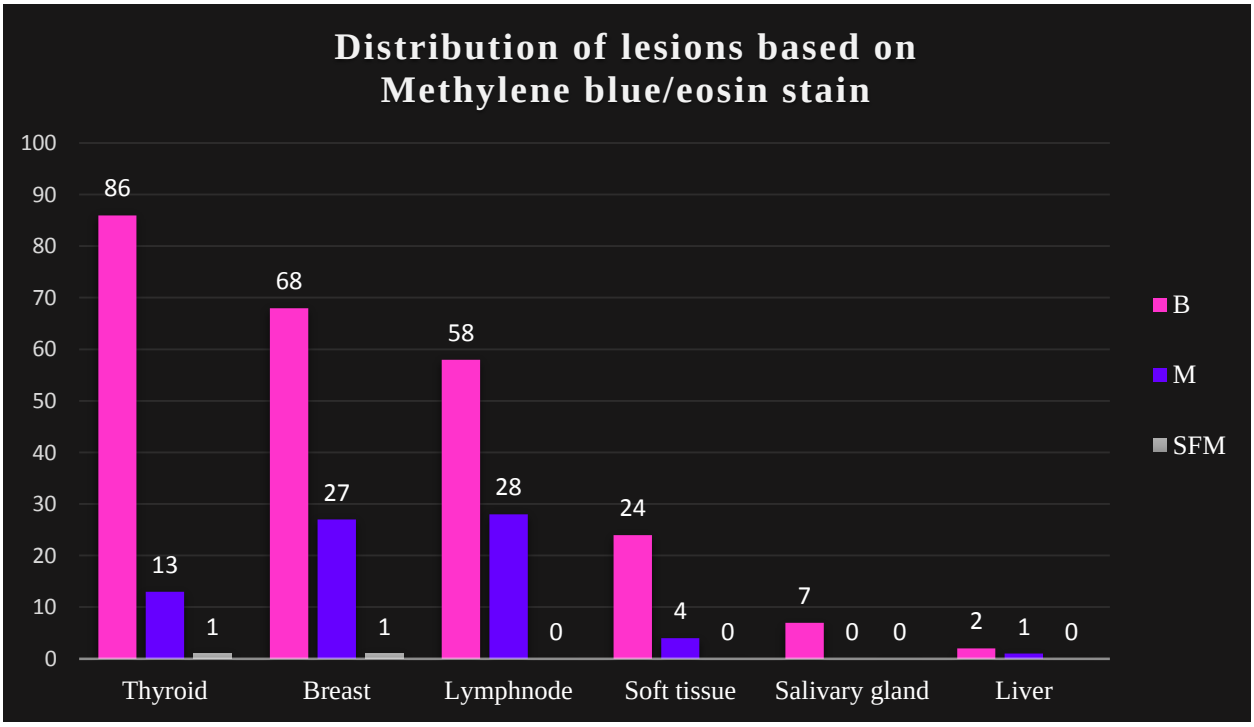


Chart 3: Distribution of lesions based on Methylene blue/Eosin stain

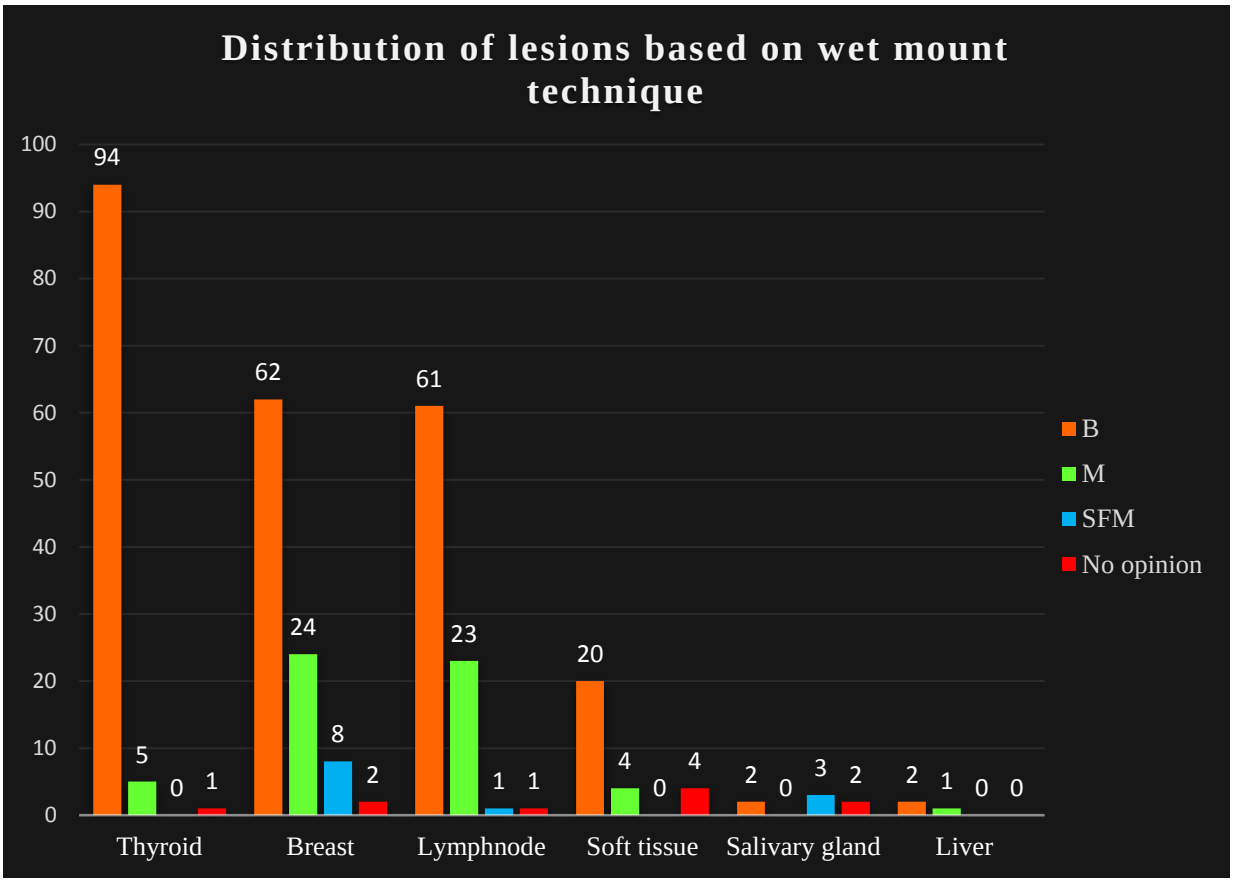


Chart 4: Distribution of lesions based on wet mount technique

THYROID LESIONS

The total number of cases were 100. Out of these 100 cases M/E stain gave the same result as compared to the conventional stains [PAP and H&E] except for 2 cases of Lymphocytic thyroiditis are diagnosed as Benign thyroid lesion. This is due to lymphocytes in these two cases were identified as naked follicular epithelial cells.

Table 5: Distribution of concordant thyroid lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS (PAP AND H&E)	METHYLENE/ EOSIN	WET MOUNT
Lymphocytic thyroiditis	34	34	33
Colloid goiter	19	19	19
Hyperplastic goitre	17	15	4
Nodular goiter	10	10	9
Benign thyroid lesion	1	1	1
de-Quervains thyroiditis	3	3	-
Acute suppurative thyroiditis	2	2	2
Suspicious for malignancy	1	1	-
Malignancy			
Papillary carcinoma thyroid	7	7	-
Follicular neoplasm	4	4	3
Anaplastic carcinoma	2	2	2
TOTAL	**100**	**98**	**73**

In TBWM there was difficulty in identifying Hyperplastic goiter due to three dimensional clusters and difficulty in identifying Hurthle cells and fire flares. The next difficulty we faced is to diagnose Papillary carcinoma of thyroid as the nuclear grooves and inclusions are not well appreciated in TBWM. Thus the total number of concordant cases by TBWM is 73.

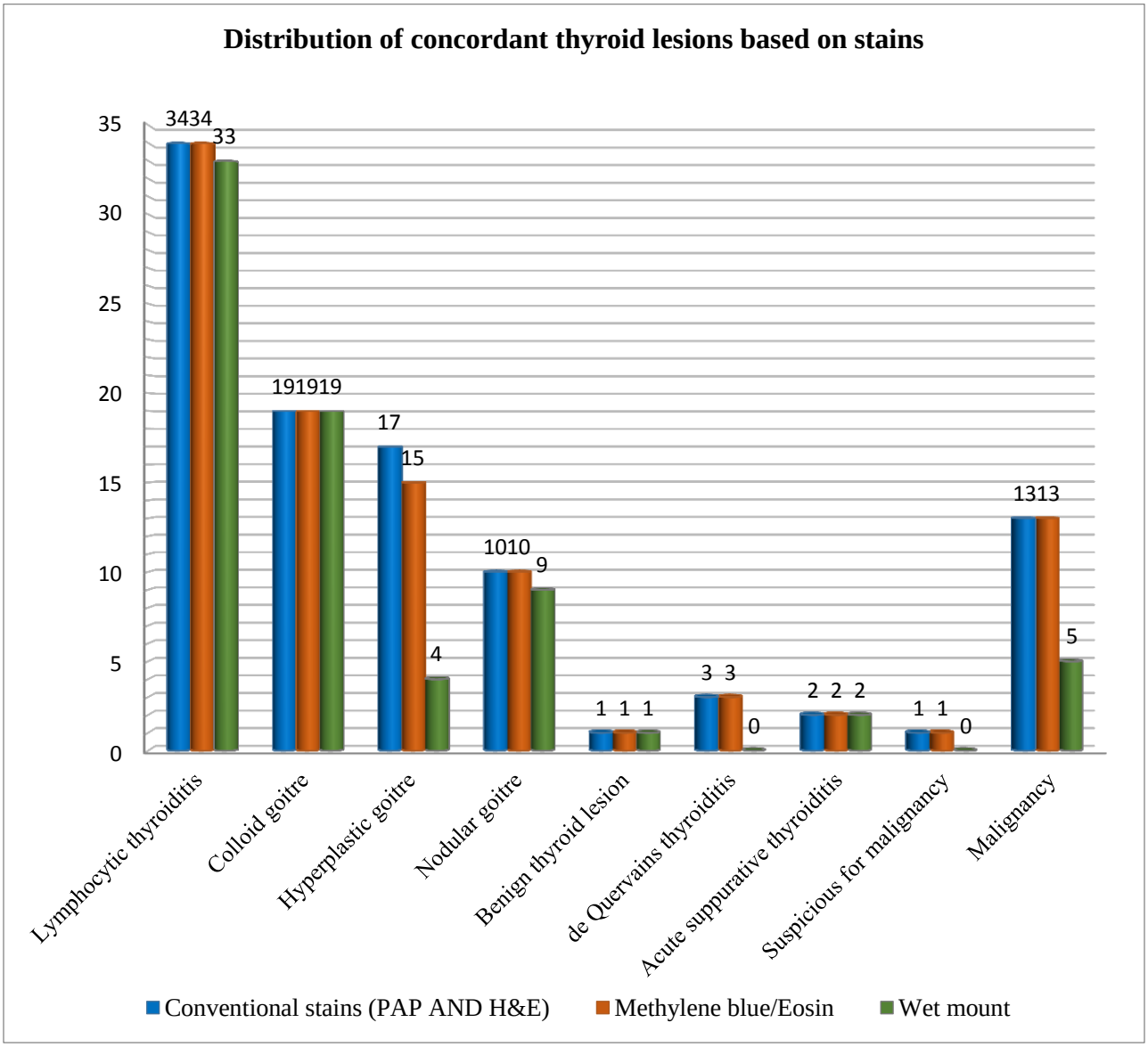


Chart 5: Distribution of concordant thyroid lesions based on stains

Table 6: Discordant thyroid lesions by wet mount technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=27)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN WET MOUNT
4	Hyperplastic goitre	Benign thyroid lesion
3	Hyperplastic goitre	Lymphocytic thyroiditis
5	Hyperplastic goitre	Nodular goitre
1	Hyperplastic goitre	Colloid goitre
3	de-Quervains thyroidtis	Benign thyroid lesion
1	Nodular goitre	Lymphocytic thyroiditis
1	Lymphocytic thyroiditis	Nodular goitre
1	Suspicious for malignancy	No opinion
7	Papillary thyroid carcinoma	Benign thyroid lesion
1	Follicular neoplasm	Hyperplastic goiter

Table 7: Discordant thyroid lesions by methylene blue/eosin technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=2)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN METHYLENE BLUE/EOSIN
1	Lymphocytic thyroiditis	Benign thyroid lesion
1	Lymphocytic thyroiditis	Benign thyroid lesion

Table 8: Statistical analysis by comparing wet mount with conventional stains in thyroid lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	66 %	46 – 100 %
Specificity	90 %	82 – 95 %
Positive predictive value	70 %	42 – 90 %
Negative predictive value	100 %	94 – 100 %

Table 9: Statistical analysis by comparing methylene blue/eosin with conventional stains in thyroid lesion

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	98 %	73 – 100 %
Specificity	100 %	94 – 100 %
Positive predictive value	98 %	73 – 100 %
Negative predictive value	100 %	94 – 100 %

Chart 6: ROC curve for Thyroid lesions by comparing M/E and TBWM with conventional stains [PAP and H&E]

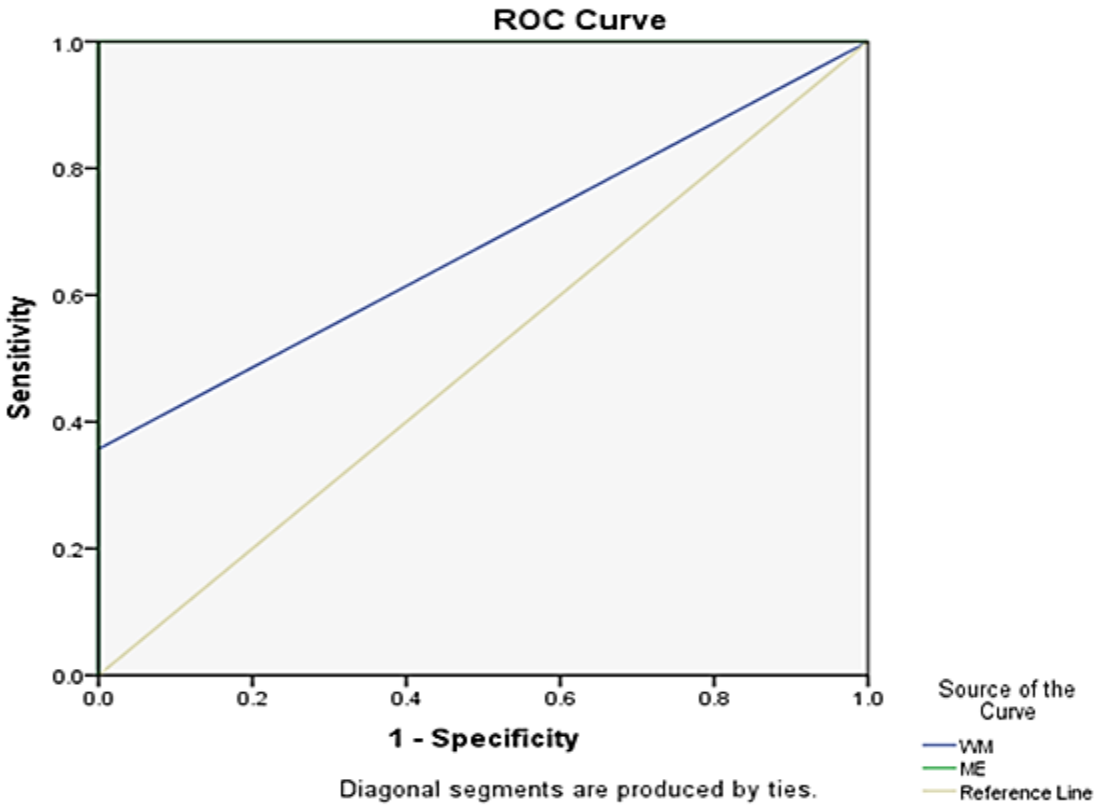


Table 10: Area Under Curve for Thyroid lesions

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	p value[b]	Asymptotic 95% Confidence Interval	
				Lower bound	Upper bound
WM	0.679	0.092	<0.033*	0.499	0.858
M/E	1.000	0.000	<.0001*	1.000	1.000

The test result variable(s): WM has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

BREAST LESIONS

The total number of cases were 96. M/E gave same result as the conventional stains [PAP and H&E] except for 1 case of fibroadenoma was diagnosed as phyllodes tumor. This is due to high cellularity and mild atypia.

The concordant cases in TBWM is 76. In TBWM, difficulty was faced in diagnosing fibrocystic change due to three dimensional clusters and cases showing atypia in fibroadenoma and atypia in Phyllodes was given as suspicious for malignancy in TBWM.

Table 11: Distribution of concordant breast lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS (PAP AND H&E)	METHYLENE/ EOSIN	WET MOUNT
Fibroadenoma	23	22	21
Fibrocystic change	19	19	14
Acute inflammatory lesion	6	6	5
Galactocele	5	5	4
Accessory breast tissue	5	5	3
Phyllodes tumor-benign	3	3	2
Keratinous cyst	3	3	3
Fat necrosis	2	2	1
Granulomatous mastitis	1	1	-
Gynecomastia	1	1	1
Suspicious for malignancy	1	1	-
Malignancy	27	27	22
TOTAL	**96**	**95**	**76**

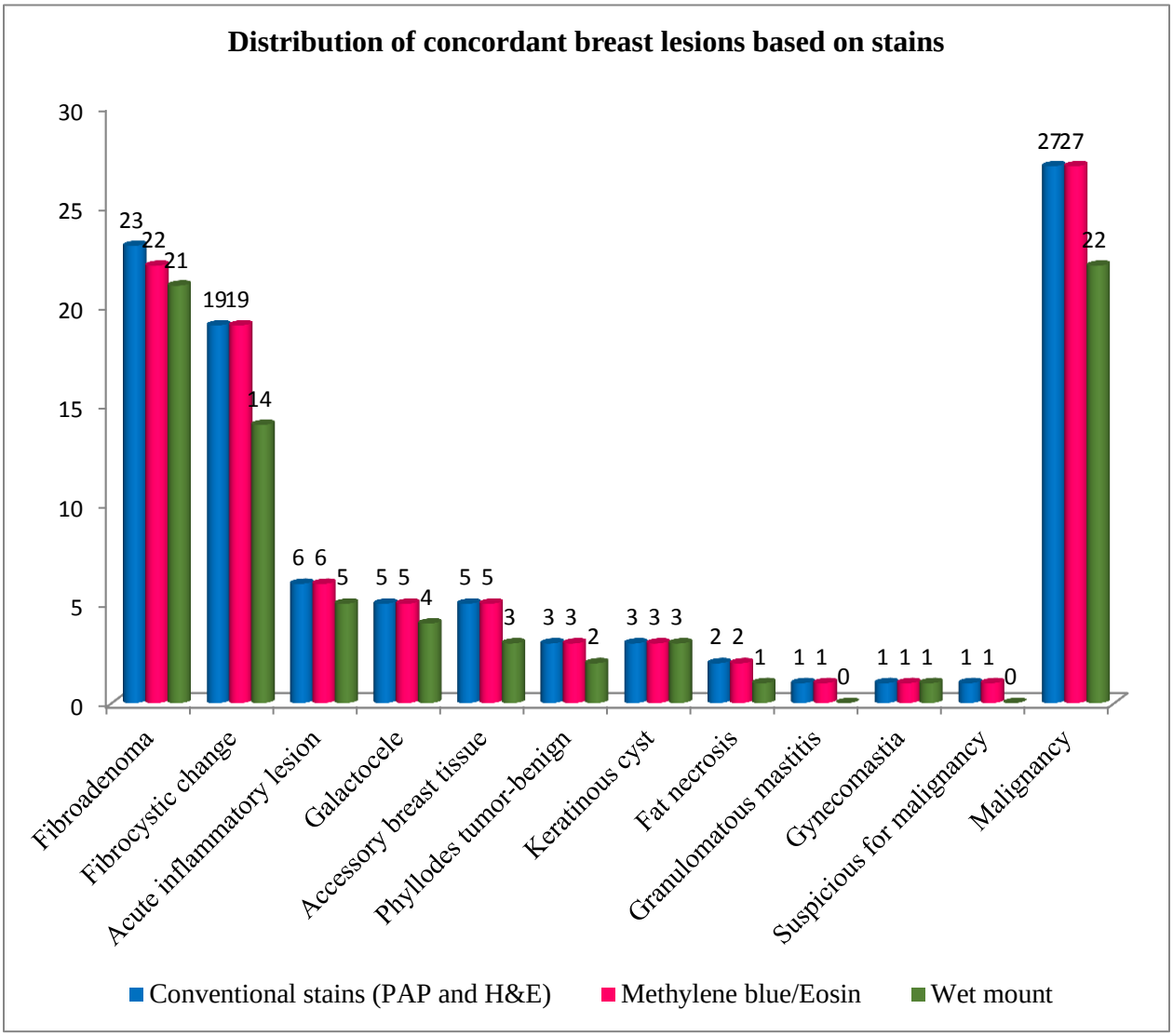


Chart 7: Distribution of concordant breast lesions based on stains

Table 12: Discordant breast lesions by wet mount technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=20)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN WET MOUNT
1	Fibrocystic change	Acute inflammatory lesion
4	Fibrocystic change	Suspicious for malignancy
1	Fibroadenoma	Suspicious for malignancy
1	Fibroadenoma	Ductal carcinoma
1	Fat necrosis	Acute inflammatory lesion
1	Acute inflammatory lesion	No opinion
1	Granulomatous mastitis	Acute inflammatory lesion
1	Galactocele	No opinion
1	Accessory breast tissue	Ductal carcinoma
1	Accessory breast tissue	Lipoma
1	Phyllodes tumor	Suspicious for malignancy
1	Suspicious for malignancy	Benign breast lesion
1	Ductal carcinoma	Fibroadenoma
2	Ductal carcinoma	Suspicious for malignancy
2	Papillary neoplasm	Benign breast lesion

Table 13: Discordant breast lesions by methylene blue/eosin technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=1)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN METHYLENE BLUE/EOSIN
1	Fibroadenoma	Phyllodes tumor - Benign

Table 14: Statistical analysis by comparing wet mount with conventional stains in breast lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	68 %	70 – 95 %
Specificity	94 %	92 – 100 %
Positive predictive value	75 %	84 – 96 %
Negative predictive value	94 %	84 – 98 %

Table 15: Statistical analysis by comparing methylene blue/eosin with conventional stains in breast lesion

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	99%	73 - 100%
Specificity	100%	94 – 100%
Positive predictive value	99%	73 – 100%
Negative predictive value	100 %	94 – 100%

Chart 8: ROC Curve for Breast lesions by comparing M/E and TBWM with conventional stains [PAP and H&E]

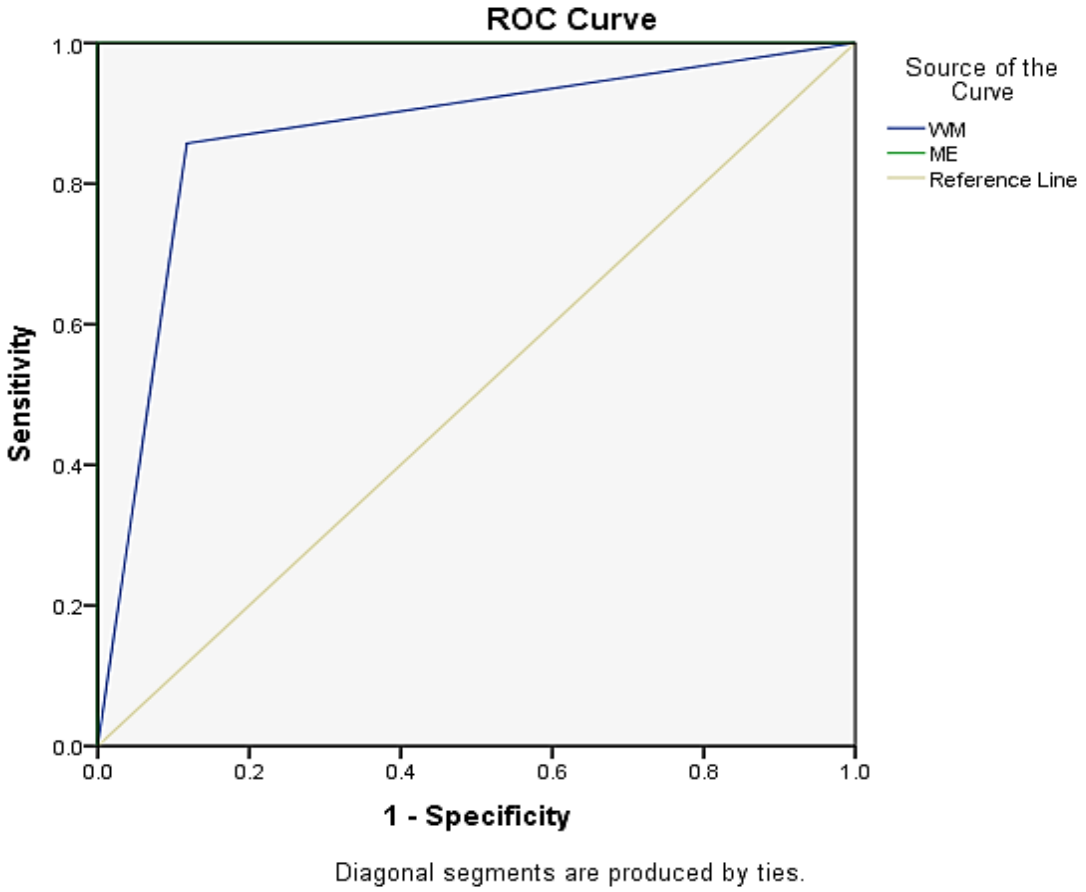


Table 16: Area Under Curve for Breast lesions

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	p value[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
WM	**0.870**	**0.045**	**<0.0001***	**0.782**	**0.957**
M/E	**1.000**	**0.000**	**<0.0001***	**1.000**	**1.000**

The test result variable(s): WM has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

LYMPH NODE LESIONS

The total number of lymph node cases were 86. The diagnosis on M/E was same as compared to the conventional stains. The concordant lesions in TBWM is 65. Only 2/15 cases of granulomatous lymphadenititis was diagnosed in TBWM. This discrepancy was mainly due to three dimensional clusters and the granulomas are not well appreciated in TBWM.

Table 17: Distribution of concordant lymph node lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS (PAP AND H&E)	METHYLENE/ EOSIN	WET MOUNT
Reactive lymphadenitis	21	21	20
Granulomatous lymphadenitis	15	15	2
Necrotising lymphadenitis	10	10	10
Suppurative lymphadenitis	7	7	7
Acute inflammatory lesion	3	3	3
Inflammed cystic lesion	1	1	-
Lymph cyst	1	1	-
Lymphoma	6	6	3
Metastasis	22	22	20
TOTAL	**86**	**86**	**65**

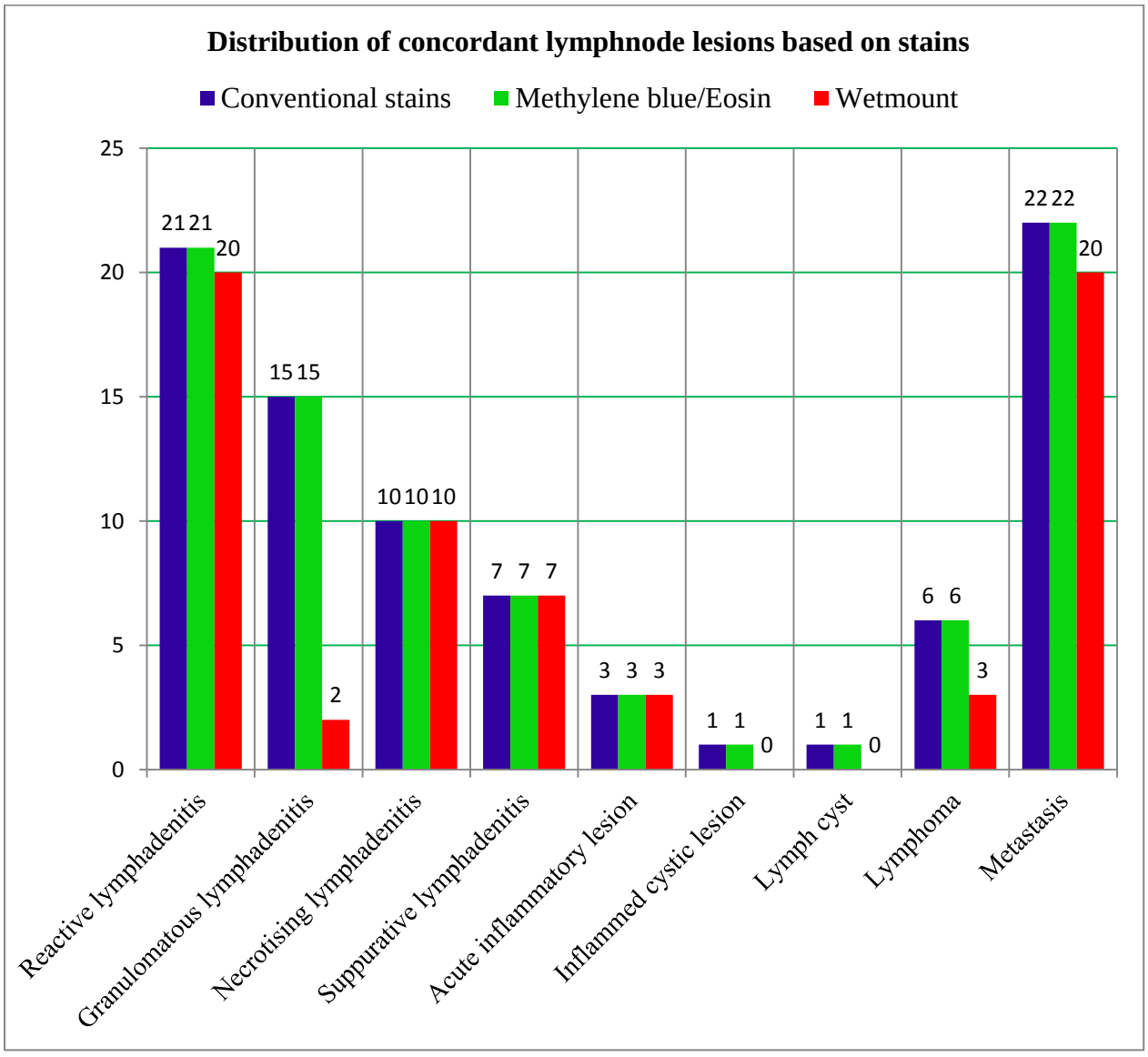


Chart 9: Distribution of concordant lymph node lesions based on stains

Table 18: Discordant lymph node lesions by wet mount technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=21)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN WET MOUNT
8	Granulomatous lymphadenitis	Necrotising lymphadenitis
5	Granulomatous lymphadenitis	Reactive lymphadenitis
1	Inflammed cystic lesion	No opinion
1	Reactive lymphadenitis	Granulomatous lymphadenitis
1	Lymph cyst	Reactive lymphadenitis
1	Non-hodgkin lymphoma	Reactive lymphadenitis
1	Squamous cell carcinoma metastasis	Acute inflammatory lesion
1	Squamous cell carcinoma metastasis	Suspicious for malignancy
1	Lymphomatous process	Suppurative lymphadenitis
1	Lymphomatous process	Necrotising lymphadenitis

Table 19: Statistical analysis by comparing wet mount with conventional stains in lymph node lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	70 %	70 – 95 %
Specificity	93 %	83 – 97 %
Positive predictive value	80 %	66 – 95 %
Negative predictive value	93 %	84 – 98 %

Table 20: Statistical analysis by comparing methylene blue/eosin with conventional stains in lymph node lesion

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	100%	73 - 100%
Specificity	100%	94 – 100%
Positive predictive value	100%	73 – 100%
Negative predictive value	100 %	94 – 100%

Chart 10: ROC Curve for Lymph node lesions by comparing M/E and TBWM with conventional stains [PAP and H&E]

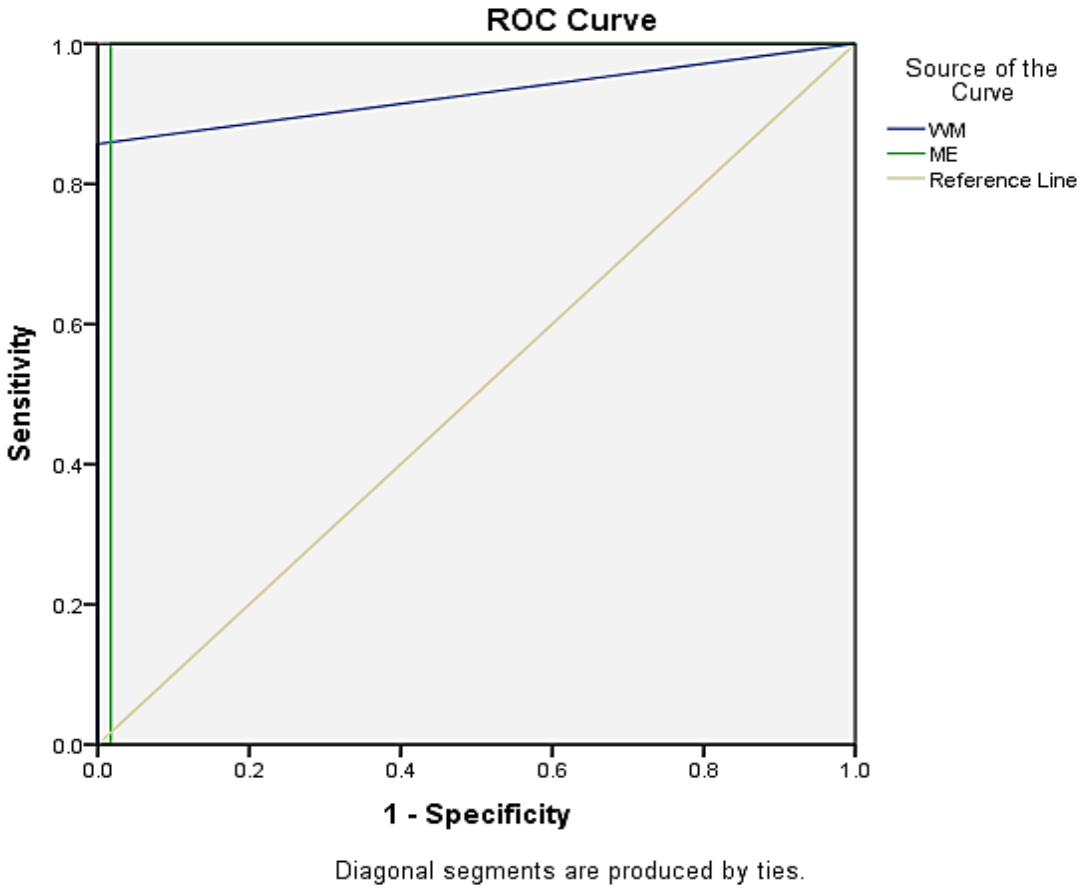


Table 21: Area Under Curve for Lymph node lesions

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	p value[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
WM	0.929	0.040	<0.0001*	0.851	1.000
M/E	0.983	0.017	<0.0001*	0.949	1.000

The test result variable(s): WM has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

SOFT TISSUE LESIONS

The total number of cases were 28. In 4 cases opinion could not be made in TBWM due to obscured morphology.

Table 22: Distribution of concordant soft tissue lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS [PAP AND H&E]	METHYLENE/EOSIN	WET MOUNT
Cystic lesions	10	10	7
Benign lesions	14	14	13
Malignant lesions	4	4	4
TOTAL	**28**	**28**	**24**

Chart 11: Distribution of concordant soft tissue lesions based on stains

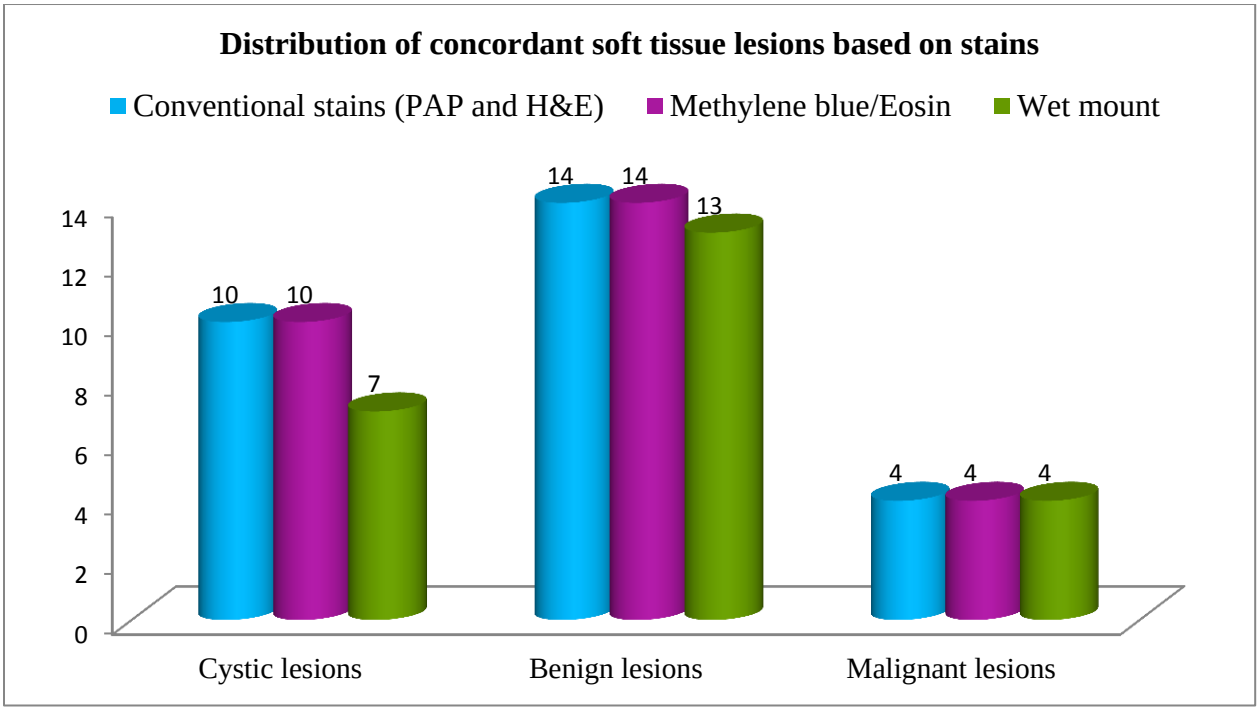
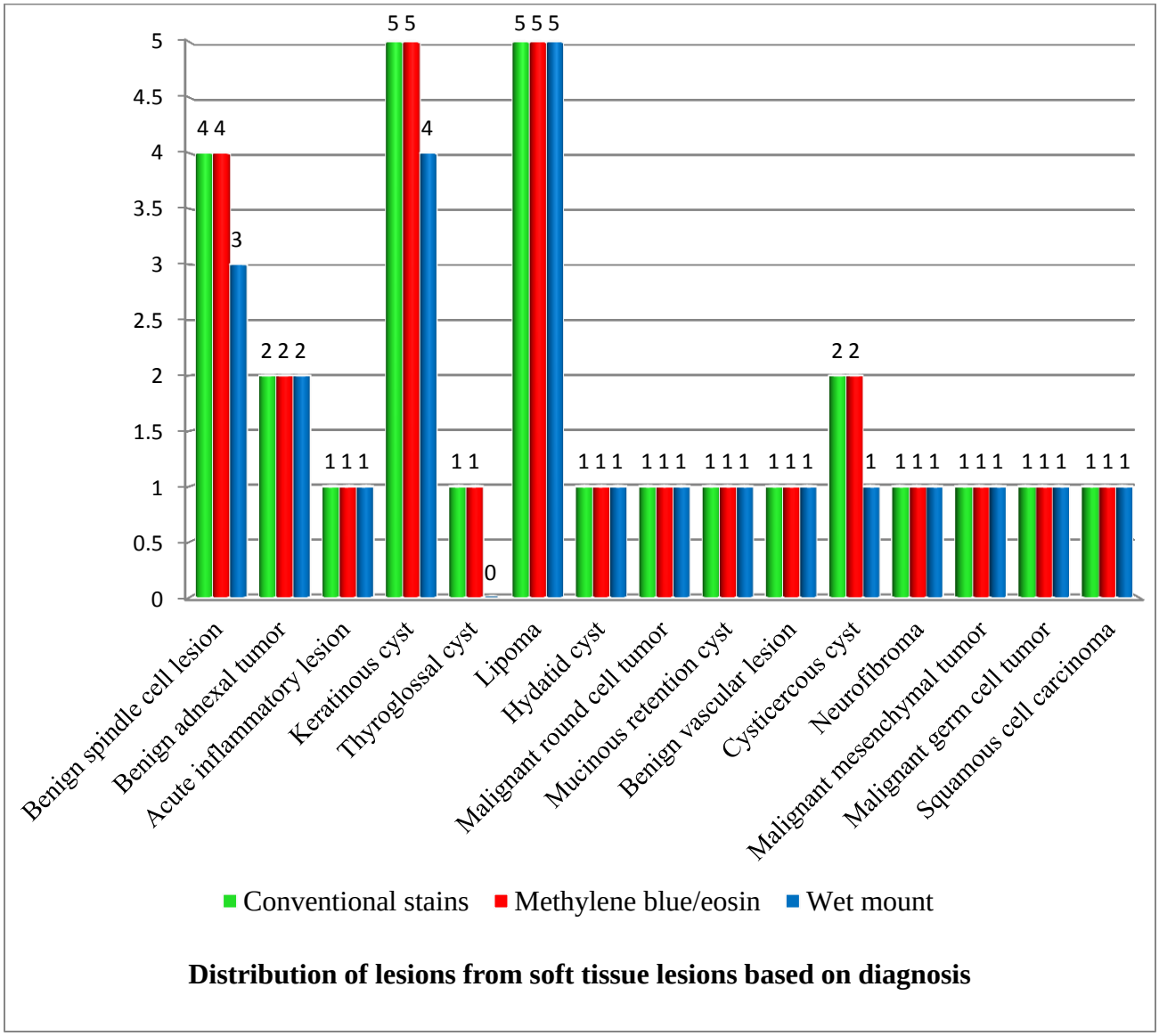


Table 23: Distribution of concordant soft tissue lesions based on diagnosis

NATURE OF LESION	CONVENTIONAL STAINS [PAP AND H&E]	METHYLENE/ EOSIN	WET MOUNT
Benign spindle cell lesion	4	4	3
Benign adnexal tumor	2	2	2
Acute inflammatory lesion	1	1	1
Keratinous cyst	5	5	4
Thyroglossal duct cyst	1	1	-
Lipoma	5	5	5
Hydatid cyst	1	1	1
Malignant round cell tumor	1	1	1
Mucinous retention cyst	1	1	1
Benign vascular lesion	1	1	1
Cysticercous cyst	2	2	1
Neurofibroma	1	1	1
Malignant mesenchymal tumor	1	1	1
Malignant germ cell tumor	1	1	1
Squamous cell carcinoma	1	1	1
TOTAL	**28**	**28**	**24**



Distribution of lesions from soft tissue lesions based on diagnosis

Chart 12: Distribution of concordant soft tissue lesions based on diagnosis

Table 24: Discordant soft tissue lesions by wet mount technique in comparison with conventional stains [PAP and H&E]

NUMBER OF CASES (n=4)	CONVENTIONAL STAINS [PAP AND H&E]	WET MOUNT
1	Keratinous cyst	No opinion
1	Thyroglossal duct cyst	No opinion
1	Benign spindle cell lesion	No opinion
1	Cysticercous cyst	No opinion

Chart 13: Discordant Soft tissue lesions shown by wet mount technique

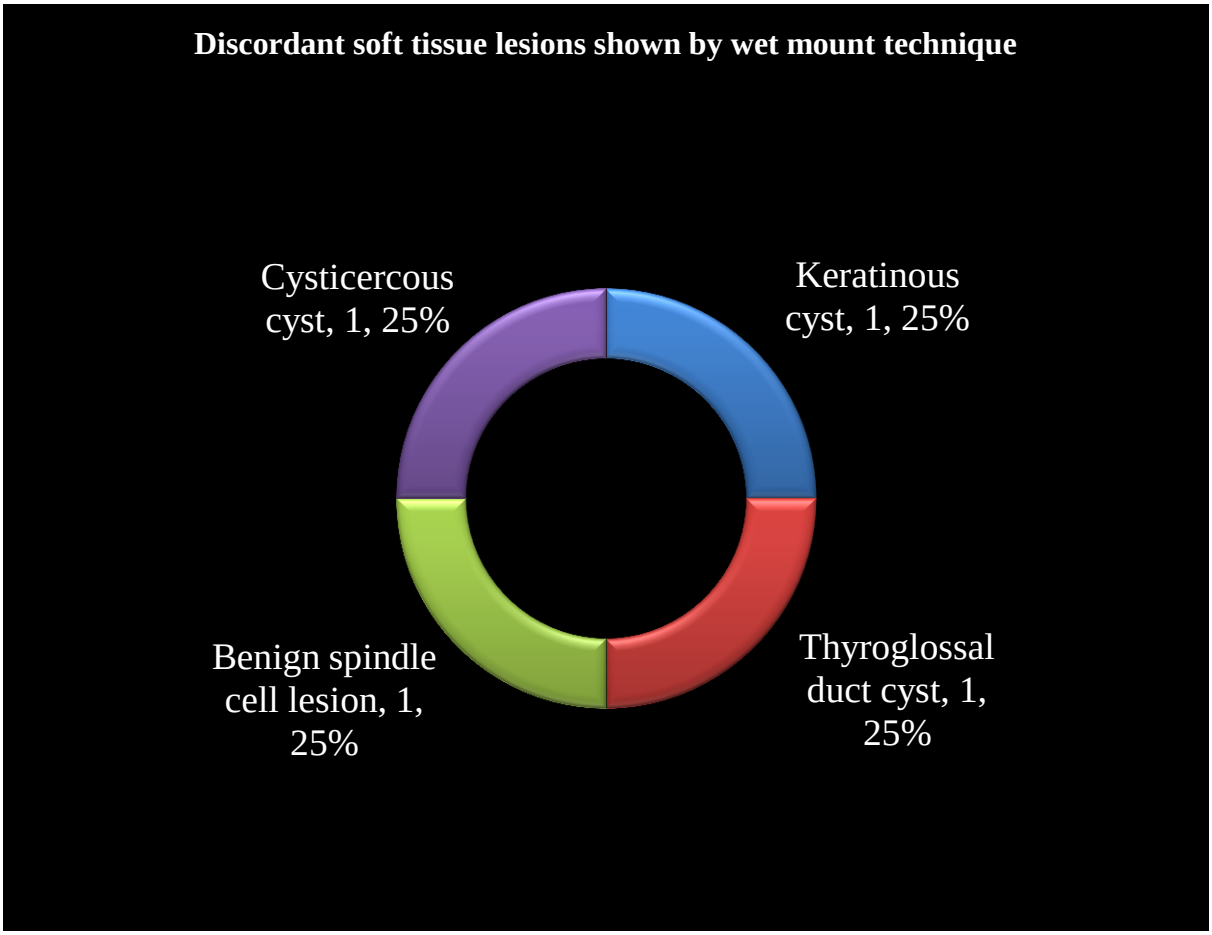


Table 25: Statistical analysis by comparing wet mount with conventional stains in soft tissue lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	84 %	83 – 100 %
Specificity	96 %	83 – 100 %
Positive predictive value	88 %	83 - 100 %
Negative predictive value	98 %	83 – 100 %

Table 26: Statistical analysis by comparing methylene blue/eosin with conventional stains in soft tissue lesions

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	100%	73 - 100%
Specificity	100%	94 – 100%
Positive predictive value	100%	73 – 100%
Negative predictive value	100 %	94 – 100%

Chart 14: ROC Curve for Soft tissue lesions by comparing M/E and TBWM with conventional stains [PAP and H&E]

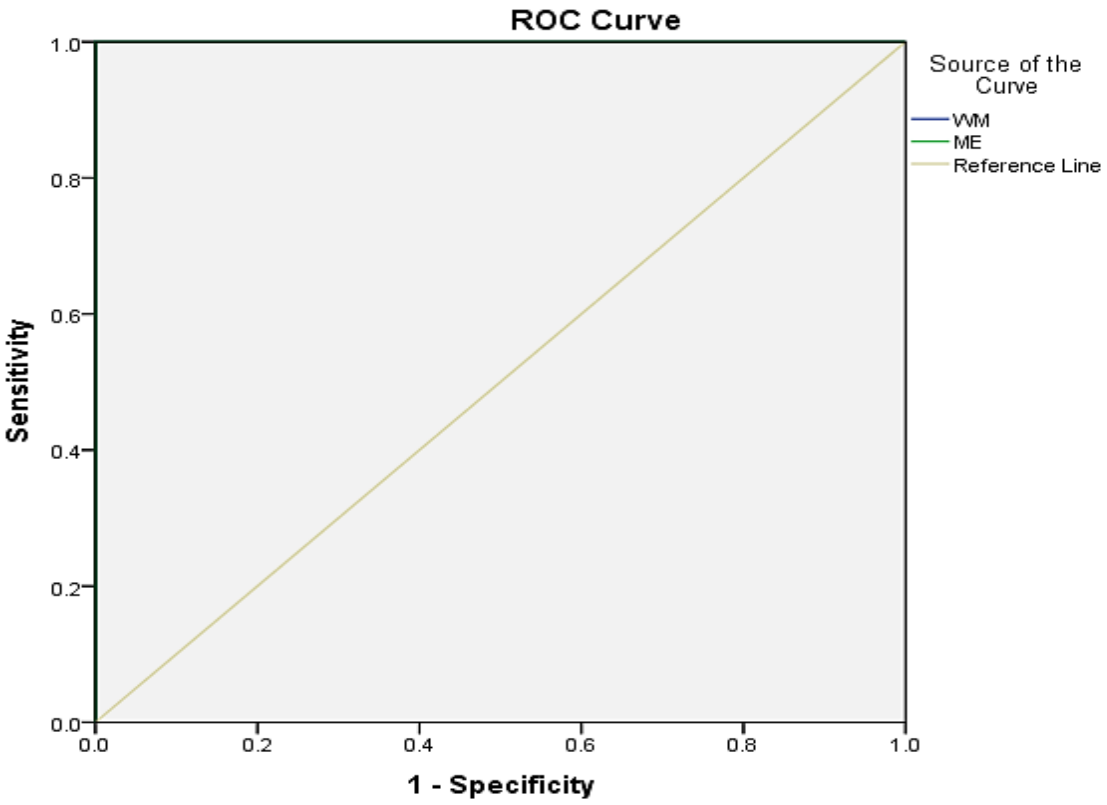


Table 27: Area Under Curve for Soft tissue lesions

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	P value.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
WM	1.000	0.000	<0.002*	1.000	1.000
M/E	1.000	0.000	<0.002*	1.000	1.000

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

SALIVARY GLAND LESIONS

The number of cases in this category is 7. M/E gave the same result as conventional stains. The concordant cases in TBWM is 2. The discrepancy was found in the cases of pleomorphic adenoma in TBWM.

Table 28: Distribution of concordant salivary gland lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS [PAP AND H&E]	METHYLENE BLUE/EOSIN	WET MOUNT
Pleomorphic adenoma	5	5	1
Monomorphic adenoma	1	1	0
Acute suppurative sialadenitis	1	1	1
TOTAL	**7**	**7**	**2**

Table 29: Discordant salivary gland lesions by wet mount technique in comparison with conventional stains [PAP and H&E]

DISCORDANT LESION NUMBER (n=5)	DIAGNOSIS IN CONVENTIONAL STAINS [PAP and H&E]	DIAGNOSIS IN WET MOUNT
1	Pleomorphic adenoma	No opinion
1	Monomorphic adenoma	No opinion
3	Pleomorphic adenoma with atypia	Suspicious for malignancy

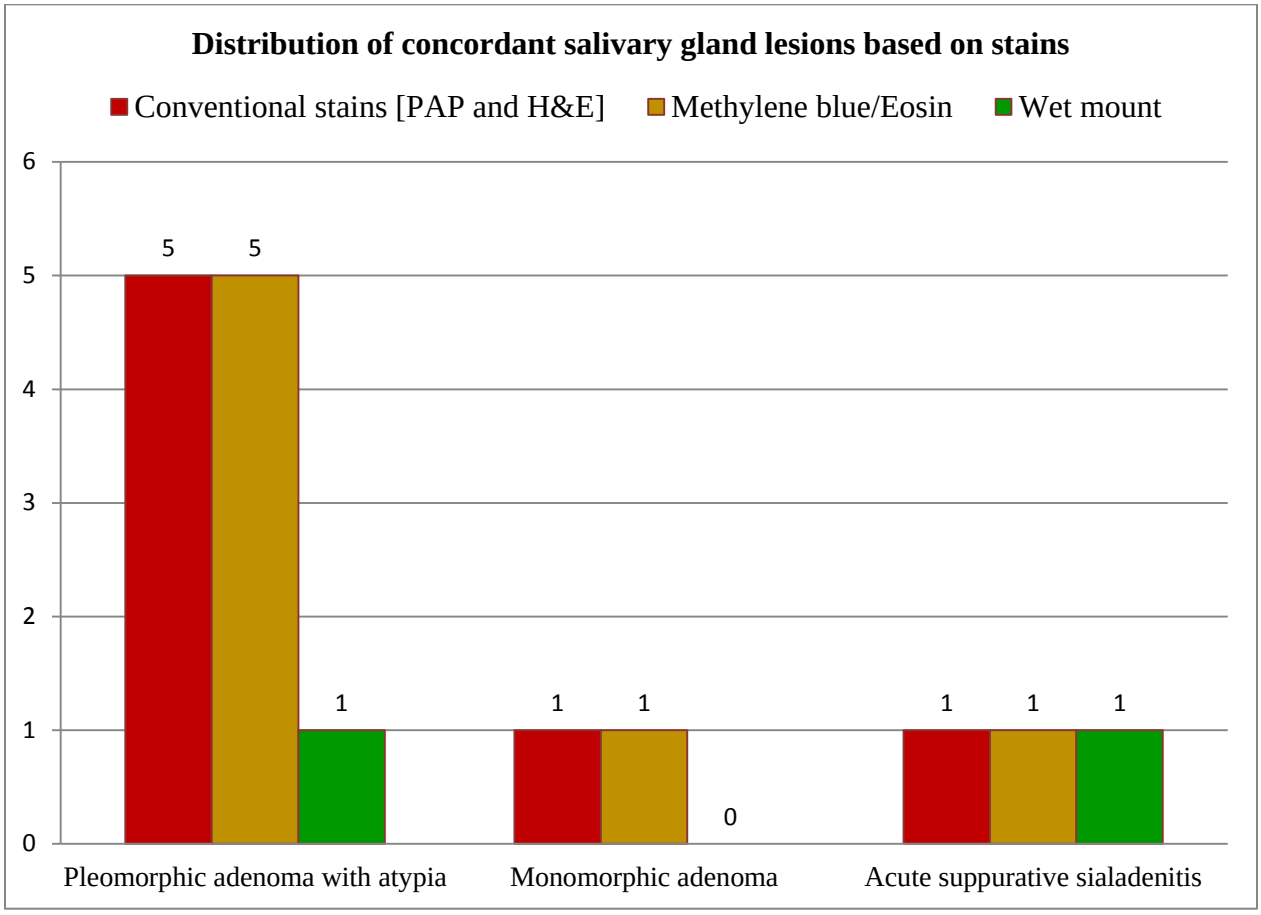


Chart 15: Distribution of concordant salivary gland lesions based on stains

Table 30: Statistical analysis by comparing wet mount with conventional stains in salivary gland lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	79 %	70 – 95 %
Specificity	93 %	83 – 97 %
Positive predictive value	85 %	66 – 95 %
Negative predictive value	93 %	84 – 98 %

Table 31: Statistical analysis by comparing methylene blue/eosin with conventional stains in salivary gland lesion

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	100%	73 - 100%
Specificity	100%	94 – 100%
Positive predictive value	100%	73 – 100%
Negative predictive value	100 %	94 – 100%

LIVER LESIONS

There were 3 cases of liver lesions. All 3 cases gives the same result in M/E and TBWM as compared to conventional stains [PAP and H&E]

Table 32: Distribution of concordant liver lesions based on stains

NATURE OF LESION	CONVENTIONAL STAINS [PAP AND H&E]	METHYLENE BLUE/EOSIN	WET MOUNT
Benign lesion	2	2	2
Malignant lesion	1	1	1
TOTAL	**3**	**3**	**3**

Table 33: Distribution of concordant liver lesions based on diagnosis

NATURE OF LESION	CONVENTIONAL STAINS [PAP AND H&E]	METHYLENE BLUE/EOSIN	WET MOUNT
Diffuse parenchymal liver disease	1	1	1
Hydatid cyst	1	1	1
Adenocarcinoma depositis	1	1	1
TOTAL	**3**	**3**	**3**

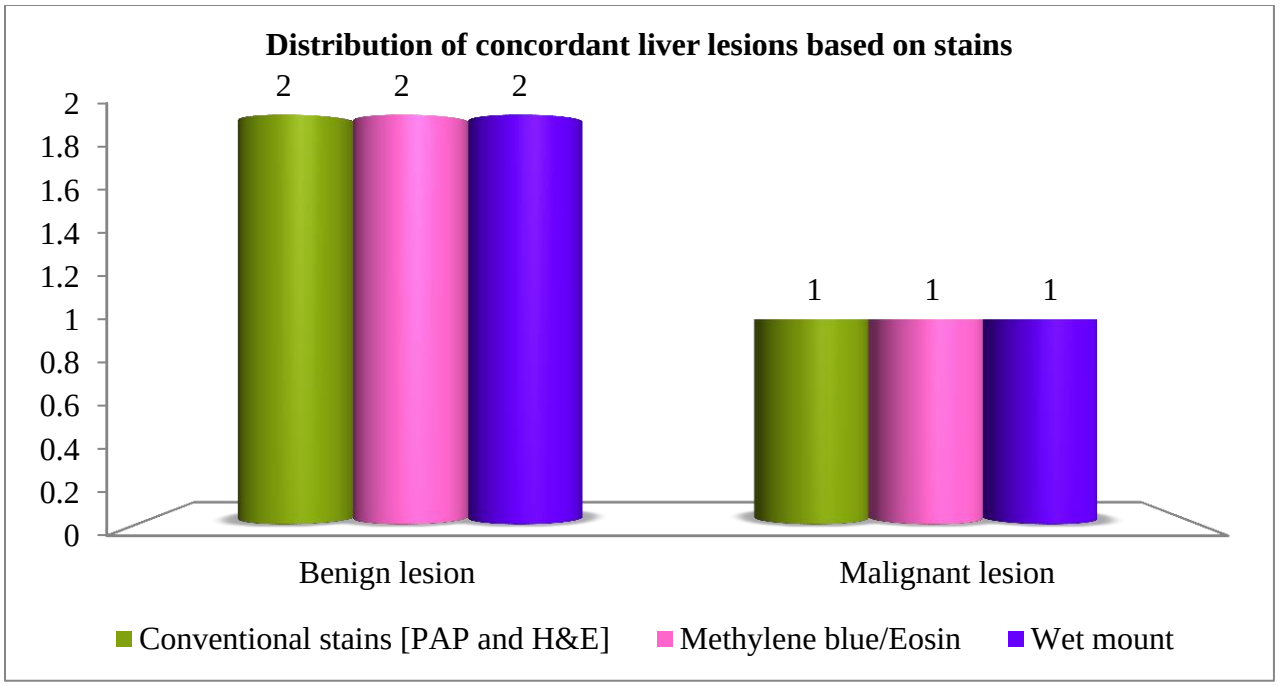


Chart 16: Distribution of concordant liver lesions based on stains

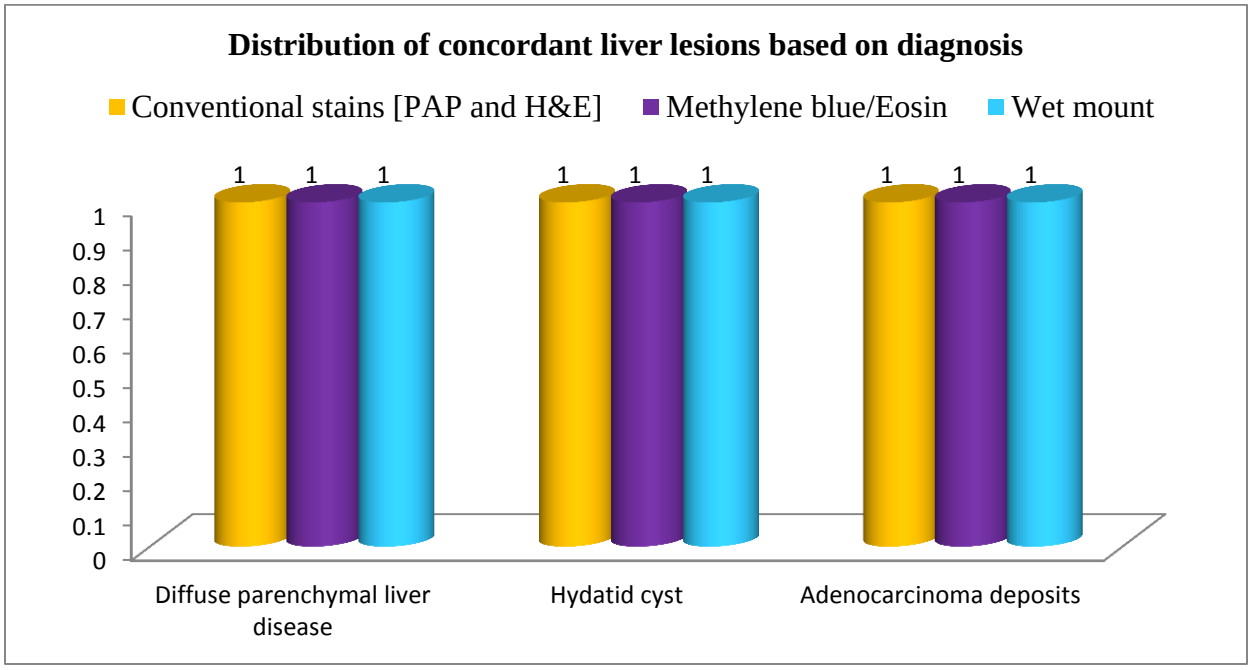


Chart 17: Distribution of concordant liver lesions based on diagnosis

Table 34: Statistical analysis by comparing wet mount with conventional stains in liver lesions

STATISTICAL PARAMETERS	WET MOUNT	CONFIDENCE INTERVAL
Sensitivity	100%	70 – 100 %
Specificity	100%	83 – 100 %
Positive predictive value	100%	86 – 100 %
Negative predictive value	100%	84 – 100 %

Table 35: Statistical analysis by comparing methylene blue/eosin with conventional stains in liver lesion

STATISTICAL PARAMETERS	METHYLENE BLUE/EOSIN	CONFIDENCE INTERVAL
Sensitivity	100%	73 - 100%
Specificity	100%	94 – 100%
Positive predictive value	100%	73 – 100%
Negative predictive value	100 %	94 – 100%

Table 36: Benign cases showing discrepancy in Wet mount technique in comparison with Conventional stains [PAP and H&E]

NUMBER OF CASES (n=48)	CONVENTIONAL STAINS [PAP AND H&E]	WET MOUNT
4	Hyperplastic goitre	Benign thyroid lesion
3	Hyperplastic goitre	Lymphocytic thyroiditis
5	Hyperplastic goitre	Nodular goitre
1	Hyperplastic goitre	Colloid goitre
3	de-Quervains thyroidtis	Benign thyroid lesion
1	Nodular goitre	Lymphocytic thyroiditis
1	Lymphocytic thyroiditis	Nodular goitre
8	Granulomatous lymphadenitis	Necrotising lymphadenitis
5	Granulomatous lymphadenitis	Reactive lymphadenitis
1	Reactive lymphadenitis	Granulomatous lymphadenitis
1	Lymph cyst	Reactive lymphadenitis
1	Fibrocystic change	Acute inflammatory lesion
4	Fibrocystic change	Suspicious for malignancy
1	Fibroadenoma	Suspicious for malignancy
1	Fibroadenoma	Ductal carcinoma
1	Fat necrosis	Acute inflammatory lesion
1	Granulomatous mastitis	Acute inflammatory lesion
1	Accessory breast tissue	Ductal carcinoma
1	Accessory breast tissue	Lipoma
1	Phyllodes tumor	Suspicious for malignancy
3	Pleomorphic adenoma	Suspicious for malignancy

Table 37: Malignant cases showing discrepancy in wet mount in comparison with conventional stains (False negative cases)

NUMBER OF CASES (n=19)	CONVENTIONAL STAINS [PAP AND H&E]	WET MOUNT
7	Papillary thyroid carcinoma	Benign thyroid lesion
1	Follicular neoplasm	Hyperplastic goiter
1	Non-hodgkin lymphoma	Reactive lymphadenitis
1	Squamous cell carcinoma metastasis	Acute inflammatory lesion
1	Squamous cell carcinoma metastasis	Suspicious for malignancy
1	Lymphomatous process	Suppurative lymphadenitis
1	Lymphomatous process	Necrotising lymphadenitis
1	Suspicious for malignancy	Benign breast lesion
1	Ductal carcinoma	Fibroadenoma
2	Ductal carcinoma	Suspicious for malignancy
2	Papillary neoplasm	Benign breast lesion

Table 38: False positive cases by wet mount in comparison with conventional stains

NUMBER OF CASES (n = 2)	CONVENTIONAL STAINS [H&E, PAP]	WET MOUNT
1	Accessory breast tissue	Ductal carcinoma
1	Fibroadenoma	Ductal carcinoma

Table 39: Lesions showing suspicious for malignancy in wet mount in comparison with conventional stains [PAP and H&E]

NUMBER OF CASES (n = 12)	CONVENTIONAL STAINS [PAP AND H&E]	WET MOUNT
4	Fibrocystic change	Suspicious for malignancy
3	Pleomorphic adenoma	Suspicious for malignancy
2	Ductal carcinoma	Suspicious for malignancy
1	Squamous cell carcinoma	Suspicious for malignancy
1	Fibroadenoma	Suspicious for malignancy
1	Phyllodes tumor	Suspicious for malignancy

Table 40: Lesions where No opinion could be made on wet mount in comparison with conventional stains [PAP and H&E]

NUMBER OF CASES (n = 10)	CONVENTIONAL STAINS [PAP AND H&E]	WET MOUNT
1	Suspicious of malignancy – Thyroid	No opinion
1	Inflammed cystic lesion – Lymph node	No opinion
1	Galactocele	No opinion
1	Acute inflammatory lesion – Breast	No opinion
1	Keratinous cyst	No opinion
1	Cysticercous cyst	No opinion
1	Benign spindle cells lesion	No opinion
1	Thyroglossal duct cyst	No opinion
1	Pleomorphic adenoma	No opinion
1	Monomorphic adenoma - Parotid	No opinion

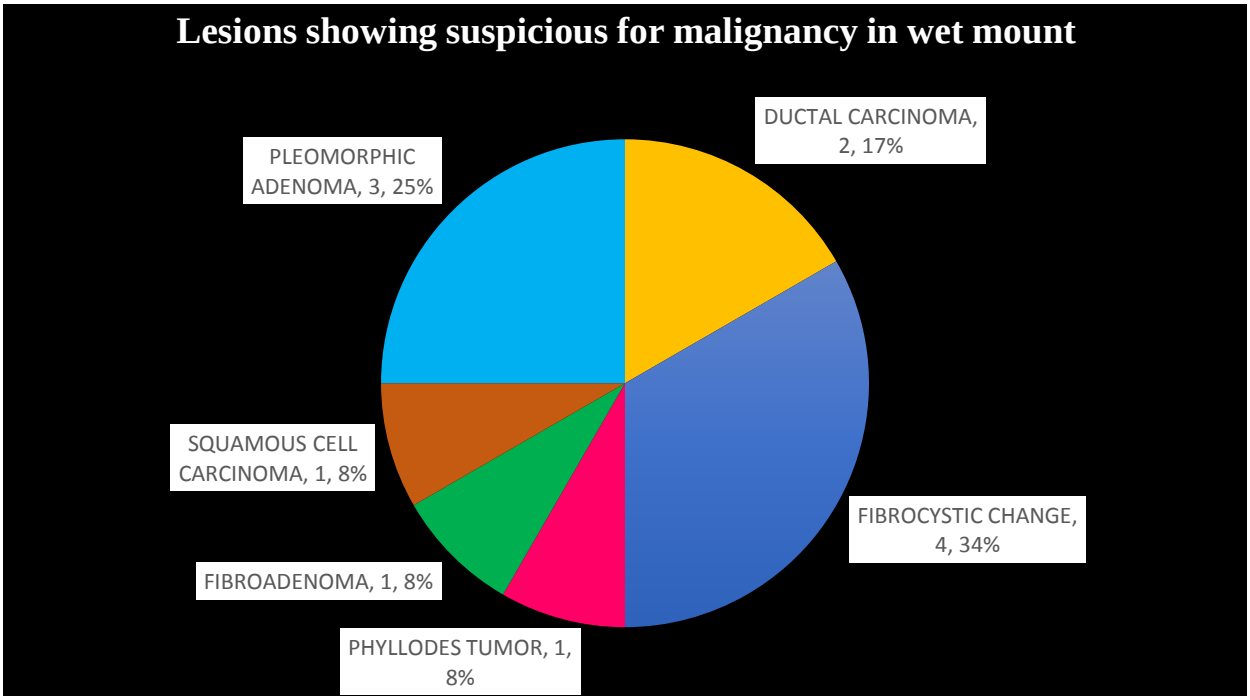


Chart 18: Lesions showing suspicious for malignancy in wet mount

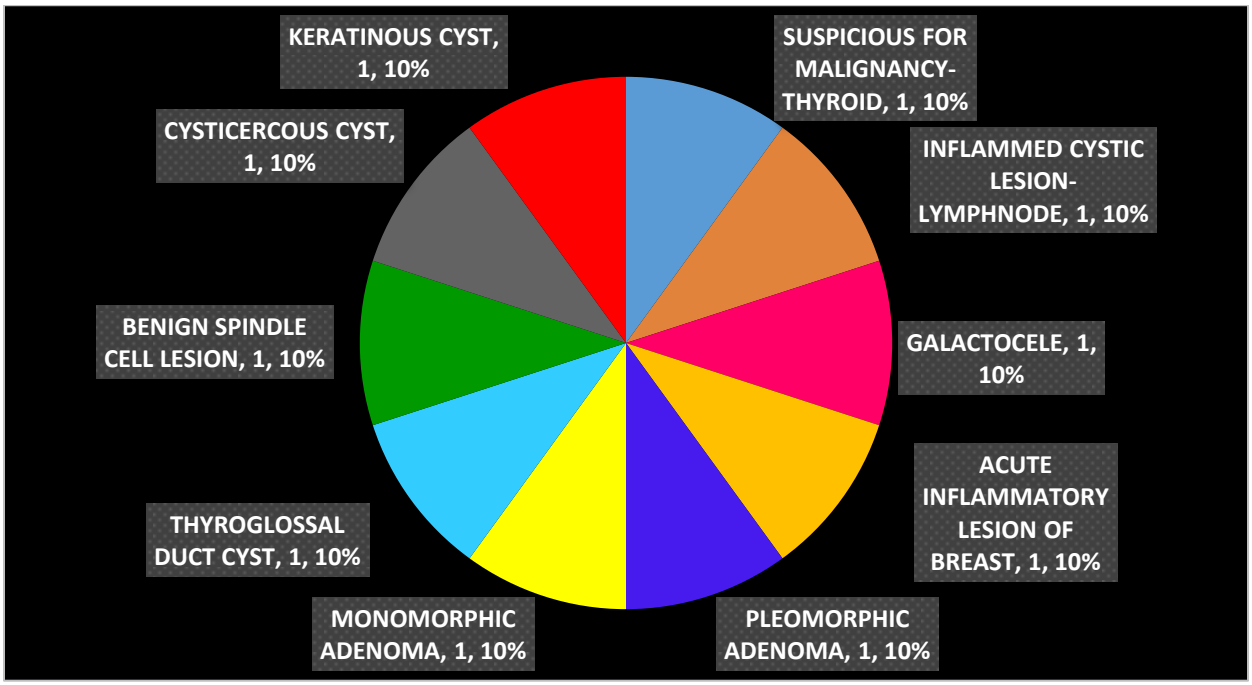


Chart 19: Lesions where no opinion could be made on wet mount

Table 41: Overall statistical analysis of M/E and TBWM in comparison with conventional stains [PAP and H&E]

STATISTICAL PARAMETERS	M/E	TBWM
Sensitivity	98 %	77 %
Specificity	100 %	94 %
Positive predictive value	98 %	83 %
Negative predictive value	100 %	96 %

M/E has good sensitivity and specificity when compared to TBWM. The following ROC curve represents all 320 cases and both the stains are statistically significant.

Chart 20: ROC curve for all 320 cases by TBWM in comparison with conventional stains.

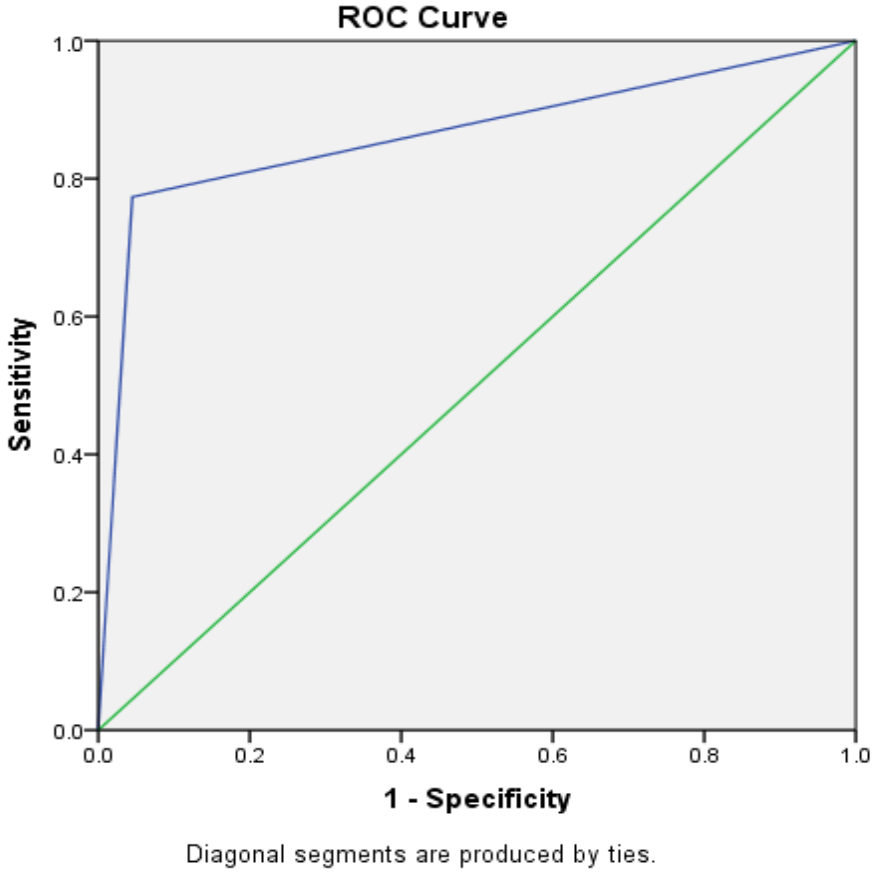


Table 42: Area Under Curve for Wet mount stain

Area Under the Curve				
Test result variable(s): WM				
Area	Std. Error[a]	p value[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.864	0.030	<0.0001*	0.806	0.923
The test result variable(s): WM has at least one tie between the positive actual state group and the negative actual state group. statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Chart 21: ROC curve for all 320 cases by M/E in comparison with conventional stains.

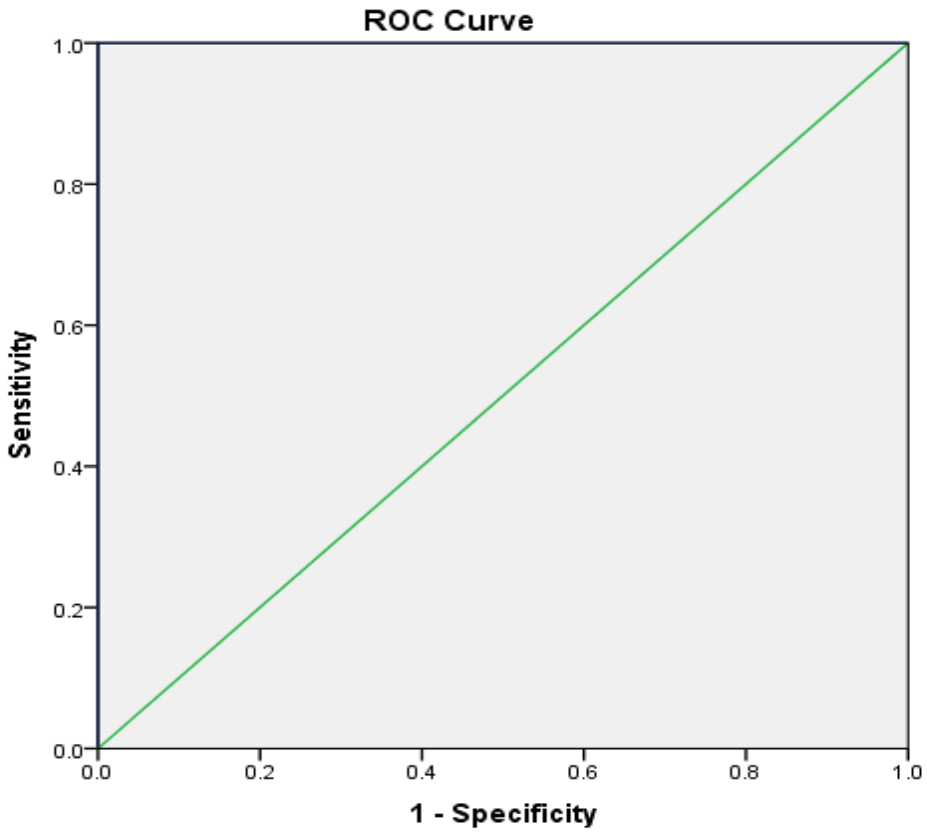


Table 43: Area Under Curve for Methylene blue/Eosin stain

Area Under the Curve				
Test result variable(s): M/E				
Area	Std. Error[a]	p value[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	0.000	<0.0001*	1.000	1.000
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

ASSESSMENT OF QUALITY INDEX

The morphology of the cells was assessed using the following scoring system for all the four stains on all 320 cases using the scoring system.

Table 44: Background: The results of estimating the efficacy of four stains (n=320)

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Hemorrhage/necrosis	38	40	46	50
Clean	282	280	274	270

Table 45: Background: Scoring of four stains

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Hemorrhage/necrosis	38	40	46	50
Clean	564	560	548	540
BACKGROUND SCORE	**602**	**600**	**594**	**590**

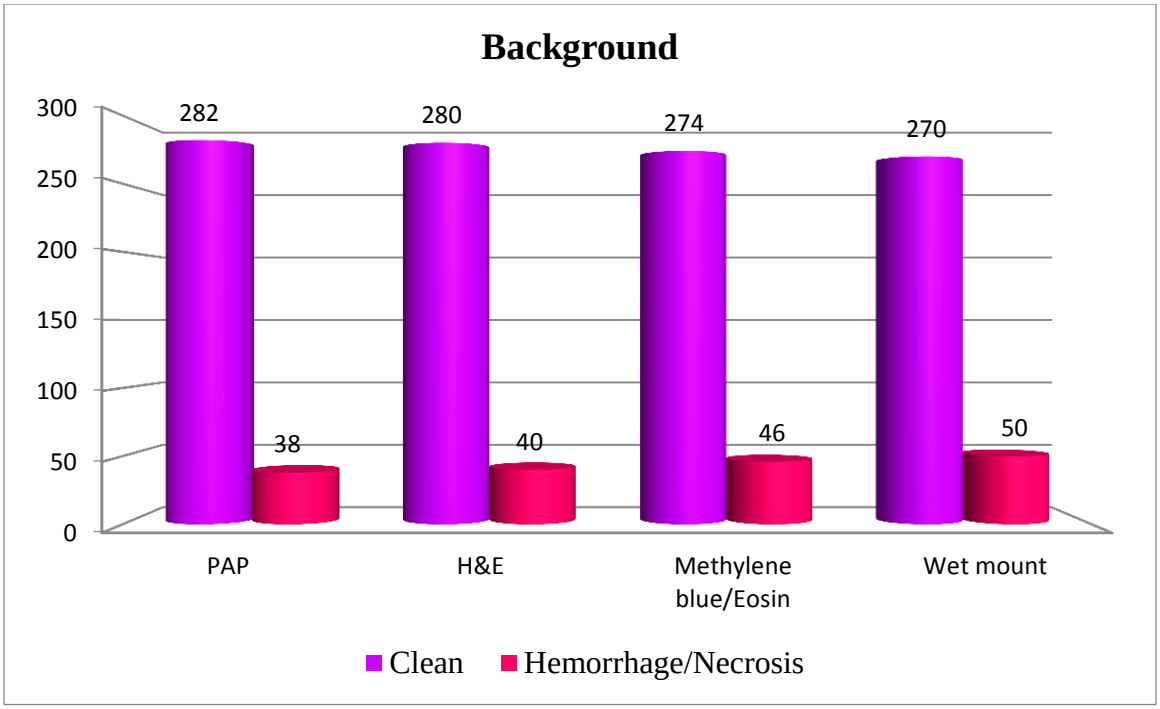


Chart 22: Background: The results of estimating the efficacy of four stains (n=320)

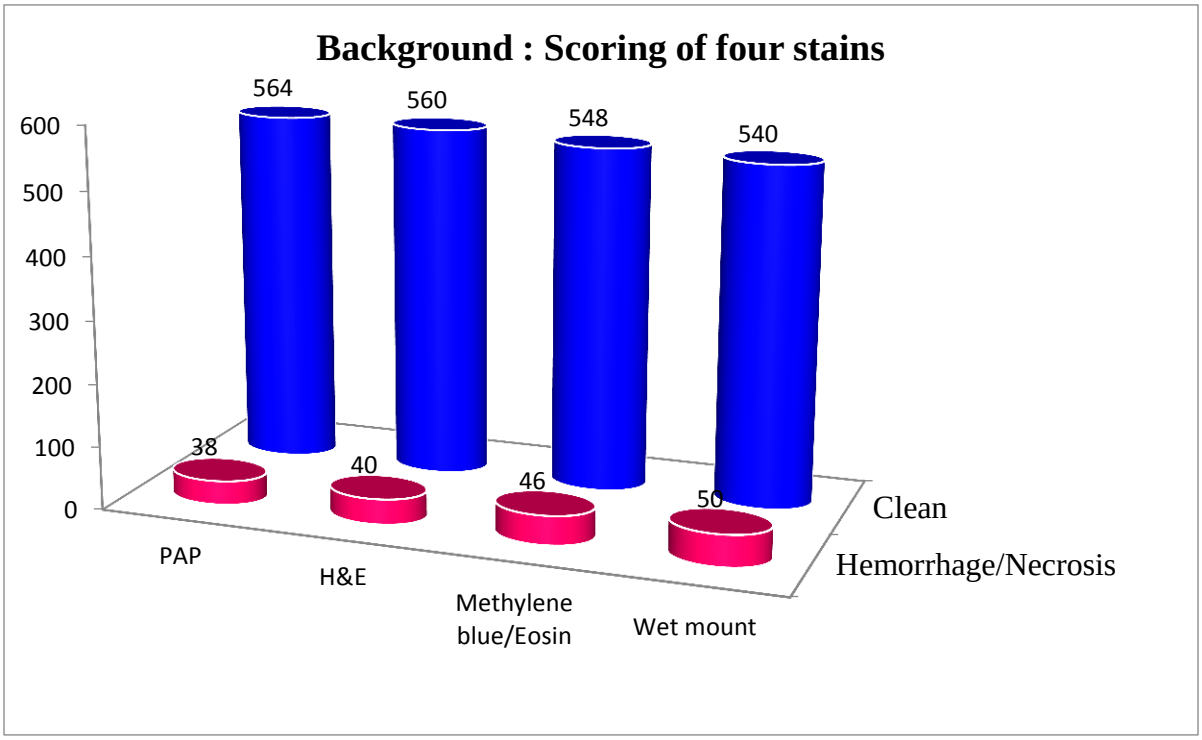


Chart 23: Background: Scoring of four stains

Table 46: Overall staining: The results of estimating the efficacy of four stains (n=320)

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Bad	26	34	58	112
Moderately good	64	90	176	150
Good	230	196	86	58

Table 47: Overall staining: Scoring of four stains

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Bad	26	34	58	112
Moderately good	128	180	352	300
Good	690	588	258	174
TOTAL SCORE	**844**	**802**	**668**	**586**

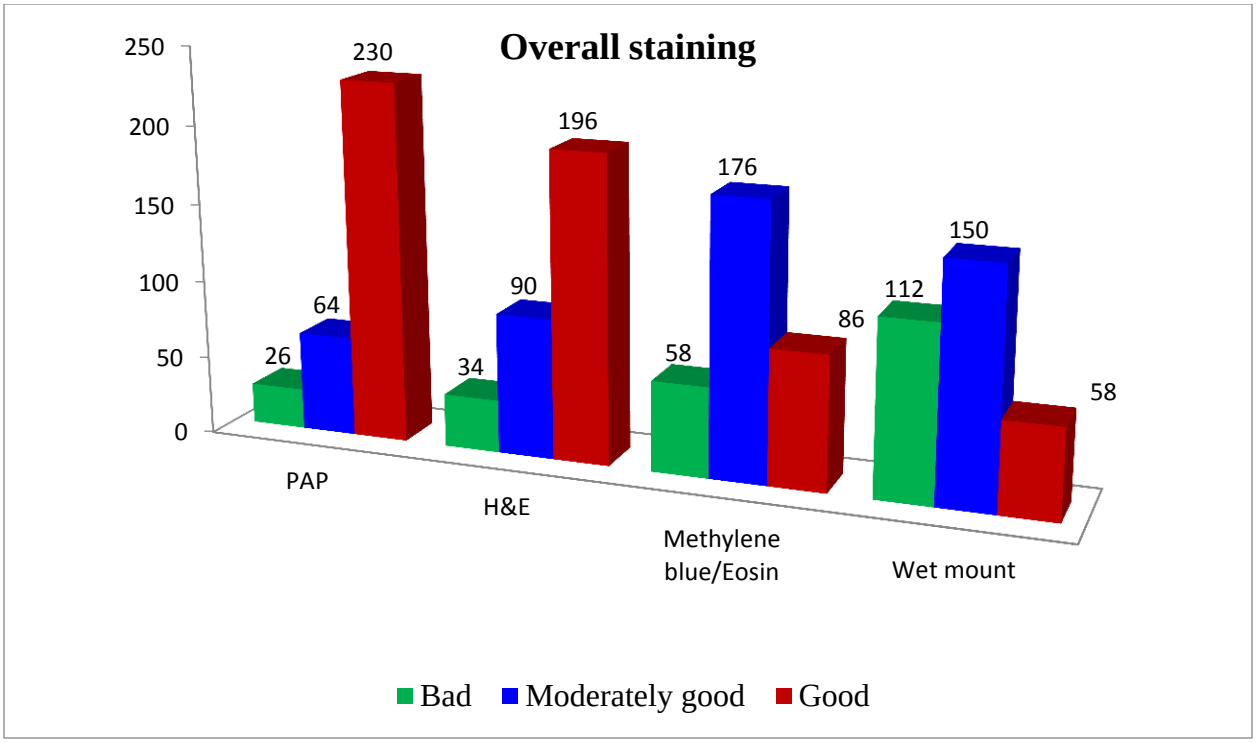


Chart 24: Overall staining: The results of estimating the efficacy of four stains (n=320)

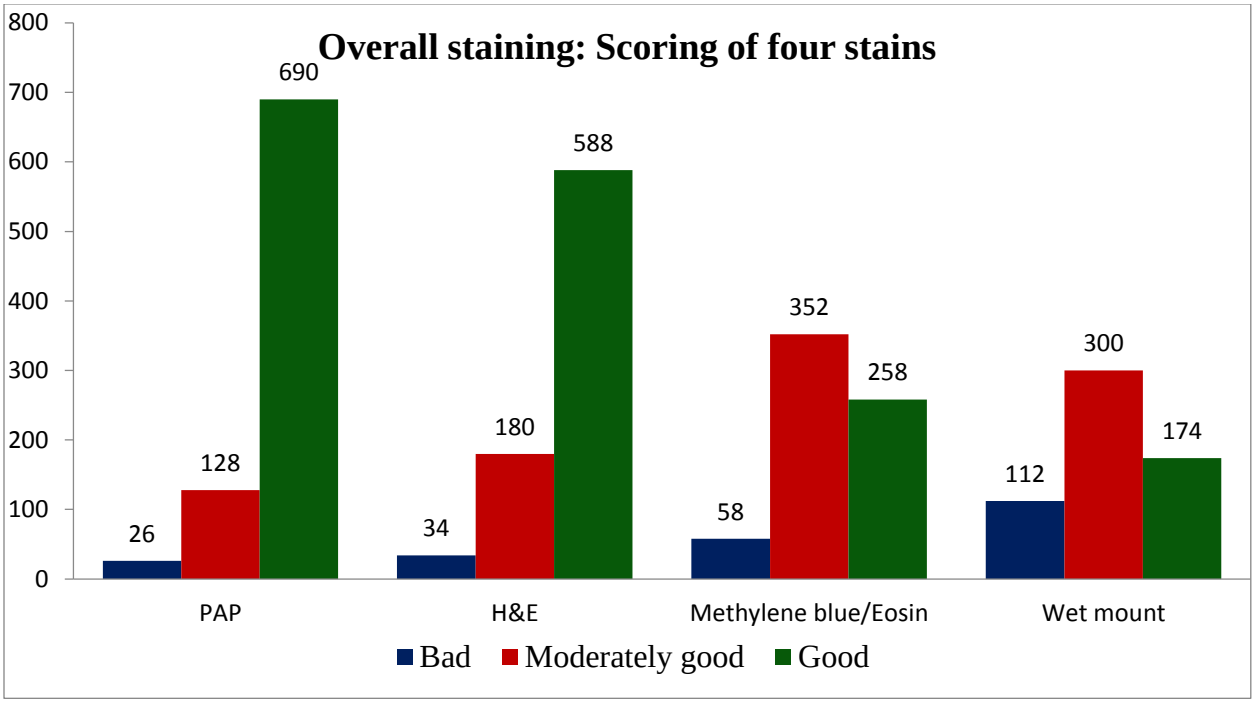


Chart 25: Overall staining: Scoring of four stains

Table 48: Cell morphology: The results of estimating the efficacy of four stains (n=320)

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Not preserved	26	28	40	57
Moderately preserved	84	108	185	207
Well preserved and crisp	210	184	95	56

Table 49: Cell morphology: Scoring of four stains

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Not preserved	26	28	40	57
Moderately preserved	168	216	370	414
Well preserved and crisp	630	552	285	168
TOTAL SCORE	**824**	**796**	**695**	**639**

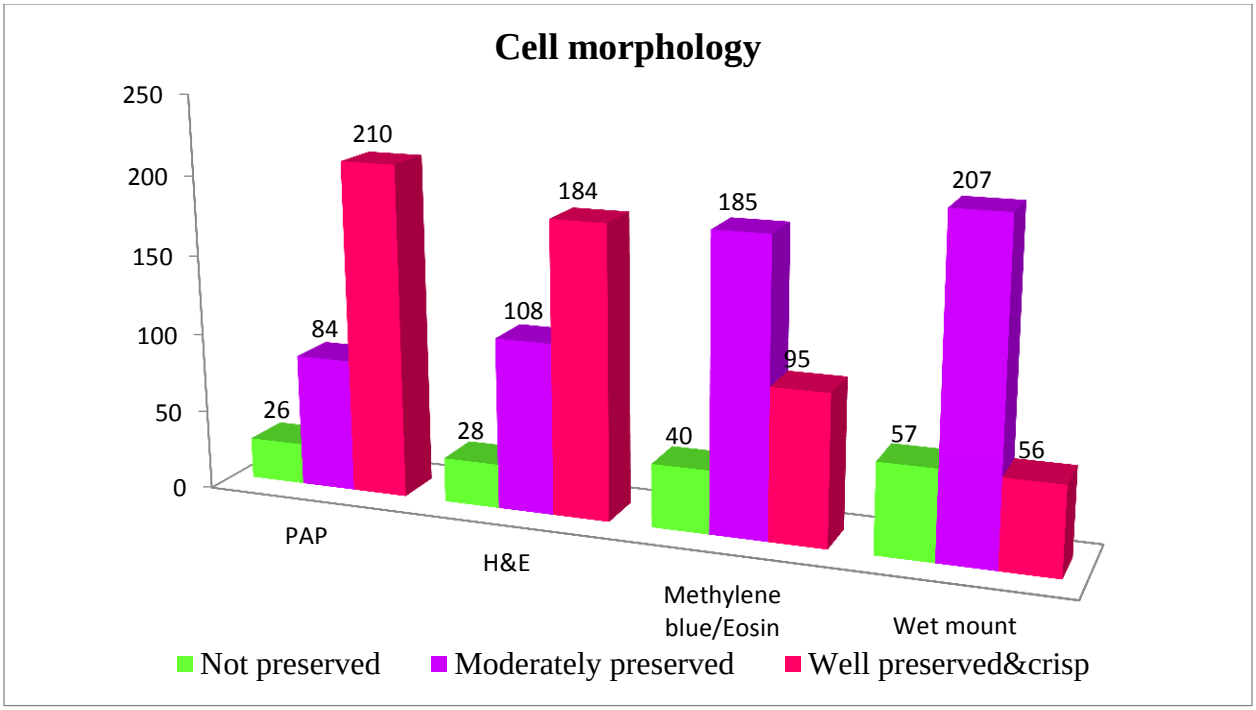


Chart 26: Cell morphology: The results of estimating the efficacy of four stains (n=320)

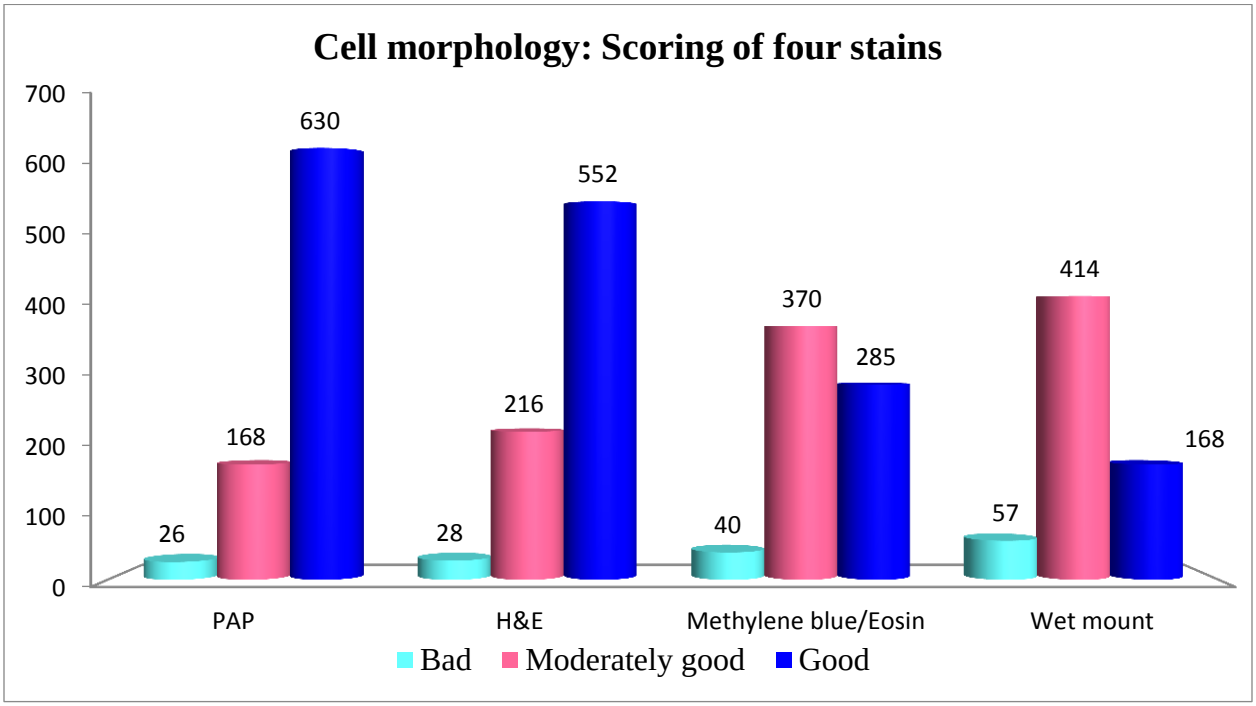


Chart 27: Cell morphology: Scoring of four stains

Table 50: Nuclear characteristics: The results of estimating the efficacy of four stains (n=320)

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Smudgy chromatin	42	50	81	92
Moderately crisp chromatin	96	128	161	170
Crisp chromatin	182	142	78	58

Table 51: Nuclear characteristics: Scoring of four stains

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Smudgy Chromatin	42	50	81	92
Moderately crisp chromatin	192	256	322	340
Crisp chromatin	546	426	234	174
TOTAL SCORE	**780**	**732**	**637**	**606**

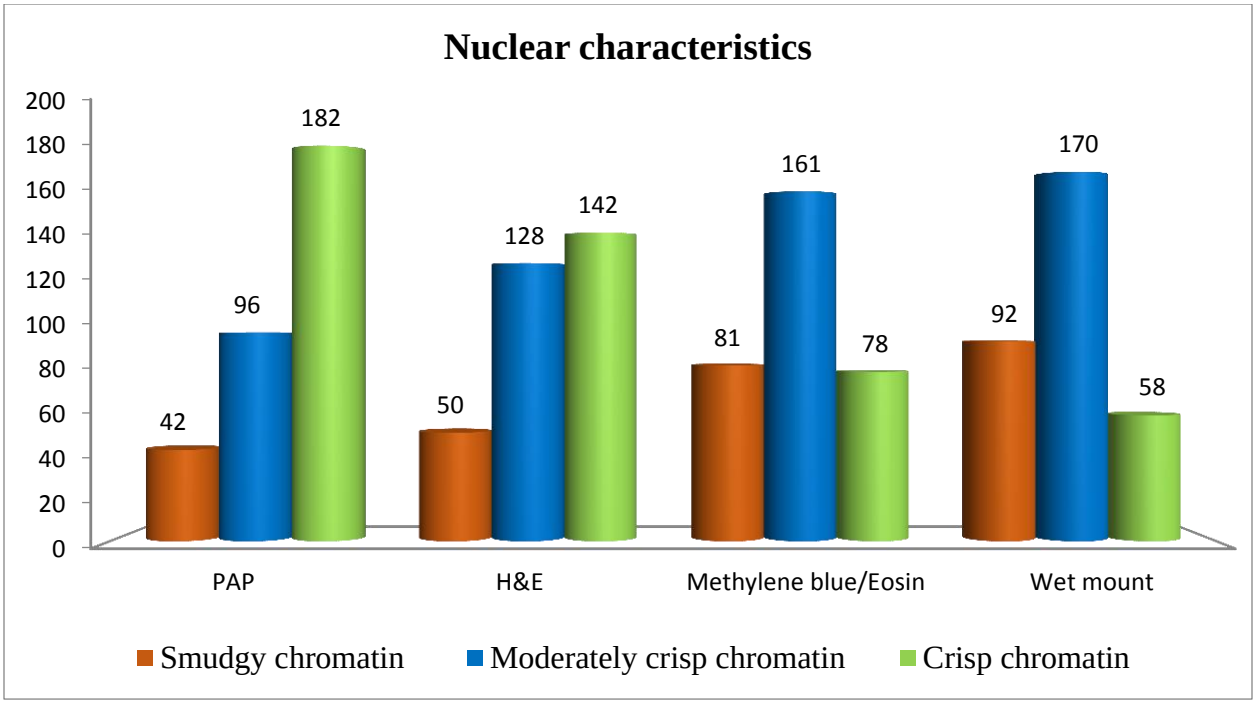


Chart 28: Nuclear characteristics: The results of estimating the efficacy of four stains (n=320)

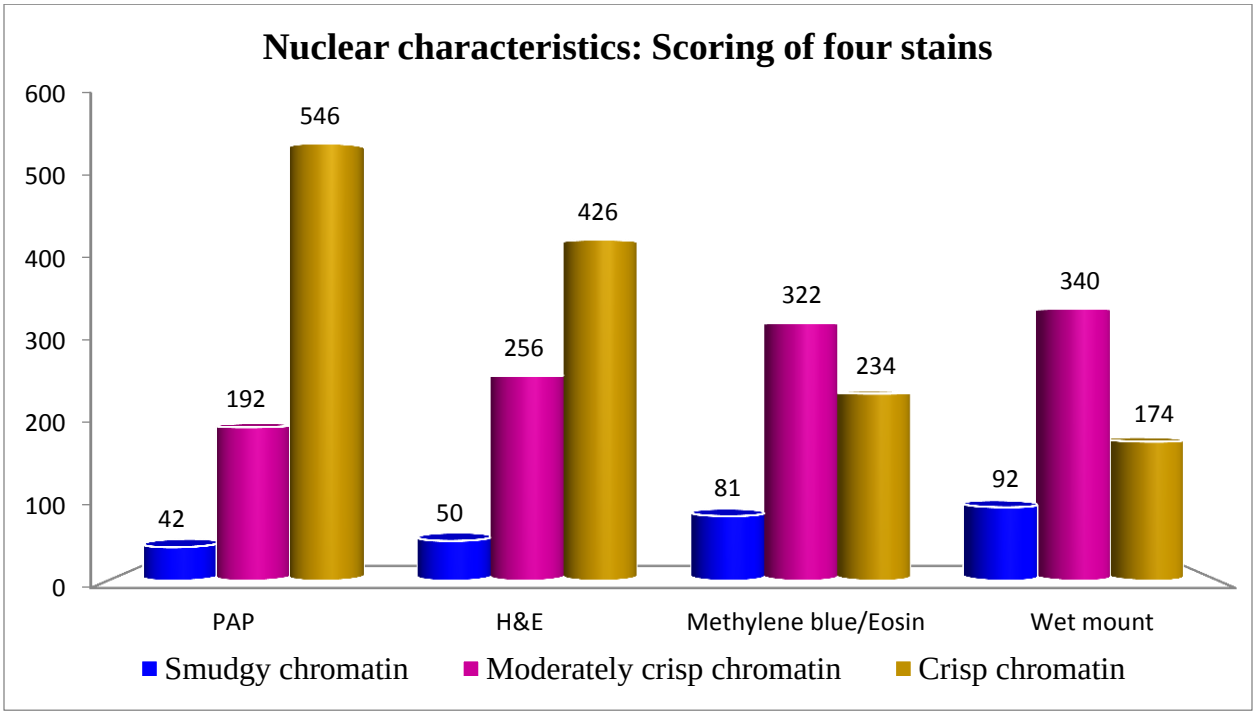
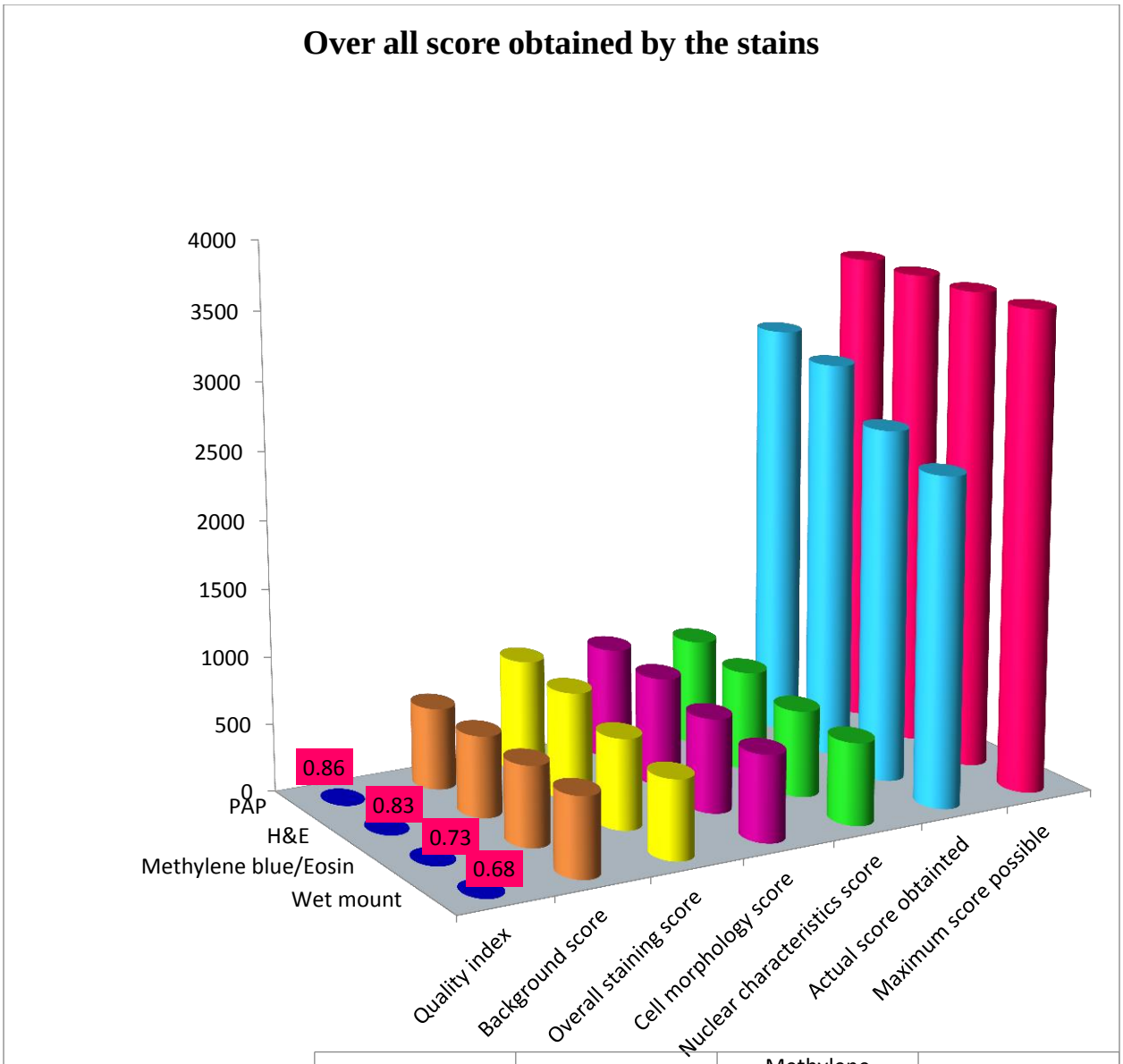


Chart 29: Nuclear characteristics: Scoring of four stains

Table 52: Overall scores obtained by all four stains with Quality Index

PARAMETER	PAP	H&E	METHYLENE BLUE/EOSIN	WETMOUNT
Background score	602	600	594	590
Overall staining score	844	802	668	586
Cell morphology score	824	796	695	639
Nuclear characteristics score	780	732	637	606
Actual score obtained	3050	2930	2594	2421
Maximum score possible	3520	3520	3520	3520
QUALITY INDEX	**0.86**	**0.83**	**0.73**	**0.68**

Thus the Quality Index of Pap stain is 0.86 which s highest when compared to other stains, followed by H&E stain with Quality Index of 0.83. The diagnostic accuracy of M/E is same as conventional stains [PAP and H&E], but this stain attained a Quality Index of 0.73. TBWM attained a Quality Index of 0.68.



	PAP	H&E	Methylene blue/Eosin	Wet mount
Quality index	0.86	0.83	0.73	0.68
Background score	602	600	594	590
Overall staining score	844	802	668	586
Cell morphology score	824	796	695	639
Nuclear characteristics score	780	732	637	606
Actual score obtainted	3050	2930	2594	2421
Maximum score possible	3520	3520	3520	3520

Chart 30: Overall scores obtained by all four stains with Quality Index

Table 53: The salient features of all four stains

FEEATURES	PAP	H&E	M/E	TBWM
Smearing technique	Important	Important	Important	Nil
Fixation	Immediate fixation	Immediate fixation	Air drying followed by fixation	Nil
Artifacts	Delay in fixation	Delay in fixation	Nil	Air bubbles
Cell loss	Due to wet fixation	Due to wet fixation	Minimal	Minimal
Cell size	Decreased, due to immediate fixation	Decreased, due to immediate fixation	Increased due to air drying	Increased
Cytoplasm	Well appreciated	Well appreciated	Moderately appreciated	Poor
Nucleus	Excellent	Excellent	Excellent	Moderately good
Nucleolus	Distinct	Excellent	Excellent	Excellent, appears immediately
Tissue fragment	Good	Good	Moderate	Moderate
Advantage	Crisp Nuclear details	Very good nuclear details, similar to PAP	Immediate assessment, Good nuclear details	Obtain material from needle hub
Disadvantage	Cell loss due to wet fixation	Cell loss due to wet fixation	Increased nuclear size due to drying	Three dimensional clusters masking the cellular details
Slide preservation	Preserved	Preserved	Preserved	Cannot be preserved
Cost	Expensive	Expensive	Cost effective	Cost effective
Rapidity	10 minutes	8 minutes	5 minutes	2 minutes

DISCUSSION

FNAC plays a vital role as a rapid diagnostic technique because of its simplicity, cost effectiveness, early availability of results, accuracy and minimal invasion. Chandler foot et al (1958), Silverman et al (1989) Verma et al (1991), Chang et al (1993), yang et al (1995), Tsou et al (1997) experimented various rapid stains such as Neutral red – Janus green, Diff Quik, rapid MGG, Liu's stain, ultra-fast PAP, Riu's stain respectively for immediate diagnosis.[16,35,38,44,45,49]

This work was inspired by the earlier work of Joy, M.P. et al in 2003 where they applied toluidine blue as a rapid stain for quick diagnosis of ultrasound guided aspiration cytology.[3]

In our study, we used supra vital stains - Toluidine blue as wet mount stain and Methylene blue/eosin as a rapid stains for rapid diagnosis.

The above authors applied this rapid stain for immediate assessment of cytology. However our study is aimed at assessing the rapidity and morphology of the cells in rapid stains in comparison with conventional H&E and PAP stained fixed smear, thus assessing the reliability of this rapid staining of toluidine blue wet mount and methylene blue/eosin.

The rapidity of the stains was assessed and found that toluidine blue wet mount stains in 2 minutes followed by methylene blue/eosin in 5 minutes. Rapid staining technique has an advantage of assessing the cellularity and adequacy of the material within few minutes and the re-aspiration can be performed immediately. This will be of much help in USG or CT guided FNAC. The rapidity of the stains used in the present study was compared with other studies and found that toluidine blue is rapid followed by methylene blue/eosin. [Table: 54]

Table 54: Comparison of rapidity of the stains with other studies

AUTHORS	YEAR	RAPID STAIN USED	TIME TAKEN FOR THE PROCEDURE
Silverma et al.[35]	1989	Diff-Quik stain	5 minutes
Kusum Verma et al.[38]	1991	Rapid MGG stain	8 minutes
Chang et al.[44]	1993	Liu's stain	5 minutes 30 seconds
Tsou et al.[49]	1997	Riu's stain	5 minutes 20 seconds
Joy MP et al.[3]	2003	Toluidine blue stain	3 minutes
Sumathi C et al.[2]	2012	Toluidine blue wet mount	2 minutes
Sumathi S et al.[1]	2013	Toluidine blue wet mount	2 minutes
Present study	2015	Toluidine blue wet mount	2 minutes
Present study	2015	Methylene blue/Eosin	5 minutes

Caya et al in 1984 reported that false negative reports were resulted from unrepresentative aspirates. False negative aspirates may include normal or reactive elements but necrotic material is an additional source of error.[64,65] This problem of sampling error cannot be eliminated entirely in FNAC but it is found reduced by rapid cytology assessment. This sampling error is reduced in our study by simultaneously doing rapid wet mount study. One problem in FNAC diagnosis is lack of adequate sample or unsatisfactory specimen in some cases. The

problem of scanty cellularity occurs in tissues with cystic degeneration and tissues with extreme desmoplasia. Cagle et al., in 1993 reported that inadequate sampling was solely responsible for 10% false negative report in lung FNAC.[66] In our study the needle and hub are rinsed with toluidine blue stain, which effectively washes all the cells collected in the lumen yielding an improved cellularity.

Degenerated cells and neoplastic cells are more fragile and distorted easily during smearing which created confusion in diagnosis. Trapping of cells within fibrin meshwork also distorted the morphology of cell. Since cytomorphology forms the basis for the cytodiagnosis, artifactual morphological distortion influences the diagnostic accuracy of FNAC.[35]

This smearing artifact is avoided in our study since we used wet mount preparations as one of the rapid stain. The disadvantage of this wet mount technique was three dimensional clusters of cells which reduces the diagnostic accuracy in our study.

One of the most important features in cytodiagnosis is the morphology of the nucleus.[12] The advantage of this supravital stain is that the cell structure is well preserved with toluidine blue stain.[33] Supravital stain has a high affinity for DNA and hence absorbed rapidly into the nucleus. As the dysplastic and anaplastic cells contain more nucleic acid, the nuclear stains of tumor cells are very prominent with toluidine blue.[67] Another advantage is the postfixation after air-drying facilitates chromatin staining in M/E stain which will improve the diagnostic accuracy in malignant cases.

The study of morphology of individual cell is on great focus in our study since individual cell morphology varies in air dried and wet smear preparation and wet mount. Cytomorphology observed in our study is compared with that described by Svante R. Orelle et al.[12]

OBSERVATION

THYROID LESIONS

Colloid stains blue to purple and form thin membrane like coat often with folds and cracks.[12] In our study thick colloid stains as patchy dark blue color material and thin colloid stains as granular purple material in both M/E and TBWM. The cyst macrophages are also well appreciated in both the stains. [Figure 16]

There was no difficulty in identifying lymphocytic thyroiditis as the morphology was well appreciated in both M/E and TBWM. [Figure 17]

The major discrepancy in TBWM was found in the cases of hyperplastic goitre, as the fire flares are not well appreciated. There was no difficulty faced in diagnosing hyperplastic goitre in M/E. [Figure 18]

In cases of follicular neoplasm the arrangement of the cells in follicular pattern and the nuclear details was well appreciated in both M/E and TBWM. [Figure 19]

Anaplastic carcinoma of thyroid shows pleomorphic cells with prominent nucleoli and multinucleated cells which was well appreciated in both the rapid stains [Figure 20]

Smear from papillary carcinoma of thyroid shows sheets of cell with enlarged ovoid nucleus, granular chromatin and prominent nucleoli with cytoplasmic inclusions and nuclear grooves. In our study papillary sheets of follicular cells showed nucleus with small prominent basophilic nucleoli and some with nuclear inclusion in M/E. But in TBWM the inclusions could not be identified. [Figure 21]

BREAST LESIONS

Fibroadenoma shows branching sheets of uniform round to oval cells with scanty to moderate cytoplasm, round to oval uniform nuclei with granular chromatin and many bare ovoid nuclei were observed in both M/E and TBWM. [Figure 22]

Cohesive fragments of highly cellular stroma composed of spindle cells with nuclear atypia or atypical bare spindle nuclei in the background along with benign sheets of epithelial cells are highly suggestive of phyllodes tumor.[12] In our study cluster of duct epithelial cells-round to oval cells with scanty cytoplasm, round uniform nuclei with single small nucleoli and cluster of stromal cells-oval to spindle cells with indistinct cytoplasmic membrane, scanty pink cytoplasm, mild anisokaryotic nuclei with granular chromatin and small nucleoli are characteristic. [Figure 23]

Ductal carcinoma aspirates showed pleomorphic cells with scanty to moderate cytoplasm, large darkly stained nucleus with smudged chromatin and prominent nucleoli. Prominent nucleoli was well appreciated in TBWM than M/E. Sometimes a necrotic material in the background was observed. [Figure 24]

Papillary carcinoma breast shows ductal epithelial cells arranged in papillary clusters with nuclear atypia. Prominent nucleoli is well appreciated in TBWM stain. [Figure 25]

LYMPH NODE LESIONS

Reactive lymphadenitis showed polymorphous population of lymphoid cells with background showing tangible body macrophages. [Figure 26]

The nucleus of epithelioid cell in granulamatous lymphadenitis is elongated and resembles sole of shoe with inconspicuous nucleoli.[12] In our study the nucleus of epithelioid cells appeared to be elongated with distinct nucleolus in M/E, but epithelioid granulomas were difficult to identify in TBWM preparation because of three dimensional clusters. [Figure 27]

Adenocarcinoma deposits shows pleomorphic cells with scanty blue cytoplasm, large round, oval, irregular hyperchromatic nucleus with prominent nucleoli. [Figure 28]

Squamous cell carcinoma shows pleomorphic cells with cytoplasmic elongation and nuclear atypia with background showing necrosis. [Figure 29]

Anaplastic large cell lymphoma shows large cells with pleomorphic nuclei, horseshow shapd nuclei, multinucleated cells, with abundant vacuolated cytoplasm. [Figure 30]

SOFT TISSUE LESIONS

Benign spindle cell lesions shows spindle cells with pale pink cytoplasm, large round to spindle hyperchromatic nuclei and invisible nucleoli. [Figure 31]

Keratinous cyst aspirates showed polyhedral purple coloured anucleate squames. [Figure 32]

Lipoma shows polyhedral to large round fat cells in lobules with abundant cytoplasm with peripherally placed nucleus. [Figure 33]

Acute inflammatory lesions shows predominantly neutrophils with no areas of necrosis.

THYROID LESIONS

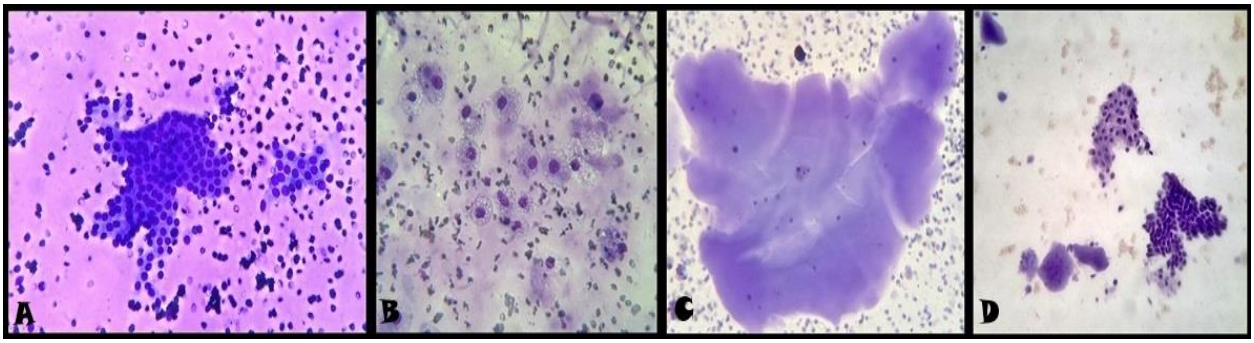


Figure 16: Colloid goiter: A – M/E, B,C,D - TBWM

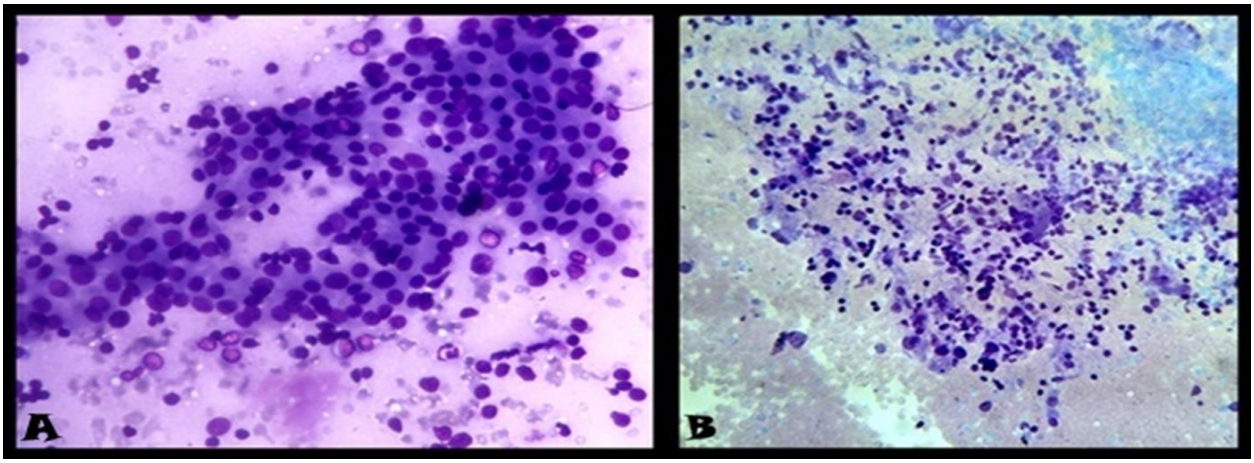


Figure 17: Lymphocytic thyroiditis: A – M/E, B - TBWM

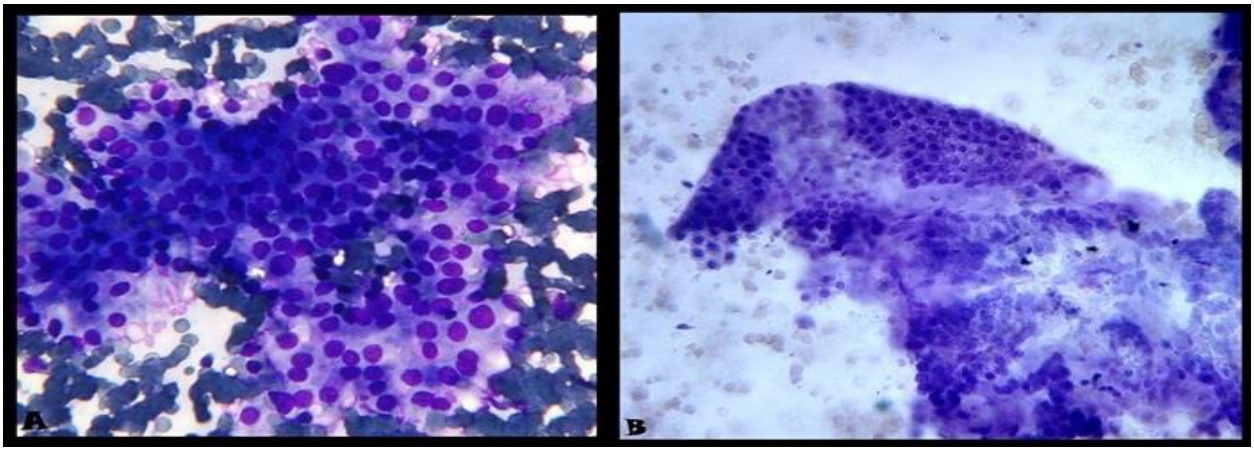


Figure 18: Hyperplastic goiter: A – M/E, B - TBWM

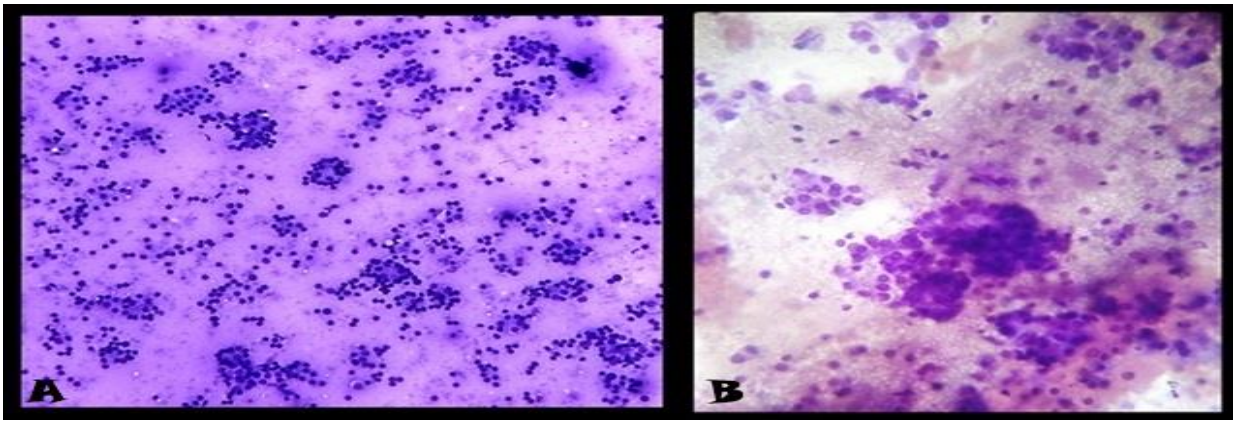


Figure 19: Follicular neoplasm: A – M/E, B - TBWM

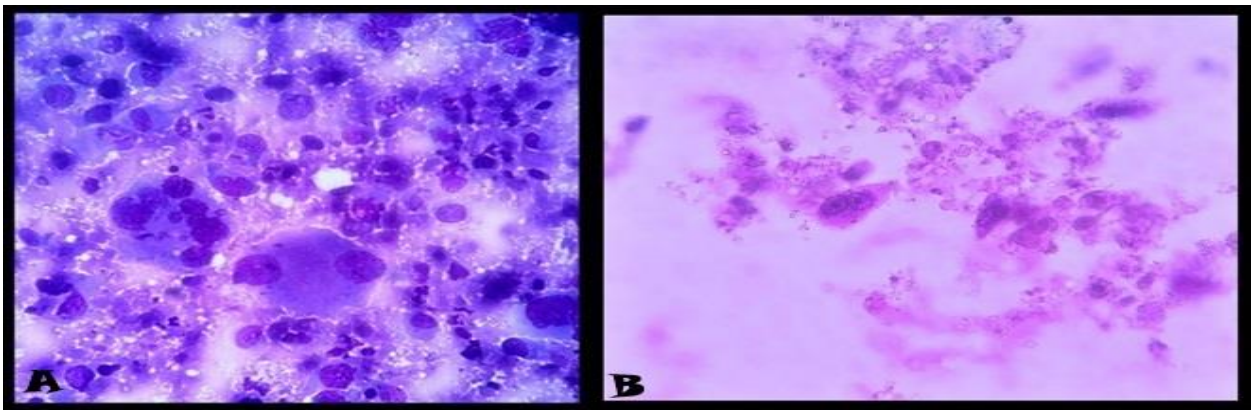


Figure 20: Anaplastic carcinoma thyroid: A – M/E, B - TBWM

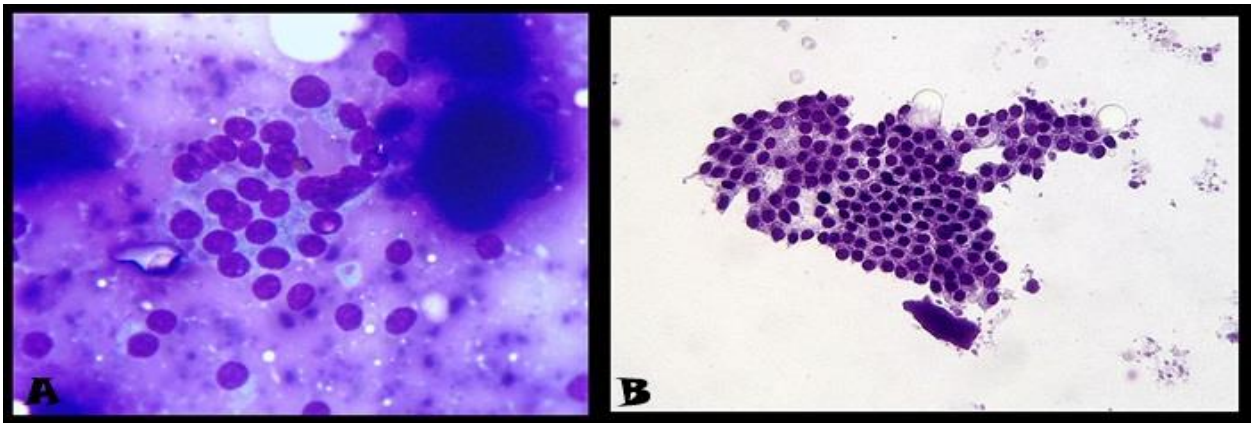


Figure 21: Papillary thyroid carcinoma: A – M/E, B - TBWM

BREAST LESIONS

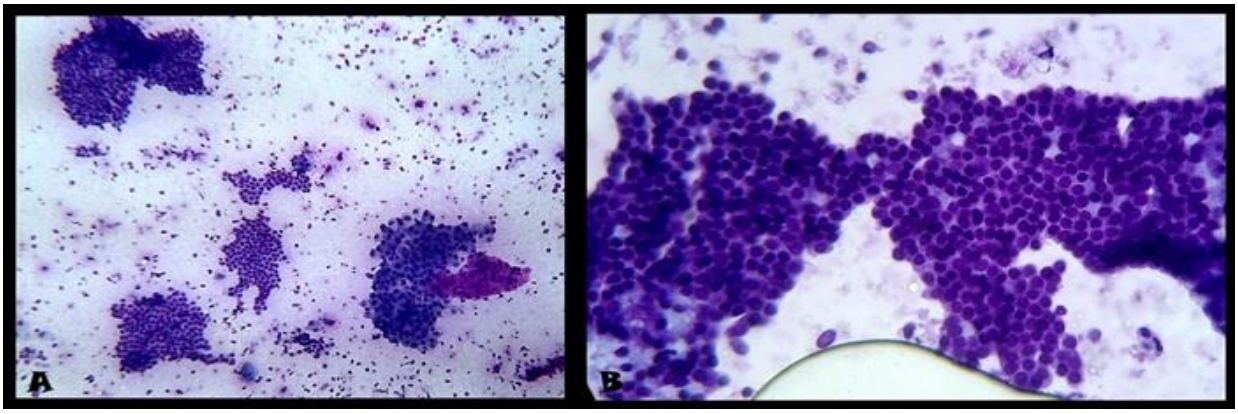


Figure 22: Fibroadenoma: A – M/E, B - TBWM

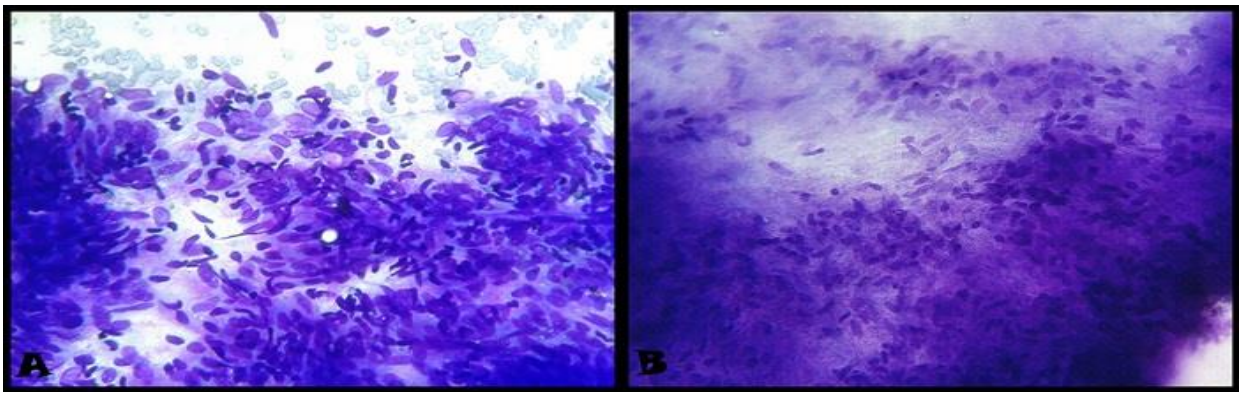


Figure 23: Phyllodes tumor: A – M/E, B - TBWM

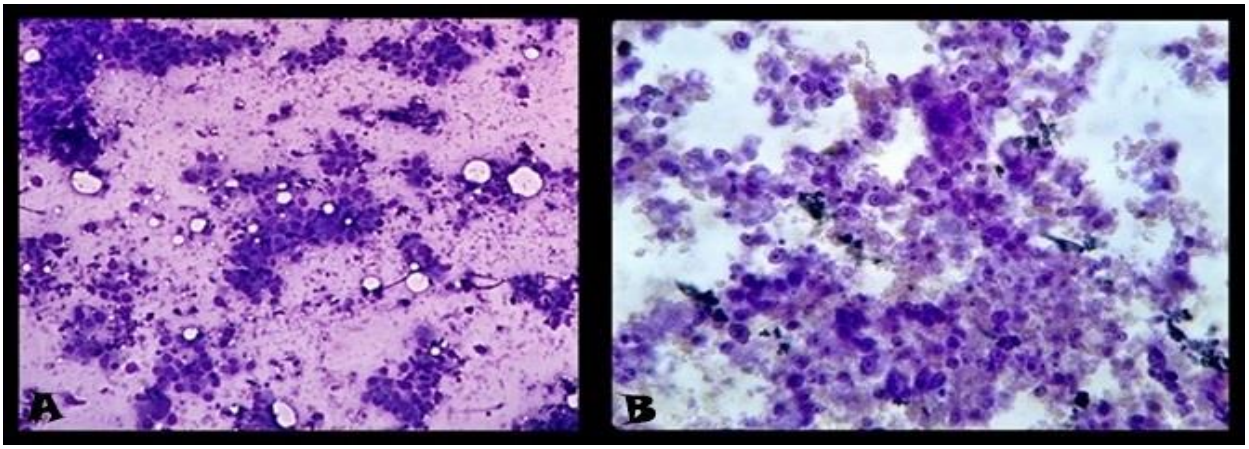


Figure 24: Ductal carcinoma: A – M/E, B - TBWM

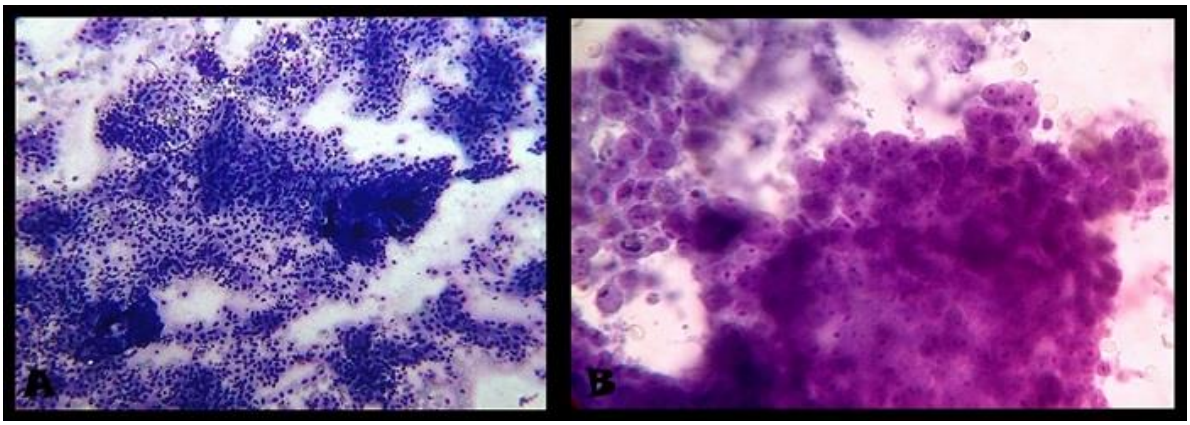


Figure 25: Papillary carcinoma of breast: A – M/E, B - TBWM

LYMPH NODE LESIONS

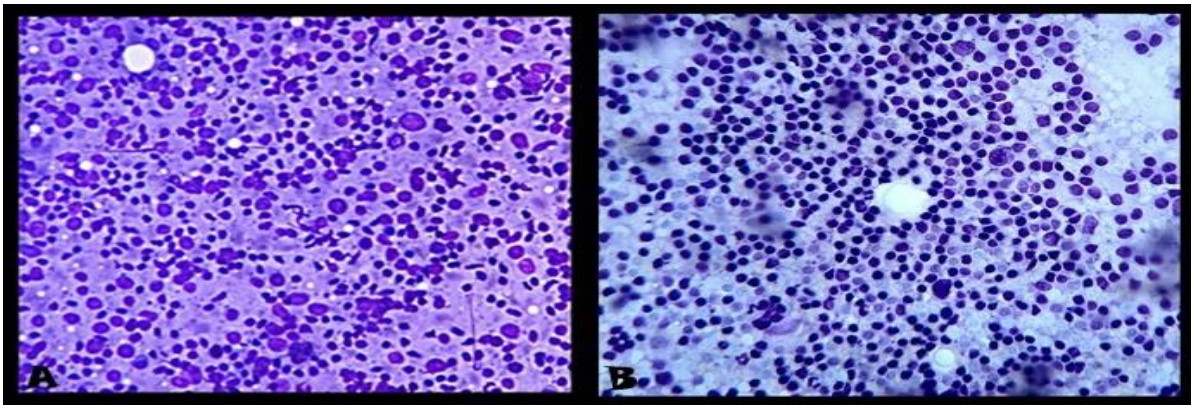


Figure 26: Reactive lymphadenitis: A – M/E, B - TBWM

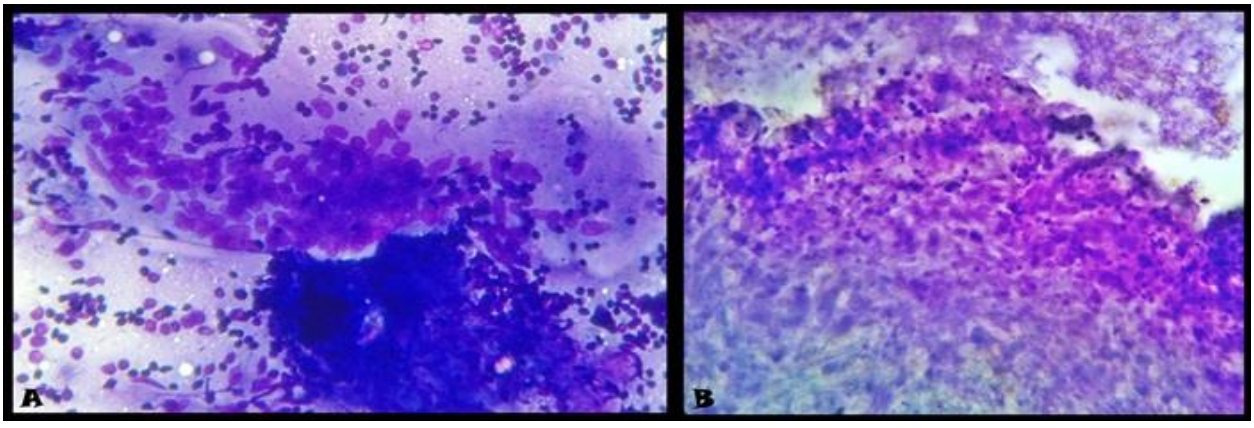


Figure 27: Granulomatous lymphadenitis: A – M/E, B - TBWM

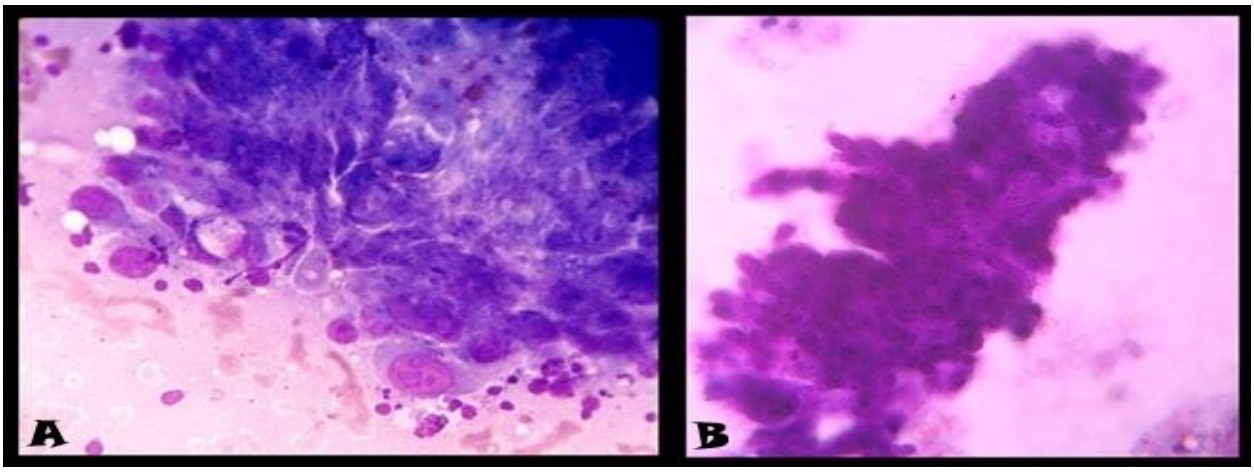


Figure 28: Adenocarcinoma deposits: A – M/E, B - TBWM

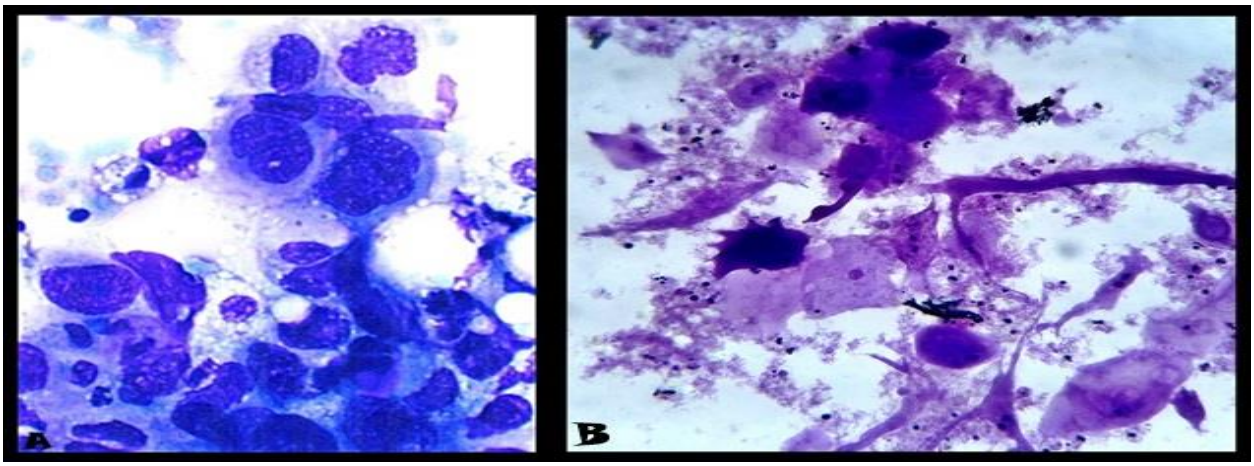


Figure 29: Squamous cell carcinoma metastasis: A – M/E, B - TBWM

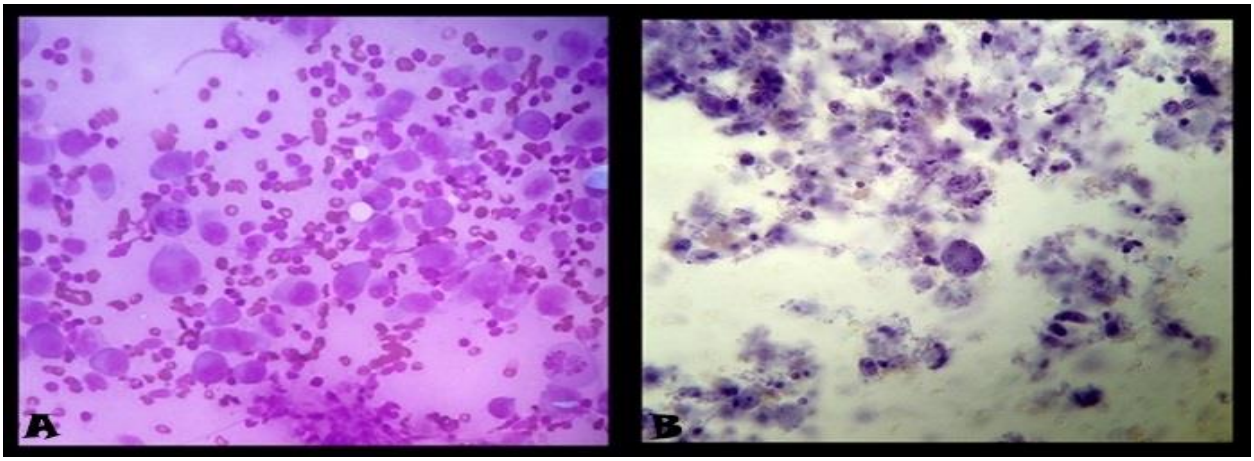


Figure 30: Anaplastic large cell lymphoma: A – M/E, B - TBWM

SOFT TISSUE LESIONS

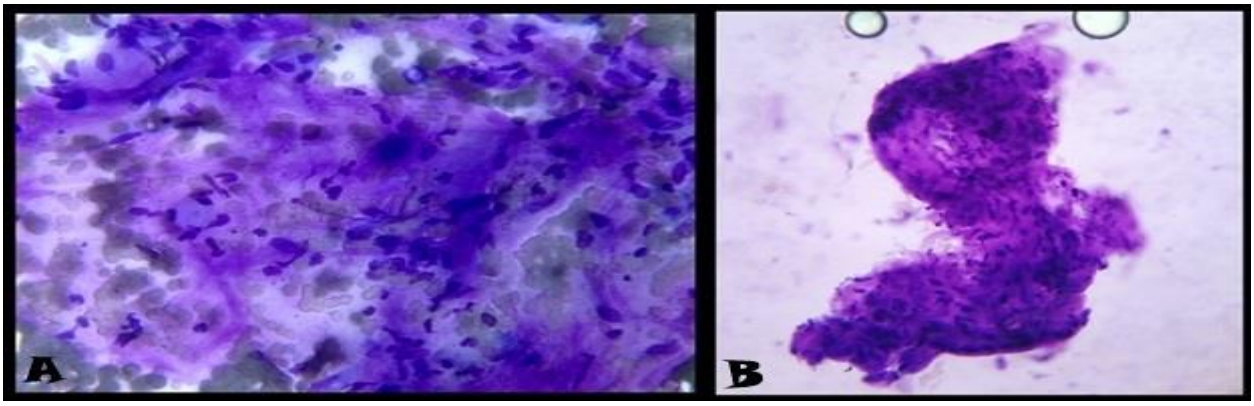


Figure 31: Benign spindle cell lesion: A – M/E, B - TBWM

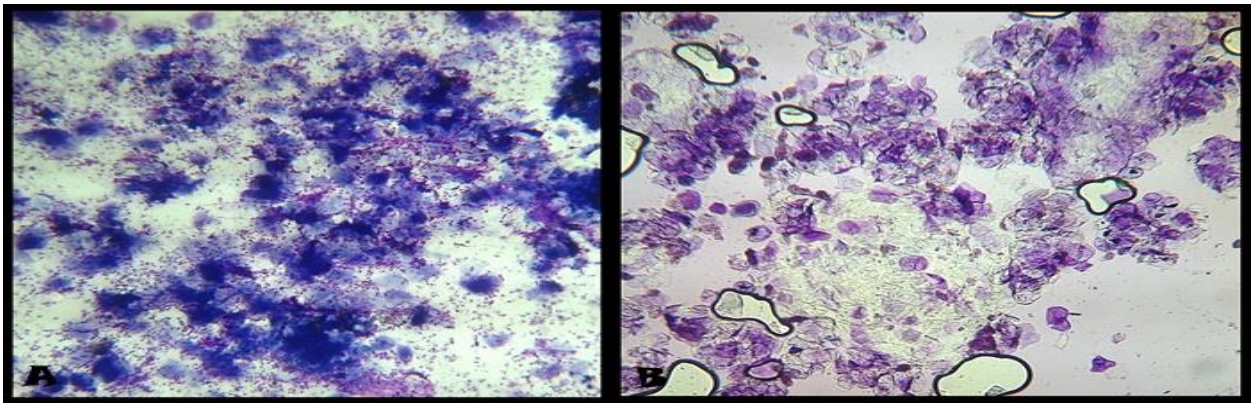


Figure 32: Keratinous cyst: A – M/E, B - TBWM

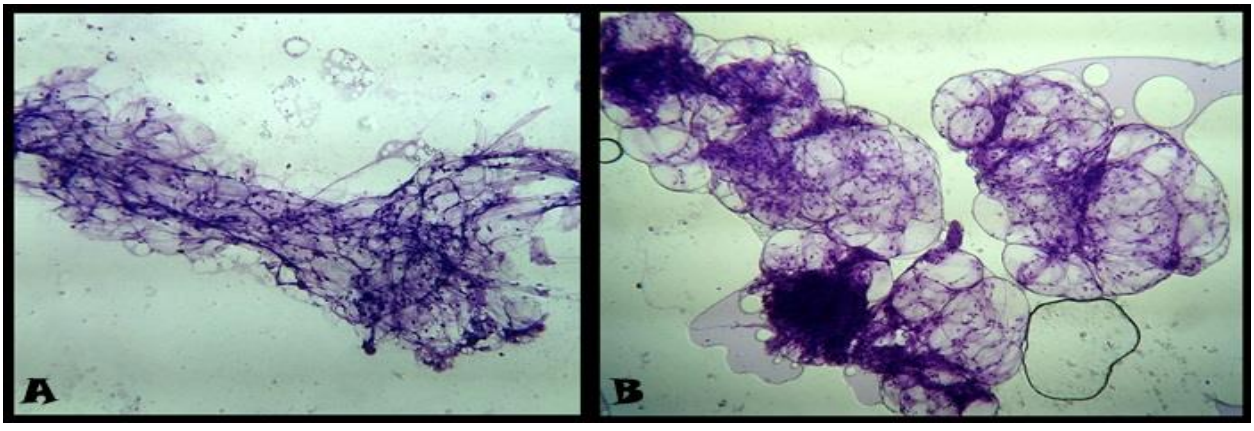


Figure 33: Lipoma: A – M/E, B - TBWM

The diagnostic accuracy of our study was compared with other studies. Sumathi C et al done a wet mount study using Toluidine blue which yields a diagnostic accuracy of 98.54%.[2] A study done by Kusem verma et al in 1991 used Rapid MGG stain which gave 97% diagnostic accuracy.[38] Diff-quik stain was tried by Silverman et al in 1989 as a rapid stain which yielded a diagnostic accuracy rate of 97%.[35] In our study methylene blue/eosin stain yielded 99% diagnostic accuracy which is more than toluidine blue wet mount stain which gave only 73.41%. The reason behind decreased diagnostic accuracy in wet mount is due to three dimensional clusters of the cells. The major difficulty was faced in diagnosing Hyperplastic goitre, Papillary carcinoma thyroid, Granulomatous lymphadenitis in TBWM. [Table: 55]

Table 55: Comparison of diagnostic accuracy of rapid stain with other studies

AUTHORS	YEAR OF THE STUDY	RAPID STAIN USED	DIAGNOSTIC ACCURACY
Chandler foot et al.[16]	1958	Neutral red Janus green stain	80%
Silverman et al.[35]	1989	Diff-quik stain	96%
Kusum verma et al.[38]	1991	Rapid MGG stain	97%
Chang et al.[44]	1993	Liu's stain	94.9%
Tsou et al.[49]	1997	Riu's stain	93.5%
Joy MP et al.[3]	2003	Toludine blue stain	98.54%
Sumathy C et al.[2]	2012	Toludine blue wet mount	89%
Sumathi S et all.[1]	2013	Toludine blue wet mount	87%
Present study	2015	Toludine blue wet mount	76%
Present study	2015	Methylene blue/eosin	98%

The morphology of the cells in thyroid lesions was compared with other studies where they found difficulty in identifying cells with definite cytoplasmic features like hurthle cells due to which sensitivity was decreased in toluidine blue wet mount stain.[1,2] This was well correlated with the present study as we had difficulty in diagnosing Hurtle cell change, Fire flares in thyroid lesions which decreased the sensitivity in Toluidine blue wet mount. This problem was not faced in M/E stain.

In breast lesions the major discrepancy in TBWM was found in diagnosing the benign cases with atypia which was given as suspicious for malignancy. This is due to three dimensional clusters of unfixed cells due to which the morphology was obscured. This was correlated with other studies where toluidine blue was used as wet mount.[2]

In lymph node lesions, granulomas were not well appreciated in TBWM which was correlated with other studies. In lesions from other site the discrepancy was less and the diagnosis can be rendered as early as possible.[1]

Though there was few discrepancy found in diagnosis in TBWM stain the diagnostic accuracy was statistically significant and can be used as a rapid stain to assess the cellularity and adequacy of FNA material. M/E had attained a good diagnostic accuracy. The diagnostic accuracy of all the lesions was compared with other studies and was depicted in the following table.

[Table: 56]

Table 56: Comparison of diagnostic accuracy of each lesions with other studies.

AUTHORS	STAIN USED	DIAGNOSTIC ACCURACY OF THE LESIONS					
		THYROID	BREAST	LN	ST	SG	LIVER
Sumathi C et al.[2]	TBWM	87.8%	92.6%	95.2%	100%	85.7%	100%
Present study	TBWM	73%	80%	76%	85%	70%	100%
Present study	M/E	98%	99%	100%	100%	100%	100%

LN* - Lymph node, ST* - Soft tissue, SG* - Salivary gland

A study done by Sumathi S et al using Toluidine blue as a wet mount yielded sensitivity rate of 93.7% and specificity rate 98%.[1] The sensitivity and specificity by using Toluidine blue in a study done by Joy MP et al was 90% and 98%.[3] In present study the sensitivity and specificity of M/E is 99% and 100% and for TBWM it is 77% and 94%. The decreased sensitivity in TBWM is due to three dimensional clusters of cells, due to which the morphology was obscured.

[Table: 57]

Table 57: Overall sensitivity and specificity of the rapid stains in comparison with other studies

ATUTHORS	YEAR OF THE STUDY	RAPID STAIN USED (SUPRAVITAL STAIN)	SENSITIVITY	SPECIFICITY
Erkilic et al.[4]	2006	Toludine blue	69%	93%
Joy MP et al.[3]	2003	Toludine blue	90%	98%
Sumathy C et al.[2]	2012	Toludine blue wet mount	90%	97%
Sumathi S et al.[1]	2013	Toludine blue wet mount	93.7%	98%
Present study	2015	Toludine blue wet mount	77%	94%
Present study	2015	Methylene blue/eosin	98%	100%

The quality of the stains was assessed using scoring system by Idris et al. The Quality Index was calculated based on the four criteria – Background score, Overall staining score, Cell morphology score and Nuclear characteristic score. In that study PAP stain attained the highest Quality Index of 0.87 followed by H&E 0.81 and MGG 0.77.[63] Another study done by Choudhary et al concluded that modified ultrafast PAP stain is rapid and better than routine PAP stain and this modified ultrafast PAP stain attained a Quality Index of 0.91.[68] [Table: 58]

Table 58: Comparison of quality index of the stains with other studies

AUTHORS	PAP STAIN	H&E STAIN	M/E STAIN	TBWM STAIN
Idris et al.[63]	0.87	0.81	-	-
Choudhary P et al.[68]	0.91	-	-	-
Shinde PB et al.[69]	0.89	-	-	-
Present study	0.86	0.83	0.73	0.68

The following table represents the comparison of three types of cytologic stains, a study done by Jourdsson et al.[70] [Table: 59]

Table 59: Comparison of staining quality for three types of cytologic stains.[70]

CELL FEATURE	H&E	PAP	ROMANOWSKY
Tissue fragments	Good	Good	Poor
Nucleus	Excellent	Excellent	Fair
Nucleolus	Distinct	Excellent	Visible
Cytoplasm	Similar to histologic specimens	Keratin	Excellent
Extracellular matrix	Poor	Poor	Excellent

The slides of TBWM could not be preserved since the cells were not fixed and should be photographed immediately. This is the major disadvantage of this stain. But it can be preserved for few hours, by sealing the cover slip by applying melted Vaseline or DPX. By this improvement the cytomorphology can be retained for a period of 2 to 3 hours without any morphological distortion and the quick drying of wet mount can also be prevented.

In most of the cases the material gets stuck in needle hub which is very difficult to obtain on the slide. This drawback was overcome by TBWM, where the toluidine blue is directly aspirated on the same needle used for FNAC and the material is expressed on the slide. This technique helps in obtaining the overall material from the needle and will improve the cellularity and helps in supplementing the conventional stains.

M/E stain helps in assessing the cellularity within few minutes which will guide us whether to proceed with the staining or to do re-aspiration. This method helps in reducing the time consumption in cases of USG guided FNAC and in intra operative cytodiagnosis.

Thus both stains can be used as a rapid stains in USG guided FNAC and in intraoperative emergency cases and can be used along with conventional stains on routine basis.

SUMMARY

This study entitled “Rapid cytodiagnosis by different staining techniques in comparison with conventional stains in fine needle aspiration cytology” was carried out at R.L. Jalappa Hospital, Tamaka, Kolar from Dec 2013 to July 2015. This study included 320 FNAC from various sites.

The role of immediate assessment of fine needle aspirate is well established in the literature for many years. However this practice has still not attracted much attention in our country mainly due to non-availability of trained personnel who can perform the spot assessment.

Moreover the preferred rapid stain in the western parts of the world is Diff-quik an imported proprietary preparation which is expensive and difficult to procure. So based on the work done by Joy MP et al., we have standardized a much simpler alternative in the form of rapid toluidine blue/eosin wet mount and methylene blue/eosin staining which is found to be cost effective.

Salient features of this study

1. Two rapid techniques – Toluidine blue wet mount and Methylene blue/Eosin was adapted in our study to assess the rapidity of the staining technique and to study the morphology of the cells in comparison with conventional stains [PAP and H&E].
2. Total number of cases were 320.
3. These cases were divided into 6 categories based on the site of lesions. Thyroid lesions (31%), Breast lesions (30%), Lymph node lesions (27%), Soft tissue lesions (9%), Salivary gland lesions (2%), Liver lesions (1%).

4. The rapidity of the stains were assessed and was found Toluidine blue wet mount is rapid and stains in 2 minutes followed by Methylene blue/Eosin in 5 minutes, H&E in 8 minutes and PAP in 10 minutes.
5. Thyroid lesions: M/E stain attained a diagnostic accuracy rate of 98%, sensitivity and specificity of 98% and 100%. TBWM attained a diagnostic accuracy of 73%, sensitivity and specificity of 66% and 90%. This decreased sensitivity in TBWM is due to difficulty in identifying Hurthle cells and fire flares.
6. Breast lesions: M/E stain attained a diagnostic accuracy of 99%, sensitivity and specificity of 99% and 200% respectively. TBWM attained a diagnostic accuracy of 80%, sensitivity and specificity rate of 68% and 94%. The diagnostic difficulty in TBWM are due to atypia in fibroadenoma and phyllodes tumor which was diagnosed as suspicious for malignancy.
7. Lymph node lesions: M/E attained a 100% diagnostic accuracy rate, sensitivity and specificity. TBWM attained a diagnostic accuracy rate of 76%, sensitivity and specificity of 70% and 93%. In TBWM we had difficulty in diagnosing Granulomatous lymphadenitis as the granulomas are not well appreciated due to three dimensional clusters of cells due to which the morphology was obscured.
8. Soft tissue lesions: M/E attained a 100% diagnostic accuracy rate, sensitivity and specificity. TBWM attained a diagnostic rate of 85%, sensitivity and specificity of 84% and 96% respectively. In TBWM, opinion could not be made in four cases due to three dimensional clusters which obscured the morphology.

9. Salivary gland lesions: M/E attained a 100% diagnostic accuracy rate, sensitivity and specificity. TBWM attained a diagnostic accuracy of 70%, sensitivity and specificity of 79% and 93% respectively.
10. Liver lesions: Both M/E and TBWM attained a 100% diagnostic accuracy rate, sensitivity and specificity.
11. Overall Diagnostic accuracy of M/E stain is 98% and for TBWM is 76%.
12. Overall Sensitivity and Specificity rate for M/E stain is 98% and 100% and for TBWM is 77% and 94%.
13. The decreased sensitivity in TBWM is mainly due to three dimensional clusters obscuring the morphology.
14. The Quality Index was calculated for all four stains based on four criteria – Background score, Overall staining score, Cell morphology score, Nuclear characteristic score. ***QUALITY INDEX = Actual score obtained / maximum score possible***
15. The Quality Index score for PAP stain is 0.86 and is found to be highest, followed by H&E stain which gave Quality Index of 0.83. The Quality Index of M/E is 0.73 and for TBWM is 0.68.
16. The advantage of M/E stains is immediate assessment of the sample and helps to find out the cellularity and adequacy of the sample. The cell loss due to immediate fixation can be avoided by this technique. The rapid assessment will help in the cases of USG guided FNAC and in intra-operative cases where re-sampling can be done without any delay.

17. TBWM stain has advantage of obtaining material from the needle hub and helps to improve the cellularity. The major disadvantage is three dimensional clusters and the slides cannot be preserved for the future reference, thus should be photographed immediately.
18. Thus both M/E and TBWM can be used as a supplementary test along with conventional stains to improve the cellularity and diagnostic accuracy.

CONCLUSION

FNAC is an important pre-operative and pre-treatment investigation. Thus rapid diagnosis will help to decide the further step in the management of the patient.

TBWM stains in 2 minutes which is rapid than other techniques and has advantage of improving the cellularity and adequacy of FNAC by obtaining the material form the needle hub. Though the diagnostic accuracy is 73%, the false negative rate (5%) is unacceptable as diagnosing malignancy as benign, has grave repercussions on the patient. Hence, TBWM should always be used as a complement stain in on site rapid diagnosis.

Methylene blue/Eosin stains in 5 minutes and attained good diagnostic accuracy than Toluidine blue wet mount. M/E has advantage of immediate assessment of the cellularity and adequacy of the material and helps in guiding the next step. Re-sampling can be done without any delay in cases of USG guided FNAC and in intra-operative cases.

Thus Toluidine blue wet mount and Methylene blue/Eosin stain can be used as a rapid diagnostic test. It is also used to assess adequacy of sample especially for deep seated lesions and in USG guided FNAC. It can be used for intra operative cytodiagnosis as an adjunct to frozen section diagnosis. Thus, both stains can be routinely undertaken as a supplementary procedure for conventional stains to improve the cellularity and to reduce the time taken for re-sampling.

This work gives a new dimension to the art of FNAC and also opens a new door for further researches in this regard.

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ANNEXURE I

PROFORMA

Name: SN no:

Age: FNAC no:

Sex:

Clinical history:

Clinical diagnosis:

Wet mount toluidine blue and eosin stain:

Methylene blue/eosin stain:

H&E stain:

PAP stain:

S. NO	STAINING TECHNIQUES	TIME TAKEN	BENIGN	MALIGNANT	SUSPICIOUS FOR MALIGNANCY	UNSATISFAC-TORY
1.	Wet mount					
2.	Methylene/Eosin					
3.	H&E stain					
4.	PAP stain					

QUALITY INDEX

SCORE	**1**	**2**	**3**
SLIDE QUALITY Background	Hemorrhage/Necrosis	Clean	
Overall staining	Bad	Moderately good	Good
Cell morphology	Not preserved	Moderately preserved	Well preserved
Nuclear characteristics	Smudgy chromatin	Moderately crisp chromatin	Crisp chromatin

SCORING FOR EACH STAIN

PARAMETERS	PAP STAIN	H&E STAIN	METHYLENE BLUE/EOSIN	TOLUIDINE BLUE WET MOUNT
Background				
Overall staining				
Cell morphology				
Nuclear characteristics				

QUALITY INDEX = Actual score obtained/maximum score possible

ANNEXURE II

INFORMED CONSENT FORM

Name of the investigator: Dr. Veena R

Name of the organization: R L Jalappa Hospital and Research centre
attached to Sri Devaraj Urs Medical College

Name of the participant: S no:

RAPID CYTODIAGNOSIS BY DIFFERENT STAINING TECHNIQUES IN COMPARISON WITH CONVENTIONAL STAINS IN FINE NEEDLE ASPIRATION CYTOLOGY

I have been invited to take part in this research study. The information in this document is meant to help me to decide whether or not to take part. I have clarified my doubts regarding this study with the principal investigator. I have been asked to participate in this study because I satisfy the eligibility criteria which is Palpable or non palpable mass requested for FNAC by concerned department.

I request and authorize Dr. Veena R to perform the designated tests for my FNAC sample. My signature below constitutes my acknowledgment that the benefits, risks and limitations of this testing have been explained to my satisfaction by a qualified health professional.

Participation is totally voluntary and there would be no payment for sample collection. All test results are treated with medical confidentiality and will not be disclosed to any outsider except if it is required by the law.

I give my consent to allow my sample to be used for medical research, test validation or education as long as my privacy is maintained.

I understand that I remain free to withdraw from this study at any time and this will not change my future care.

I have read and received a copy of patient information sheet. I understand the information provided in this document and I have had the opportunity to ask questions I might have about the testing, the procedure, the associated risk and alternatives.

Subject name and signature/ Thumb impression DATE:

Parents / Guardians name / Thumb impression DATE:

Signature of the person taking consent DATE:

ANNEXURE III

PREPERATION OF STAINING SOLUTIONS

PREPARATION OF TOLUIDINE BLUE STAIN

i. Toluidine blue 0.5 g

ii. 95% alcohol 20 ml

iii. Distilled water 80 ml

Toluidine blue was dissolved in alcohol. Then distilled water was added and filtered through the Whatman No. 1 filter paper. Solution was stored in refrigerator.

PREPARATION OF AQUOUS EOSIN

i. Eosin Y 0.5 g

ii. Distilled water 100 ml

Aqueous eosin stain was prepared by dissolving 0.5 gm of eosin in distilled water. Then the solution was filtered using Whatman No. 1 filter paper and stored.

PREPARATION OF LOFFLER'S METHYLENE BLUE ALKALINE STAIN

SOLUTION A: Methylene blue alkaline (90%) dye content – 0.3 g

Ethanol (95%) - 30 ml

SOLUTION B: Diluted potassium hydroxide (0.01%) – 100 ml

Mix solution A and B

ANNEXURE IV

KEY TO MASTER CHART

COLUMN H OT J

ABT	-	ACCESSORY BREAST TISSUE
ACD	-	ADENOCARCINOMA DEPOSITS
ACT	-	ANAPLASTIC CARCINOMA THYROID
AIL	-	ACUTE SUPPURATIVE LESION
ALCL	-	ANAPLASTIC LARGE CELL LYMPHOMA
ASS	-	ACUTE SUPPURATIVE SIALADENITIS
AST	-	ACUTE SUPPURATIVE THYROIDITIS
BAT	-	BENIGN ADNEXAL LESION
BBL	-	BENIGN BREAST LESION
BCL	-	BENIGN CYSTIC LESION
BL	-	BENIGN LESION
BSCL	-	BENIGN SPINDLE CELL LESION
BTL	-	BENIGN THYROID LESION
BVL	-	BENIGN VASCULAR LESION
CC	-	CYSTICERCOUS CYST
DC	-	DUCTAL CARCINOMA
de-Q	-	de-QUERVAINS THYROIDITIS
DPLD	-	DIFFUSE PARENCHYMAL LIVER DISEASE
FA	-	FIBROADENOMA
FCC	-	FIBROCYSTIC CHANGE
FN	-	FAT NECROSIS
FNT	-	FOLLICULAR NEOPLASM THYROID
GC	-	GALACTOCELE
GL	-	GRANULOMATOUS LYMPHADENITIS

GM	-	GRANULOMATOUS MASTITIS
GYM	-	GYNECOMASTIA
HC	-	HYDATID CYST
HG	-	HYPERPLASTIC GOITRE
ICL	-	INFLAMMED CYSTIC LESION
KC	-	KERATINOUS CYST
LC	-	LYMPH CYST
LP	-	LYMPHOMATOUS PROCESS
LT	-	LYMPHOCYTIC THYROIDITIS
MA	-	MONOMORPHIC ADENOMA
MGCT	-	MALIGNANT GERM CELL TUMOR
MM	-	MALIGNANT MELANOMA
MMT	-	MALIGNANT MESENCHYMAL TUMOR
MRC	-	MUCOUS RETENTION CYST
MRCT	-	MALIGNANT ROUND CELL TUMOR
NF	-	NEUROFIBROMA
NG	-	NODULAR GOITRE
NHL	-	NON-HODGKIN LYMPHOMA
NIL	-	NO OPINION
NL	-	NECROTISING LYMPHADENITIS
PA	-	PLEOMORPHIC ADENOMA
PCB	-	PAPILLARY CARCINOMA BREAST
PCT	-	PAPILLARY CARCINOMA THYROID
PDC	-	POORLY DIFFERENTIATED CARCINOMA
PT	-	PHYLLODES TUMOR
RL	-	REACTIVE LYMPHADENITIS
SFM	-	SUSPICIOUS FOR MALIGNANCY
SL	-	SUPPURATIVE LYMPHADENITIS
SCC	-	SQUAMOUS CELL CARCINOMA
TDC	-	THYROGLOSSAL DUCT CYST

COLUMN K TO N

1 - HEMORRHAGE/NECROSIS

2 - CLEAN

COLUMN O TO R

1 - BAD

2 - MODERATELY GOOD

3 - GOOD

COLUMN S TO V

1 - NOT PRESERVED

2 - MODERATELY PRESERVED

3 - WELL PRESERVED

COLUMN W TO Z

1 - SMUDGY CHROMATIN

2 - MODERATELY CRISP CHROMATIN

3 - CRISP CHROMATIN

S.NO	OP/IP NO	AGE	SEX	FNAC SITE	FNAC NO	CLINICAL DIAGNOSIS	IMPRESSION			BACKGROUND				OVERALL STAINING				CELL MORPHOLOGY				NUCLEAR CHARACTERISTICS			
							PAP AND H&E	M/E	TBWM	PAP	H&E	M/E	WM	PAP	H&E	M/E	WM	PAP	H&E	M/E	WM	PAP	H&E	M/E	WM
1	971037	20	M	SUBMANDIBULAR SWELLING	C/2046/13	LYMPHADENITIS	RL	RL	RL	1	1	1	1	1	1	1	1	2	2	3	2	2	2	3	2
2	971338	26	M	AXILLARY LYMPHNODE	C/2054/13	?TBLN	GL	GL	NL	1	1	1	1	2	2	2	2	3	3	3	2	3	2	2	2
3	972629	38	F	NECK SWELLING	C/2074/13	?MULTINODULAR GOITRE	HG	HG	BTL	2	2	2	2	3	3	3	3	2	2	2	2	3	3	3	2
4	971092	24	F	THYROID NODULE	C/2075/13	GOITRE	LT	LT	LT	1	1	1	1	3	2	2	2	3	3	3	2	2	2	2	2
5	972689	48	F	LEFT SUPRACLAVICULAR SWELLING	C/2076/13	?NEUROFIBROMA	BSCL	BSCL	BSCL	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
6	971880	53	F	THYROID SWELLING	C/2085/13	THYROTOXICOSIS	BTL	BTL	BTL	2	2	1	1	1	1	2	2	2	2	2	2	3	3	3	2
7	975235	34	F	CERVICAL LN	C/2119/13	?TBLN	RL	RL	RL	2	2	2	2	2	2	1	1	2	2	1	1	3	2	1	1
8	975464	63	F	LEFT INGUINAL LN	C/2129/13	?SECONDARIES NODE	NHL	NHL	RL	1	1	1	1	3	3	2	2	3	3	2	2	2	2	2	2
9	975611	47	F	THYROID SWELLING	C/2136/13	MULTINODULAR GOITRE	LT	LT	LT	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
10	977374	49	F	RIGHT BREAST LUMP	C/06/14	?FIBROADENOMA	DC	DC	DC	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
11	977228	25	F	THYROID SWELLING	C/09/14	MULTINODULAR GOITRE	LT	BTL	LT	2	2	1	1	3	3	3	2	2	2	2	2	2	2	2	2
12	974375	48	F	LN IN NECK PROVISION	C/13/14	POSTCRICOID CARCINOMA	SCC	SCC	SCC	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
13	977717	80	M	SUBMANDIBULAR SWELLING	C/14/14	?TBLN	GL	GL	RL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
14	978529	22	F	LEFT BREAST LUMP	C/30/14	?GALACTOCELE	GC	GC	NIL	2	2	2	2	3	2	2	1	1	1	1	1	1	1	1	1
15	978553	22	F	THYROID SWELLING	C/31/14	GOITRE	NG	NG	NG	2	2	2	2	3	3	2	2	2	2	3	2	2	2	2	2
16	978764	72	M	RIGHT EPIDIDYMAL SWELLING	C/37/14	?KOCH'S	AIL	AIL	AIL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
17	980292	85	M	NECK SWELLING RIGHT SIDE	C/68/14	CA THYROID	ACT	ACT	ACT	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
18	982713	46	F	LEFT SUPRACLAVICULAR LYMPHNODE	C/95/14	ABSCESS	ACD	ACD	ACD	1	1	1	1	2	2	2	2	3	2	2	2	3	2	3	2
19	982719	40	F	RIGHT BREAST LUMP	C/96/14	?FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2
20	796976	62	M	CERVICAL LN	C/98/14	?CARCINOMA	SCC	SCC	SCC	1	1	2	1	3	3	3	3	3	3	3	3	3	3	3	3
21	984204	37	F	THYROID SWELLING	C/124/14	NODULAR GOITRE	LT	LT	LT	2	2	2	2	3	3	2	2	3	3	3	2	3	3	2	2
22	984906	70	M	PREAURICULAR SWELLING	C/138/14	LYMPHADENITIS	KC	KC	NIL	2	2	2	2	2	2	1	1	3	2	1	1	2	2	1	1
23	754737	29	M	SUBMANDIBULAR SWELLING	C/139/14	LYMPHADENITIS	PA	PA	NIL	2	2	2	2	2	1	2	1	2	2	2	1	3	3	3	2
24	568524	51	F	THYROID SWELLING	C/140/14	?CARCINOMA	PCT	PCT	BTL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
25	642487	26	F	RIGHT BREAST LUMP	C/141/14	?CARCINOMA	DC	DC	SFM	2	2	2	2	3	3	3	2	2	2	2	2	3	2	2	2
26	623591	54	F	LEFT BREAST LUMP	c/143/14	CARCINOMA BREAST	DC	DC	DC	1	1	1	1	3	3	2	2	3	2	3	2	2	2	2	2
27	623615	36	F	NECK SWELLING	C/144/14	GOITRE	TDC	TDC	NIL	2	2	2	2	2	2	1	1	2	1	2	1	1	1	2	1
28	638612	45	F	RIGHT THYROID SWELLING	C/145/14	GOITRE	HG	HG	HG	2	2	2	2	2	2	2	1	2	2	2	2	2	2	1	1
29	769950	34	F	AXILLARY SWELLING-RIGHT SIDE	C/148/14	?TBLN	ABT	ABT	ABT	2	2	2	2	3	3	2	2	3	3	3	2	3	3	2	2
30	747010	25	F	RIGHT SUBAREOLAR SWELLING	C/149/14	DUCT PAPILLOMA	AIL	AIL	AIL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
31	770764	32	F	RIGHT BREAST LUMP	C/150/14	?CARCINOMA	DC	DC	SFM	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
32	710441	56	F	RIGHT BREAST LUMP	C/159/14	CARCINOMA BREAST	DC	DC	DC	2	2	2	2	1	1	1	1	2	2	2	2	3	3	3	3
33	760583	28	F	THYROID SWELLING	C/160/14	GOITRE	de-Q	de-Q	BTL	2	2	2	2	3	3	2	2	3	3	3	3	2	2	3	2
34	985569	44	M	LUMP AT NAPE OF NECK	C/161/14	?LIPOMA	LIPOMA	LIPOMA	LIPOMA	2	2	2	2	3	3	3	3	3	3	3	3	3	2	2	2
35	985657	50	F	LEFT BREAST LUMP	C/162/14	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
36	796852	33	F	LEFT BREAST LUMP	C/163/14	FIBROADENOMA	FN	FN	AIL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
37	616751	50	F	RIGHT BREAST LUMP	C/166/14	CARCINOMA BREAST	DC	DC	DC	2	2	2	2	3	3	2	2	3	3	3	2	3	3	3	3
38	618146	54	M	GLUTEAL CLEFT SWELLING	C/168/14	LIPOMA	KC	KC	KC	2	2	2	2	3	3	3	3	2	2	2	2	2	2	2	2
39	787301	38	M	GUIDED FNAC-ABDOMINAL SWELLING	C/173/14	CYSTICERCOUS CYST	HC	HC	HC	2	2	2	2	2	2	2	1	3	3	2	2	2	2	3	2
40	778119	42	F	LEFT BREAST LUMP	C/174/14	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	3	2	2	2	1	1	2	1	1	1
41	754890	43	F	NECK SWELLING	C/179/14	?GOITRE	HG	HG	BTL	2	2	2	2	3	3	2	2	3	3	3	2	3	2	2	2
42	791953	32	F	NECK SWELLING	C/180/14	HYPOTHYROIDISM	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	3	2	3	3	2	2
43	613685	40	F	RIGHT BREAST LUMP	C/189/14	FIBROADENOMA	PT	PT	PT	2	2	2	2	1	1	1	1	3	3	2	2	1	1	1	1
44	776984	48	F	LEFT BREAST LUMP	C/191/14	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	2	2	2	1	1	1	1	1	2	2	2	2
45	799858	46	F	RIGHT BREAST LUMP	C/193/14	CARCINOMA BREAST	PCB	PCB	PCB	2	2	2	2	3	3	2	2	3	2	2	2	3	2	1	1
46	986935	28	F	THYROID SWELLING	C/196/14	?MULTINODULAR GOITRE	PCT	PCT	BTL	2	2	2	2	3	3	3	2	2	2	1	1	3	3	3	2
47	602387	52	F	LEFT BREAST LUMP	C/201/14	CARCINOMA BREAST	DC	DC	DC	1	1	1	1	2	2	1	1	3	3	3	2	3	2	1	1
48	718653	36	F	RIGHT BREAST LUMP	C/208/14	?FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	2	2	2	1	3	3	2	2
49	786329	39	F	NECK SWELLING	C/218/14	?TBLN	GL	GL	NL	2	2	1	1	3	3	2	2	2	2	2	2	3	3	2	2
50	774076	36	F	THYROID SWELLING	C/222/14	GOITRE	LT	LT	LT	2	2	2	2	3	2	2	1	3	3	2	2	2	2	1	1
51	523366	56	M	PAROTID SWELLING	C/224/14	PLEOMORPHIC ADENOMA	MA	MA	NIL	1	1	1	1	1	1	1	1	2	1	2	1	3	2	1	1
52	555622	58	M	GUIDED FNAC-SWELLING IN THE LIVER	C/230/14	?SECONDARIES	DPLD	DPLD	DPLD	2	1	2	1	2	2	2	1	3	2	3	2	1	1	1	1
53	604287	53	F	RIGHT BREAST LUMP	C/232/14	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	3	2	3	3	3	2	3	2	2	2
54	987456	65	F	GUIDED FNAC-SWELLING IN THE LIVER	C/250/14	?SECONDARIES	ACD	ACD	ACD	2	2	2	2	3	2	1	1	2	2	2	2	2	2	3	2
55	694724	28	F	RIGHT BREAST LUMP	C/254/14	FIBROADENOMA	FA	FA	FA	2	2	2	2	1	1	2	1	2	2	1	1	3	3	2	2
56	689911	45	M	CERVICAL LN	C/255/14	?TBLN	RL	RL	RL	2	2	2	2	3	3	2	2	3	3	3	2	3	3	2	2
57	770184	42	F	THYROID SWELLING	C/257/14	GOITRE	HG	HG	BTL	2	2	2	2	3	3	2	2	3	3	3	2	1	1	1	1

58	998554	8	M	SWELLING IN RIGHT HIP	C/479/14	MALIGNANCY	MRCT	MRCT	MRCT	2	2	2	2	2	2	1	1	2	2	2	2	3	2	2	2
59	517612	26	F	BILATERAL CERVICAL SWELLING	C/08/15	?TBLN	GL	GL	NL	2	2	2	2	3	3	2	2	1	1	2	1	1	1	1	1
60	675563	7	M	BILATERAL CERVICAL SWELLING	C/09/15	LYMPHADENITIS	RL	RL	RL	2	2	1	1	2	3	3	2	3	3	2	2	3	3	2	2
61	694824	25	F	RIGHT CERVICAL SWELLING	C/18/15	CERVICAL LYMPHADENITIS	NL	NL	NL	2	2	2	2	1	1	2	1	3	2	1	1	2	2	1	1
62	799079	48	F	LEFT BREAST LUMP	C/19/15	?CARCINOMA	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
63	633769	24	F	SWELLING IN NAPE OF NECK	C/29/15	LIPOMA	LIPOMA	LIPOMA	LIPOMA	2	2	2	2	3	3	2	2	2	2	2	2	2	2	2	2
64	510654	32	M	RIGHT CERVICAL SWELLING	C/30/15	TBLN	NL	NL	NL	1	1	1	1	2	2	1	1	1	1	2	1	3	3	2	2
65	727190	48	F	BILATERAL CERVICAL SWELLING	C/44/15	LYMPHADENITIS	GL	GL	RL	2	2	2	2	3	3	2	2	3	3	3	2	2	1	1	1
66	849436	25	M	RIGHT BREAST LUMP	C/80/15	GYNAECOMASTIA	AIL	AIL	AIL	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2
67	771080	34	F	LEFT CERVICAL LYMPHADENOPATHY	C/81/15	TBLN	RL	RL	RL	2	2	2	2	3	3	3	3	2	2	3	2	3	3	3	2
68	892120	32	M	THYROID SWELLING	C/82/15	GOITRE	HG	HG	LT	1	1	2	1	1	1	1	1	3	3	2	2	3	2	2	2
69	624299	54	F	SWELLING IN NAPE OF NECK	C/94/15	LIPOMA	LIPOMA	LIPOMA	LIPOMA	2	2	2	2	3	3	2	2	3	3	2	2	2	2	1	1
70	706190	28	F	THYROID SWELLING	C/95/15	MULTINODULAR GOITRE	LT	LT	LT	2	2	2	2	3	3	3	2	3	3	2	2	2	2	2	2
71	620144	35	F	THYROID SWELLING	C/96/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	2	2	2	1	2	2	1	1	2	2	2	2
72	567828	60	M	LEFT CERVICAL LN	C/104/15	LYMPHADENITIS	AIL	AIL	AIL	2	2	2	2	3	3	3	3	3	3	3	2	3	3	3	2
73	791932	32	F	RIGHT SUPRACLAVICULAR SWELLING	C/105/15	TBLN	GL	GL	GL	2	2	2	2	3	2	1	1	3	3	3	2	3	3	2	2
74	892099	39	M	RIGHT CERVICAL SWELLING	C/106/15	TBLN	GL	GL	GL	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	1
75	510425	57	F	RIGHT BREAST LUMP	C/112/15	?CARCINOMA	AIL	AIL	NIL	2	2	2	2	3	3	2	2	2	2	2	1	3	2	2	2
76	574484	40	F	LEFT BREAST LUMP	C/113/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
77	781579	35	M	SUBLINGUAL SWELLING	C/115/15	TBLN	MRC	MRC	MRC	2	2	2	2	2	2	2	1	1	1	2	1	1	1	1	1
78	849258	65	F	LEFT BREAST LUMP	C/116/15	?CARCINOMA	DC	DC	DC	2	2	2	2	2	2	1	1	3	3	2	2	3	3	2	2
79	892341	5	M	SUBMENTAL SWELLING	C/117/15	?TBLN	RL	RL	RL	2	2	2	2	3	3	3	3	3	3	3	2	3	3	3	3
80	892107	56	F	THYROID SWELLING	C/136/15	GOITRE	NG	NG	NG	2	2	2	2	2	1	2	1	2	2	1	1	3	2	1	1
81	891784	28	F	LEFT BREAST LUMP	C/137/15	FIBROADENOMA	FCC	FCC	SFM	2	2	2	2	3	2	1	1	1	1	1	1	3	3	2	2
82	849407	45	F	RIGHT BREAST LUMP	C/138/15	FIBROADENOMA	FCC	FCC	AIL	2	2	2	2	3	3	2	2	3	3	2	2	1	1	1	1
83	849106	32	F	RIGHT BREAST LUMP	C/146/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	3	3	3	2	2	2	2	2
84	892119	23	F	LEFT CERVICAL LN	C/147/15	LYMPHADENITIS	ICL	ICL	NIL	2	2	2	2	2	2	2	1	3	3	3	2	2	2	1	1
85	892361	70	M	LEFT SUPRACLAVICULAR LN	C/148/15	MALIGNANCY	SCC	SCC	SCC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	2
86	849592	33	F	THYROID SWELLING	C/149/15	GOITRE	NG	NG	NG	2	2	2	2	2	1	1	1	2	2	1	1	3	3	2	2
87	849556	28	F	THYROID SWELLING	C/150/15	GOITRE	CD	CD	CD	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	1
88	891809	28	F	RIGHT BREAST LUMP	C/151/15	?CARCINOMA	DC	DC	FA	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
89	849599	53	F	THYROID SWELLING	C/167/15	GOITRE	PCT	PCT	BTL	2	2	2	2	3	3	3	3	3	3	3	3	2	2	2	2
90	892262	21	M	LEFT AXILLARY SWELLING	C/168/15	MALIGNANCY	ALCL	ALCL	ALCL	1	1	1	1	3	3	2	2	3	3	3	2	3	3	3	3
91	892465	45	M	LEFT CERVICAL LN	C/169/15	MALIGNANCY	SCC	SCC	AIL	1	1	2	1	2	2	2	1	1	1	1	1	1	1	1	1
92	892432	43	M	RIGHT SUPRACLAVICULAR LN	C/213/15	MALIGNANCY	SCC	SCC	SFM	2	2	2	2	3	3	3	2	3	3	2	2	3	3	2	2
93	849519	33	M	LEFT CERVICAL LYMPHADENOPATHY	C/214/15	TBLN	RL	RL	RL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
94	849572	26	F	THYROID SWELLING	C/215/15	GOITRE	CG	CG	CG	2	2	2	2	1	1	1	1	3	3	2	2	1	1	1	1
95	892481	28	F	THYROID SWELLING	C/230/15	GOITRE	HG	HG	NG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
96	849549	46	F	LEFT BREAST LUMP	C/231/15	CARCINOMA BREAST	DC	DC	DC	2	2	2	2	3	3	2	2	3	2	3	2	2	2	1	1
97	892395	24	F	CERVICAL LN	C/232/15	LYMPHADENITIS	RL	RL	RL	1	1	1	1	2	2	2	1	2	2	2	2	1	1	1	1
98	892304	18	F	THYROID SWELLING	C/234/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	2	1	1	1	3	2	2	2	3	2	2	2
99	892438	37	F	RIGHT CERVICAL LN	C/235/15	LYMPHADENITIS	KC	KC	KC	2	2	2	2	3	3	2	2	2	2	3	2	2	2	2	2
100	892284	45	F	RIGHT SUBMANDIBULAR LN	C/243/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
101	892100	26	F	THYROID SWELLING	C/244/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	2	2	3	3	3	3	3	2	1	1
102	849490	48	F	RIGHT BREAST LUMP	C/245/15	CARCINOMA BREAST	DC	DC	DC	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
103	891778	37	M	LEFT CERVICAL LN	C/247/15	?TBLN	SCC	SCC	SCC	2	2	2	2	2	2	1	1	2	2	1	1	2	1	2	1
104	892210	28	F	LEFT CERVICLA LN	C/249/15	TBLN	GL	GL	NL	1	1	1	1	1	1	2	1	1	1	2	1	3	2	2	2
105	849568	32	F	RIGHT AXILLARY SWELLING	C/251/15	LYMPHADENITIS	ABT	ABT	DC	2	2	2	2	3	3	2	2	3	2	2	2	3	3	2	2
106	849317	35	F	THYROID SWELLING	C/272/15	GOITRE	NG	NG	NG	2	2	2	2	3	3	2	2	3	3	3	2	2	2	1	1
107	849639	40	F	RIGHT BREAST LUMP	C/273/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
108	849218	29	F	LEFT CERVICAL LN	C/274/15	TBLN	GL	GL	RL	2	2	2	2	3	3	3	2	3	3	2	2	3	2	2	1
109	892523	37	M	LEFT CERVICAL LN	C/286/15	LYMPHADENITIS	RL	RL	RL	1	1	1	1	2	2	1	1	2	2	1	1	1	1	1	1
110	849501	28	F	RIGHT BREAST LUMP	C/287/15	FIBROADENOMA	AIL	AIL	AIL	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
111	849337	45	F	THYROID SWELLING	C/289/15	GOITRE	CG	CG	CG	2	2	2	2	2	1	1	1	3	2	2	1	2	2	3	2
112	849703	50	F	LEFT BREAST LUMP	C/290/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	3	2	2	2	3	2	3	3	2	2
113	849635	22	F	LEFT BREAST LUMP	C/292/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	2	2	2	1	3	3	2	2	1	1	1	1
114	892020	55	M	SWELLING NEAR SACRUM	C/319/15	?NEUROFIBROMA	BSCL	BSCL	NIL	2	2	2	2	3	2	1	1	1	1	1	1	3	3	2	2
115	892124	32	F	THYROID SWELLING	C/320/15	GOITRE	SFM	SFM	NIL	2	2	2	2	1	1	2	1	3	2	2	1	2	2	2	1
116	849193	30	F	THYROID SWELLING	C/322/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	1	1	1	1	1	1

117	892502	27	M	LEFT SUBMANDIBULAR SWELLING	C/323/15	LYMPHADENITIS	GL	GL	NL	1	1	1	1	2	2	1	1	3	2	2	1	3	2	2	2
118	849475	56	F	RIGHT AXILLARY SWELLING	C/325/15	LYMPHADENITIS	DC	DC	DC	2	2	2	2	2	2	2	1	1	1	1	1	2	2	3	2
119	849270	28	F	RIGHT CERVICAL LN	C/326/15	TBLN	GL	GL	NL	2	2	1	1	3	2	1	1	3	2	1	1	2	2	2	2
120	849687	20	F	LEFT BREAST LUMP	C/327/15	FIBROADENOMA	KC	KC	KC	2	2	2	2	2	1	2	1	2	2	2	2	2	1	1	1
121	849187	58	F	LEFT SUPRACLAVICULAR LN	C/329/15	TBLN	SCC	SCC	SCC	2	2	2	2	3	3	2	2	3	3	3	2	3	3	2	2
122	891871	53	M	LEFT INGUINAL LN	C/342/15	?LYMPHOMA	RL	RL	RL	2	2	2	2	3	3	2	2	3	2	2	2	3	3	2	2
123	849196	65	F	THYROID SWELLING	C/343/15	GOITRE	CG	CG	CG	1	1	1	1	2	2	1	1	1	1	1	1	2	2	1	1
124	892459	48	F	LEFT BREAST LUMP	C/344/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	3
125	847852	40	F	GUIDED FNAC-SWELLING IN THE LIVER	C/346/15	CYSTIC LESION	HC	HC	HC	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
126	849119	48	F	CERVICAL LN	C/347/15	TBLN	RL	RL	RL	2	2	2	2	3	3	2	2	2	2	2	2	3	2	2	2
127	849138	48	F	LEFT MANDIBULAR SWELLING	C/353/15	?SECONDARIES	BVL	BVL	BVL	2	2	2	2	3	3	2	2	3	2	3	2	2	2	3	2
128	849250	30	F	MIDLINE CERVICAL SWELLING	C/354/15	LYMPHADENITIS	GL	GL	NL	1	1	1	1	3	3	2	2	3	3	2	2	3	3	2	2
129	849168	28	F	THYROID SWELLING	C/355/15	GOITRE	CG	CG	CG	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1
130	892417	56	F	LEFT BREAST LUMP	C/428/15	CARCINOMA	DC	DC	DC	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
131	891819	48	M	MIDLINE NECK SWELLING	C/429/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
132	891802	35	M	FOREHEAD SWELLING	C/436/15	CYSTIC LESION	CC	CC	NIL	2	2	2	2	2	2	1	1	2	2	1	1	2	2	1	1
133	815103	32	F	THYROID SWELLING LEFT LOBE	C/437/15	GOITRE	HG	HG	BTL	2	2	2	2	3	3	3	2	3	3	2	2	2	2	2	2
134	892187	18	M	LEFT CERVICAL LYMPHADENOPATHY	C/438/15	TBLN	GL	GL	RL	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
135	892266	28	M	FOREHEAD SWELLING	C/458/15	LIPOMA	LIPOMA	LIPOMA	LIPOMA	2	2	2	2	3	3	2	2	3	2	3	2	2	2	3	2
136	892196	40	F	THYROID SWELLING LEFT LOBE	C/459/15	GOITRE	HG	HG	NG	2	2	2	2	3	3	2	2	3	2	2	2	3	3	2	2
137	892303	10	F	LEFT SUPRACLAVICULAR SWELLING	C/460/15	?CARCINOMA	CC	CC	CC	2	2	2	2	2	1	2	1	1	1	1	1	1	1	2	1
138	891964	24	F	RIGHT CERVICAL LN	C/672/15	TBLN	AIL	AIL	AIL	2	2	2	2	2	2	1	1	3	3	2	2	3	3	2	2
139	892170	26	F	LEFT BREAST LUMP	C/673/15	FIBROADENOMA	FA	FA	FA	1	1	1	1	2	2	2	1	3	2	2	1	2	2	1	1
140	891821	38	M	RIGHT SUBMANDIBULAR SWELLING	C/675/15	TBLN	SCC	SCC	SCC	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
141	849160	18	F	LEFT BREAST LUMP	C/677/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	1	1	1	1	3	2	1	1	3	3	2	2
142	891854	32	M	RIGHT CERVICAL LN	C/686/15	TBLN	GL	GL	NL	1	1	1	1	3	2	2	1	2	2	2	1	2	2	2	2
143	891848	37	M	LEFT ARM SWELLING	C/687/15	LIPOMA	NF	NF	NF	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
144	891832	49	F	THYROID SWELLING	C/689/15	GOITRE	CG	CG	CG	2	2	2	2	1	1	2	1	3	3	3	2	2	2	2	2
145	891875	25	F	THYROID SWELLING RIGHT LOBE	C/691/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	2	2	2	2	3	2
146	913647	27	F	RIGHT BREAST LUMP	C/692/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	2	3	2	3	2	2	2	2	2
147	912595	28	F	THYROID SWELLING LEFT LOBE	C/700/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	3	3	2	2	3	2	3	3	2	2
148	918671	43	M	RIGHT SUPRACLAVICULAR SWELLING	C/701/15	?CARCINOMA	NL	NL	NL	1	1	1	1	2	2	1	1	3	2	2	2	1	1	1	1
149	69746	49	F	RIGHT BREAST LUMP	C/702/15	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
150	906769	36	M	LEFT BREAST LUMP	C/751/15	?CARCINOMA	GYM	GYM	GYM	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
151	916861	40	M	OCCIPITAL SWELLING	C/752/15	LIPOMA	LIPOMA	LIPOMA	LIPOMA	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	1
152	916849	45	F	THYROID SWELLING RIGHT LOBE	C/754/14	GOITRE	HG	HG	NG	2	2	2	2	1	1	1	1	2	2	3	2	3	3	2	2
153	916869	72	M	LEFT LEG SWELLING	C/755/15	LIPOMA	MMT	MMT	MMT	1	1	1	1	2	2	3	2	3	2	2	2	3	3	3	3
154	916348	36	M	THYROID SWELLING LEFT LOBE	C/759/15	?CARCINOMA	PCT	PCT	NG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
155	913642	26	F	THYROID SWELLING RIGHT LOBE	C/760/15	GOITRE	LT	LT	LT	2	2	2	2	3	2	1	1	2	2	3	2	1	1	1	1
156	916733	28	F	LEFT BREAST LUMP	C/761/15	FIBROADENOMA	FCC	FCC	SFM	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
157	954981	42	F	THYROID SWELLING LEFT LOBE	C/762/15	GOITRE	LT	LT	LT	2	2	2	2	2	2	1	1	3	3	2	2	3	2	1	1
158	914050	23	F	LEFT BREAST LUMP	C/770/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	3	3	2	2	2	3	3	3	2
159	914000	26	F	LEFT BREAST LUMP	C/771/15	FIBROADENOMA	GM	GM	AIL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
160	913301	55	M	THYROID SWELLING RIGHT LOBE	C/774/15	GOITRE	CG	CG	CG	2	2	2	2	1	1	1	1	2	2	2	2	1	1	2	1
161	914056	39	F	RIGHT BREAST LUMP	C/775/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
162	955215	54	F	LEFT SUPRACLAVICULAR SWELLING	C/962/15	?CARCINOMA	GL	GL	RL	2	2	2	2	3	3	2	2	1	1	1	1	2	2	1	1
163	914048	47	F	THYROID SWELLING RIGHT LOBE	C/963/15	GOITRE	CG	CG	CG	2	2	2	2	2	2	1	1	3	3	2	2	3	3	2	2
164	914274	50	F	THYROID SWELLING RIGHT LOBE	C/964/15	?CARCINOMA	PCT	PCT	BTL	2	2	2	2	1	1	2	1	3	2	2	2	2	2	2	2
165	94401	82	M	RIGHT INGUINAL SWELLING	C/967/15	?METASTASIS	MM	MM	MM	2	2	2	2	3	3	3	2	2	2	2	2	3	3	3	3
166	913547	45	F	RIGHT BREAST LUMP	C/968/15	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	3
167	937821	48	F	THYROID SWELLING LEFT LOBE	C/989/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	3	3	3	3	3	3	3	2	2	2
168	916882	56	F	LEFT BREAST LUMP	C/990/15	?CARCINOMA	PCB	PCB	BBL	2	2	2	2	3	3	2	2	3	3	2	2	2	1	1	1
169	953693	57	F	LEFT CERVICAL SWELLING	C/991/15	?METASTASIS	PDC	PDC	PDC	1	1	1	1	3	3	3	2	3	3	3	3	3	3	3	3
170	913627	45	F	THYROID SWELLING RIGHT LOBE	C/992/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
171	914271	28	F	THYROID SWELLING RIGHT LOBE	C/993/15	DIFFUSE SWELLING	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	2	2	2	2	2	2
172	953730	49	F	LEFT BREAST LUMP	C/1037/15	?CARCINOMA	DC	DC	DC	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
173	955290	48	F	RIGHT BREAST LUMP	C/1038/15	FIBROADENOMA	FCC	FCC	SFM	1	1	1	1	3	3	3	2	3	3	2	2	3	3	2	2
174	913864	66	M	LEFT SUPRACLAVICULAR SWELLING	C/1039/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	3	2	2	3	2	2	2	2	2	2	2
175	954613	43	F	THYROID SWELLING RIGHT LOBE	C/1040/15	DIFFUSE SWELLING	LT	LT	LT	2	2	2	2	3	2	2	2	3	3	3	2	1	1	1	1

176	913527	38	M	RIGHT SUBMANDIBULAR SWELLING	C/1110/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
177	914020	70	M	LEFT CERVICAL SWELLING	C/1111/15	?CARCINOMA	ACT	ACT	ACT	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
178	913284	27	M	LEFT CERVICAL SWELLING	C/1112/15	?METASTASIS	ALCL	ALCL	ALCL	2	2	2	2	3	3	3	2	3	3	2	2	3	3	2	2
179	937697	44	F	RIGHT BREAST LUMP	C/1113/15	?CARCINOMA	DC	DC	DC	2	2	2	2	2	2	1	1	2	2	2	2	2	2	1	1
180	955349	26	F	THYROID SWELLING LEFT LOBE	C/1127/15	GOITRE	HG	HG	CG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
181	954954	28	F	LEFT BREAST LUMP	C/1128/15	FIBROADENOMA	FA	FA	SFM	2	2	2	2	3	2	2	1	1	1	1	1	1	1	1	1
182	955361	42	F	LEFT BREAST LUMP	C/1129/15	FIBROADENOMA	FCC	FCC	SFM	2	2	2	2	1	1	1	1	3	3	2	2	3	3	2	2
183	919463	60	F	LEFT CERVICAL SWELLING	C/1130/15	?CARCINOMA	PDC	PDC	PDC	2	2	2	2	3	2	2	1	3	2	2	2	3	2	1	1
184	955517	43	M	LEFT CERVICAL SWELLING	C/1151/15	?CARCINOMA	PCT	PCT	NG	2	2	2	2	2	2	1	1	3	3	2	2	3	3	3	2
185	953776	48	F	THYROID SWELLING LEFT LOBE	C/1152/15	?CARCINOMA	FNT	FNT	FNT	2	2	2	2	3	2	2	2	1	2	2	2	1	1	2	1
186	913874	30	M	RIGHT POSTERIOR CERVICAL LYMPHNO	C/1153/15	TBLN	NL	NL	NL	1	1	1	1	3	2	1	1	1	1	1	1	2	2	2	2
187	954599	29	F	THYROID SWELLING RIGHT LOBE	C/1154/15	GOITRE	LT	LT	LT	2	2	2	2	1	1	2	1	3	3	2	2	2	2	2	1
188	954635	24	F	RIGHT AXILLARY SWELLING	C/1531/15	?METASTASIS	ABT	ABT	ABT	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
189	955265	28	F	RIGHT BREAST LUMP	C/1532/15	ABSCESS	AIL	AIL	AIL	2	2	2	2	3	3	3	3	2	2	2	2	3	3	3	3
190	953740	39	F	RIGHT BREAST LUMP	C/1533/15	FIBROADENOMA	FCC	FCC	FCC	2	1	1	1	3	3	2	2	3	3	2	2	2	2	2	2
191	953778	48	F	THYROID SWELLING RIGHT LOBE	C/1534/15	GOITRE	HG	HG	NG	2	2	2	2	2	2	1	1	3	3	3	2	1	1	1	1
192	904156	25	F	THYROID SWELLING RIGHT LOBE	C/1535/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	3	2	3	3	2	2	3	3	2	2
193	913475	23	M	RIGHT CERVICAL LN	C/1554/15	TBLN	SL	SL	SL	2	2	2	2	3	3	2	2	3	3	2	2	3	2	2	2
194	916976	39	M	RIGHT GBS SWELLING	C/1555/15	?CARCINOMA	PA	PA	PA	1	1	1	1	3	3	2	2	3	3	3	3	3	3	2	2
195	913343	30	M	RIGHT CERVICAL LN	C/1557/15	TBLN	NL	NL	NL	2	2	2	2	1	1	2	1	2	2	1	1	2	2	1	1
196	955633	43	M	THYROID SWELLING LEFT LOBE	C/1558/15	GOITRE	NG	NG	NG	2	2	2	2	3	2	1	1	3	3	2	2	3	2	1	1
197	102167	58	M	RIGHT SUBMANDIBULAR SWELLING	C/1560/15	ABSCESS	ASS	ASS	ASS	2	2	2	2	3	3	3	2	3	3	3	3	3	3	2	2
198	955311	37	F	LEFT BREAST LUMP	C/1580/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
199	954355	26	F	LEFT BREAST LUMP	C/1581/15	CYSTIC LESION	GC	GC	GC	2	2	2	2	2	2	1	1	2	2	2	2	1	1	1	1
200	914023	20	F	LEFT BREAST LUMP	C/1582/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
201	913598	45	M	LEFT CERVICAL LYMPHADENOPATHY	C/1583/15	TBLN	NL	NL	NL	2	2	1	1	3	3	2	2	3	3	3	2	3	3	2	2
202	918345	28	F	RIGHT BREAST LUMP	C/1584/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	3	3	3	3	3	3	3	2	2	2
203	953766	46	F	RIGHT CERVICAL LYMPHADENOPATHY	C/1585/15	TBLN	NL	NL	NL	2	2	2	2	3	3	2	2	3	3	3	3	2	2	2	2
204	955958	48	F	THYROID SWELLING RIGHT LOBE	C/1609/15	GOITRE	LT	LT	LT	2	2	2	2	1	1	2	1	3	3	2	2	3	3	3	3
205	955934	32	F	THYROID SWELLING RIGHT LOBE	C/1610/15	GOITRE	LT	LT	NG	2	2	2	2	2	2	1	1	2	2	2	2	2	1	1	1
206	955968	34	F	RIGHT AXILLARY SWELLING	C/1611/15	LYMPHADENITIS	ABT	ABT	LIPOMA	2	2	2	2	3	3	3	3	3	3	3	3	3	3	2	2
207	955605	27	F	RIGHT BREAST LUMP	C/1612/15	ABSCESS	AIL	AIL	AIL	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
208	955616	28	F	RIGHT BREAST LUMP	C/1613/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
209	955235	57	F	THYROID SWELLING RIGHT LOBE	C/1979/15	GOITRE	HG	HG	NG	1	1	1	1	3	2	2	1	1	1	2	1	1	1	1	1
210	914180	18	M	THYROID SWELLING RIGHT LOBE	C/1980/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	2	2	3	2	2	2
211	919491	19	M	RIGHT CERVICAL LYMPHADENOPATHY	C/1981/15	TBLN	SL	SL	SL	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
212	955652	36	F	RIGHT CERVICAL LYMPHADENOPATHY	C/1982/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	2	2	1	1	3	3	2	2	2	2	1	1
213	913351	21	M	THYROID SWELLING RIGHT LOBE	C/1983/15	GOITRE	NG	NG	NG	2	2	2	2	3	2	2	1	3	2	1	1	3	3	2	2
214	955671	30	F	RIGHT CERVICAL LYMPHADENOPATHY	C/1984/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	3	3	3	2	3	3	2	2	3	2	2	2
215	955892	44	F	THYROID SWELLING RIGHT LOBE	C/1985/15	GRAVES DISEASE	HG	HG	LT	2	2	2	2	3	3	2	2	2	2	2	2	1	1	1	1
216	955880	26	F	RIGHT BREAST LUMP	C/1986/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	2	2	2	2	2	2
217	955528	26	F	THYROID SWELLING RIGHT LOBE	C/2139/15	?CARCINOMA	FNT	FNT	FNT	1	1	1	1	3	3	2	2	2	2	2	2	2	2	2	2
218	914268	55	M	LEFT CERVICAL SWELLING	C/2140/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	3	2	2	2	2	2	2	3	3	3	3
219	919372	27	F	LEFT BREAST LUMP	C/2141/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
220	186548	15	M	RIGHT CERVICAL LYMPHADENOPATHY	C/2200/15	CYSTIC LESION	LC	LC	RL	2	2	1	1	3	3	3	3	3	3	3	3	2	2	2	2
221	186587	25	F	LEFT BREAST LUMP	C/2201/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	2	2	2	3	2	2	1	1	1	1
222	186723	37	M	THYROID SWELLING RIGHT LOBE	C/2202/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	2	2	1	1	3	2	2	2	3	3	2	2
223	186650	26	F	RIGHT BREAST LUMP	C/2203/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	1	1	2	1	3	3	2	2	3	3	3	2
224	186302	56	F	THYROID SWELLING RIGHT LOBE	C/2205/15	GOITRE	LT	BTL	LT	2	2	2	2	3	2	1	1	1	1	1	1	3	2	1	1
225	186624	4	M	RIGHT CERVICAL LYMPHADENOPATHY	C/2206/15	TBLN	RL	RL	RL	1	1	1	1	3	3	2	2	3	3	2	2	2	2	2	2
226	916941	61	M	LEFT CERVICAL SWELLING	C/2207/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	2	2	1	2	2	2	2	3	3	2	2
227	186001	45	M	LEFT AXILLARY SWELLING	C/2209/15	LYMPHADENITIS	SL	SL	SL	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
228	186100	61	F	THYROID SWELLING RIGHT LOBE	C/2210/15	GOITRE	NG	NG	NG	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
229	186398	25	M	THYROID SWELLING RIGHT LOBE	C/2212/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
230	186224	36	M	LEFT ARM SWELLING	C/2213/15	NEUROFIBROMA	BSCL	BSCL	BSCL	2	2	2	2	3	2	2	1	2	2	2	2	3	2	1	1
231	186257	56	F	THYROID SWELLING RIGHT LOBE	C/2221/15	CYSTIC LESION	PCT	PCT	BCL	2	2	2	2	3	2	1	1	3	3	2	2	3	3	2	2
232	186027	45	F	LEFT BREAST LUMP	C/2222/15	BENIGN BREAST DISEASE	FCC	FCC	FCC	2	2	2	2	3	3	3	2	3	3	2	2	3	2	2	2
233	186315	58	M	LEFT CERVICAL SWELLING	C/2223/15	?METASTASIS	PDC	PDC	PDC	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
234	186133	50	F	LEFT BREAST LUMP	C/2224/15	BENIGN BREAST DISEASE	PCB	PCB	BBL	2	2	2	2	3	2	1	1	2	2	2	2	2	2	1	1

235	186633	29	F	RIGHT BREAST LUMP	C/2226/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
236	186748	34	M	THYROID SWELLING RIGHT LOBE	C/2227/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	2	2	3	3	2	2	3	2	2	2
237	186686	3	M	RIGHT CERVICAL LYMPHADENOPATHY	C/2228/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	1	1	2	1	2	2	2	2	1	1	1	1
238	186871	45	F	THYROID SWELLING RIGHT LOBE	C/2230/15	GRAVES DISEASE	HG	HG	LT	1	1	1	1	3	3	2	2	3	3	2	2	3	3	2	2
239	186264	30	M	LEFT CERVICAL SWELLING	C/2231/15	TBLN	SL	SL	SL	2	2	2	2	2	2	1	1	1	1	1	1	2	2	2	2
240	184213	35	F	LEFT BREAST LUMP	C/2232/15	?CARCINOMA	SFM	SFM	BBL	2	2	2	2	3	3	2	2	3	3	3	3	3	3	3	3
241	186323	37	F	LEFT BREAST LUMP	C/2240/15	MALIGNANCY	DC	DC	DC	2	2	2	2	3	3	3	3	2	2	2	2	3	3	3	3
242	186218	27	M	THYROID SWELLING RIGHT LOBE	C/2241/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
243	186779	40	F	LEFT BREAST LUMP	C/2242/15	?CARCINOMA	FCC	FCC	FCC	2	2	2	2	3	3	2	2	2	2	2	2	1	1	1	1
244	184232	35	F	RIGHT BREAST LUMP	C/2244/15	BENIGN BREAST DISEASE	KC	KC	KC	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2
245	186655	60	M	RIGHT SUBMANDIBULAR SWELLING	C/2245/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	3	3	3	3	3	2	2	3	3	2	2
246	186423	63	M	RIGHT CHEEK SWELLING	C/2246/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	3	2	2	3	3	2	2	2	2	1	1
247	186729	24	F	LEFT BREAST LUMP	C/2247/15	PHYLLODES TUMOR	PT	PT	SFM	2	2	2	2	3	3	3	3	2	3	3	3	3	3	3	3
248	186007	24	M	LEFT PREAURICULAR SWELLING	C/2249/15	CYSTIC LESION	SL	SL	SL	2	2	2	2	3	3	2	2	2	2	2	2	3	3	2	2
249	186727	43	M	LEFT SUBMANDIBULAR SWELLING	C/2251/15	LYMPHADENITIS	RL	RL	GL	2	2	1	1	2	2	1	1	2	2	2	2	1	1	1	1
250	185740	39	M	RIGHT CERVICAL SWELLING	C/2252/15	LYMPHADENITIS	BAT	BAT	BL	2	2	2	2	1	1	2	1	3	3	2	2	2	2	2	2
251	186344	38	F	LEFT BREAST LUMP	C/2283/15	BENIGN BREAST DISEASE	FCC	FCC	FCC	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
252	184351	24	F	THYROID SWELLING LEFT LOBE	C/2284/15	GOITRE	LT	LT	LT	1	1	1	1	3	2	1	1	1	1	1	1	2	2	2	2
253	186585	1	M	CHEST WALL SWELLING	C/2286/15	ABSCESS	KC	KC	KC	2	2	2	2	3	3	2	2	2	2	2	2	3	3	2	2
254	185960	55	M	THYROID SWELLING LEFT LOBE	C/2287/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	3	3	3	3	2	2	3	2	1	1
255	185758	42	M	RIGHT CERVICAL SWELLING	C/2292/15	ABSCESS	KC	KC	KC	2	2	2	2	3	3	3	3	3	3	2	2	3	3	2	2
256	186318	30	M	THYROID SWELLING LEFT LOBE	C/2293/15	CYSTIC LESION	AST	AST	AST	2	2	2	2	3	3	3	2	2	2	3	3	2	2	2	2
257	186503	55	M	RIGHT NECK SWELLING	C/2297/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	2	2	2	1	2	2	2	2	3	3	2	2
258	186525	38	F	THYROID SWELLING LEFT LOBE	C/2298/15	GOITRE	de-Q	de-Q	BTL	1	1	1	1	3	3	2	2	2	3	2	2	2	2	1	1
259	186696	28	F	RIGHT BREAST LUMP	C/2300/15	GALACTOCELE	GC	GC	GC	2	2	2	2	3	2	2	1	3	3	2	2	3	3	2	2
260	186326	32	M	LEFT TESTICULAR SWELLING	C/2301/15	?MALIGNANCY	MGCT	MGCT	MGCT	2	2	2	2	3	3	2	2	3	2	1	1	1	1	1	1
261	184813	30	F	LEFT BREAST LUMP	C/2332/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
262	185287	22	F	LEFT BREAST LUMP	C/2333/15	GALACTOCELE	GC	GC	GC	2	2	2	2	3	3	2	2	2	2	2	2	3	2	2	2
263	186168	30	M	THYROID SWELLING RIGHT LOBE	C/2334/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	3	3	2	2	2	2	2	2	3	3	2	2
264	185374	52	F	THYROID SWELLING RIGHT LOBE	C/2336/15	GRAVES DISEASE	HG	HG	HG	2	2	2	2	3	3	3	3	3	3	3	3	2	2	2	2
265	186725	27	F	RIGHT BREAST LUMP	C/2337/15	FIBROADENOMA	FA	FA	FA	2	2	2	2	3	3	3	2	3	3	2	2	1	1	1	1
266	186931	39	F	RIGHT BREAST LUMP	C/2338/15	?CARCINOMA	DC	DC	DC	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
267	184406	21	F	THYROID SWELLING LEFT LOBE	C/2340/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	2	2	2	2	2	2	2	2	2	2
268	185147	40	F	RIGHT BREAST LUMP	C/2341/15	?CARCINOMA	FN	FN	FN	2	2	2	2	2	1	1	1	3	3	2	2	2	2	1	1
269	185917	8	M	RIGHT AXILLARY SWELLING	C/2342/15	LYMPHADENITIS	NL	NL	NL	1	1	1	1	3	3	2	2	2	2	1	1	3	3	2	2
270	186403	26	M	RIGHT AXILLARY SWELLING	C/2344/15	LYMPHADENITIS	BAT	BAT	BL	2	2	2	2	2	2	2	1	1	1	2	1	2	1	2	1
271	186731	24	F	RIGHT BREAST LUMP	C/2365/15	FIBROADENOMA	FCC	FCC	FCC	2	2	2	2	3	3	3	3	3	3	2	2	3	3	2	2
272	185341	19	F	THYROID SWELLING RIGHT LOBE	C/2366/15	GOITRE	LT	LT	LT	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	3
273	186606	60	F	THYROID SWELLING	C/2367/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	3	2	2	2	2	2	3	2	1	1
274	186386	25	F	THYROID SWELLING LEFT LOBE	C/2367/15	GOITRE	HG	HG	HG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
275	186122	65	M	LEFT CERVICAL SWELLING	C/2368/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	3	2	1	1	3	3	2	2	3	3	2	2
276	186609	24	F	THYROID SWELLING LEFT LOBE	C/2370/15	GOITRE	FNT	FNT	HG	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
277	184360	24	F	THYROID SWELLING LEFT LOBE	C/2371/15	GRAVES DISEASE	HG	HG	HG	2	2	2	2	3	3	2	2	3	3	2	2	3	2	2	2
278	186750	31	M	RIGHT CHEST SWELLING	C/2372/15	ABSCESS	SL	SL	SL	2	2	1	1	3	3	2	2	2	2	2	2	1	1	1	1
279	186387	26	M	RIGHT CERVICAL SWELLING	C/2373/15	ABSCESS	LP	LP	SL	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
280	186320	36	F	RIGHT CERVICAL SWELLING	C/2375/15	ABSCESS	SL	SL	SL	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
281	186643	20	F	RIGHT GBS SWELLING	C/2383/15	?MALIGNANCY	PA	PA	SFM	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
282	186671	11	F	RIGHT CERVICAL SWELLING	C/2384/15	LYMPHADENITIS	NL	NL	NL	2	2	1	1	2	2	2	1	3	3	2	2	2	2	2	2
283	186936	56	F	THYROID SWELLING LEFT LOBE	C/2385/15	GOITRE	NG	NG	NG	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1
284	186749	68	F	LEFT BREAST LUMP	C/2386/15	?CARCINOMA	FA	PT	DC	2	2	2	2	3	3	3	3	2	2	2	2	2	2	2	2
285	186577	20	F	LEFT BREAST LUMP	C/2387/15	GALACTOCELE	GC	GC	GC	2	2	2	2	3	2	1	1	3	3	2	2	2	2	1	1
286	186660	55	M	LEFT CERVICAL SWELLING	C/2388/15	?METASTASIS	SCC	SCC	SCC	1	1	1	1	3	3	2	2	3	3	2	2	3	3	3	3
287	186649	52	F	THYROID SWELLING RIGHT LOBE	C/2389/15	CYSTIC LESION	CG	CG	CG	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1
288	186209	24	F	THYROID SWELLING LEFT LOBE	C/2390/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	2	2
289	186357	30	M	THYROID SWELLING RIGHT LOBE	C/2391/15	GOITRE	de-Q	de-Q	BTL	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
290	186670	17	F	THYROID SWELLING RIGHT LOBE	C/2392/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	2	2	2	1	2	2	2	2	2	2	1	1
291	186445	50	M	THYROID SWELLING RIGHT LOBE	C/2399/15	GOITRE	NG	NG	LT	2	2	2	2	3	3	2	2	3	3	3	3	3	3	2	2
292	186447	55	M	LEFT KNEE SWELLING	C/2400/15	BENIGN LESION	BSCL	BSCL	BSCL	2	2	2	2	3	3	2	2	3	3	3	3	3	2	2	2
293	186234	66	M	RIGHT BUCCAL SWELLING	C/2401/15	?MALIGNANCY	PA	PA	SFM	2	2	2	2	3	3	3	3	3	3	2	2	2	2	2	2

294	186775	72	F	RIGHT BREAST LUMP	C/2403/15	?CARCINOMA	PT	PT	PT	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
295	186297	67	F	LEFT BREAST LUMP	C/2405/15	MALIGNANCY	DC	DC	DC	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	3
296	185772	19	F	LEFT BREAST LUMP	C/2407/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	3	3	2	2	2	2
297	185346	24	F	RIGHT SUBMANDIBULAR SWELLING	C/2408/15	LYMPHADENITIS	PA	PA	SFM	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
298	184882	18	F	RIGHT AXILLARY SWELLING	C/2410/15	LYMPHADENITIS	ABT	ABT	ABT	2	2	2	2	1	1	2	1	1	1	2	1	1	1	1	1
299	184884	55	F	RIGHT CERVICAL LYMPHADENOPATHY	C/2411/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	3	3	2	2	3	3	1	2	3	2	1	1
300	186998	47	M	THYROID SWELLING LEFT LOBE	C/2414/15	GOITRE	CG	C	CG	2	2	2	2	3	3	2	2	3	3	2	2	3	3	2	2
301	186678	9	F	RIGHT CERVICAL LYMPHADENOPATHY	C/2423/15	TBLN	NL	NL	NL	2	2	2	2	3	2	2	1	3	3	2	2	2	1	1	1
302	186754	49	M	RIGHT CERVICAL LYMPHADENOPATHY	C/2424/15	TBLN	LP	LP	NL	2	2	2	2	3	3	2	2	3	3	3	3	2	2	2	2
303	186604	20	F	RIGHT BREAST LUMP	C/2425/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	3	2	3	3	2	2	3	3	2	2
304	186685	65	M	RIGHT CERVICAL SWELLING	C/2426/15	LYMPHADENITIS	NHL	NHL	NHL	2	2	2	2	3	2	1	1	2	2	1	1	3	3	2	2
305	186740	53	M	RIGHT CERVICAL SWELLING	C/2428/15	?METASTASIS	SCC	SCC	SCC	2	2	2	2	2	2	2	1	3	3	2	2	1	1	1	1
306	186722	48	F	LEFT AXILLARY SWELLING	C/2429/15	LYMPHADENITIS	SL	SL	SL	2	2	2	2	3	3	3	3	3	3	2	2	2	2	2	2
307	184097	57	F	THYROID SWELLING RIGHT LOBE	C/2430/15	GOITRE	NG	NG	NG	1	1	1	1	3	3	2	2	3	3	2	2	2	2	2	2
308	185578	55	F	THYROID SWELLING RIGHT LOBE	C/2432/15	GOITRE	LT	LT	LT	2	2	2	2	3	2	3	2	3	3	2	2	3	3	2	2
309	186870	45	M	THYROID SWELLING RIGHT LOBE	C/2433/15	GOITRE	CG	CG	CG	2	2	2	2	3	3	2	2	3	3	2	2	2	2	1	1
310	185364	60	F	RIGHT BREAST LUMP	C/2434/15	CYSTIC LESION	KC	KC	KC	2	2	2	2	3	2	2	2	3	3	2	2	3	3	2	2
311	186767	63	M	RIGHT NECK SWELLING	C/2459/15	?METASTASIS	SCC	SCC	SCC	1	1	1	1	3	3	3	2	3	3	2	2	3	3	3	3
312	186559	21	F	THYROID SWELLING LEFT LOBE	C/2460/15	GOITRE	AST	AST	AST	2	2	2	2	3	3	2	2	3	2	3	2	3	3	2	2
313	186595	29	F	THYROID SWELLING RIGHT LOBE	C/2461/15	GRAVES DISEASE	LT	LT	LT	2	2	2	2	3	3	2	2	2	3	2	2	2	2	2	2
314	186262	73	F	RIGHT CERVICAL LYMPHADENOPATHY	C/2462/15	LYMPHADENITIS	RL	RL	RL	2	2	2	2	3	3	3	3	3	3	2	2	3	2	2	2
315	186396	30	F	LEFT BREAST LUMP	C/2463/15	BENIGN BREAST DISEASE	FA	FA	FA	2	2	2	2	3	3	3	3	3	3	2	2	3	3	3	3
316	186806	55	F	THYROID SWELLING LEFT LOBE	C/2464/15	GOITRE	CG	CG	CG	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1
317	186221	76	F	THYROID SWELLING RIGHT LOBE	C/2465/15	GOITRE	FNT	FNT	FNT	2	2	2	2	3	2	2	1	3	3	2	2	2	2	2	2
318	186311	47	M	RIGHT CERVICAL SWELLING	C/2466/15	TBLN	RL	RL	RL	2	2	2	2	3	3	3	2	3	3	2	2	3	3	2	2
319	186617	18	F	THYROID SWELLING RIGHT LOBE	C/2467/15	GOITRE	LT	LT	LT	1	1	1	1	3	2	2	1	3	3	2	2	3	2	1	1
320	186314	72	F	RIGHT BREAST LUMP	C/2468/15	?CARCINOMA	DC	DC	DC	2	2	2	2	3	2	2	2	3	3	2	2	3	3	2	2