COMPARISON OF CYTOMORPHOLOGICAL FEATURES OF CONVENTIONAL SMEAR WITH LIQUID BASED CYTOLOGY OF REMNANTS IN THE NEEDLE HUB OF FINE NEEDLE ASPIRATION CYTOLOGY

By

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Under the Guidance of

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List of Abbreviations used	
ASCUS	Atypical Squamous Cells of Undetermined Significance
CS	Conventional Smear
FDA	Food and Drug Administration
FNA	Fine needle aspiration
FNAC	Fine needle aspiration cytology
G	Gauge
HE	Hematoxylin and Eosin
LBC	Liquid Based Cytology
LSIL	Low-grade Squamous Intraepithelial Lesion
MLBC	Manual liquid-based cytology
NS	Not Significant
OPD	Outpatient Department
PAP	Papanicolaou
RBC	Red Blood Cell
SP	Surepath
TP	Thinprep

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ABSTRACT

BACKGROUND:

Fine Needle Aspiration Cytology (FNAC) had become a first line investigation and prerequisite procedure for certain conditions prior to surgery. With the advancing techniques for demonstration and documentation of diagnosis in an objective manner with the aid of ancillary techniques both during and after the procedure, had increased the need for processing all the possible material obtained from the patient. On the contrary, the material in the needle hub after preparing smears is usually discarded even though material is visible macroscopically.

OBJECTIVE:

To assess the efficacy of Liquid Based Cytology (LBC) smears prepared by Cytospin technique from residual material in the needle hub in fine needle aspiration cytology by comparing with the cytomorphological features of conventional cytology smears.

MATERIALS AND METHODS:

The study was done on patients who were referred for FNAC of various lesions to the Cytology section in the department of Pathology, B.L.D.E.U.'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapur. The study period was from 1st December, 2015 – 30th June, 2017.

Standard FNAC procedure was performed, conventional smears were prepared by expressing the material onto clean glass slides and the LBC smears were prepared after collecting the hub remnants into 95% ethanol and subjecting this material to cytocentrifugation at 900rpms for a duration of 4minutes. Comparison of both the smears was done based on the

parameters like cellularity, staining quality, background, cellular degeneration and nuclear preservation.

RESULTS:

A total of 103 cases were included in the study, with mean age of 38.7 years and M;F ratio of 0.49. The predominant sites for FNA were of thyroid followed by lymph node, soft tissue swelling and breast. Cellularity (p-value =0.0001), 32 cases of CS were acellular,but the corresponding smears of LBC technique were having cells and cell clusters which aided and augmented the diagnostic and adequacy criteria. Background was haemorrhagic in 27.2% of LBC smears in contrast to CS which had 60.1% haemorrhagic smears. Staining quality, cellular degeneration was comparable in both the techniques, whereas nuclear preservation was better in LBC with a significant p-value of 0.008.

CONCLUSION:

Needle hub Cytospin LBC smears have added to the diagnosis and also yielded additional material which can be subjected to ancillary tests like IHC and special stains. The processing of the hub remnants adds a significant amount of additional diagnostic information. Utilisation of cheaper alternatives like post-it tissue paper cytofilt cards instead of manufacturer provided cards might help in doing this in a cost-effective manner too.

Keywords: Cytospin, FNAC, LBC, Needle hub remnants.

INTRODUCTION

Fine needle aspiration cytology (FNAC) is a minimally invasive procedure commonly used for preoperative diagnosis of neoplastic and non-neoplastic masses. The collected sample is processed using the conventional method by expressing the aspirated material on clean glass slides. Then the material is smeared with the help of another glass slide (spreader) using a rapid and regular motion so that a thin layer of cells is formed on the slide.[1]

With innovations in technology and advent of automatized faster processing techniques, Liquid Based Cytology (LBC) has been invented. In LBC, the sample is collected into the liquid fixative medium. Commonly used LBC techniques are Thin Prep and Sure path.[1]

LBC is well accepted and approved technique for the gynaecological smears. The overall performance of this technique in gynaecological PAP smear test was widely studied and thoroughly reviewed by various investigators across the globe with varied practices and protocols. LBC is superior to conventional smears (CS) in case of obtaining a clear background and a monolayer of cells.[1]

The utility of LBC in non-gynaecological smears is being actively investigated. LBC has added the advantage of reducing the screening time as the area to be screened for the material is less compared to CS. LBC also offers the additional advantage of aggregating the available material in case of scant aspirates. This helps in reducing the laborious process of carefully screening the CS slides with scant material which is often scattered in multiple slides, thus providing more time for better and at the same time faster evaluation. Unlike conventional method, LBC has the ability to provide additional material for ancillary techniques.[2]Along with showing

better morphology and appreciation of the cells in the clear background, LBC will be an adjunct to the CS in cases of scant aspirates.[1,3]

The main inhibiting factor for full-scale use of LBC technique is the high cost of fully automated equipment. Apart from the initial cost of procuring the equipment, the need for the specialised machine, specific reagents, devices and cost of maintenance has added a significant cost to the processing of a sample. This has been overcome by certain users by using Cytospin. Manual methods of liquid-based cytology like Manual liquid-based cytology (MLBC) and Surepath hand method, where the collection, concentration and smear preparation are done manually is bridging the gap by providing the LBC smears at a much lower cost.[1-4]

Hence, the present study was done to analyse the utility of cytospin smear preparation technique for residual material in needle hub in FNAC samples.

OBJECTIVES OF THE STUDY

To assess the efficacy of Liquid Based Cytology (LBC) smears prepared by Cytospin technique from residual material in the needle hub in fine needle aspiration cytology by comparing with the cytomorphological features of conventional cytology smears.

REVIEW OF LITERATURE

FNAC History

Ward suggested aspiration of lymph nodes in lymphoblastomas in the year 1912 for the first time. Over a period of next 15 years, Guthrie and Goeller published their findings of aspiration studies from lymph nodes and prostate respectively. Goeller in his study used a trocar for obtaining and securing the sample, whereas Forkner used dental broach inserted in an 18-gauge needle. These specialised needles used by the said authors have disadvantages like not being available easily and were delicate to operate or caused more trauma to the patient.[5]

Martin H E and EllisB E[5] in their paper titled "Biopsy by needle puncture and aspiration" described needle aspiration for the first time using a record syringe and ordinary 18-gauge needle in the study done in 1926.[5]In their study, along with a20ml syringe and 18-gaugeneedlethey also used10% formalin to preserve cells on the glass slides. Prior to the procedure, Iodine was applied on the skin and 1% novocaine was used as a local anaesthetic. A small cut was made at the point of entry using a number 11 blade, this was done to avoid skin contamination. Then the needle was introduced, once it enters the region of interest negative pressure was applied and passes were given.[5] These steps are followed even today except for the initial skin nick which is not a routine practice nowadays.

Indications expressed by Martin & Ellis for biopsy by needle puncture and aspiration were tumour masses below the surface of the normal tissue and in conditions where surgical exposure is contraindicated. Other indications being, disadvantages of an open biopsy by surgical exposure like dissemination of disease

and interference with a definitive surgical procedure on a later date formed indication for this newer technique.[5]

The material obtained in their study was fragments of tissue bits or blood mixed cells depending on the organ. Material obtained was further processed by following two methods

1. Immediate Method
2. Longer method

In the immediate method, fragment or cells obtained were smeared on the glass slide. Gentle heating was used to fix the cells. Haematoxylin and Eosin staining was done. This method takes about 6-8 minutes.

In the longer method, the remainder of the tissue was processed and paraffin embedding was done. A preference to the histological preparations was given over the cytological smears. A definite group of atypical cells were given diagnostic significance in direct smears. They had reported in their study that it was possible to make the correct distinction of benign and malignant in all cases. Follow up surgical procedure diagnosis was correlating in 6% of cases.[5]

Martin & Ellis[5]alsostressed the need for interpreting the smears by correlating with the clinical scenario and tissue plane of the swelling. A success rate of 80% was reported in their study and the predominant cause of failure was observed in fibrous tumours. They concluded that needle aspiration technique will be of immense value in routine diagnostic practice and also elucidated few limitations of the method like loss of architectural arrangement and diameter of the tissue obtained was small respectively.

FNAC evolution as a routine diagnostic modality:

In the 1930s, the technique described by Martin & Ellis was followed by Fred W Stewart[6] and Hoffman WJ[7]in their study of diagnosis of a tumour by aspiration and new technique and instrument for obtaining biopsy specimen at the Memorial Hospital, New York respectively.

The new needle used by Hoffman was 14cm in length and had an outer diameter twice that of the 18-gauge needle. The needle was electrically insulated to coagulate the track once the tissue was obtained.[7]

Sharp GS[8] used the technique described by Martin & Ellis in lung lesions by bronchoscopy and he found that it was useful in such instances.[8] Stewart FW[6] followed the smear preparation method described by Martin & Ellis and Stewart FW too used the bistoury blade to give a nick at puncture site to prevent skin contamination and gentle heating of the smears in smear preparation.[6]The main aim of Stewart FW was to establish the practical value of the procedure with a particular focus on the cancerous cervical node.[6]Avast majority of the cases metastasising to the cervical nodes were inoperable, so a histological proof was necessary to establish the diagnosis and these cases formed the major portion of his study material. With the evaluation of 725cases of neck nodes, Stewart FW established the usefulness of the procedure, and concluded: “for most cases, it was simple to distinguish cancer cells”.[6] FNAC thus found a place of extreme utility in diagnostic evaluation.

Stewart FW et al,[6] with a wide variety of lesions and a significant sample size of various organs, established indications for the aspiration in breast lesions and to differentiate few bone lesions in their study further cementing the utility of FNAC. Considerable success with bone lesions was also documented by the authors. There

was no untoward incident in 2500 cases and thus the authors stated that safety of the procedure was proved beyond doubt.

MacCarty WC[9,10] from Mayo clinic, in his paper and his presentation at Pathology and Physiology at the Eighty-Seventh Annual Session of the American Medical Association, Kansas City, in 1936 stated that many cancer campaigns were being organised, however only 25%, 50% and 58% stomach, breast and large intestine were operable respectively in a total of 7,179 cases indicating the need for an early diagnostic modality and the increased need for utilisation of needle aspiration in such cases.

MacCarty WC[9] also highlighted the need for a newer method of observation of unfixed fresh cells to be taught to medical students who were studying fixed histopathology sections alone. He and his colleagues from surgical pathology laboratories had studied the characteristics of cells and measured the sizes of nucleus and nucleoli of various organs and tabulated them for regenerative, benign and malignant cells.

They proved that the nucleoli of the cancer cells are larger than any other cell and the importance of the ratio of nucleus and nucleoli and their size comparison. He also emphasised the need for pathologists to study the fresh unembedded tissues if they expect to recognise cancer before it reaches the late stage.[9]

With the increasing utilisation of cancer programs and intriguing research in the field of cytology, cellular characteristics of malignant tumours were widely studied by comparing the cytological diagnosis with the gold standard histopathological diagnosis.[6,9-11]

Hauptmann E[11] compared the cytological characteristics from 188 cases involving 268 regions with the histological diagnosis of specimens by preparing direct smears from the unfixed tissue. The measurements and illustrations were documented in diagrams and comparative tables establishing the importance of nucleoli and stated that in the majority of the cases the dimensions were 1-2 microns. Among 90 histologically proven cancers, 86 had one or the other atypical cells. Atypical cells were also found in four cases of which belonged to the non-cancerous group.

The advantages mentioned by these authors include that the procedure was fast and also mentioned the avoidance of a surgical procedure for open biopsy.[5-8] which were studied along with the disadvantages and safety aspects of aspiration extensively.[12]

Berg JW et al[12] studied whether FNAC made a difference in case management and prognosis by matching breast cancer patients who have undergone FNAC prior to surgery and controls who did not have FNAC, during the years 1940-1943. Control and aspiration groups comprising of 370 patients each were matched. After a 5year follow-upperiod,106 patients with aspiration were not alive while the control patient was still alive. But, even higher number of patients died n=116 while the patient with aspiration was still alive and this has shown that patients who underwent aspiration biopsy had a better than average prognosis.

With the gradual introduction of the procedure at various centres across United States, Sweden and all over the world it has quickly turned into one of the initial investigations before the surgical procedure.[4]

Franzen et al[13] had reviewed the breast aspiration in a large series of 3479 cases, over a ten-year period in their study about the safety, accuracy and correlation

with histopathology diagnosis. Evaluation of FNAC in terms of safety and accuracy continued as many authors have studied the procedure and its utility in detail in a large sample size spanning over a decade or more and concluding that the procedure is safe and reliable in providing a rapid diagnosis.

Many articles have been published over the years analysing the procedure in the form of systematic reviews and meta-analysis. The diagnostic value of FNA in the form of meta-analysis in thebreast,[14]head and neck,[15] metastatic melanoma[16]and thyroid[17] were studied and established that the procedure to be safe, rapid and a diagnostic tool of extreme importance.

FNAC was further investigated and studied in a much wider anatomical regions and varied applications than offering the initial diagnosis. Fine needle aspiration in Testis was studied by Con Mallidis in comparison with open biopsy in 26 cases and recommended the procedure as "quick, easy, repeatable and reproducible".[18] Further studies stated that FNA testis had an useful role in the diagnosis of non – neoplastic and neoplastic lesions such as tuberculous orchitis and seminoma respectively.[19,20]

Agnese Assi et al[21] studied the local recurrences or inguinal lymph node metastasis due to FNA for a 5-year period and found that there were no local recurrences or metastasis indicating the safety of the procedure. FNA testis had played a substantial role in infertility assessment and sperm retrieval thus expanding the reach and utilisation of the procedure.[22]

Review of FNA with a particular focus on disease-specific analysis was also widely done. Michael J Costa et al[23] from University of California-Davis Medical centre did a retrospective review of diagnostic utility and specificity of FNAC of

Sarcoma in bone and soft tissue over a span of ten years. They also studied the utility of FNA in diagnosing recurrences and 5 out of 5 recurrent cases were identified correctly by FNA.

Needle size and the pain were studied by Angelo C et al in case of thyroid nodule by comparing FNAC alone with FNAC plus large needle aspiration biopsy group and found that large needle aspiration did not add any discomfort or pain.[24]

With the approval of the FNAC as an established procedure of choice in many easily accessible locations, the need for utilising this rapid technique for deep seated locations were investigated. Advanced imaging technologies, for example ultrasonography and computed tomography were incorporated into FNAC procedure. This guided FNAC has made the previously impractical and dangerous sites for blind FNAC to be performed with high precision and safety.[25]

The role of FNA in large thyroid nodules was evaluated in 6921 USG guided aspirations at Mayo clinic from January 2002 to December 2006 retrospectively. After this extensive analysis, Porterfield et al suggested that resection is not necessary for diagnosis in large nodules and the prior criteria/concept of “more than 3cm size should not be an independent indication for resection.” Thus, FNAC has played a pivotal role in changing patient management with regard to diagnostic approach.[26]

Many studies were conducted using various gauge sizes of needles and their effects.[27,28]The authors evaluated the impact of the needle sizes in case of endoscopic guided biopsies. 21G vs 22G in 185 patients retrospectively by Jeyabalan et al[27]using endobronchial guided aspirations. In the randomised control trial by Carrara et al[28] 22 and 25G needles were evaluated in case of 144 patients comprising of solid pancreatic

masses and lymph nodes. The sample was adequate in 25G needles when compared with 22G.

With the validation by randomised trials, comparative studies and meta-analysis establishing utility in various organs and diseases, FNA has carved a place of its own in the diagnostic arena and has become one of the pre-operative diagnostic modalities. The aiding of imaging technologies in increasing the accuracy and adequacy has refined the procedure further. Improvement of the technique, in the form of LBC has entered the field of non-gynaecologic cytology after crossing over from LBC gynaecologic cytological examination.

LBC- A brief overview in gynaecologic cytology, to its implementation in Non-gynaecologic cytology:

LBC technology Thinprep (TP) was approved for the gynaecological smears by Food and Drug Administration in the year 1996.[2,29] Whereas, Surepath (SP) was approved in 1999.[29]

Principle Thinprep and Surepath:

Thinprep: With the help of polycarbonate filters, custom designed technology for immersion and rotation of these filters into the proprietary fixative solution in the vial containing the specimen achieves homogenisation of the sample. Then vacuum is applied and cells in the sample adhere to the filter which is then pressed against a slide creating a circular smear with a diameter of 20mm.

Whereas in case of the Surepath method, vortexing, straining and layering of the sample onto a density gradient and centrifuged to create a 12.5mm diameter

circular smear. Robotic pipette controlled through a computer is used along with the centrifuge.

The smears prepared by automated LBC have advantages like

- monolayer of cells,
- no obscuring material, air drying, smearing artefacts and
- clean background.

All of these parameters help in the better interpretation of the LBC smears in comparison to the conventional smears.[3,30]

However, it is an expensive procedure requiring the initial set up of machine technology which is custom made by the manufacturer and further requirement of usage of consumables and special reagents provided by the individual manufacturer adding to the overall cost of sample preparation. This made this technology out of reach for many smaller institutions and countries with limited resources alike. This cost factor also acted as a hindrance in a widespread and rapid uptake of this very useful and superior technique.[31]

Validation of LBC techniques:

Large multicentre studies were done involving six laboratories and 35 gynaecologists with 5428 included cases. Comparison of Thinprep technique with the conventional method has shown a significant increase in detection of ASCUS and LSIL.[32]

Nationwide comparison[33] of Thinprep[34], Surepath, Cytospin[35] and Conventional pap smear amongst each other and altogether was done in Canada,[33] Dutch,[36] China,[37] New Zealand,[38] Denmark,[39]Australia,[40] and Scotland.[41]

In the early introduction period, there were split sample studies[32,42] which evaluated the specimen adequacy and found that LBC is having an advantage and had better adequacy parameters.[43]

With a two year follow up of over 4499 cases, among which Thinprep cohort comprised of 2288 cases and conventional PAP tests were done in 2211 cases. Data analysis showed that Thinprep was superior to conventional smear with a 50% higher yield of confirmed tests in two years follow up. 6% lower in the normal and benign category in case of Thinprep and a 6.8% higher in case of ASCUS. Authors concluded that the LBC Thinprep method was superior.[33]

Approval of LBC by FDA

In continuation to such high yield of adequacy and improved detection rates, FDA approved the utilisation of LBC for routine diagnostic use in PAP smears. Over a period of time automation has been incorporated into the analysis of the smears. Modalities like PAPNET, Thinprep Imager[38,41,44] have found hold in various centres and they are being used as routine diagnostic tools[39] and in Quality assurance.[44]

LBC in Non-gynaecologic smears:

With the success of LBC in gynaecologic cytology due to its superior smear quality and improved detection rates, in spite of its high cost, it had made inroads into the Non-gynaecologic cytology spectrum and is widely studied in this arena too.[45] The additional advantage by adopting LBC in the field of non-gynaecologic cytology is that the residual material can be used for ancillary testing.[30,46]

As with the PAP smear, the initial adoption of LBC in non-gynaecologic cytology has also seen the split-sample method of evaluation.[47] With significant

number of studies found in evaluation of thyroid[48,49] and breast[30,50] followed by lymph nodes.[51]

Diana E et al[49] studied the diagnostic efficacy of LBC in comparison with a conventional method in thyroid lesions of 10,360 FNA spanning over multiple time periods and methods with CS alone CS and LBC and only LBC. The parameters evaluated were inadequacy, indeterminacy and rate of malignancy.

Meta-analysis comparing the thyroid FNA conventional smears with LBC accounting for 599 unique articles with 24,307 aspirations in 19,433 patients concluded that 12 studies didn't show any difference with respect to inadequate for evaluation and 13 studies have shown no difference with respect to indeterminate smears and final recommendation of a method amongst the two boiled down to cost, feasibility and accuracy.[52]

LBC and Guided FNAC

Debasis G et al[53] evaluated the role of FNA in 130 cases of spleen over a period of five years with the help of ultrasonography with a definite diagnosis in case of 88 cases and no complications were encountered. With a special focus on head and neck region, studies have been done evaluating the FNA utility in neck masses,[54]salivary glands.[55] Deepa G et al[56] from 2001-2006 studied the capability of FNA as a diagnostic tool in mediastinal lesions as a substitute to core biopsy.

LBC technique was also applied for endoscopic ultrasonography guided aspirations[57,58] with the same interest as that of direct aspirations.[49,59,60] Multicentre

studies have increased the understanding of these procedures in a better way over years in Brussels.[59]

Like any other new technology, LBC is not immune to its process specific disadvantages like altered morphology and artefacts which are attributed to fixation and processing techniques.[29] To mention a few, altered or reduced background, breakage of papillae, smaller cell size, loss of myoepithelial cells, stromal elements and more 3D clusters.[29,30]

However, rather than these disadvantages, the main inhibiting factor for full-scale use of LBC technique by Thin Prep and Sure path is the high cost of fully automated equipment and reagents. This has been overcome by certain users by using the Cytospin, Manual methods of liquid based cytology like MLBC and Sure path hand method. In these economical methods collection, concentration and smear preparation are done manually.[1-4,61]

Among the Manual Liquid based cytology, semi-automated technique cytocentrifugation has been widely used to bring the advantages of LBC into many laboratories at a much lower cost in comparison to fully automated machines.[61]

Cytocentrifuge Historical Milestones:

Newton coined the term “centrifuge”, which means “flee from the centre”, in the year 1685. Dore & Balfour description of a device for preparation of cell spreads was followed a year later by Watson describing a Slide centrifuge: apparatus for preparing a cell suspension on a microscope slide”

Centrifuge was used for creating a thin layer cervical cytology in 2000 followed by a renewal of the trademark registration by Thermo Electron for Cytospin. Cytocentrifuge concentrates cells by using centrifugal force and cells flattened directly onto a microscope slide avoiding the use of Millipore membrane filters. Once the centrifugation force is applied, the fluid part is absorbed into the filter paper and the cells are flattened on the slide creating a monolayer. FDA described cytocentrifuge as "a centrifuge which concentrates cells from suspensions and deposits them on slides for assessment."[62]

The funnels used are of single, double and mega funnel type which creates single, double and large smear with an area of 28.3mm^2, 56.6 mm^2 and 325 mm^2 respectively. Depending on the requirement and the need for processing and preparing smears the suitable funnels are used as applicable. The funnels have a horizontal and vertical portion in its sample recipient area. The horizontal portion is a straight cylinder with uniform size and holds a volume of 500 microliters. Whereas the larger vertical portion of the funnel is conical in shape with a broad opening and a narrow base joining with the horizontal part. The horizontal portion opens onto the slide with a cytofilt card sandwiched between the two. This cytofilt card absorbs the excess fluid from the periphery while the cells are forced by the centrifugal force onto the slide creating a monolayered smear with a clear background. An upper limit of 0.5ml or 500microliters in case of single and double funnels irrespective of the manufacturer is suggested due to the reason that fluid beyond this volume will remain in the conical portion during centrifugation and thereby not adding to the smear formation.[62]

Cytospin was adopted by users who want to have the advantages of LBC without the higher cost of equipment as well as reagents and at the same time

achieved comparable results in breast FNA[63]and hormone receptor evaluation by immunocytochemistry.[64] Cytocentrifugationwith economical fixative Easyfix and technologies like Papspin and Turbitec was evaluated by Christian et al.[61]Thai Yen et al[65] compared the cytospin preparations with Thinprep in case of breast lesions.

Parallel to the evaluation of cytospin method, there was interest in Manual LBC method too.[66] which was studied in comparison with conventional cytology in oral squamous cell carcinoma,[66] cervical smears[67] and breast[45] concluding that there is a good concordance between the two methods and this Manual LBC technique formed an economical alternative in resource constrained settings.

In MLBC method, centrifugation, the smear preparation is done manually. Paraffin wax conforming to the bottom of a test tube upon cooling a precisely cut filter paper is placed upon it and the fluid is transferred which is then centrifuged. The filter paper is then gently touched on the slide after decanting the supernatant creating a smear.[1]

LBC by Cytocentrifuge method also helped in the preparation of monolayered smears with less overlapping along with the added advantage of processing the remnants in the needle hub as a low-cost alternative[3]as compared to automated LBC techniques such as Sure Path and Thin Prep.

Gupta *et al*[3] in their study on "Cytospin preparation from residual material in needle hub: Does it add to fine needle aspiration diagnosis", after performing FNAC prepared smears by expressing the material onto clean glass slides. Then the needle hub remnants were collected into a tube by aspirating 2.5ml of normal saline. One or more smears were prepared and stained with Giemsa stain. They concluded that

cytocentrifugation of the residual material in the needle hub after FNAC has improved the diagnostic yield, which was 16% of the total cases. These 16 cases were inadequate for opinion in CS method.

Pawar *et al*[1] also concluded that MLBC technique will ensure adequacy due to the remnant in the needle hub getting processed which had the same diagnosis as the CS but with better cellular morphology and absence of haemorrhage in the background.

There are factors like presence of RBCs and overlapping of the cells which interfere with the interpretation of smears. Presence of these obscuring factors are not largely operator dependent and are unavoidable in case of vascular lesions like thyroid in CS.[2] Addition of glacial acetic acid to the solution lyses the RBCs and helps in visualising cells in a clean background eliminating overlapping by RBCs. Thus, techniques like LBC which are being used these days produce better results and has improved efficiency in the diagnosis and decreased rates of inadequate to opine.[68,69]

Further, different approaches have been used like metastable alcoholic gel transfer method,[70] CytoSEDTM[71] which does not require any special instrumentation and not affecting turnaround times were evaluated.[70] The residual material after the preparation of the Thinprep smears was used to rescreen by using MLBC in case of ASCUS smears to offset the costs suggesting increased utilisation of the alternate techniques.

As observed in various studies, the main intention was to utilise and integrate the better technologies into FNAC technique for providing an accurate, informative and at the same time having an objective justification of the diagnosis offered at an economically feasible price. The focus now is on incorporating the LBC technologies

and their advantages at lower price and processing entire material obtained in the FNAC, to make it better than it is today.

MATERIALS AND METHODS

Source of data

The study was done on patients who were referred for FNAC of various lesions to the Cytology section in the Department of Pathology, B.L.D.E.U.'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Study period: 1st December, 2015 – 30th June, 2017

Methods of collection of data.

FNAC procedure was performed with a10ml disposable syringe and 22 – 23 gauge needle. The mass was fixed with the non-dominant hand and with the dominant hand, a needle with attached syringe secured in a Cameco piston syringe holder was used to give multiple and rapid passes in various directions within the swelling so that the representative sample was obtained. Negative pressure was released and the syringe was removed, pressure applied over the entry site to stop bleeding. Routine conventional smears were prepared from aspirated material by expressing the material on clean glass slides. After this, the residual material in needle hub was collected into ethanol and processed by cytocentrifugation.

An initial assessment of ten cases with the prior settings of 2000rpms and 5 minutes duration, glacial acetic acid to the tune of 1 ml for 3ml of ethanol was changed as mentioned below to have a better morphology.

1. One to 1.5ml of 95% ethanol was aspirated into the syringe
2. The material was flushed into single use plain tube to avoid contamination. Then the sample was allowed to settle for one hour.
3. The additional unprocessed material was further processed by preparing additional smears.

4. Glacial acetic acid was added in the proportion of 20 microliters for 100 microliters of haemorrhagic material.

Cytofilt cards were aligned with the cytofunnels corresponding to the opening of the horizontal portion of the sample holding channel. These were placed on a labelled slide and the entire setup is placed into a metallic holder as depicted in Figure 5. Both the single funnels and double funnels were used as per the need and depending on a case to case basis. The cytospin (Figure 1) used in this study has the capability to process six funnels at a time providing six smears with a single funnel and twelve smears with double funnels. Thus, double funnels enhanced the processing capacity of the cytospin by 100%.

After an hour, using a micropipette, 100 microliters from the sediment portion was taken and distributed into the cytofilt funnels. The preprogrammed settings of 900 rotations per minute for 4 minutes was used in the cytospin. The smears were stained using Giemsa, PAP and HE staining.

Conventional smears and LBC smears by Cytospin method were assessed for the following parameters as per the study done by Pawar *et al*[1] and Gupta *et al*[3]as mentioned in Table 1.

Table 1 Parameters for comparison of cytomorphology for CS and LBC smears

S.no	**PARAMETERS**	**LBC**	**CS**
1.	**Cellularity**		
	Low		
	Medium		
	High		
2.	**Staining quality**		
	Poor		
	Average		
	Good		
3.	**Background**		
	Haemorrhage		
	Clear		
4.	**Cellular degeneration**		
	Present		
	Absent		
	Poor		
5.	**Nuclear Preservation**		
	Poor		
	Average		
	Good		

Figure 2 Various needle sizes

Figure 1 Cameco Piston Syringe holder and cytocentrifuge

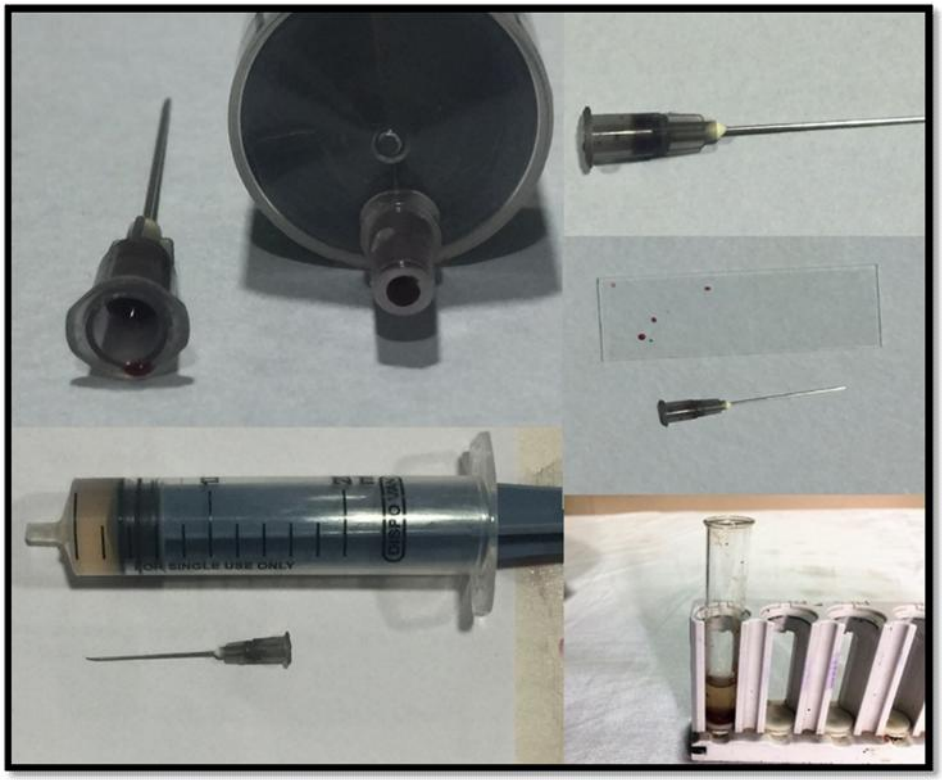


Figure 3 Collecting needle hub remnants into the tube

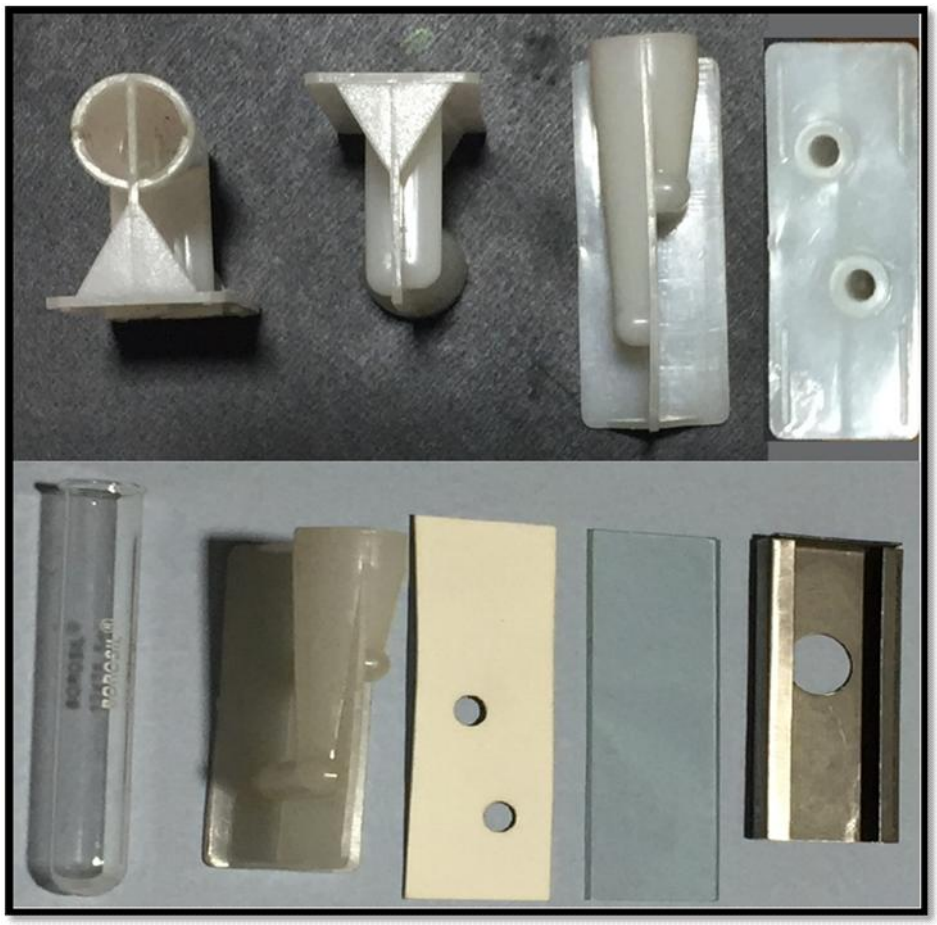


Figure 4 Cytofunnels and cytofilt cards

Figure 5 Assembled Cytofunnel, Pipettes and cytofunnels in the cytocentrifuge ready for processing.

Sample Size:

In the study done by Gupta *et al*[3] it was found that the number of cases with diagnostic material by the cytospin LBC method alone was 16% and by the conventional method, it was inadequate for opinion in these cases.

Considering the common proportion of inadequate to opine cases as 16%, at 99%confidence level and 90% power in the study the calculated sample size was 84.

By the following formula, $n=\frac{(Z_\alpha+Z_\beta)\times 2\times p\times q}{d^2}$

Where,

$Z\alpha$ = Z value for α level is 99%

P = common proportion between two groups

q = 100- p

d = difference between two groups.

Hence 103 samples were included in the study.

Statistical analysis:

The following statistical analysis was done:

- Percentage and graphical presentation.
- Chi-square test.

Inclusion criteria:

- All patients referred for FNAC to the cytology section for cytological evaluation during the study period were included.

Exclusion criteria:

- FNAC smears with inadequate material and only haemorrhagic material by both techniques (CS and Cytospin LBC Smear) were excluded from the study.
- FNAC smears where cytospin LBC smears were not prepared.

RESULTS

A total of 103 cases were included in the present study. Among the study group, 67% were female (n=69) and 33% were male (n=34) with a male to female ratio of 0.49. The mean age of the study population was 38.7 years. A significant number of study subjects were from the third, fourth and fifth decade with 25, 20 and 15 cases respectively.

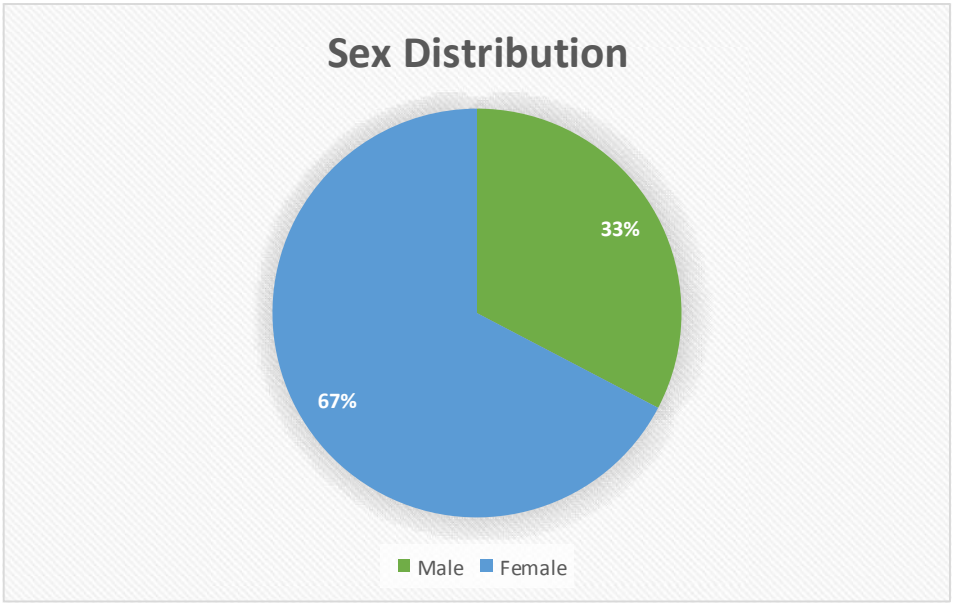


Figure 6 Pie chart representing Sex distribution.

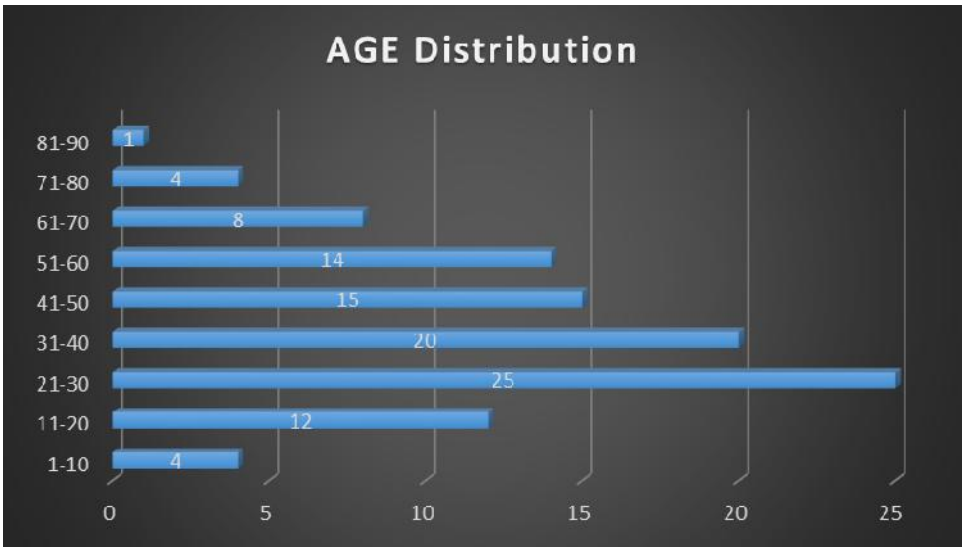


Figure 7 Bar diagram representing Age distribution

Table 2 Distribution of cases according to sex and age in years				
Age in Years	Male		Female	
	n	%	n	%
1-10	2	5.9	2	2.9
11-20	4	11.8	8	11.6
21-30	2	5.9	23	33.3
31-40	6	17.6	14	20.3
41-50	5	14.7	10	14.5
51-60	6	17.6	8	11.6
61-70	7	20.6	1	1.4
71-80	2	5.9	2	2.9
81-90	0	0.0	1	1.4
Total	34	100	69	100

The distribution of the cases as per the site of involvement or swelling at the time of presentation included Thyroid with 32.0% (n= 33), Lymph node 29.2%(n=30), Soft tissue 13.6%(n=14), Breast 12.6%(n=13). Whereas the miscellaneous group 12.6% (n=13) comprised of 4 cases from skin and 3 cases from Salivary gland (2 from parotid and one from submandibular gland). One case each from Liver, Tongue, External auditory canal, Oral Cavity and Epididymis.

Table 3 Distribution of cases according to the site of involvement.

Sr. No	Site	Number of cases	% of cases
1	Thyroid	33	32.0
2	Lymph node	30	29.2
3	Soft tissue swelling	14	13.6
4	Breast	13	12.6
5	Miscellaneous	13	12.6
	Total	103	100.0

Based on the type of the diagnosis offered the cases were categorised into benign, inflammatory, tubercular aetiology and malignant.

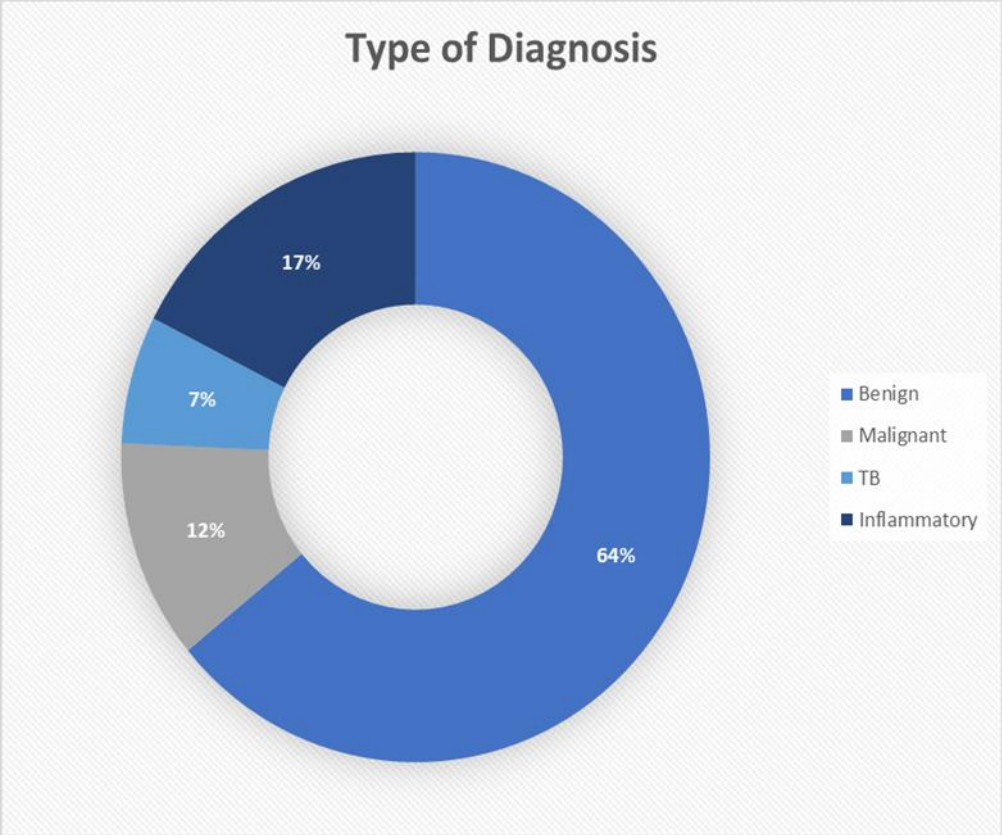


Figure 8 Pie chart representing type of Diagnosis

The entire spectrum of cases from all the locations is represented in

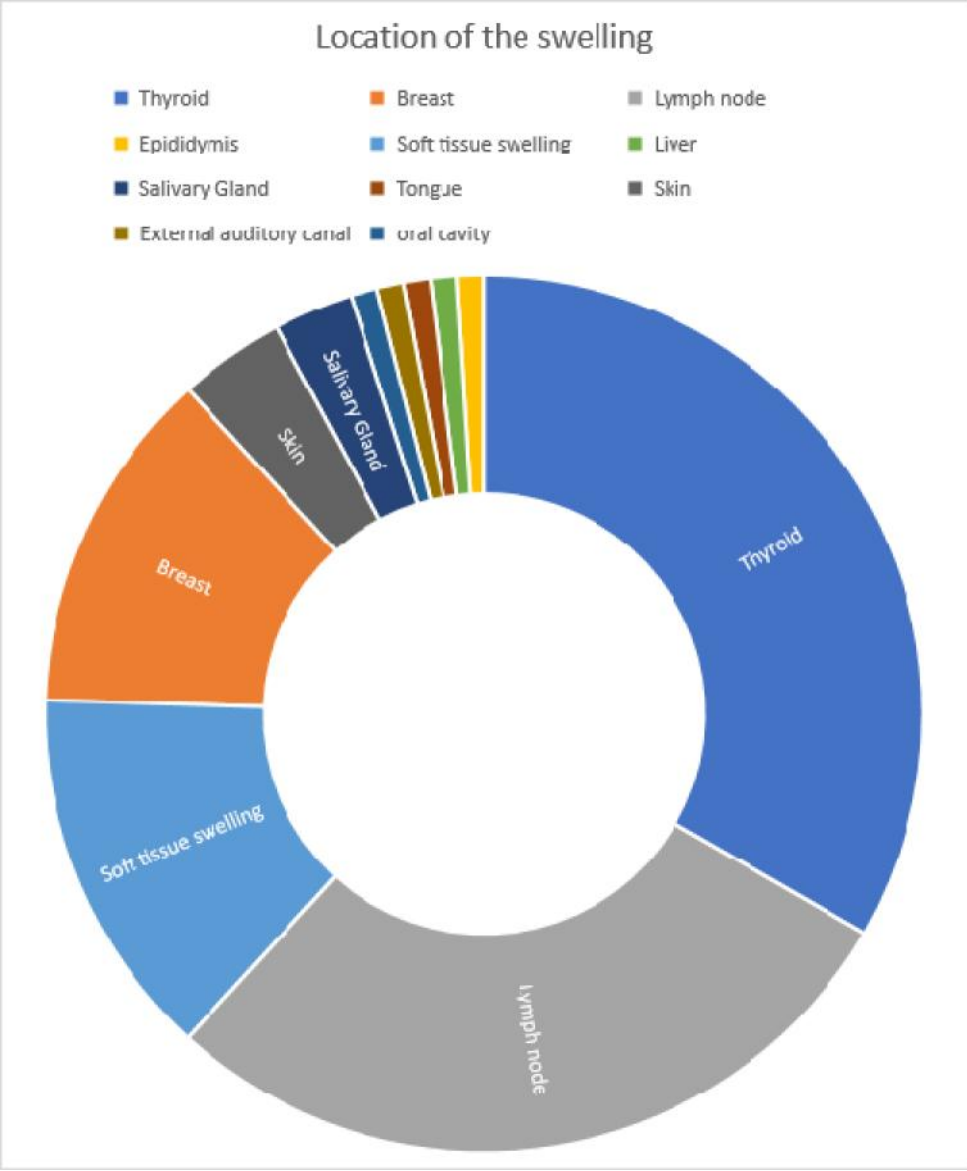


Figure 9 Chart representing Location of the swelling

The overall evaluation of LBC and CS smears for the parameters like cellularity, staining quality, background, nuclear preservation and cellular degeneration was done.

Cellularity:

The overall cellularity was better in case of LBC as compared to conventional smears. In LBC smears cellularity was low in 33 cases and in CS the number of cases with low cellularity was 29. Medium cellularity was noticed in 44 cases in LBC whereas 26 cases of CS had medium cellularity. High cellularity was observed in 21 cases of LBC but in CS the high cellularity was seen in 16 cases. LBC smears did not yield any cellularity in 5 cases and the number of CS smears with no cellularity was 32 cases. Analysis of the data using chi square test has a p-value of 0.0001 which was highly statistically significant. The percentages were documented in Table 4.

Table 4 Overall comparison of cellularity in LBC & CS (n=103)

LBC Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 .0%	2 6.9%	3 11.5%	0 .0%	5 4.9%	P=0.0001*
Low	15 46.9%	12 41.4%	5 19.2%	1 6.3%	33 32.0%	
Medium	16 50.0%	12 41.4%	14 53.8%	2 12.5%	44 42.7%	
High	1 3.1%	3 10.3%	4 15.4%	13 81.3%	21 20.4%	
Total	32 100.0%	29 100.0%	26 100.0%	16 100.0%	103 100.0%	

Staining Quality:

The overall staining quality was comparable in both the LBC and CS smears. It was poor in 4 cases of LBC whereas the number of cases with poor staining quality in the CS category was 2. The staining characteristics were average to good in 92.3% of the cases in LBC and 68.9% of the cases in CS. Staining quality could not be assessed in 30 cases of CS smears.

Table 5 Overall comparison of Staining quality in LBC & CS (n=103)

LBC Staining quality	CS Staining quality				Total	
	Could not be assessed	Poor	Average	Good		Chi square test
Could not be assessed	0 .0%	0 .0%	1 7.1%	3 5.3%	4 3.9%	P=0.082 NS
Poor	3 10.0%	0 .0%	1 7.1%	0 .0%	4 3.9%	
Average	14 46.7%	1 50.0%	5 35.7%	12 21.1%	32 31.1%	
Good	13 43.3%	1 50.0%	7 50.0%	42 73.7%	63 61.2%	
Total	30 100.0%	2 100.0%	14 100.0%	57 100.0%	103 100.0%	

Background:

The background was haemorrhagic in 27.2% of the cases of LBC whereas in CS it was 60.1% of cases. Clear background was seen in 64.1% of the LBC smears.

Table 6 Overall comparison of Background in LBC & CS (n=103)						
LBC Background	CS Background					
	Could not be assessed	Haemorrhage	Clear	Colloid	Total	Chi square test
Could not be assessed	0 .0%	4 6.5%	0 .0%	0 .0%	4 3.9%	P=0.107 NS
Haemorrhage	10 33.3%	17 27.4%	1 11.1%	0 .0%	28 27.2%	
Clear	20 66.7%	38 61.3%	8 88.9%	0 .0%	66 64.1%	
Colloid	0 .0%	3 4.8%	0 .0%	2 100.0%	5 4.9%	
Total	30 100.0%	62 100.0%	9 100.0%	2 100.0%	103 100.0%	

Cellular degeneration:

Cellular degeneration was noticed in 3.9% of the LBC smears whereas CS smears there was no cellular degeneration. Cross tabulation of the data was presented in Table 7. The p-value obtained was 0.174 which was statistically not significant.

Table 7 Overall comparison of cellular degeneration in LBC & CS (n=103)

LBS Cellular degeneration	CS Cellular degeneration				Chi square test
	Could not be assessed	Absent	Poor	Total	
Could not be assessed	1 3.3%	4 5.6%	0 .0%	5 4.9%	P=0.174 NS
Present	3 10.0%	1 1.4%	0 .0%	4 3.9%	
Absent	23 76.7%	66 91.7%	1 100.0%	90 87.4%	
Poor	3 10.0%	1 1.4%	0 .0%	4 3.9%	
Total	30 100.0%	72 100.0%	1 100.0%	103 100.0%	

Nuclear preservation:

LBC had good to average nuclear preservation in 89.3% of cases and in CS smears the nuclear preservation was 66.9% with a statistically significant p-value of 0.006.

Table 8 Overall comparison of Nuclear preservation in LBC & CS

LBS Nuclear Preservation	CS Nuclear Preservation				Total	Chi square test
	Could not be assessed	Poor	Average	Good		
Could not be assessed	3 9.1%	0 .0%	0 .0%	4 6.1%	7 6.8%	P=0.006*
Poor	4 12.1%	0 .0%	0 .0%	0 .0%	4 3.9%	
Average	11 33.3%	0 .0%	2 66.7%	8 12.1%	21 20.4%	
Good	15 45.5%	1 100.0%	1 33.3%	54 81.8%	71 68.9%	
Total	33 100.0%	1 100.0%	3 100.0%	66 100.0%	103 100.0%	

Evaluation of cytomorphological parameters based on organ or location of the swelling:

Thyroid:

Thyroid was the most common organ among the study group, n=33. The predominant presenting symptom among this group was diffuse midline neck swelling. The age range was from 18 to 80 years, with a mean age of 38.8 years. Male to female ratio was 0.09. The diagnoses offered on FNA were represented in Figure 10. Among 33 cases, 54.5% of the cases were of nodular goitre, 6 cases of which were having associated cystic change and a case each with papillary hyperplasia and Hurthle cell change. Conditions like Hashimotos thyroiditis, colloid goitre, papillary carcinoma of thyroid and follicular neoplasm were also reported. All the three male patients had a diagnosis of nodular goitre with cystic change.

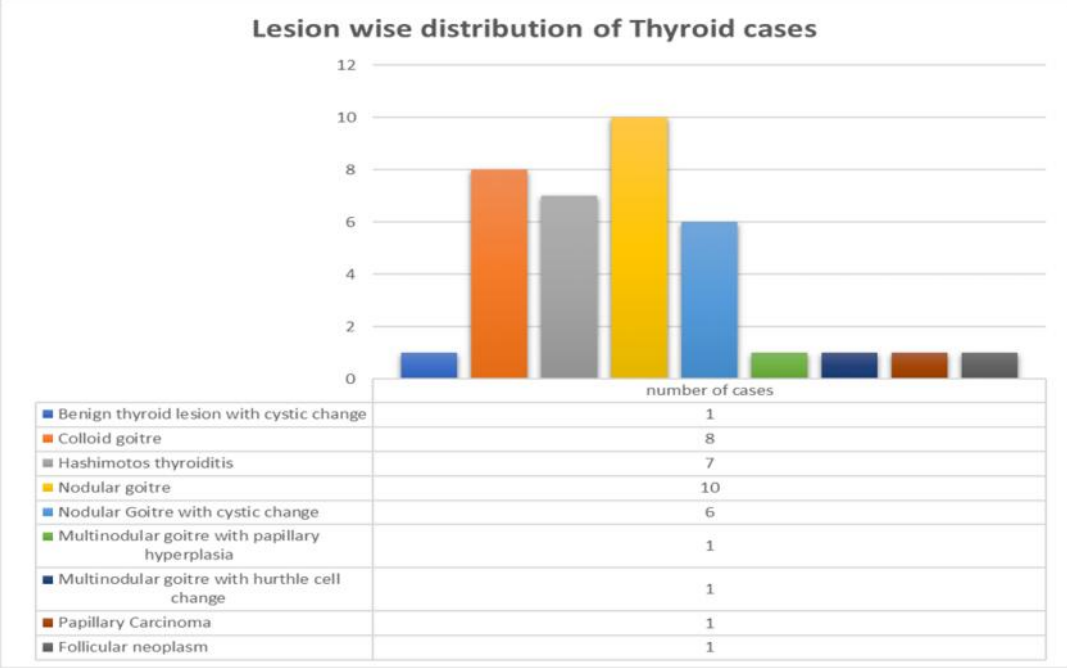


Figure 10 Bar diagram representing Distribution of cases in Thyroid FNA

Table 9 Comparison of cellularity in LBC & CS in Thyroid FNA(n=33)						
LBC Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 0.0%	1 6.7%	1 16.7%	0 .0%	2 6.1%	P=0.0001 *
Low	6 66.7%	4 26.7%	1 16.7%	0 .0%	11 33.3%	
Medium	3 33.3%	9 60.0%	4 66.7%	0 .0%	16 48.5%	
High	0 .0%	1 6.7%	0 .0%	3 100.0%	4 12.1%	
Total	9 100.0%	15 100.0%	6 100.0%	3 100.0%	33 100.0%	

Among 33 cases, 31 (93.9%) were having cellular smears and 2 cases (6.1%) were having no cellularity in LBC, whereas in conventional method 9 cases had no cellularity and only 24 cases were showing cellularity.

With a significant p-value of 0.0001, cellularity in LBC smears was better in comparison to CS. There was no cellularity in 9 cases of CS. In these 9 cases LBC smears showed low and medium cellularity in 6 and 3 cases respectively. Three cases were found to be having high cellularity in both CS and LBC smears. In LBC medium cellularity was observed in 16 cases. Corresponding CS smears showed low and medium cellularity in 9 and 4 cases.

Staining quality was comparable with good staining in 22 and 20 cases of LBC and CS respectively. Staining quality was average in 9 cases of LBC and 4 cases of CS. The detailed tabulation of number of cases along with the percentages is represented in Table 10.

Table 10 Comparison of Staining quality in LBC vs CS Thyroid FNA(n=33)						
LBC Staining quality	CS Staining quality				Total	
	Could not be assessed	Poor	Average	Good		Chi square test
Could not be assessed	0 .0%	0 .0%	0 .0%	1 5.0%	1 3.0%	P=0.812 NS
Poor	1 12.5%	0 .0%	0 .0%	0 .0%	1 3.0%	
Average	2 25.0%	0 .0%	2 50.0%	5 25.0%	9 27.3%	
Good	5 62.5%	1 100.0%	2 50.0%	14 70.0%	22 66.7%	
Total	8 100.0%	1 100.0%	4 100.0%	20 100.0%	33 100.0%	

Table 11 Comparison of Background in LBC Vs CS in Thyroid FNA(n=33)						
LBC Background	CS Background					
	Could not be assessed	Haemorrhage	Clear	Colloid	Total	Chi square test
Could not be assessed	0 .0%	1 5.3%	0 .0%	0 .0%	1 3.0%	P=0.107 NS
Haemorrhage	2 25.0%	5 26.3%	1 25.0%	0 .0%	8 24.2%	
Clear	6 75.0%	10 52.6%	3 75.0%	0 .0%	19 57.6%	
Colloid	0 .0%	3 15.8%	0 .0%	2 100.0%	5 15.2%	
Total	8 100.0%	19 100.0%	4 100.0%	2 100.0%	33 100.0%	

In LBC smears of thyroid FNA the background was clear and having abundant colloid in 19 and 5 cases respectively. Haemorrhagic background was noted in 19 cases of CS smears. Ten cases of CS smears with a haemorrhagic background had a clear background in LBC smears. Colloid was seen in clear background in LBC smears in 5 cases whereas only 2 cases of CS smears had colloid in the background.

Table 12 Comparison of Cellular degeneration in LBC Vs CS in Thyroid FNA(n=33)				
LBC Cellular degeneration	Cs Cellular degeneration		Total	Chi square test
	Could not be assessed	Absent		
Could not be assessed	2 25.0%	1 4.0%	3 9.1%	P=0.033*
Present	1 12.5%	0 .0%	1 3.0%	
Absent	5 62.5%	24 96.0%	29 87.9%	
Total	8 100.0%	25 100.0%	33 100.0%	

Cellular degeneration was not present in 29 and 25 cases of LBC and CS, with a statistically significant p-value of 0.033. The nuclear preservation was better in LBC smears with 29 cases having good and average preservation whereas in CS the corresponding number was 24.

Table 13 Comparison of Nuclear preservation in LBC Vs CS in Thyroid FNA(n=33)					
LBC Nuclear Preservation	CS Nuclear Preservation			Total	Chi square test
	Could not be assessed	Average	Good		
Could not be assessed	2 25.0%	0 .0%	1 4.3%	3 9.4%	p=0.055
Poor	1 12.5%	0 .0%	0 .0%	1 3.1%	
Average	2 25.0%	2 100.0%	3 13.0%	7 18.8%	
Good	3 37.5%	0 .0%	19 82.6%	22 68.8%	
Total	8 100%	2 100%	23 100%	33 100%	

Lymph node:

With the age range from 5-72 years and a mean age of 33.6 a total of 30 cases of lymph node FNA were included in the present study. Male to female ratio was 0.81, predominant group of lymph nodes aspirated were of cervical region followed by axillary and inguinal. Majority of the cases were of reactive lymphadenitis (n=14). The distribution of cases based on diagnosis was represented in the Figure 11.

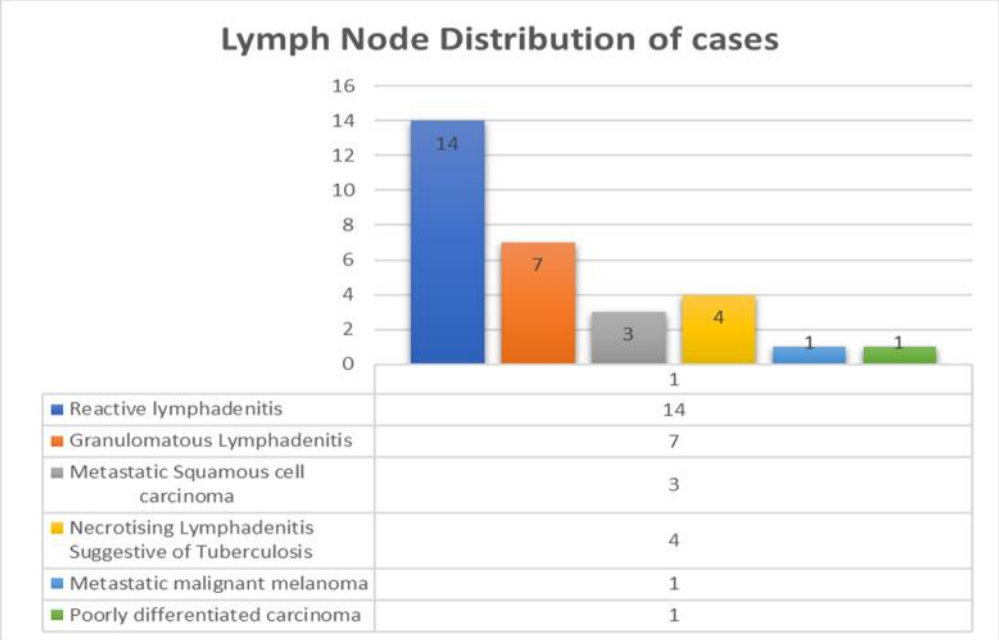


Figure 11 Bar diagram representing lesion wise distribution of Lymph node FNA

Table 14 Comparison of Cellularity in LBC vs CS Lymph node FNA (n=30)						
LBC Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 0.0%	0 0.0%	1 9.1%	0 0.0%	1 3.3%	P=0.428 NS
Low	4 50.0%	4 57.1%	3 27.3%	0 0.0%	11 36.7%	
Medium	3 37.5%	2 28.6%	3 27.3%	1 25.0%	9 30.0%	
High	1 12.5%	1 14.3%	4 36.4%	3 75.0%	9 30.0%	
Total	8 100.0%	7 100.0%	11 100.0%	4 100.0%	30 100.0 %	

Table 15 Comparison of Staining quality in LBC vs CS Lymph node FNA (n=30)						
LBC Staining quality	CS Staining quality				Total	
	Could not be assessed	Poor	Average	Good		Chi square test
Could not be assessed	0 0.0%	0 0.0%	0 0.0%	1 5.9%	1 3.3%	P=0.521 NS
Poor	1 12.5%	0 0.0%	0 0.0%	0 0.0%	1 3.3%	
Average	3 37.5%	1 100.0%	2 50.0%	3 17.6%	9 30.0%	
Good	4 50.0%	0 0.0%	2 50.0%	13 76.5%	19 63.3%	
Total	8 100.0%	1 100.0%	4 100.0%	17 100.0%	30 100.0%	

Staining quality in LBC and CS smears was good in 19 and 17 cases respectively. Nine cases of LBC and 4 cases of CS had average staining quality.

Coming to the background, 17 smears of CS had RBCs but the corresponding smears in LBC were having a clear background as represented in Table 16.

Table 16 Comparison of Background in LBC vs CS Lymph node FNA (n=30)			
LBC Background	CS Background		Total
	Could not be assessed	Haemorrhage	
Could not be assessed	0 0.0%	1 4.5%	1 3.3%
Haemorrhage	3 37.5%	4 18.2%	7 23.3%
Clear	5 62.5%	17 77.3%	22 73.3%
Total	8 100.0%	22 100.0%	30 100.0%

Table 17 Comparison of Cellular Degeneration in LBC vs CS Lymph node FNA (n=30)					
LBC Cellular degeneration	CS Cellular degeneration				
	Could not be assessed	Absent	Poor	Total	Chi square test
Could not be assessed	0 0.0%	1 4.8%	0 0.0%	1 3.3%	P=0.522 NS
Absent	7 87.5%	20 95.2%	1 100.0%	28 93.3%	
Poor	1 12.5%	0 0.0%	0 0.0%	1 3.3%	
Total	8 100.0%	21 100.0%	1 100.0%	30 100.0%	

Four cases had low cellularity in both LBC and CS, three cases of CS with medium cellularity had low cellularity in LBC. Four cases of LBC had low cellularity which were found to have no cellularity with only haemorrhagic smears on CS.

Cellular degeneration as represented in Table 17 showed absent cellular degeneration in 21 cases of CS and 28 cases of LBC. With respect to nuclear preservation, 29 smears of LBC and 21 smears in case of Cs were having acceptable nuclear preservation. The detailed analysis and comparison was represented in Table 14 to Table 18.

Table 18 Comparison of Nuclear preservation in LBC vs CS Lymph node FNA (n=30)						
LBC Nuclear Preservation	CS Nuclear Preservation				Total	Chi square test
	Could not be assessed	Poor	Average	Good		
Could not be assessed	0 0.0%	0 0.0%	0 0.0%	1 5.3%	1 3.3%	P=0.948 NS
Average	3 37.5%	0 0.0%	1 50.0%	5 26.3%	9 30.0%	
Good	5 62.5%	1 100.0%	1 50.0%	13 68.4%	20 66.7%	
Total	8 100.0%	1 100.0%	2 100.0%	19 100.0%	30 100.0%	

Soft tissue swelling:

With a mean age of 42.5 years and range of 3-70years, the male to female ratio was 2.5. Various soft tissue lesions were depicted in Figure 12.

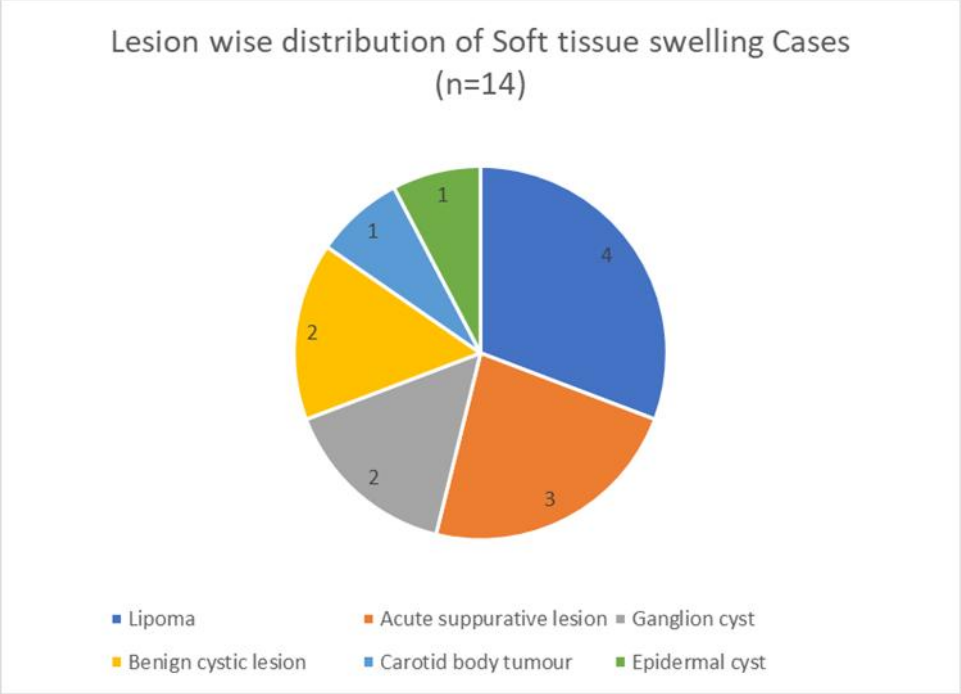


Figure 12 Pie chart representing Lesion wise distribution of Soft tissue swelling Cases

Table 19 Comparison of Cellularity in LBC vs CS Soft tissue swelling FNA (n=14)						
LBC Cellularity	CS Cellularity				Total	
	No Cellularity	Low	Medium	High		Chi square test
Low	3 60.0%	2 66.7%	0 0.0%	0 0.0%	5 35.7 %	P=0.058
Medium	2 40.0%	0 0.0%	3 100.0%	1 33.3%	6 42.9 %	
High	0 0.0%	1 33.3%	0 0.0%	2 66.7%	3 21.4 %	
Total	5 100.0%	3 100.0%	3 100.0%	3 100.0%	14 100.0 %	

Cellularity was seen in 5 cases of LBC which were having acellular haemorrhagic smears in CS amongst which 3 cases were having low cellularity and 2 were of medium cellularity.

Table 20 Comparison of Staining quality in LBC vs CS Soft tissue swelling FNA (n=14)					
LBC Staining quality	CS Staining quality			Total	
	Could not be assessed	Average	Good		Chi square test
Poor	1 25.0%	0 0.0%	0 0.0%	1 7.1%	P=0.080 NS
Average	2 50.0%	1 100.0%	1 11.1%	4 28.6%	
Good	1 25.0%	0 0.0%	8 88.9%	9 64.3%	
Total	4 100.0%	1 100.0%	9 100.0%	14 100.0%	

Staining quality was comparable among the two with 8 cases each having good staining quality. Staining quality was average in 4 LBC smears and one CS smear as represented in Table 20.

Table 21 Comparison of Background in LBC vs CS Soft tissue swelling FNA (n=14)					
LBC Background	CS Background			Total	Chi square test
	Could not be assessed	Haemorrhage	Clear		
Haemorrhage	1 25.0%	1 16.7%	0 0.0%	2 14.3%	P=0.586 NS
Clear	3 75.0%	5 83.3%	4 100.0%	12 85.7%	
Total	4 100.0%	6 100.0%	4 100.0%	14 100.0%	

LBC smears had a clear background in 85.7% of cases whereas the clear background was noted in 4 cases of CS and haemorrhagic background in 6 cases. There was no cellular degeneration noted in soft tissue swelling FNA both in LBC and CS.

Table 22 Comparison of Cellular degeneration in LBC vs CS Soft tissue swelling FNA (n=14)			
LBC Cellular degeneration	CS Cellular degeneration		Total
	Could not be assessed	Absent	
Absent	4 100.0%	10 100.0%	14 100.0%
Total	4 100.0%	10 100.0%	14 100.0%

Note: Chi square test could not be applied because in LBC cellular degeneration observations are only in Absent category.

With a significant p-value of 0.032 nuclear preservation was good in 11 cases and 9 cases of LBC and CS respectively. The detailed cross tabulation of nuclear preservation was depicted in Table 23

Table 23 Comparison of Nuclear preservation in LBC vs CS Soft tissue swelling FNA (n=14)				
LBS Nuclear Preservation	CS Nuclear Preservation		Total	
	Could not be assessed	Good		Chi square test
Could not be assessed	1 20.0%	0 0.0%	1 7.1%	P=0.032*
Average	2 40.0%	0 0.0%	2 14.3%	
Good	2 40.0%	9 100.0%	11 78.6%	
Total	5 100.0%	9 100.0%	14 100.0%	

Breast:

With a predominant complaint of lump in the breast followed by pain in the breast, a total of 13 cases were included and the age range was 18 – 85 years with a mean age of 40.9 years.

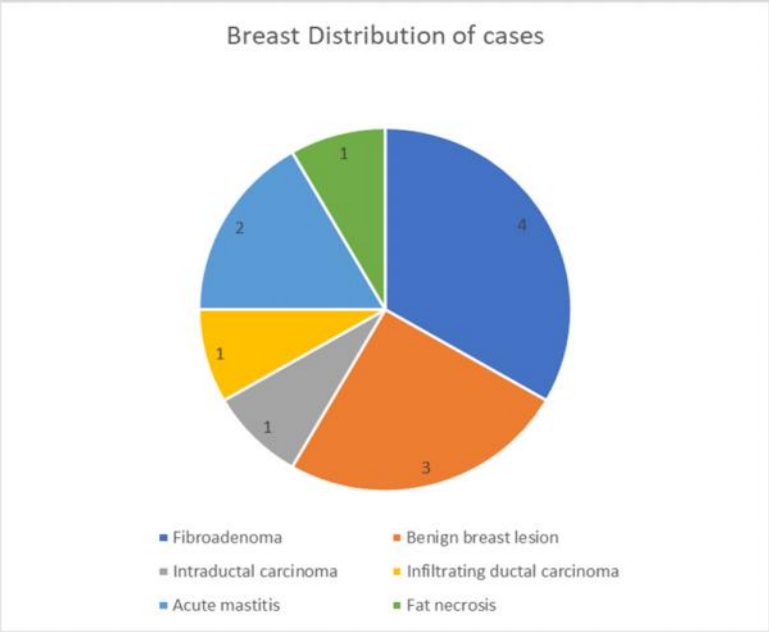


Figure 13 Pie chart representing lesion wise distribution of Breast FNA cases.

The analysis of breast FNA cases had shown that in LBC smears there was no cellularity in 7.7% (n=1) of the cases in comparison to CS with no cellularity in 38% (n=5). The 5 cases with no cellularity in CS had low cellularity in one case and medium cellularity in the other 4. The one case with no cellularity in LBC had low cellularity in CS. Forty six percent of the cases had medium cellularity and 15.4% had high cellularity in LBC. A p-value of 0.044 was obtained which was statistically significant. The cross tabulation of data comparing LBC and CS was represented in Table 24.

Table 24 Comparison of Cellularity in LBC vs CS Breast FNA (n=13)						
LBC Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 .0%	1 25.0%	0 .0%	0 .0%	1 7.7%	P=0.044 *
Low	1 20.0%	2 50.0%	1 50.0%	0 .0%	4 30.8%	
Medium	4 80.0%	1 25.0%	1 50.0%	0 .0%	6 46.2%	
High	0 .0%	0 .0%	0 .0%	2 100.0%	2 15.4%	
Total	5 100.0%	4 100.0%	2 100.0%	2 100.0%	13 100.0%	

Table 25 Comparison of Staining quality in LBC vs CS Breast FNA (n=13)					
LBC Staining quality	CS Staining quality			Total	Chi square test
	Could not be assessed	Average	Good		
Could not be assessed	0 .0%	0 .0%	1 20.0%	1 7.7%	P=0.392 NS
Poor	0 .0%	1 33.3%	0 .0%	1 7.7%	
Average	2 40.0%	0 .0%	1 20.0%	3 23.1%	
Good	3 60.0%	2 66.7%	3 60.0%	8 61.5%	
Total	5 100.0%	3 100.0%	5 100.0%	13 100.0%	

Eight cases of LBC had good staining quality and 5 cases of CS were found to have good staining quality. However, there was no statistical significance with respect to staining quality as both CS and LBC had acceptable staining quality in 8 and 11 cases respectively.

Table 26 Comparison of Background in LBC vs CS Breast FNA (n=13)

LBC Background	CS Background			Total	
	Could not be assessed	Haemorrhage	Clear		Chi square test
Could not be assessed	0 .0%	1 14.3%	0 .0%	1 7.7%	
Haemorrhage	2 40.0%	4 57.1%	0 .0%	6 46.2%	P=0.584 NS
Clear	3 60.0%	2 28.6%	1 100.0%	6 46.2%	
Total	5 100.0%	7 100.0%	1 100.0%	13 100.0%	

In case of background, LBC had 6 cases with a clear background and CS had haemorrhagic background in 7 cases.

Cellular degeneration was absent in 11 cases of LBC and 8 cases of CS. Single case of LBC had poor cellular morphology whereas the corresponding smear of CS had no cellularity to assess the cellular morphology.

Table 27 Comparison of cellular degeneration in LBC vs CS Breast FNA (n=13)

LBC Cellular degeneration	CS Cellular degeneration		Total	
	Could not be assessed	Absent		
Could not be assessed	0 .0%	1 12.5%	1 7.7%	
Absent	4 80.0%	7 87.5%	11 84.6%	P=0.325 NS
Poor	1 20.0%	0 .0%	1 7.7%	
Total	5 100.0%	8 100.0%	13 100.0%	

Nuclear preservation was comparable among LBC and CS with good nuclear preservation in 9 and 8 cases respectively. Further stratification of data along with p-value was represented in Table 28.

Table 28 Comparison of Nuclear preservation in LBC vs CS Breast FNA (n=13)

LBC Nuclear Preservation	CS Nuclear Preservation		Total	
	Could not be assessed	Good		Chi square test
Could not be assessed	0 .0%	1 12.5%	1 7.7%	P=0.040 NS
Average	3 60.0%	0 .0%	3 23.1%	
Good	2 40.0%	7 87.5%	9 69.2%	
Total	5 100.0%	8 100.0%	13 100.0%	

Miscellaneous:

A total of 14 cases were included in the miscellaneous category with age range from 18 – 70 years, mean age of 44.3 years and a male to female ratio of 1.3:1. The distribution of cases as per the diagnosis offered was represented in

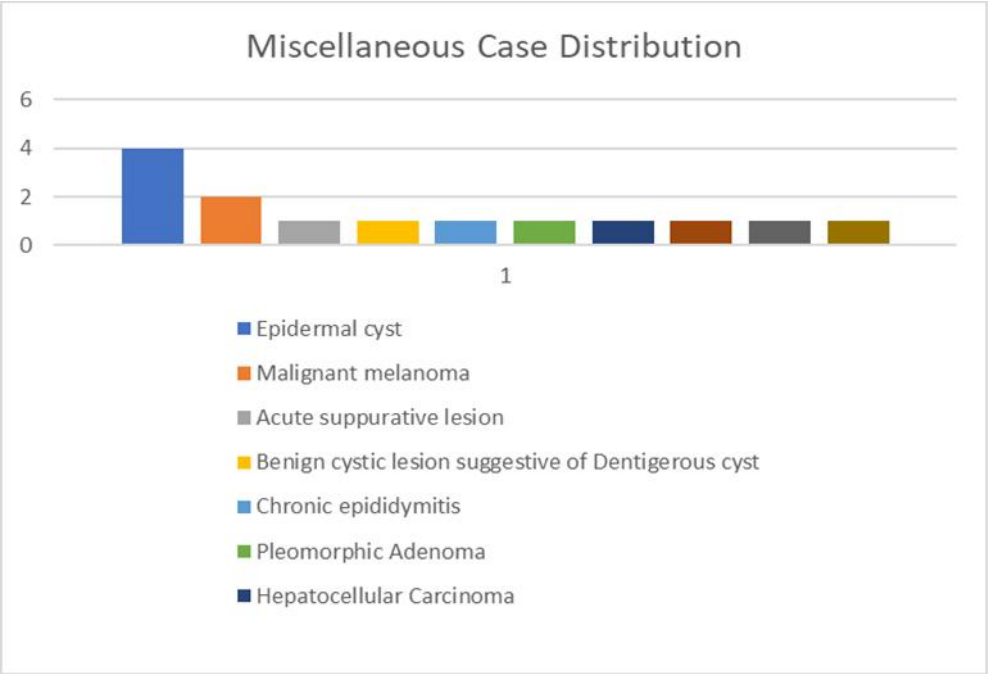


Figure 14 Bar diagram representing lesion wise distribution of Miscellaneous FNA

Three cases were not having cellularity in CS which were having medium cellularity in LBC. One case of LBC was acellular which was having medium cellularity in CS. The cellularity comparison using chi square test yielded a statistically significant p-value of 0.012 and the three smears with high cellularity on CS were also having high cellularity in LBC.

Table 29 Comparison of Cellularity in LBC vs CS Miscellaneous FNA (n=13)

LBC Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 .0%	0 .0%	1 25.0%	0 .0%	1 7.7%	P=0.012*
Low	0 .0%	2 100.0%	0 .0%	1 25.0%	3 23.1%	
Medium	3 100.0%	0 .0%	3 75.0%	0 .0%	6 46.2%	
High	0 .0%	0 .0%	0 .0%	3 75.0%	3 23.1%	
Total	3 100.0%	2 100.0%	4 100.0%	4 100.0%	13 100.0%	

Table 30 Comparison of Staining quality in LBC vs CS Miscellaneous FNA (n=13)					
LBS Staining quality	CS Staining quality			Total	Chi square test
	Could not be assessed	Average	Good		
Could not be assessed	0 .0%	1 33.3%	0 .0%	1 7.7%	P=0.196 NS
Average	2 66.7%	0 .0%	2 28.6%	4 30.8%	
Good	1 33.3%	2 66.7%	5 71.4%	8 61.5%	
Total	3 100.0%	3 100.0%	7 100.0%	13 100.0%	

Staining quality was comparable with 8 and 7 cases having good staining quality in LBC and CS respectively. In 3 cases where staining quality could not be assessed, two cases had average staining and one case had good staining quality in corresponding LBC smears.

Table 31 Comparison of Background in LBC vs CS Miscellaneous FNA (n=13)				
LBS Background	CS Background		Total	Chi square test
	Could not be assessed	Haemorrhage		
Could not be assessed	0 .0%	1 10.0%	1 7.7%	P=0.296 NS
Haemorrhage	0 .0%	4 40.0%	4 30.8%	
Clear	3 100.0%	5 50.0%	8 61.5%	
Total	3 100.0%	10 100.0%	13 100.0%	

Background was clear in 8 cases of LBC of which 5 smears had haemorrhagic background in CS.

There was no cellular degeneration in majority of the cases 76.9% of CS and 69.2% of LBC smears. Two cases of LBC had cellular degeneration, one of which had no cellular degeneration in CS and in the other case cellular degeneration could not be assessed due to the absence of cellularity.

Detailed representation of the cellular degeneration was tabulated using cross tabulation analysis and chi square test in Table 32.

Table 32 Comparison of Cellular degeneration in LBC vs CS Miscellaneous FNA (n=13)				
LBS Cellular degeneration	CS Cellular degeneration		Total	Chi square test
	Could not be assessed	Absent		
Could not be assessed	0 .0%	1 10.0%	1 7.7%	P=0.701 NS
Present	1 33.3%	1 10.0%	2 15.4%	
Absent	2 66.7%	7 70.0%	9 69.2%	
Poor	0 .0%	1 10.0%	1 7.7%	
Total	3 100.0%	10 100.0%	13 100.0%	

Table 33 Comparison of Nuclear preservation in LBC vs CS Miscellaneous FNA (n=13)				
LBS Nuclear Preservation	CS Nuclear Preservation		Total	Chi square test
	Average	Good		
Average	1 20.0%	1 12.5%	2 15.4%	P=0.365 NS
Poor	1 20.0%	0 .0%	1 7.7%	
Good	3 60.0%	7 87.5%	10 76.9%	
Total	5 100.0%	8 100.0%	13 100.0%	

Nuclear preservation was good (87.5%) in both the methods and CS method had an average nuclear preservation in 5 cases whereas in LBC 2 cases were having average nuclear preservation.

Analysis of the results in FNA cases with a diagnosis Other than Malignancy:

The detailed results were cross tabulated in the following tables from Table 34 to Table 38

Table 34 Comparison of Cellularity in LBC vs CS Non-Malignant FNA Cases (n=91)						
LBS Cellularity	CS Cellularity				Total	Chi square test
	No Cellularity	Low	Medium	High		
No Cellularity	0 .0%	2 6.7%	3 13.0%	0 .0%	5 6.5%	P=0.0001*
Low	14 51.8%	13 43.3%	4 17.4%	1 9.1%	32 34.8%	
Medium	13 48.2%	12 40.0%	13 56.5%	2 18.2%	40 43.5%	
High	0 .0%	3 10.0%	3 13.0%	8 72.7%	14 15.2%	
Total	27 100.0%	30 100.0%	23 100.0%	11 100.0%	91 100.0%	

Table 35 Comparison of Staining quality in LBC vs CS Non-Malignant FNA Cases (n=91)					
LBS Staining quality	CS Staining quality			Total	Chi square test
	Could not be assessed	Average	Good		
Could not be assessed	0 .0%	1 7.7%	3 5.7%	4 4.4%	P=0.019*
Poor	3 12.0%	0 .0%	0 .0%	3 3.3%	
Average	10 40.0%	5 38.5%	10 18.9%	25 27.5%	
Good	12 48.0%	7 53.8%	40 75.5%	59 64.8%	
Total	25 100.0%	13 100.0%	53 100.0%	91 100.0%	100.0%

Table 36 Comparison of Cellular degeneration in LBC vs CS Non-Malignant FNA Cases					
LBS Cellular degeneration	CS Cellular degeneration				Chi square test
	Could not be assessed	Absent	Poor	Total	
Could not be assessed	2 8.0%	4 6.2%	0 .0%	6 6.6%	P=0.392 NS
Present	2 8.0%	0 .0%	0 .0%	2 2.2%	
Absent	20 80.0%	60 92.3%	1 100.0%	81 89.0%	
Poor	1 4.0%	1 1.5%	0 .0%	2 2.2%	
Total	25 100.0%	65 100.0%	1 100.0%	91 100.0%	

Table 37 Comparison of Background in LBC vs CS Non-Malignant FNA Cases (n=91)						
LBS Background	CS Background				Total	Chi square test
	Could not be assessed	Haemorrhage	Clear	Colloid		
Could not be assessed	0 .0%	4 7.1%	0 .0%	0 .0%	4 4.4%	P=0.0001*
Haemorrhage	6 24.0%	15 26.8%	1 12.5%	0 .0%	22 24.2%	
Clear	19 76.0%	34 60.7%	7 87.5%	0 .0%	60 65.9%	
Colloid	0 .0%	3 5.4%	0 .0%	2 100.0%	5 5.5%	
Total	25 100.0%	56 100.0%	8 100.0%	2 100.0%	91 100.0 %	

Table 38 Comparison of Nuclear preservation in LBC vs CS Non-Malignant FNA Cases (n=88)					
LBS Nuclear Preservation	CS Nuclear Preservation			Total	Chi square test
	Could not be assessed	Average	Good		
Could not be assessed	4 15.4%	0 .0%	4 6.7%	8 9.1%	P=0.006*
Poor	1 3.8%	0 .0%	0 .0%	1 1.1%	
Average	9 34.6%	2 100.0%	8 13.3%	19 21.6%	
Good	12 46.2%	0 .0%	48 80.0%	60 68.2%	
Total	26 100.0%	2 100.0%	60 100.0 %	88 100.0%	

Analysis of the results in FNA cases with a diagnosis of Malignancy: The detailed results were cross tabulated in the following tables from Table 39 to Table 43.

Table 39 Comparison of Cellularity in LBC vs CS Malignant FNA Cases (n=12)							
LBS Cellularity		CS Cellularity				Total	Chi square test
		No Cellularity	Low	Medium	High		
	Low	0 .0%	1 100.0%	1 33.3%	0 .0%	2 16.7%	P=0.054
	Medium	2 66.7%	0 .0%	1 33.3%	0 .0%	3 25.0%	
	High	1 33.3%	0 .0%	1 33.3%	5 100.0%	7 58.3%	
Total		3 100.0%	1 100.0%	3 100.0%	5 100.0%	12 100.0%	

Table 40 Comparison of Staining quality in LBC vs CS Malignant FNA Cases (n=12)						
LBS Staining quality	CS Staining quality				Total	Chi square test
	Could not be assessed	Poor	Average	Good		
Poor	0 .0%	0 .0%	1 50.0%	0 .0%	0 .0%	P=0.425 NS
Average	1 33.3%	1 50.0%	0 .0%	2 40.0%	2 40.0%	
Good	2 66.7%	1 50.0%	1 50.0%	3 60.0%	3 60.0%	
Total	3 100.0%	2 100.0%	2 100.0%	5 100.0 %	12 100%	

Table 41 Comparison of Background in LBC vs CS Malignant FNA Cases (n=12)					
LBS Background	CS Background			Total	Chi square test
	Could not be assessed	Haemorrhage	Clear		
Haemorrhage	2 66.7%	3 37.5%	0 .0%	5 41.7%	P=0.462 NS
Clear	1 33.3%	5 62.5%	1 100.0%	7 58.3%	
Total	3 100.0%	8 100.0%	1 100.0%	12 100.0%	

Table 42 Comparison of Cellular degeneration in LBC vs CS Malignant FNA Cases (n=12)				
LBS Cellular degeneration	CS Cellular degeneration		Total	Chi square test
	Could not be assessed	Absent		
Present	0 .0%	1 11.1%	1 8.3%	P=0.177 NS
Absent	2 66.7%	8 88.9%	10 83.3%	
Poor	1 33.3%	0 .0%	1 8.3%	
Total	3 100.0%	9 100.0%	12 100.0%	

Table 43 Comparison of Nuclear preservation in LBC vs CS Malignant FNA Cases (n=12)						
	CS Nuclear Preservation				Total	Chi square test
	Could not be assessed	Poor	Average	Good		
Average	1 33.3%	0 .0%	0 .0%	0 .0%	1 8.3%	P=0.351 NS
Good	2 66.7%	1 100.0%	1 100.0%	7 100.0%	11 91.7%	
Total	3 100.0%	1 100.0%	1 100.0%	7 100.0%	12 100.0%	

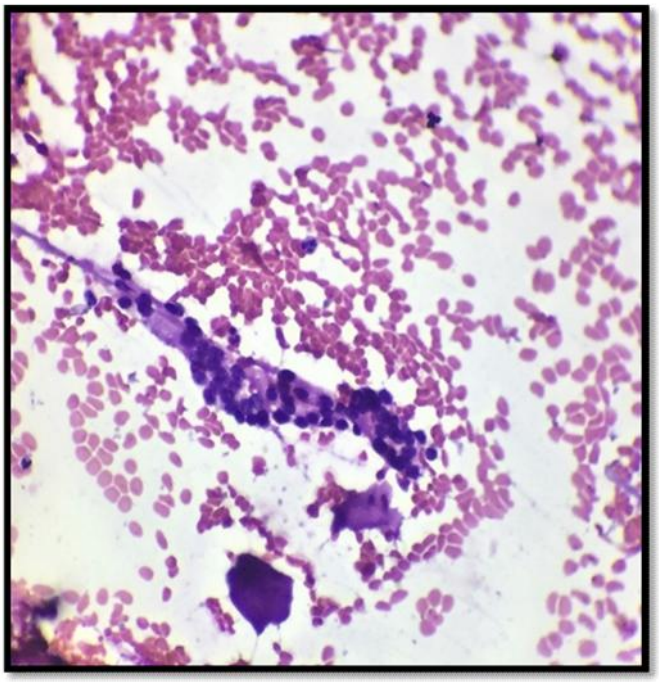


Figure 16 Thyroid FNA, CS smear, H & E 400X

Cluster of thyroid follicular epithelial cells seen in a background of plenty of RBCs.

Diagnosis: Multi nodular goitre with papillary change

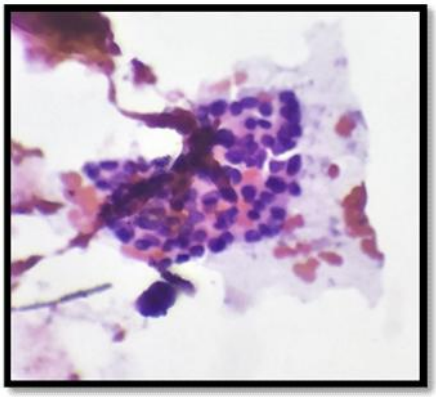
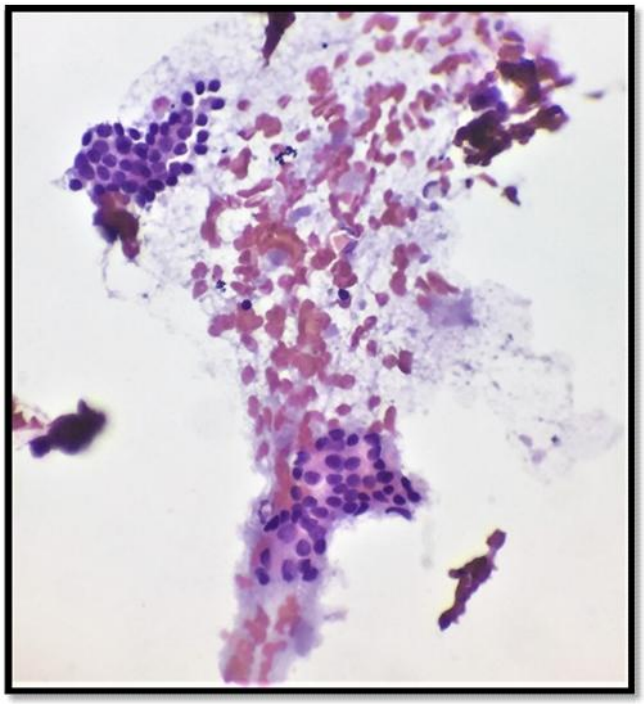


Figure 15 Thyroid FNA, LBC Smear, H & E 400X

Clusters of thyroid follicular epithelial cells with a clean background and relatively less RBCs



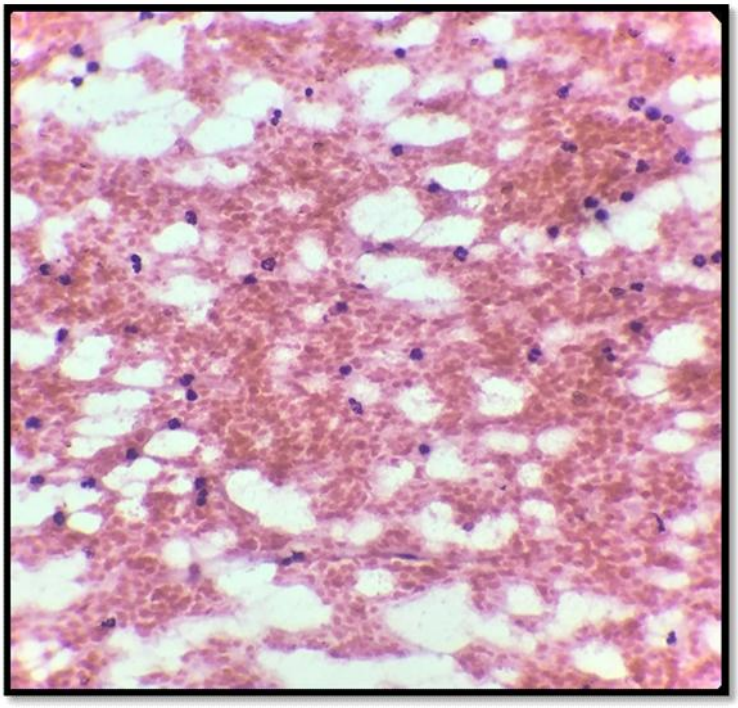


Figure 17 Soft tissue FNA, CS smear H & E 400X,

Singly scattered Inflammatory cells in a haemorrhagic background

Diagnosis:
Acute Suppurative lesion

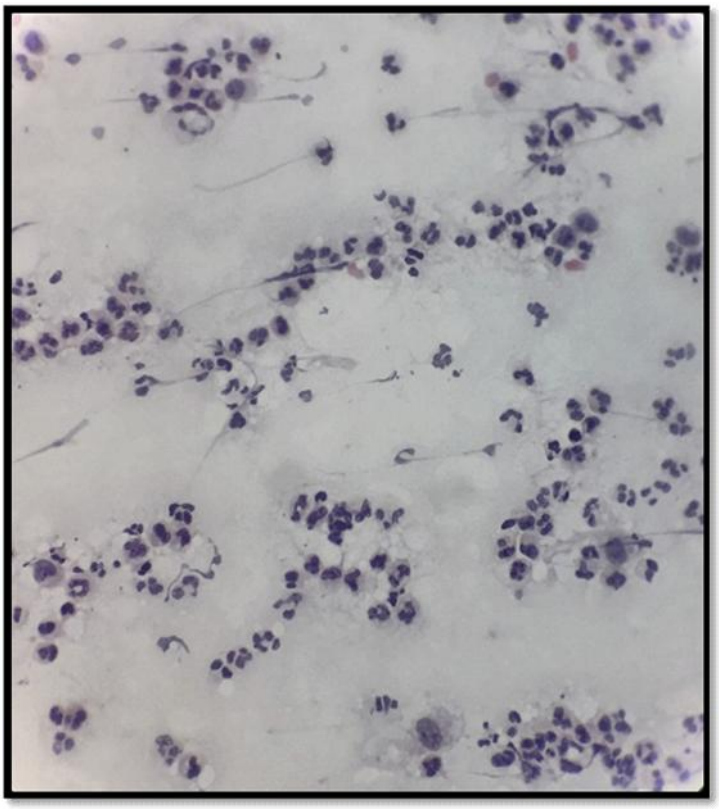


Figure 18 Soft tissue FNA, LBC smear H & E 400X,

Aggregates and singly scattered mixed inflammatory cells in a clean background

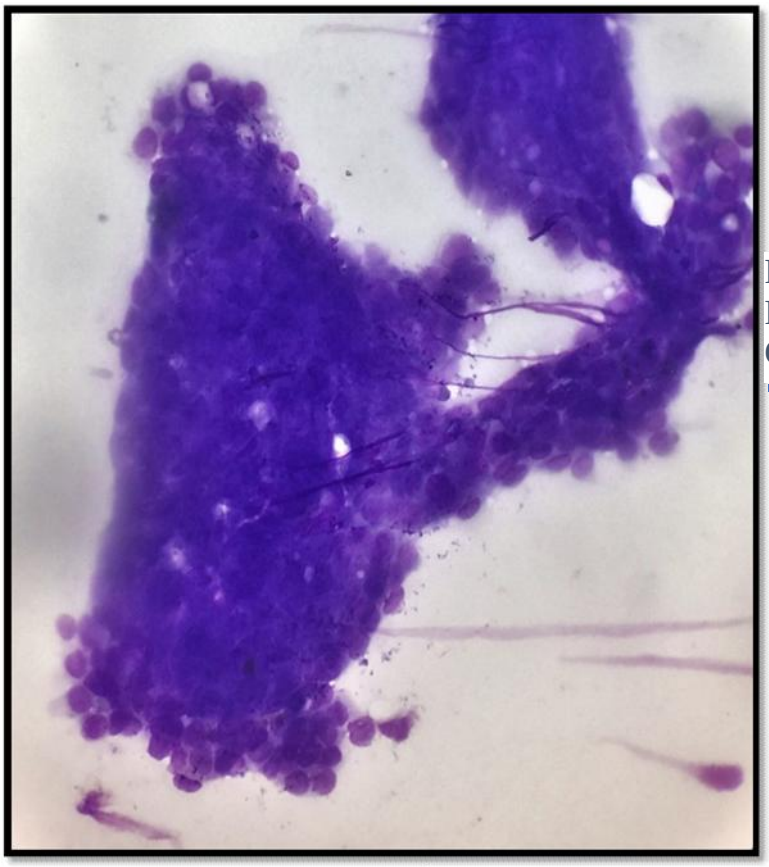


Figure 20 Breast FNA, CS Smear, Giemsa 400X

Cluster of tight cohesive cells, overlapping of cells is noted.

Diagnosis: Fibroadenoma

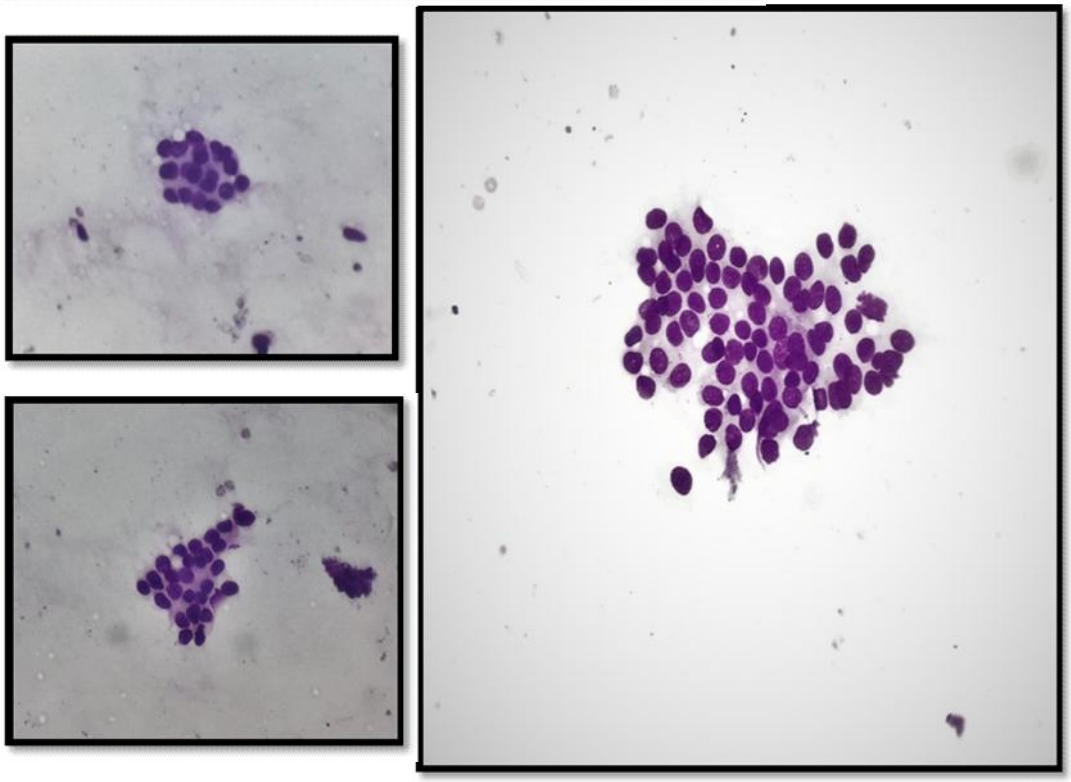


Figure 19 Breast FNA, LBC smear, Giemsa 400X, Inset clusters

Cohesive clusters of monolayered cells in a clean background with no overlapping

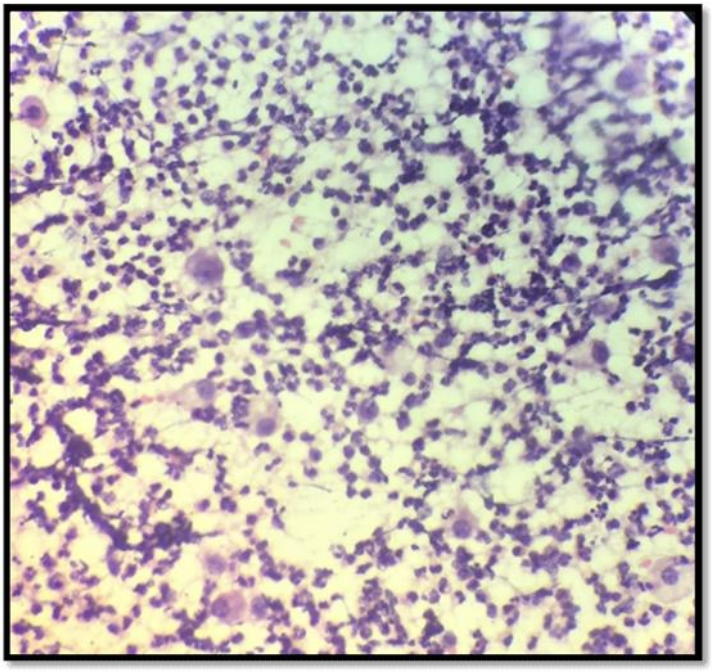


Figure 22 Breast FNA, CS smear, Giemsa 400x

Dense and diffuse inflammatory cell infiltrate with scattered ductal epithelial cells

Diagnosis:
Acute mastitis

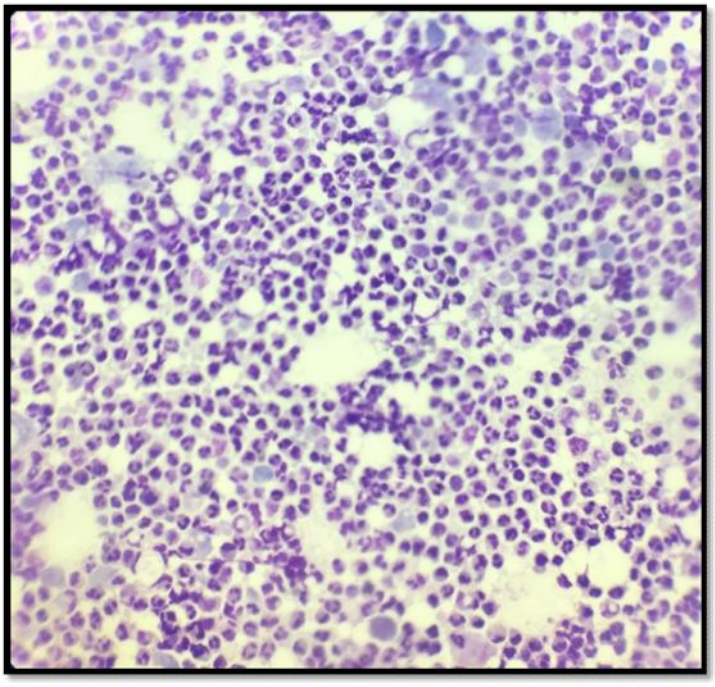


Figure 21 Breast FNA, LBC smear, Giemsa 400X

Dense and diffuse inflammatory cell infiltrate with singly scattered ductal epithelial cells

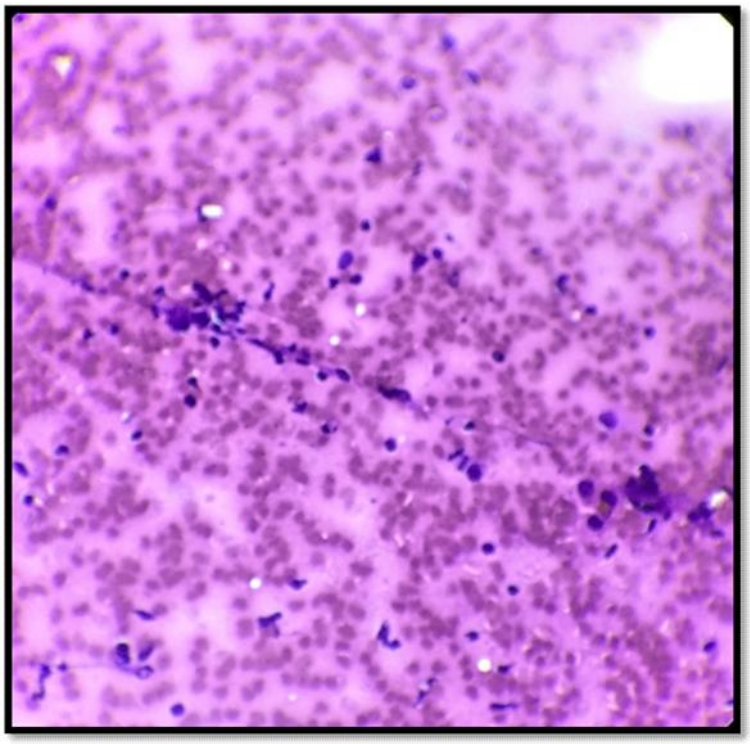


Figure 23 Thyroid FNA, CS Smear, H &E 400X

Singly scattered follicular epithelial cells in a background of RBCs and colloid material.

Diagnosis:
Colloid Goitre

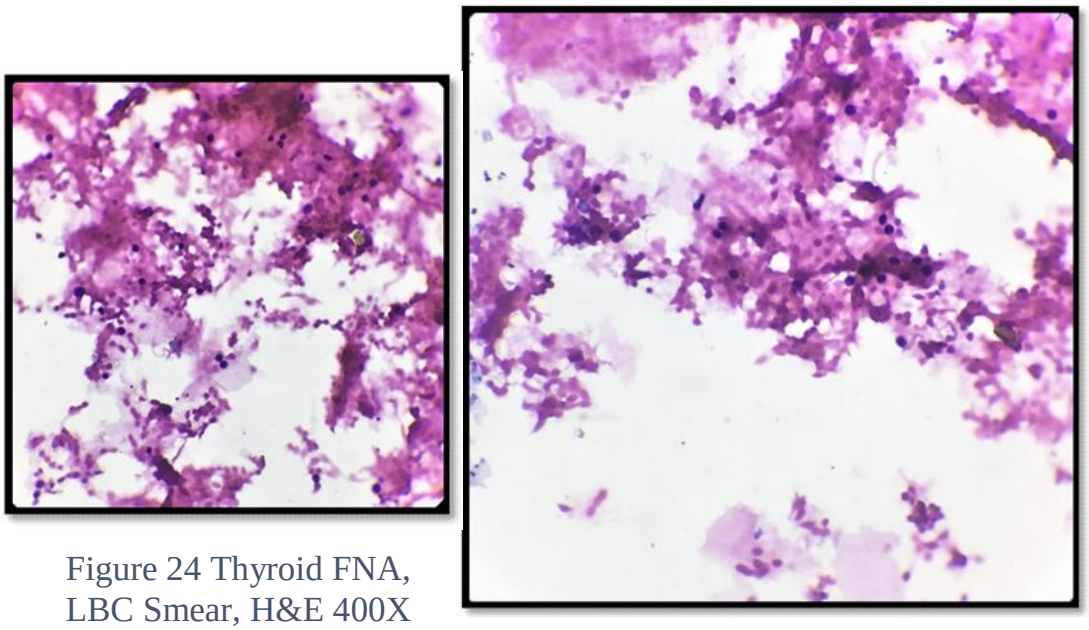


Figure 24 Thyroid FNA, LBC Smear, H&E 400X

Singly scattered follicular epithelial cells in a clean background with few RBCs and colloid

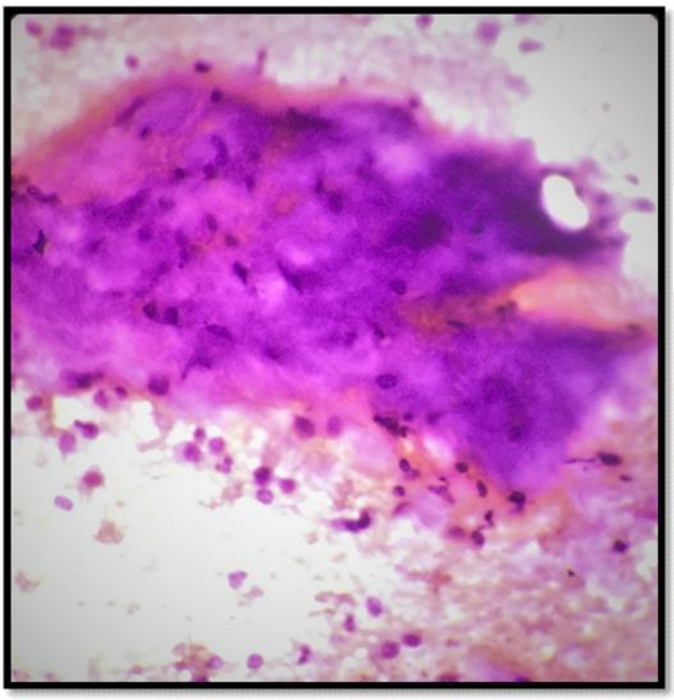
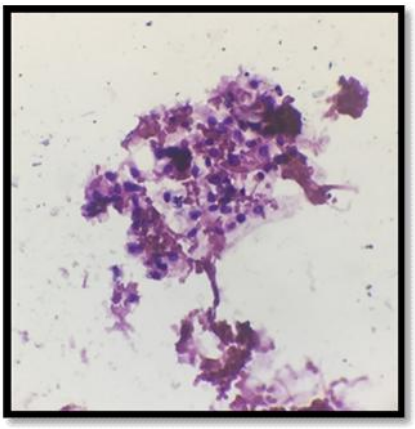


Figure 25 Salivary gland (Parotid) FNA, CS, PAP 400X

Chondromyxoid matrix and singly scattered ductal epithelial cells in a haemorrhagic background

Diagnosis:
Pleomorphic adenoma



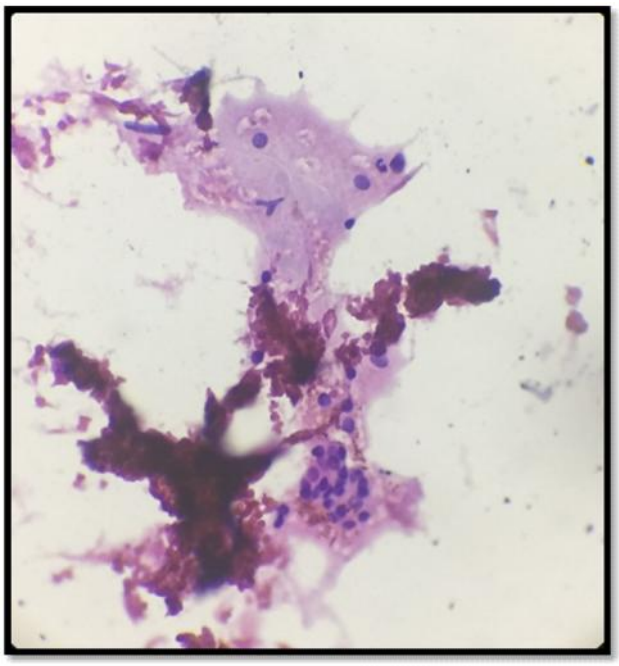


Figure 26 Salivary gland (Parotid) FNA, LBC PAP 400X

Chondromyxoid matrix, poorly cohesive clusters and singly scattered cells in a clean background.

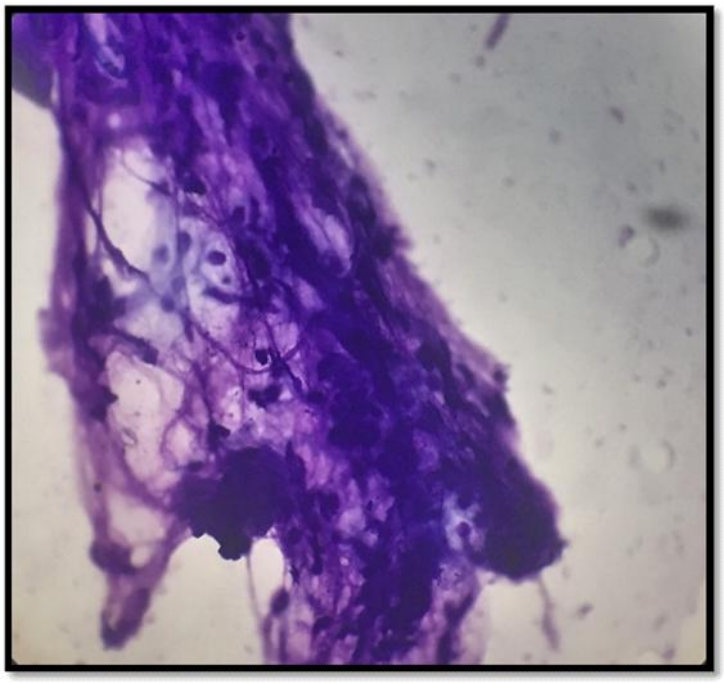


Figure 27 Soft tissue FNA, CS smear, Giemsa 400X

Fragments of mature adipose tissue comprised of abundant vacuolated cytoplasm with central to eccentric nucleus.

Diagnosis:
Lipoma

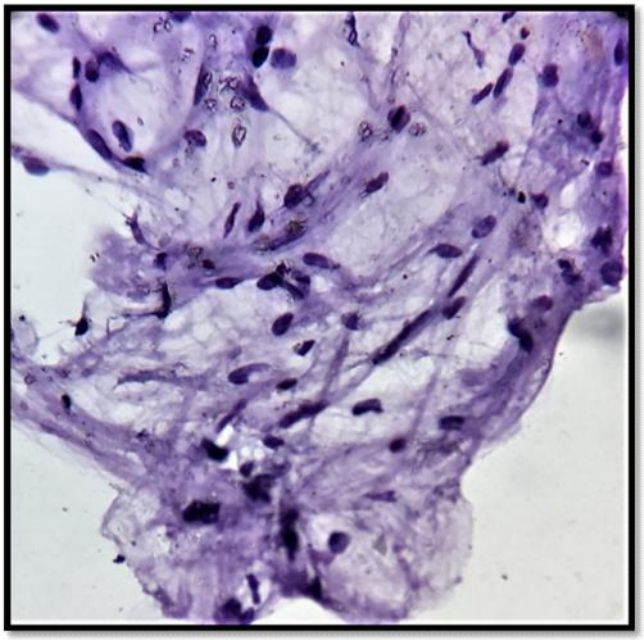


Figure 28 Soft tissue FNA, LBC, Giemsa, 400X

Fragments of mature adipocytes with minimal overlapping

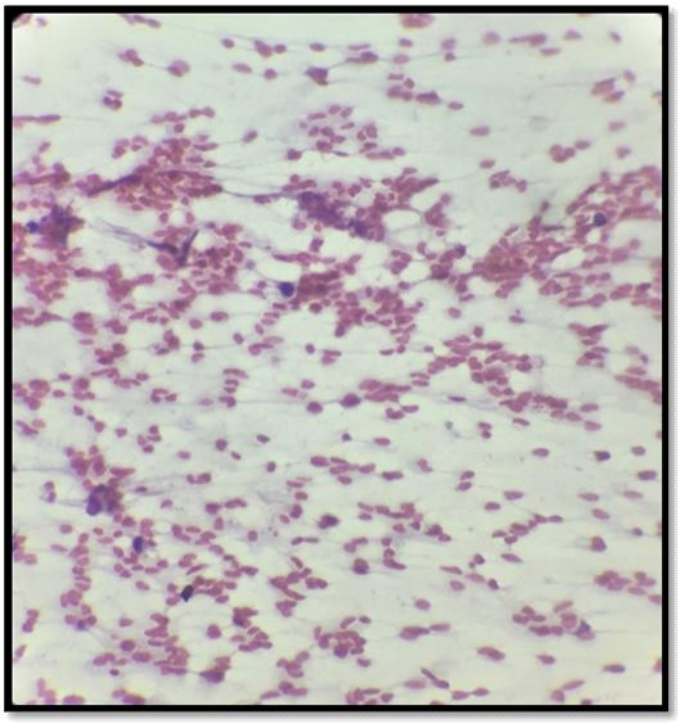


Figure 29Thyroid FNA, CS Smear, PAP 400X

Singly scattered follicular epithelial cells in a background of RBCs.

Diagnosis:
Colloid Goitre

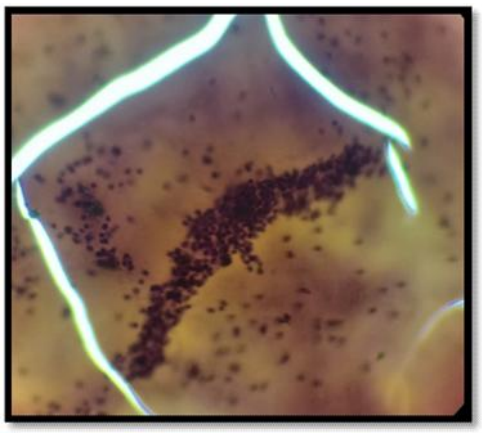
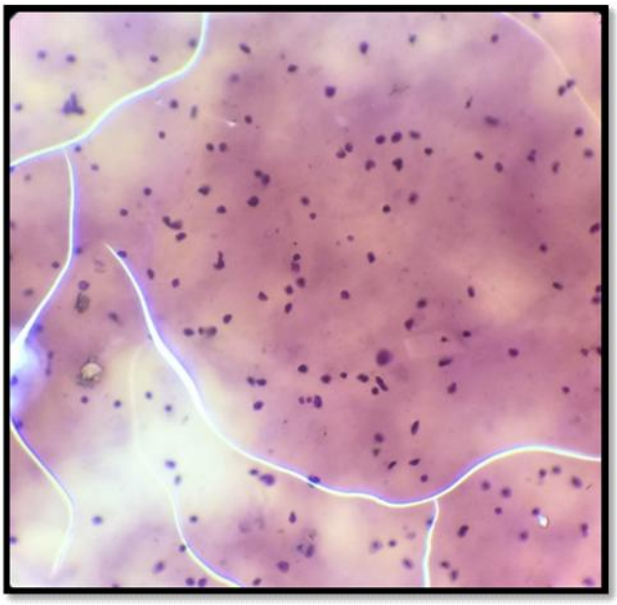


Figure 30 Thyroid FNA, LBC smear, PAP 400X

Singly scattered thyroid follicular epithelial cells in a background of colloid. Inset: Clusters of thyroid follicular cells in a different field of view.



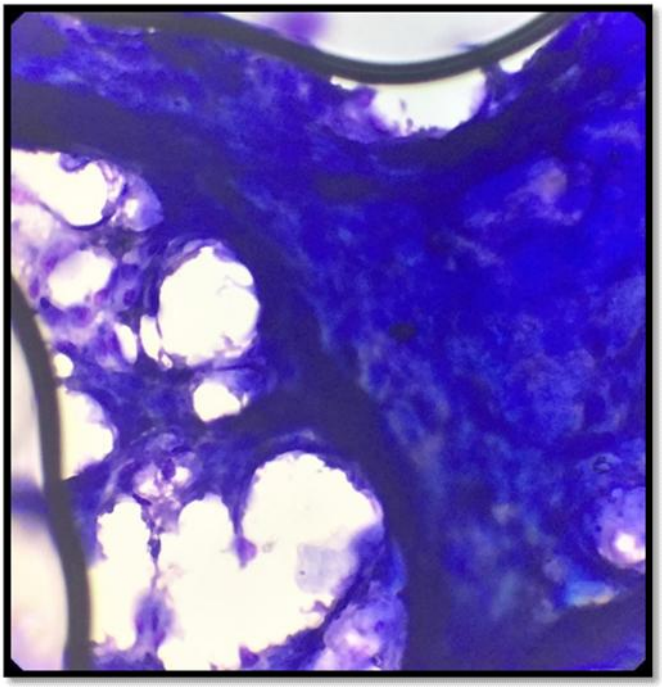


Figure 32 Guided Liver FNA, CS Smear Giemsa 400X

Cluster of cells with overlapping.

Diagnosis:
Hepatocellular carcinoma

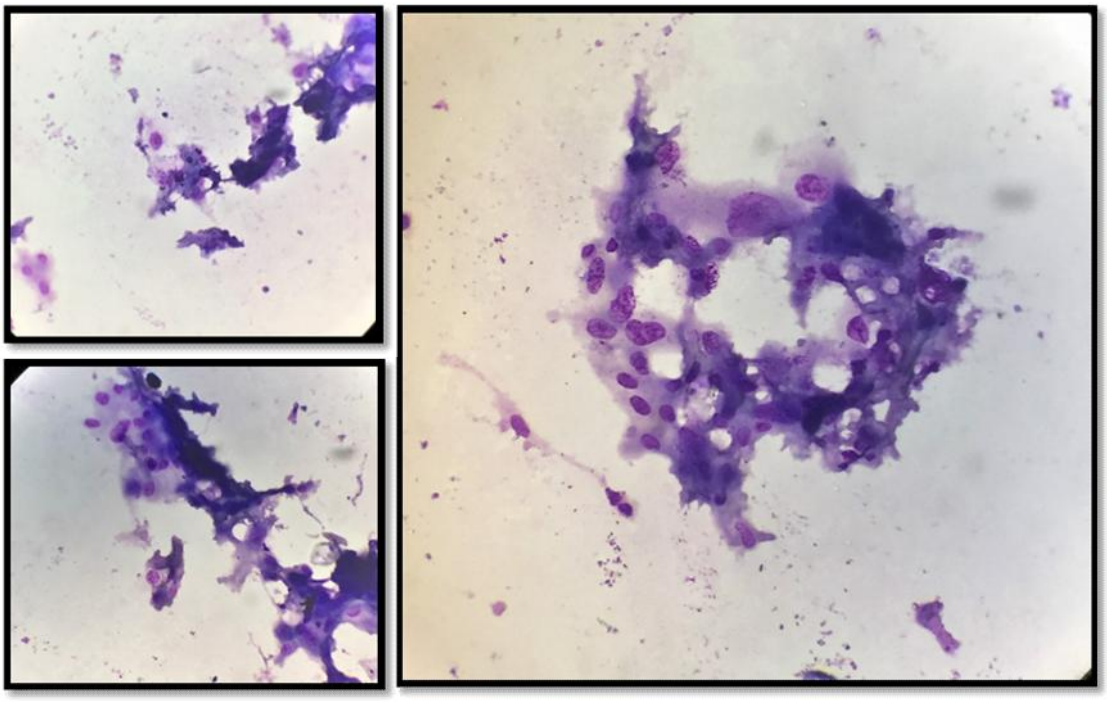


Figure 31 Guided Liver FNA, LBC, Giemsa 400X

Monolayered sheets of cells

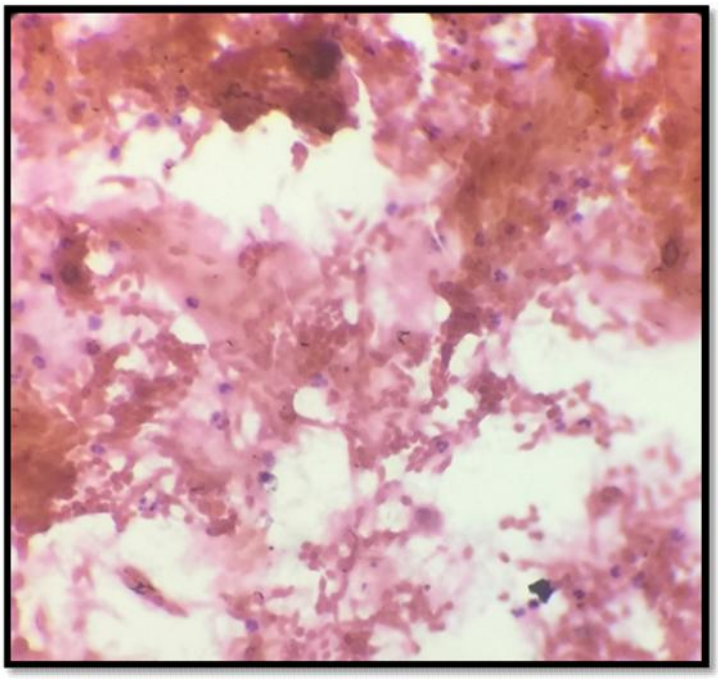


Figure 33 Skin FNA
CS Smear, PAP 400X

Singly scattered round to oval cells in a background of RBCs

Diagnosis (Cell block preparation)
Benign skin adnexal tumour

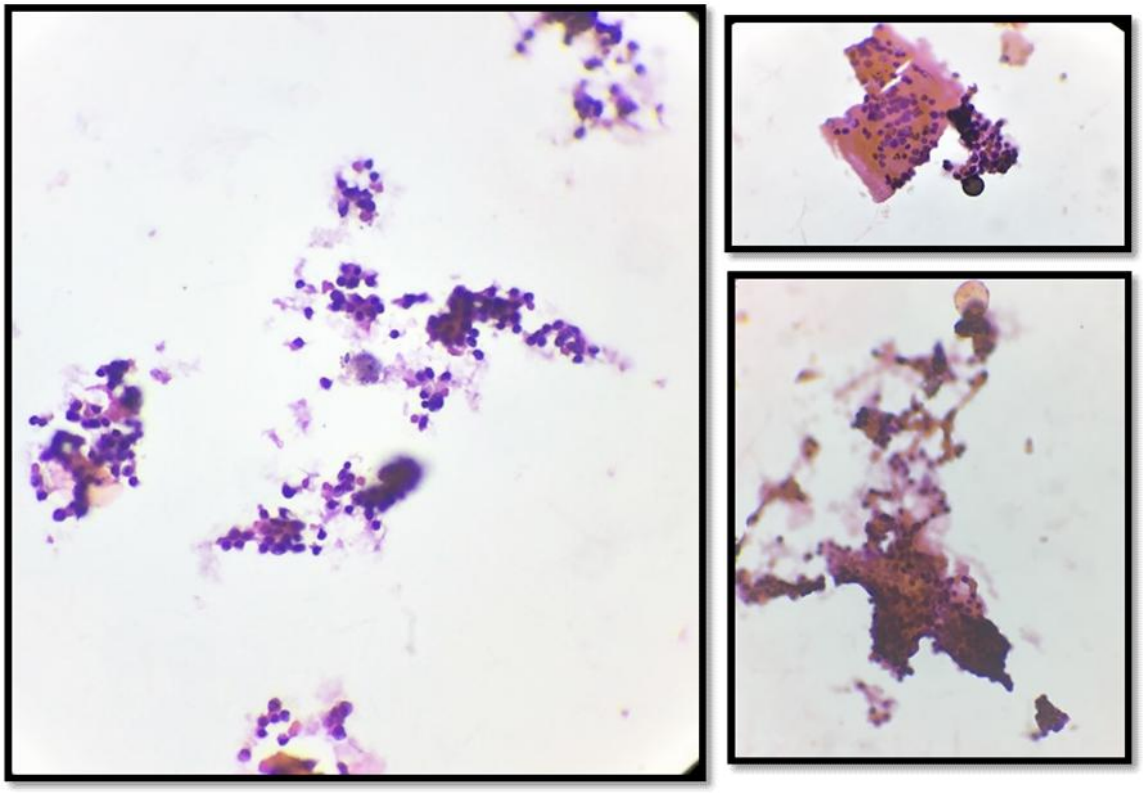


Figure 34 Skin FNA LBC Smear, PAP 400X

Clusters and singly scattered cells in a clean background. Inset clusters of cells in other fields.

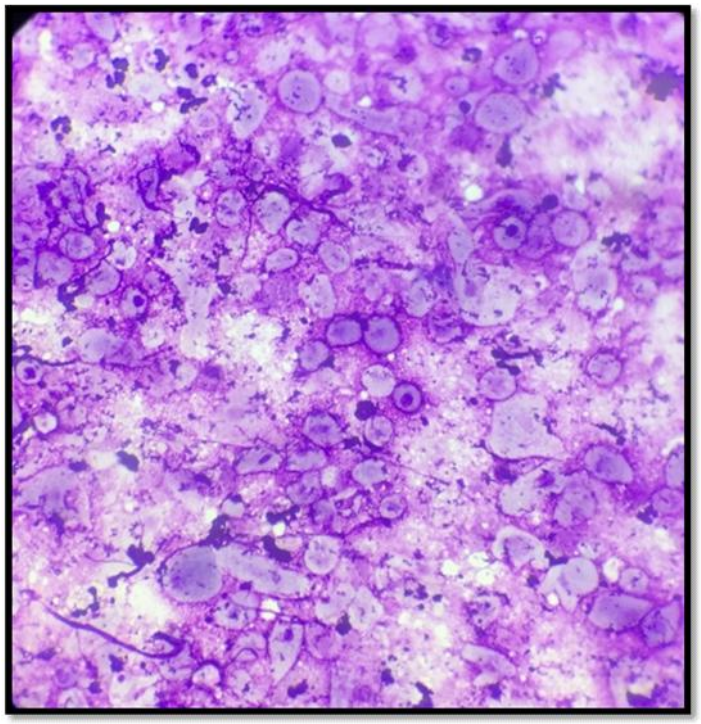


Figure 36 Skin FNA, CS Smear Giemsa 400X

Smear with high cellularity showing pleomorphic cells, intra and extra cellular pigment.

Diagnosis:
Malignant melanoma

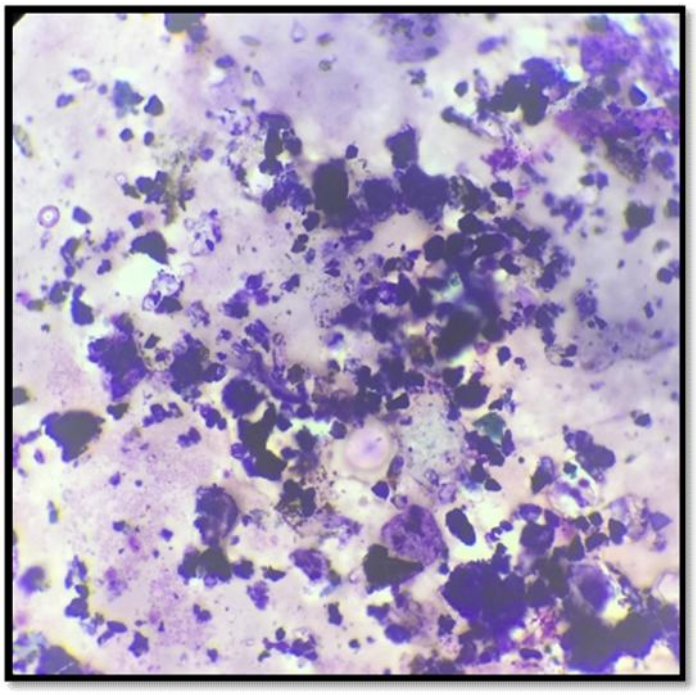


Figure 35 Skin FNA, LBC Smear, Giemsa 400X

Black coloured pigment noted intra and extracellularly.

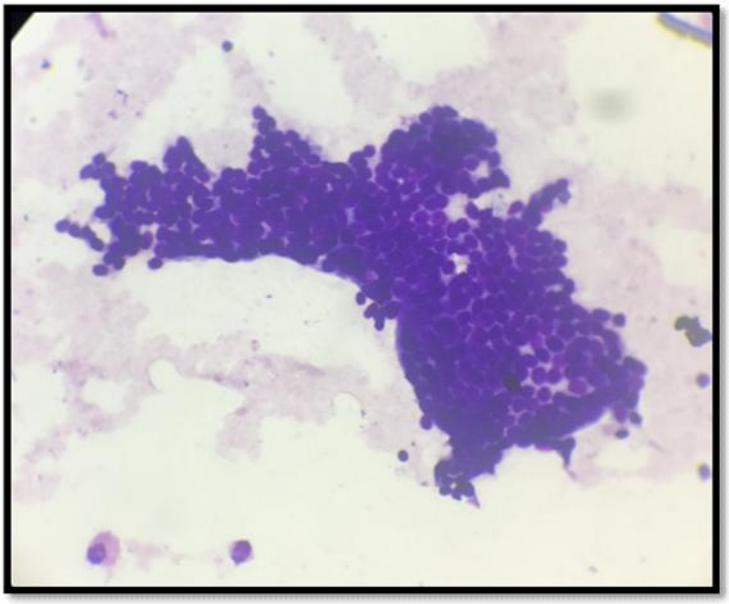


Figure 38 Thyroid FNA, CS Smear, Giemsa 400X

Cluster of cells with papillary pattern, anatomical borders and overcrowding.

Diagnosis:
Papillary carcinoma Thyroid

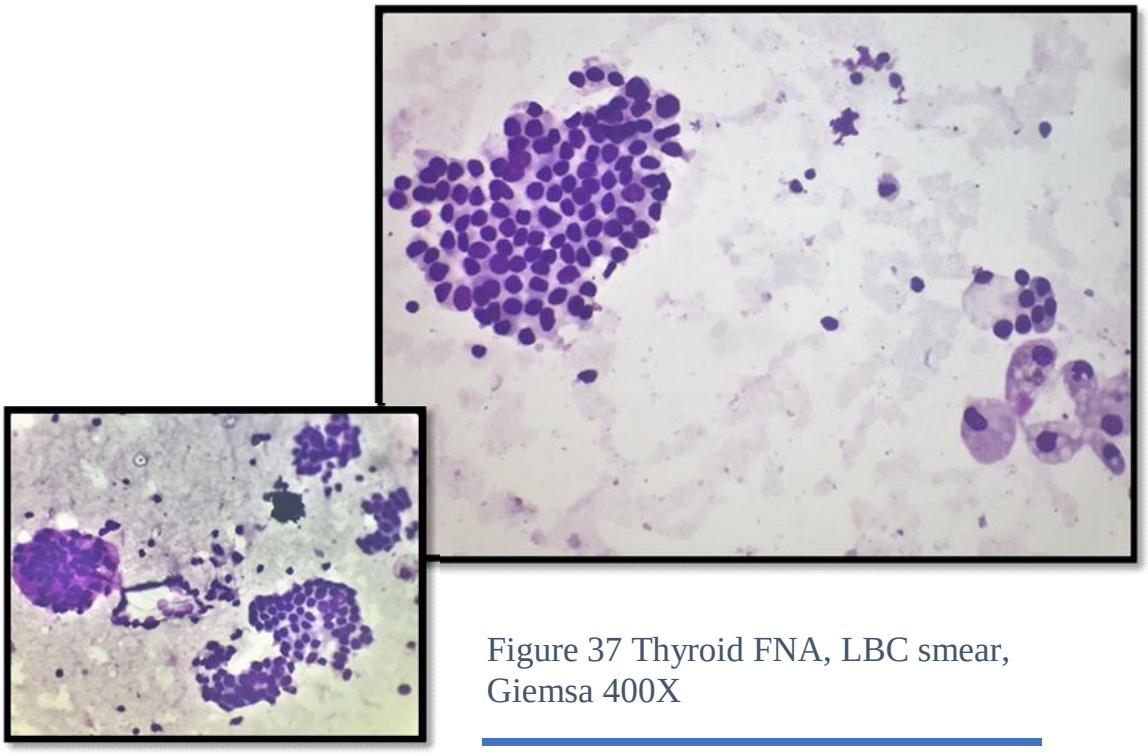


Figure 37 Thyroid FNA, LBC smear, Giemsa 400X

Cellular smears with cluster of cells with anatomical borders (Inset) and cyst macrophages.

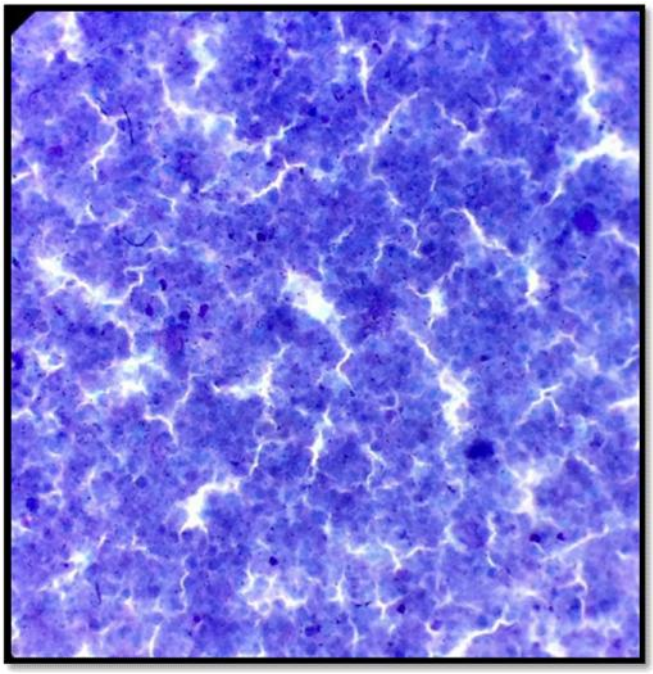


Figure 40 Lymph node FNA, CS Smear, Giemsa 400X

Sheets of degenerated and singly scattered cells with necrotic debris.

Diagnosis:
Necrotising lymphadenitis

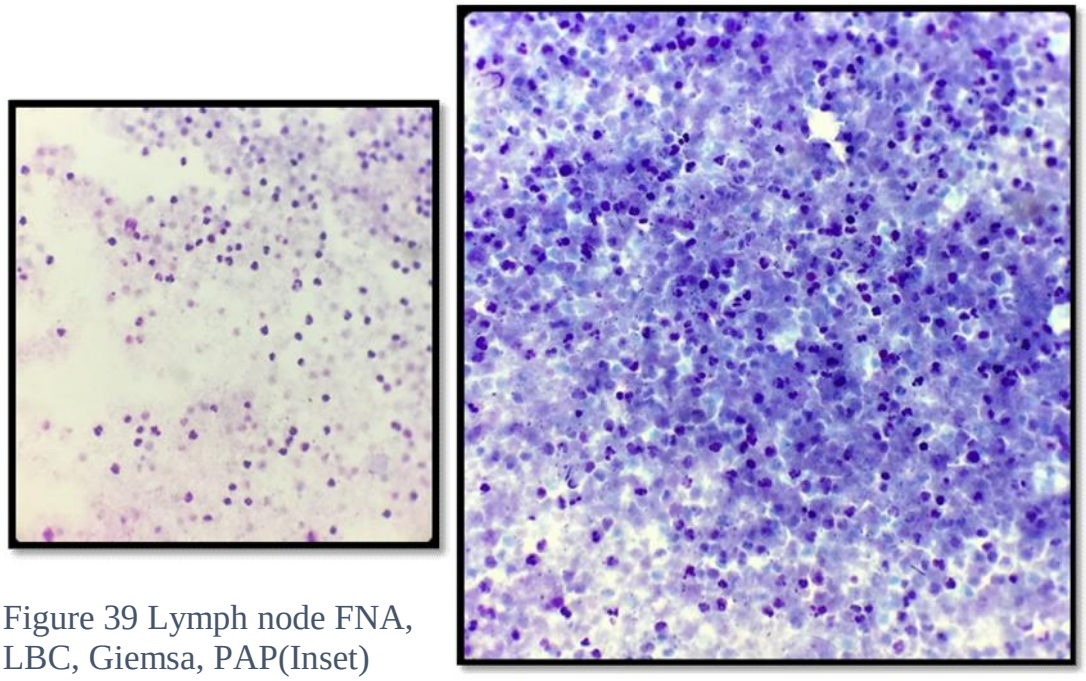


Figure 39 Lymph node FNA, LBC, Giemsa, PAP(Inset) 400X

Singly scattered lymphocytes in a necrotic background with minimal overlapping.

DISCUSSION

FNAC has proven to be a reliable, repeatable and rapid diagnostic OPD procedure which is minimally invasive but at the same time provides maximum information in cases where it is indicated or is a procedure of choice. It has gained solid ground as a routine pre-operative investigation and decides the line of patient management too based on the result. With the advent of the liquid based cytology, objective immunocytochemistry and other ancillary techniques the procedure has gained further importance to the extent that it has replaced biopsy confirmation in certain conditions and the definitive treatment procedures are carried out adding a significant benefit to the patient in terms of earlier diagnosis and minimising discomfort.[12]

Even though a fine needle is used to obtain the material, this is still an uncomfortable situation/procedure for the patient if the test has to be repeated. There are criteria for adequacy of the sample obtained like minimum number of passes required and number of clusters seen to call a sample as inadequate for opinion or non-diagnostic. For example, sample will be reported as inadequate for opinion/ non-diagnostic in case of lesions like thyroid if the minimum adequacy criteria of six groups of follicular cells with ten cells each were not met. In addition to this problem, the rate of the inadequate for opinion in case of thyroid swellings as per various studies from different countries is around 30%. With such statistics and the need for processing the specimen material in entirety finds an area of interest in the present scenario. The needle remnants which were otherwise discarded added to the diagnosis in the study done by Gupta et al highlighting the need for processing the material in the hub.[3]

The integration of imaging guidance into FNA has increased the access to many deep-seated organs too. This has increased the utility of the FNA to the extent that it has become indispensable to ignore or omit prior to surgery in case of solid masses/lesions. With such continuous increasing importance for the technique, both to the clinicians and pathologists alike has enabled them to provide the best possible treatment protocol for the patient at the earliest.

As it is being used in wide variety of clinical scenarios the needle length too varies ranging from half an inch to one and half inch in blind procedures and much lengthier needles like lumbar puncture and specialised needles causing retention of the useful diagnostic material within the needle.[3] The interest in processing this material is steadily increasing and is gaining traction with the utilisation of LBC in toto for entire FNA sample where resources like Thinprep and Surepath technologies are available and utilisation of economical cytocentrifuge and even MLBC techniques where the expensive LBC techniques are not available . Various authors studied the application of the technologies such as LBC cytocentrifuge and MLBC with respect to utilisation of needle hub material in this era of rapidly evolving ancillary techniques for better characterisation and diagnosis of the lesions and making Pathology more objective.[1,3]

The needle hub material might vary from few scattered cells to clusters which helps in augmenting the adequacy criteria and providing the additional information aiding in diagnosis.[1] With the comparatively increased availability of the cytocentrifuge LBC in laboratories this technique has become accessible. Using LBC techniques (Thinprep, Surepath) per se for the processing of entire sample material

after FNA is not economical at many institutes presently, due to the high single time overhead and recurring operating costs in the form of consumables and reagents.

Unlike in the last three to four decades, FNA aspirate is being subjected to multiple stains and immunological investigations in current cytology practice wherein every possible chance to cost effectively utilise all the material available is being made. Authors have even studied the utilisation of scraping the material from the stained FNA slides when there is a need for performing additional investigations but the sample was not available due to various reasons.[72]

In such scenarios, interest in needle hub remnants has peaked to the extent that Manual liquid based cytology is being used to process these materials without adding significant cost on a per case basis. However, the disadvantage of the MLBC technique is that there is a learning curve for the procedure and additional steps in the processing which might add to the turnaround time. So, using cytocentrifuge brings the advantages of LBC at a relatively lower cost per case without the need for special training even though it is not as economical as CS and manual methods of LBC. Processing of hub remnants, be it by cytocentrifuge or MLBC increases the data needed to correctly evaluate the advantages and disadvantages in terms of various cytological parameters and cost effectiveness.

Many of the institutions practice the conventional method for preparation of smears after FNA by expressing the material on to the glass slides. The remaining material in the needle hub is partly taken out, although not completely, by using another needle or tapping on to the clean glass slide. The needle is then discarded even though there is grossly visible blood mixed material in most of the cases which might contain diagnostically important material. The evaluation of the LBC protocols

provides data which helps in analysing whether processing the remnant material is meaningful in terms of presence of useful diagnostic material and also helps in analysing whether the process is cost effective and is it optimal use of valuable human resources.[1,3]

Gupta et al[3] considered that processing this material using cytospin adds to the diagnosis. To evaluate this, they have studied the rinsing of hub remnants after FNAC procedure where in the average number of passes were 2-3. Once FNA was done using a 20-ml syringe, smears were prepared by conventional method by expressing the material on to a glass slide. Then the remnants in the needle hub were processed using 2.5ml of normal saline. The material was collected into a tube and the suspension was further processed by cytocentrifugation. The authors mentioned that they were able to prepare one or more LBC smears which were stained using Giemsa stain.

Among a total of 100 cases included in their study, FNA of lymph node were 52% with more than half of the sample size. Followed by breast (n=24), soft tissue (n=12) and thyroid (n=8).[3] This distribution of cases in terms of the most numerous lesions is in line with the present study except for the change that thyroid FNA is the predominant group.

Pawar et al[1] in their study from a rural tertiary care centre made an innovative effort by using MLBC technique without adding to the cost of the processing significantly. This method can be used in centres without access to the cytocentrifuge to obtain LBC smears using a table top centrifuge. They have evaluated the utility of processing the needle hub remnants using routine FNA procedure to study their technique and procedure of MLBC. Once FNA was done the CS smears were

prepared by spreading the material expressed from the needle on clean glass slides. The material in the hub was used for preparing MLBC smears.

They have created the test tubes for processing in the centrifuge by using routinely stocked items in the laboratory i.e. paraffin wax and filter papers. The paraffin wax formed the bottom most part of the test tube. Upon this paraffin wax a precisely cut filter paper was placed carefully. Such paraffin and filter paper stacked test tube formed the receptive holder for the needle hub remnants. Using phosphate buffer (2ml, pH – 7.4) in a syringe to which the FNA needle after preparation of CS smears was attached and this buffer solution was expressed into the test tube there by collecting hub remnants.

Once the material was expressed into the tube, they were processed in a routine centrifuge. They have tested various speeds and found that 1200rpm for a duration of 8 minutes was ideal. This method and the utility of processing of hub remnants were evaluated in 50 cases. Majority of the cases were from the breast FNA with 21 cases.

In the present study, processing of hub remnants (LBC Smears) was done in a total of 103 cases. In the initial 10 cases, the residual material in needle hub was collected by aspirating 3ml of 95% ethanol into the syringe and the material was flushed into the plain test tube. Glacial acetic acid was added in the proportion of one ml for three ml of 95% ethanol in cases of haemorrhagic aspirates. Then the sample was allowed to settle for one hour. Using micropipette 100 microliters from the sediment portion was aspirated and smears were prepared using Cytospin with the settings 1500 rotations per minute for a period of 5minutes. The smears were stained using PAP and Giemsa staining techniques. In these cases, it was observed that after

pipetting 200 microliters, material was still present in the tube. The other problem identified was centrifugation induced enlargement of cell size and poor nuclear staining quality.

Hence, after 10 cases the following changes were made in the technique of needle hub cytospin smear preparation. Aspiration of 1-1.5ml of 95% ethanol instead of 3ml of ethanol. Additional unprocessed material found in the tube after aspirating 200 microliters was further processed by preparing additional smears. The glacial acetic acid concentration (1ml for 3ml of ethanol and residual material) has yielded in changes of the chromatin which has distorted nuclear morphology. This was an observation made in comparison with the smears without the glacial acetic acid. So, 20 microliters of glacial acetic acid were added to 100 microliters of rinse material in all the later cases. The settings of the cytocentrifuge were changed from 1500 to 900 rpm and the duration was reduced from 5 minutes to 4 minutes.[3]

After making the said changes, the nuclear morphology was better and cellular distortion due to centrifugation was reduced at 900 rpm and 4 minutes.

Table 44 Comparison of cytomorphological features of present study with other author studies.				
Parameters	Gupta et al[3]		Present Study	
	LBC	CS	LBC	CS
Cellularity	90%	74%	95.1%	68.9%
Satisfactory Staining quality	100%	100%	92.3%	97.2%
No Cellular Degeneration	72%	68%	87.4%	69.9%

In comparison to the study done by Gupta et al[3] who also used cytocentrifuge to process the needle hub remnants, the cellularity yield in the present study was at 95.1% whereas the LBC smears in their study had 90% cellularity. In the present study with the significant number of cases from thyroid FNA LBC has proven to be of immense value by adding to the diagnosis in 9 cases where CS smears had no cellularity. However, Pawar et al while evaluating MLBC technique for processing hub remnants had documented the decreased cellularity in comparison to conventional method all the while maintaining that there was good material which aided in the diagnosis. The stated reason for this was that material was used to prepare CS smears followed by LBC smears. [3,1]

Gupta et al[3] found that there was 100% good staining characteristics in LBC. Whereas in the present study, the staining characteristics were good in 92.3% of the cases. This was due to the addition of glacial acetic acid in the proportion of 1ml for three ml of rinse material which amounts to 33% of rinse material, in the initial ten cases whereas after bringing down the proportion to 20% after trying various

combinations below the 33% like 30%, 25%, 20 and 10%. 10% of the glacial acetic acid was not lysing the RBCs completely whereas the 20% proportion was ideal in providing clean background without interfering the staining process.

In the present study, cellular preservation was good in 87.4% of the cases which was at 72% in the study done by Gupta et al. The nuclear morphology and background were on par with the CS smears in the present study, which was also a similar finding in studies done by Gupta et al and Pawar et al.[1,3]

In three cases, two were of ultrasound guided FNA and one case of malignant melanoma, the number of smears prepared was 8-12. Thus a 50% increase in the availability of additional smears from the needle rinse material was noted in case of guided and malignant FNA. However, in malignant melanoma there was overlapping of cells and the pigment accumulation in LBC smears in comparison to CS smears. This pigment accumulation was interfering with the interpretation of cellular morphology.

The cellularity in malignant smears was high in both LBC and CS smears, but the overlapping was less and staining quality was optimal in LBC smears in comparison with CS smears as seen in Figure 32 and Figure 31. This could be due to the monolayer formation in LBC, a technique based advantage and also better control in terms of titrating the number of microliters of sample material that can be added to the Cytofunnel. With the varied range of 100 to a maximum of 500 microliters per channel in the Cytofunnel gives a better morphology and to avoid overlapping.[62]

In lymph node FNA as stated by previous studies, the partial loss of necrotic debris and lymphoglandular bodies in background of LBC smears was observed in the present study too as represented in Figure 39 and Figure 40. In epidermal cyst, the CS

smears were having better cellularity in comparison to LBC smears. The haemorrhagic aspirates in the CS smears had plenty of RBCs obscuring the cellular morphology whereas this was not an issue in LBC smears which had the additional advantage to titrate the glacial acetic acid along with the collecting fixative medium.

Limitations of this study: Histopathological correlation was not done, addition of glacial acetic acid only to the LBC smears due procedural advantage of LBC which was not found in CS smear preparation and distribution of material during smear preparation in the order of CS smears followed by LBC without randomisation.

LBC smear preparation from the hub remnants added to the diagnosis and had better cellularity and comparative morphological features in terms of cellular degeneration, nuclear morphology and staining quality. However, while analysing the LBC smears and processing of the needle hub remnants the one significant part which adds to the total cost of processing is cytofilt cards. A box of cytofilt cards containing 200nos retails at 5000Rs. This brings the cost per smear to 25Rs which drains on the resources available. So, we have developed the post it and tissue paper cytofilt card (Figure 41)during the process of this study to reduce the cost. Post it tissue paper cytofilt card brings down the cost of a smear to under a rupee(40Paise).

LBC smears prepared by using the double cytofunnels have the added advantage of performing two different IHC stains as two smears are prepared further apart. With the comparable smear quality and morphology and slightly better cellularity there is a need to further evaluate the processing of needle hub remnants with a larger sample size and cost analysis to be done along with cytomorphological analysis.

Figure 41 Steps of Preparation of Post it and tissue paper cytofilt card, extreme right Manufacturer provided Cytofilt card for comparison.

SUMMARY

A total of 103 cases were included in the study. The predominant age group was in the 20-30 years with 40% of cases. The significant location of the cases included were from thyroid followed by breast, lymph node and soft tissue swellings. Both conventional and LBC (Cytocentrifuge) smears from needle hub remnants were prepared for all cases. The smears were compared for parameters like cellularity, staining quality, nuclear preservation, cellular morphology and background.

The needle hub remnants yielded cellularity in 95.1% of LBC smears in comparison to conventional smears having 68.9% cellularity with a significant statistical difference. The advantage of LBC which provides a clean background which was seen in the present study too. The other parameters like cellular degeneration, nuclear preservation and staining quality were comparable to CS smears. The needle hub remnants which were discarded otherwise added to the diagnosis in thyroid FNA where one third of cases had a final impression of inadequate for opinion in CS as stated by various studies.

By processing the needle hub remnants using cytocentrifuge, enabled us to have advantages of LBC, which have been carried over to the FNA smears which wouldn't have been possible if discarding them was followed. The cellularity in malignant and the non- malignant lesions was better in LBC technique indicating that processing of the hub remnants has better chance of yielding cells.

However, even with the advantages of LBC, it has added to the cost in the form of cytofilt cards which amounts to a minimum of Rs.25 per smear which makes the option of processing the specimen not so attractive even if it helps in providing

material for ancillary techniques. This disadvantage can be warded off by using Post-it tissue paper cards which has been utilised during the process of this study and which brings down the cost per smear from Rs.25 to 40Paise.

CONCLUSION:

FNAC is now the first line of investigation in many of the easily accessible and superficial swellings. It has retained its importance as one of the fastest and minimally invasive procedure providing a rapid diagnosis in most of the cases over the years.

Additional material from this needle hub remnants forms a significant source to increase the adequacy of the material and thus aids in the diagnosis and also provides material for ancillary investigations which are seeing a steady rise over the years. Thus, the processing of needle hub remnants is finding its importance and place in the Cytology investigation.

This argument loses the impact when the economics of processing the remnants is taken into account along with the potential benefits. LBC techniques like Surepath and Thinprep are beyond the reach of many of the institutions to be used as a method of analysis of aspirated material. Whereas in case of cheaper alternative cytospin technique, with some of the advantages of the LBC techniques, also had a disadvantage of cost of the consumables in the form of cytofilt cards. Cost of this manufacturer provided cytofilt cards is matching with the cost of the FNAC procedure itself. In our study, we found that utilisation of the cheaper alternatives like Post-it tissue paper cytofilt cards bring the processing of the needle hub remnants within reach and brings the advantage of processing the material and augmenting the adequacy criteria and diagnosis.

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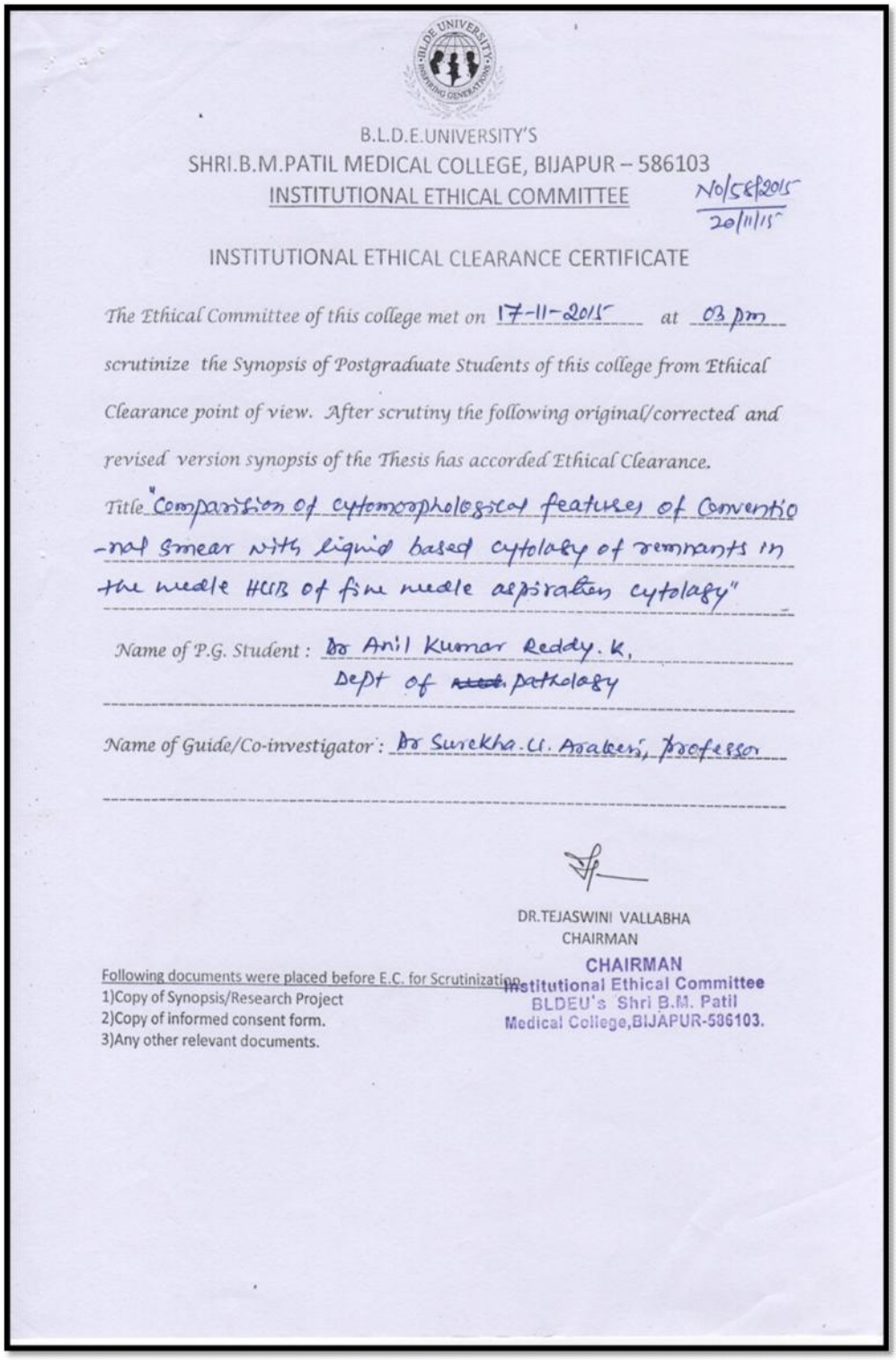
Annexure – I

INSTITUTIONAL ETHICAL COMMITTEE CLEARANCE CERTIFICATE



B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE

No/58/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "Comparision of cytomorphological features of Conventio -nal smear with liquid based cytology of remnants in the needle HUB of fine needle aspiration cytology"

Name of P.G. Student : Dr Anil Kumar Reddy. K, Dept of ~~Med.~~ Pathology

Name of Guide/Co-investigator : Dr Surekha. M. Asaker, Professor

DR.TEJASWINI VALLABHA
CHAIRMAN

CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

Annexure - II

B.L.D.E.U's SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTER, VIJAYAPUR - 586103

RESEARCH INFORMED CONSENT FORM

I, the undersigned,_____ , S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and

not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date: Place

Annexure - III

PROFORMA FOR STUDY

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		
Presenting Complaints	:		
Past history	:		
Personal history	:		
Family history	:		
Treatment history	:		

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent

Edema present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

FNAC

CS Report:

LBC report:

Comparison of cytomorphology for CS and LBC smears

S.no	PARAMETERS	CS	LBC
1.	**Cellularity**		
	Low		
	Medium		
	High		
2.	**Staining quality**		
	Poor		
	Average		
	Good		
3.	**Background**		
	Haemorrhage		
	Clear		
4.	**Cellular degeneration**		
	Present		
	Absent		
	Poor		
5.	**Nuclear Preservation**		
	Poor		
	Average		
	Good		

ANNEXURE-IV

KEY TO MASTERCHART

S.No	Serial Number
OP	Out Patient Number
IP	In Patient Number
FNAC No	Fine Needle Aspiration Cytology Number
LBC	Liquid Based Cytology
CS	Conventional Smear
Cellularity 1	Low Cellularity
Cellularity 2	Medium Cellularity
Cellularity 3	High Cellularity
Cellularity 0	No cellularity
Staining Quality 1	Poor Staining Quality
Staining Quality 2	Average Staining Quality
Staining Quality 3	Good Staining Quality
Staining Quality 0	Staining Quality could not be assessed
Background 1	Haemorrhagic Background
Background 2	Clear Background
Background 3	Colloid
Cellular degeneration 1	Cellular degeneration Present
Cellular degeneration 2	Cellular degeneration Absent
Cellular degeneration 3	Cellular degeneration Poor
Nuclear Preservation 1	Poor Nuclear Preservation
Nuclear Preservation 2	Average Nuclear Preservation
Nuclear Preservation 3	Good Nuclear Preservation

MASTER CHART

												LBC					CS				
S.No	OP/IP No	Lab No	FNAC No	Year	Patient Name	Age	Sex	Chief complaint	Organ	Diagnosis	Type	Cellularity	Staining Quality	Background	Cellular Degeneration	Nuclear Preservation	Cellularity	Staining Quality	Background	Cellular Degeneration	Nuclear Preservation
1	142007	68126	1566	2017	Laxmi Madagond	11	F	Pre auricular swelling	Lymph node	Granulomatous Lymphadenitis	TB	2	3	2	2	3	2	3	1	2	3
2	411088	185076	2810	2016	Sham Chavan	42	M	Mass over left lateral border	Tongue	Squamous cell carcinoma	Malignant	2	2	1	1	3	2	3	1	2	3
3	413033	186048	2821	2016	Vivekanand Patil	36	M	Left cervical swelling	Lymph node	Granulomatous Lymphadenitis	TB	1	3	1	2	3	2	3	1	2	3
4	39369	186580	2882	2016	Kallappa Gugadaddi	85	F	Lump breast	Breast	Intraductal carcinoma	Malignant	2	3	1	3	3	0	0	0	0	0
5	37704	177581	2689	2016	Rakamabai	60	F	Right breast lump	Breast	Fat necrosis	Inflammatory	2	3	1	2	2	0	0	0	0	0
6	37276	176993	2678	2016	Somanath	32	M	Left thigh swelling	Soft tissue swelling	Lipoma	Benign	1	1	1	2	2	0	0	0	0	0
7	402962	182111	2579	2016	Lokesh Math	27	M	Right Cervical swelling	Lymph node	Reactive lymphadenitis	Benign	1	1	1	2	2	0	0	0	0	0
8	391301	178263	2724	2016	Sangamesh	20	M	Left epididymal cyst	Epididymis	Chronic epididymitis	Inflammatory	2	2	2	2	3	0	0	0	0	0
9	397217	178665	2703	2017	Parvati	50	F	Right lobe sweling	Thyroid	Colloid goitre	Benign	1	3	2	0	0	0	0	0	0	0
10	402166	18583	2760	2016	Anita Basri	23	F	Supraclavicular Right	Lymph node	Necrotising Lymphadenitis Suggestive of Tuberculosis	TB	0	0	0	0	0	2	3	1	2	3
11	133679	64016	1477	2017	Sumakka	37	F	Right Breast Lump	Breast	Benign breast disease	Benign	0	0	0	0	0	1	3	1	2	3
12	11238	57346	1346	2017	Yamanawwa	75	F	Left Breast	Breast	Atypical ductal hyperplasia	Benign	1	3	2	2	3	1	2	1	2	3
13	133933	64186	1479	2017	Chandrayya alagur	40	M	Swelling infront of neck	Thyroid	Colloid goitre	Benign	2	2	2	2	3	1	3	2	2	3
14	39336	186482	2893	2015	Nagawwa	26	F	Axillary swelling	Lymph node	Granulomatous lymphadenitis	Inflammatory	1	3	2	2	3	1	2	1	2	3
15	449117	187731	2921	2015	Ramya K	5	F	Post auricular swelling	Lymph node	Reactive lymphadenitis	Inflammatory	2	3	2	2	3	1	2	1	2	3
16	148774	71963	1629	2017	Mananda	30	F	Pain in the breast	Breast	Acute mastitis	Inflammatory	3	3	2	2	3	3	3	2	2	3
17	30897	144355	2220	2015	Pramod	3	M	Neck swelling	Soft tissue swelling	Acute suppurative lesion	Inflammatory	3	3	2	2	3	3	3	1	2	3
18	154436	74880	1687	2017	Kaveri Guttadar	18	F	Swelling infront of neck	Thyroid	Hashimotos Thyroiditis	Inflammatory	2	2	2	2	2	1	2	1	2	3
19	155053	75027	1688	2017	Vajirbee Patiwale	70	F	Swelling over left eyebrow	Eye brow	Skin adnexal tumour	Benign	3	3	2	2	3	3	3	1	2	3
20	156649	75774	1712	2017	Narasappa	45	F	Swelling infront of neck	Thyroid	Multinodular goitre with papillary hyperplasia	Benign	2	3	2	2	3	1	3	2	2	3
21	157692	76348	1729	2017	Nanasab Dhanawadw	70	M	Swelling right Sternoclavicular joint swelling	Soft tissue swelling	Acute suppurative lesion	Benign	2	3	1	2	3	3	3	1	2	3
22	15108	76855	1744	2017	Shivashankar	70	M	Neck swelling left side	Parotid swelling	Sialadenosis	Benign	0	0	0	0	0	2	2	1	2	3

23	15063	76527	1743	2017	Shantabai	50	F	Swelling infront of neeck	Thyroid	benign cystic lesion	Benign	1	3	2	2	3	0	0	0	0	0
24	15108	76855	1744-B	2017	Shivashankar-B	70	M	Neck side left side-E	Lymph node	Metastatic squamous cell carcinoma	Malignant	3	3	1	2	3	0	0	0	0	0
25	6066	30967	746	2016	Harini S N	40	F	Neck swelling	Thyroid	Colloid goitre	Benign	1	2	1	2	2	0	0	0	0	0
26	163333	79187	1786	2017	Basamma	25	F	Right side neck swelling	Parotid swelling	Pleomorphic Adenoma	Benign	2	3	2	2	3	0	0	0	0	0
27	163317	79178	1787	2017	Laxmi laxman Rao	55	F	Swelling in the right iliac crest region	Soft tissue swelling	Ganglion cyst	Benign	1	2	2	2	3	1	2	2	2	3
28	163350	79235	1788	2017	Malappa	50	M	Right inguinal region swelling	Lymph node	Reactive lymphadenitis	Benign	1	2	2	2	2	1	2	1	2	2
29	165645	80374	1815	2017	Shantabai Hadimani	53	F	Swelling infront of Neck	Thyroid	Colloid goitre	Benign	2	3	1	2	3	2	3	1	2	3
30	15579	81538	1848	2017	Siddamma B P	30	F	Swelling in the submandibular region	Sub mandibular gland	Sialadenosis	Benign	1	3	1	2	3	1	3	1	2	3
31	167231	81271	1842	2017	Rajashree	30	F	Right breast lump	Breast	Fibroadenoma	Benign	2	3	1	2	3	1	2	1	2	3
32	15598	81617	1859	2017	Laxmi Chandrakanth N	25	F	Swelling in Posterior triangle	Posterior triangle	Benign cystic lesion	Benign	1	3	2	2	3	1	2	1	2	0
33	344123	141676	2177	2016	Kavya Guddi	7	F	Right cervical region	Lymph node	Reactive lymphahdenitis	Benign	2	3	2	2	3	3	3	1	2	3
34	170105	82505	1873	2017	Shabana	23	F	Right breast Lump	Breast	Acute Mastitis	Inflammatory	3	3	1	2	3	3	3	1	2	3
35	170267	82614	1874	2017	Lalita Bosale	45	F	Swelling near right sternoclavicular joint	Soft tissue swelling	Benign cystic lesion	Benign	1	3	2	2	3		3	1	2	3
36	16257	82656	1875	2017	Laxmi N P	25	F	Left breast lump	Breast	Fibroadenoma	Benign	2	2	1	2	3	2	3	1	2	3
37	142007	68126	1566	2017	Laxmi Madagond	11	F	Left preauricular swelling	Lymph node	Granulomatous lymphadenitis suggestive of Tuberculosis	TB	3	3	1	2	3	3	3	1	3	3
38	413033	186048	2821	2016	Vivekananda Patil	26	M	Cervical Lymphnode	Lymph node	Granulomatous lymphadenitis suggestive of Tuberculosis	TB	2	3	2	2	3	2	3	1	2	3
39	402166	182583	2769	2016	Supriya H	23	F	Cervical region swelling	Lymph node	Reactive lymphadenitis	Benign	1	3	2	2	3	1	3	1	2	3
40	230443	113795	2503	2017	Yallappa	42	M	Swelling infront of neck	Thyroid	Nodular Goitre with cystic change	Benign	2	3	3	2	2	2	3	3	2	3
41	220821	108640	2406	2017	Lakshmibai Biradar	48	F	Swelling infront of neck	Thyroid	Nodular Goitre with cystic change	Benign	1	2	3	2	2	1	3	3	2	3
42	22520	112772	2482	2017	Lakshmibai Biradar	60	F	Swelling infront of neck	Thyroid	Hashimotos thyroiditis	Benign	1	3	3	2	3	2	3	1	2	3
43	220641	108248	2405	2017	Sharada Shetty	33	F	Swelling left side of neck	Lymph node	Reactive lymphadenitis	Benign	3	3	1	2	2	1	3	1	2	3
44	238701	117671	2577	2017	Rudresh Hunshagi	12	M	Left side of neck Swelling	Lymph node	Reactive lymphadenitis	Benign	1	2	2	2	3	0	0	0	0	0
45	234511	115883	2544	2017	Savita S Malli	36	F	Midline neck swelling	Thyroid	Benign thyroid lesion with cystic change	Benign	2	2	2	2	3	1	3	1	2	3
46	23078	115336	2543	2017	Devindramma Mallappa Metri	60	F	Midline neck swelling	Thyroid	Nodular Goitre	Benign	2	3	1	2	3	2	3	1	2	3
47	21851	1097790	2437	2017	Sanganagowda Awappa Biradar	65	M	Swelling in the right ankle	Soft tissue swelling	Features are of acute inflammation	Benign	1	3	2	2	3	1	3	1	2	3
48	233829	115169	2532	2016	Usamabanu Mujawar	19	F	Midline neck swelling	Thyroid	Hashimotos Thyroiditis	Benign	2	3	2	2	3	1	3	1	2	3

49	258095	127989	2801	2016	Anushabai	22	F	Left side thyroid nodule	Thyroid	Papillary Carcinoma	Malignant	3	3	2	2	3	3	1	2	2	3
50	257879	127991	2800	2017	Laxmibai	80	F	Midline neck swelling	Thyroid	Follicular neoplasm	Benign	1	3	3	2	3	1	3	1	2	3
51	253153	128217	2813	2017	Saraswati	32	F	Swelling in the anterior wall of left external auditory canal	Ear	Epidermal cyst	Benign	2	3	1	2	0	2	3	1	2	0
52	255411	126499	2768	2017	Ramavva Kuri	45	F	Midline neck swelling	Thyroid	Hashimotos Thyroiditis	Benign	2	3	1	2	3	2	3	1	2	3
53	249950	123597	2714	2017	Iranna Math	18	M	Swelling over left lower jaw	Oral cavity	Benign cystic lesion suggestive of Dentigerous cyst	Benign	2	3	2	2	3	2	3	1	2	3
54	253516	125655	2745	2017	Jyoti Karuti	25	F	Midline neck swelling	Thyroid	Nodular goitre	Benign	1	3	2	2	3	0	0	0	0	0
55	237944	118270	2593	2017	Kundan PrathamShetty	40	F	Lump in the left breast	Breast	Benign breast lesion	Benign	1	3	2	2	3	2	3	1	2	3
56	2451526	1192287	2611	2017	Mallayya hiremath	66	M	Growth over posterior aspect of right foot	Skin	Malignant melanoma	Malignant	3	2	2	2	3	3	3	1	2	3
57	413033	186048	2821	2017	Vivekanand Patil	36	M	Left cervical region	Lymph node	Reactive lymphadenitis	Benign	1	3	2	2	3	1	3	1	2	3
58	260617	92436	2831	2017	Kavitha	21	F	Bilateral Cervical lymphnode swelling	Lymph node	Necrotising Lymphadenitis Suggestive of Tuberculosis	TB	2	3	2	2	3	0	0	0	0	0
59	26163	130902	2863	2017	Shantamma	50	F	Lump in right breast	Breast	Infiltrating ductal carcinoma	Malignant	1	1	1	2	3	1	2	1	2	3
60	218625	107167	2397	2017	Kamalabai	55	F	Swelling in the neck	Soft tissue swelling	Carotid body tumour	Benign	2	2	2	2	3	0	0	0	0	0
61	215954	105695	2352	2017	Mahadevi	30	F	Swelling in front of neck	Thyroid	Nodular goitre	Benign	2	3	2	2	3	1	3	1	2	3
62	206026	100876	2272	2017	Shobha	32	F	Neck swelling	Soft tissue swelling	Benign cystic lesion	Benign	1	3	2	2	3	0	0	0	0	0
63	221480	108680	2407	2017	Swapna	30	F	Swelling neck	Thyroid	Nodular goitre with cystic change	Benign	2	2	2	2	3	1	3	1	2	3
64	224336	110458	2451	2017	Kiran	6	M	Swelling in popliteal region	Soft tissue swelling	Ganglion cyst	Benign	2	3	2	2	3	2	3	2	2	3
65	12342	63025	1460	2017	Kashimath	42	M	Soft tissue swelling	Soft tissue swelling	Lipoma	Benign	2	3	2	2	3	2	3	2	2	3
66	1654	10675	164	2016	Valu Jamali L	65	M	Right inguinal region	Lymph node	Metastatic malignant melanoma	Malignant	3	3	2	2	3	3	3	1	2	3
67	380292	170780	2578	2016	Imabai	60	F	Cervical region	Lymph node	Reactive Lymphadenitis	Benign	1	3	2	2	3	0	0	0	0	0
68	8104	96524	139	2017	Priya G	22	F	Left breat lump	Breast	Fibroadenoma	Benign	1	3	2	2	3	0	0	0	0	0
69	247874	135465	2700	2017	Rakesh	52	M	Cervical region	Soft tissue swelling	Epidermal cyst	Benign	2	2	2	2	0	2	3	2	2	0
70	247825	93085	2702	2017	Netravati	26	F	Neck swelling	Thyroid	Nodular goitre	Benign	3	3	2	2	3	3	3	1	2	3
71	31151	14893	2323	2015	Dattatray R Ingale	60	M	Right cervical region	Lymph node	Metastatic Squamous cell carcinoma/Primary	Malignant	1	3	2	2	3	2	3	1	2	3
72	362061	149676	2321	2015	Savita Naik	25	F	Swelling infront of neck	Thyroid	Colloid goitre	Benign	1	2	3	2	3	1	2	1	2	3
73	362717	150063	2325	2015	Sushila Rathod	31	F	Swelling infront of neck	Thyroid	Nodular goitre	Benign	3	3	2	2	2	1	3	1	2	2
74	351209	146130	2249	2015	Basavaraj	14	M	Cervical region	Lymph node	Reactive lymphadenitis	Inflammatory	1	3	2	2	3	2	3	1	2	3

75	352701	143055	2246	2015	Neelappa	60	M	Cervical region	Lymph node	Poorly differentiated carcinoma	Malignant	2	2	2	2	2	0	0	0	0	0
76	440030	184026	2855	2015	Dipika Singh	30	F	Right cervical lymph node swelling	Lymph node	Necrotising lymphadenitis	Inflammatory	3	2	1	2	2	2	3	1	2	3
77	389104	16130	2486	2015	Sunanda Mudagal	35	F	Swelling infront of neck	Thyroid	Hashimotos Thyroiditis	Inflammatory	0	0	0	0	0	2	3	1	2	3
78	389114	161700	2487	2015	Ashabi Mulla	18	F	Right breast lump	Breast	Fibroadenoma	Benign	2	2	2	2	2	0	0	0	0	0
79	38217	180367	2809	2015	Rehana Shekh	31	F	Cervical region swelling	Lymph node	Reactive lymphadenitis	Inflammatory	2	3	2	2	2	2	3	1	2	3
80	432040	180717	2819	2015	Shakuntala Badiger	31	F	Swelling infront of neck	Thyroid	Nodular goitre	Benign	1	3	2	2	3	1	3	1	2	3
81	432049	180723	2820	2015	Shankaramma Bavakod	24	F	Swelling infront of neck	Thyroid	Nodular goitre	Benign	2	3	2	2	3	1	2	1	2	3
82	37714	180377	2810	2015	Shushila N Kanakaraddi	58	F	Cervical swelling	Lymph node	Reactive lymphadenitis	Inflammatory	3	2	2	2	3	2	3	1	2	3
83	37596	178887	2788	2015	Shankar Amagond Biradar	16	F	Sub mandibular region	Lymph node	Reactive lymphadenitis	Inflammatory	1	2	2	2	2	0	0	0	0	0
84	410050	171482	2750	2015	Rajeshwari D K	18	F	Swelling infront of Neck	Thyroid	Colloid goitre	Benign	1	3	2	2	3	0	0	0	0	0
85	38073	184539	2867	2015	Shanthawwa Gundappa Nagur	42	F	Swelling infront of neck	Thyroid	Nodular Goitre with cystic change	Benign	2	3	2	2	3	1	2	1	2	3
86	432742	182954	2853	2015	Shrishail	38	M	Swelling over the back	Soft tissue swelling	Lipoma	Benign	3	3	2	2	3	1	3	1	2	3
87	274303	165421	1632	2015	Veena	32	F	Left cervical region	Lymph node	Reactive lymphadenitis	Inflammatory	2	2	2	2	2	1	3	1	2	3
88	24947	127146	2832	2017	Basappa S Devoor	74	M	Right Lobe of Liver	Liver	Positive for malignancy, Hepatocellular carcinoma	Malignant	3	3	2	2	3	0	0	0	0	0
89	253516	125655	2745	2017	Jyoti Karuti	25	F	Swelling infront of neck	Thyroid	Nodular Goitre	Benign	2	2	2	2	2	0	0	0	0	0
90	255411	126499	2768	2017	Ramawwa Kuri	45	F	Swelling infront of neck	Thyroid	Hashimotos Thyroiditis	Inflammatory	2	2	2	2	2	0	0	0	0	0
91	344608	142354	2201	2015	Kalavalhi	40	F	Neck swelling	Thyroid	Nodular Goitre	Benign	1	1	1	1	1	0	0	0	0	0
92	345342	142331	2190	2015	Devalamma	24	F	Right parotid region	Parotid	Acute suppurative lesion	Inflammatory	2	2	2	1	1	0	0	0	0	0
93	34704	143049	2206	2015	Chatabai	20	F	Cervical region swelling	Lymph node	Reactive lymphadenitis	Benign	3	2	2	2	2	2	2	1	2	3
94	241526	119287	2611	2015	Shivappa M	68	M	Growth over posterior aspect over right foot	Skin	Malignant Melanoma	Malignant	3	3	1	2	3	3	2	1	2	3
95	26464	132723	2910	2015	Neelappa G	72	M	Cervical region swelling	Lymph node	Metastatic Squamous cell carcinoma	Malignant	3	3	2	2	3	3	3	1	2	1
96	121127	57961	1347	2017	Chandapatel	55	M	Swelling left lobe of Thyroid	Thyroid	Nodular goitre with cystic change	Benign	2	3	1	2	3	0	3	2	2	0
97	135050	64862	1499	2017	Reshma	30	F	Swelling	Thyroid	Colloid goitre	Benign	2	3	2	2	3	0	0	0	0	0
98	133679	64016	1477	2017	Sumakka	37	F	Diffuse lump in the breast	Breast	Fibroadenoma	Benign	2	2	2	2	2	0	0	0	0	0
99	329169	180540	2280	2016	Hanumanth	56	M	Lower back swelling	Soft tissue swelling	Lipoma	Benign	2	2	2	2	2	0	0	0	0	0
100	170320	82691	1881	2017	Bhimappa	39	M	Left axillary region swelling	Lymph node	Tubercular lymphadenitis	TB	2	3	1	3	3	0	0	0	0	0

101	170948	83215	1882	2017	Basanna S K	56	M	Right sub mandibular region swellin	Skin	Epidermalcyst	benign	1	3	2	3	3	3	3	1	2	3
102	171558	83353	1883	2017	H K Ingale	44	M	Swelling in the suboccipital region	Soft tissue swelling	Lipoma	Benign	3	3	2	2	3	3	3	1	2	3
103	345113	142167	2188	2015	Bangarawwa	45	F	Neck swelling	Thyroid	Hashimotos thyroiditis	Inflammatory	3	3	1	2	3	3	3	1	2	3