CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNAECOLOGICAL SAMPLES

Dissertation submitted in partial fulfilment
of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

INSTITUTE OF PATHOLOGY,
MADRAS MEDICAL COLLEGE,
CHENNAI – 600 003.

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"CONVENIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNAECOLOGICAL SAMPLES"** is the bonafide original work of **Dr.J.MAHESWARI,** in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr. M.G.R Medical University to be held in April 2015.

Prof. Dr. R.VIMALA, M.D.,
DEAN,
Madras Medical College and
Rajiv Gandhi Government General Hospital,
Chennai – 600003.

Prof.Dr.M.SARASWATHI, M.D.,
DIRECTOR,
Institute of pathology,
Madras Medical College,
Chennai – 600003.

DECLARATION

I **Dr.J.MAHESWARI,** solemnly declare that the dissertation titled **"CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNECOLOGICAL SAMPLES"** is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr. M. SARASWATHI, M.D.,**Professor and Director of Institute of Pathology, Madras Medical College. The dissertation is submitted to the TamilnaduDr.M.G.R Medical University towards partialfulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place : Chennai

Date : **Dr.J.MAHESWARI**

ACKNOWLEDGEMENT

I express my sincere thanks to **Prof.Dr.R.VIMALA, M.D.**,Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, forpermitting me to utilize the facilities of the Institution.

I take this opportunity to express my heartfelt sincere gratitude to **Prof.Dr. M. SARASWATHI, M.D.,** Professor and Director of Institute of Pathology, Madras Medical College, Chennai, for her constantencouragement, wholehearted support, valuable suggestions and expert guidance throughout the study, without which this study would not have ever been possible.

I am thankful to **Prof.Dr.P.KARKUZHALI, M.D.,** Professor and former Director of Institute of Pathology, Madras Medical College for her initial guidance and valuable suggestions during the study.

I am truly thankful to **Prof. Dr. SHANTHA RAVISANKAR M.D. D.C.P, Prof. Dr.R.PADMAVATHI, M.D. D.G.O., Prof. Dr. V.RAMAMURTHY M.D., Prof. Dr. GEETHA DEVADAS, M.D., D.C.P., Prof. Dr. M. P. KANCHANA M.D., Prof. Dr. K. RAMA M.D., Prof. Dr.RAJAVELU INDIRA, M.D., Prof. Dr. SUDHA VENKATESH M.D.,** and **Prof.Dr. S.PAPPATHI M.D., D.C.H** for their valuable suggestions and encouragement throughout the study.

I express my heartfelt sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I would like to thank the Institutional Ethics Committee for approving my study. I acknowledge my thanks to our Statistician, Madras Medical college for his help during my study in statistical analysis.

I am indebted to my husband Mr.J.Mahendrakumar & my son M.Sharvesh and my parents &all family members who have been solid pillars of everlasting support and encouragement and for their heartfelt blessings.

Finally I thank all my colleagues, especially Dr. K. Indumathi for her valuable suggestions, my junior postgraduates and all the staffs of the Institute of Pathology, at Madras Medical College, for their immense support through out my study period.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. J. Maheswari,
PG in MD Pathology,
Institute of Pathology,
Madras Medical College, Chennai-3.

Dear **Dr. J. Maheswari,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Conventional cytological smear versus Liquid Based Preparation (E-PREP) in Non-Gynaecological Samples"** No.16112013

The following members of Ethics Committee were present in the meeting held on 13.11.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. R. Nandini, MD Director, Instt. of Pharmacology, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Prof. Kalai Selvi, MD Prof. of Pharmacology, MMC, Ch-3	-- Member
6. Thiru. S. Govindasamy, BABL	-- Lawyer
7. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

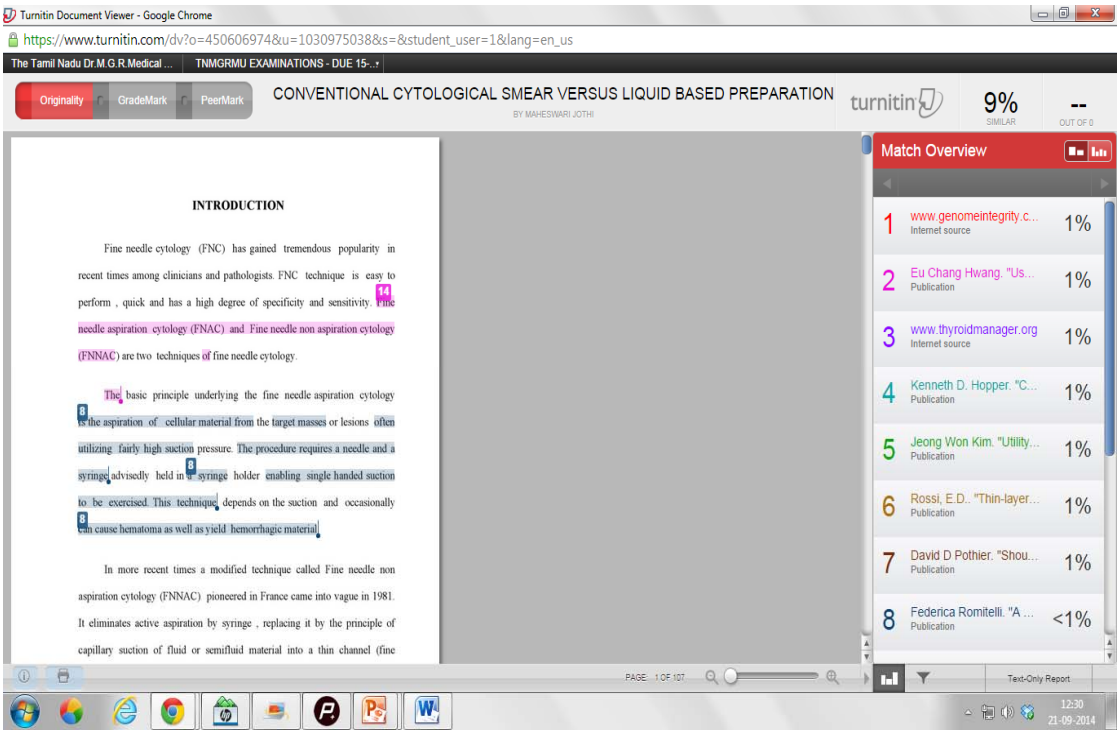
Sd/Chairman & Other Members

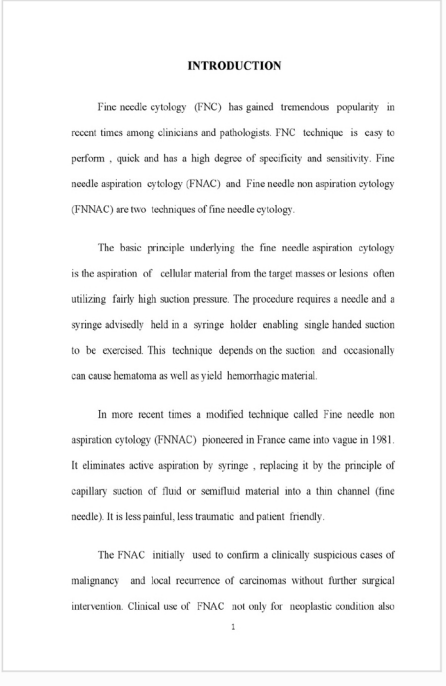
The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

19/6/13

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003


Turnitin Document Viewer - Google Chrome
https://www.turnitin.com/dv?o=450606974&u=1030975038&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical ...
TNMGRMU EXAMINATIONS - DUE 15-..
Originality
GradeMark
PeerMark
CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION
BY MAHESWARI JOTHI
turnitin
9%
SIMILAR
--
OUT OF 0
INTRODUCTION
Fine needle cytology (FNC) has gained tremendous popularity in recent times among clinicians and pathologists. FNC technique is easy to perform , quick and has a high degree of specificity and sensitivity. Fine needle aspiration cytology (FNAC) and Fine needle non aspiration cytology (FNNAC) are two techniques of fine needle cytology.
The basic principle underlying the fine needle aspiration cytology is the aspiration of cellular material from the target masses or lesions often utilizing fairly high suction pressure. The procedure requires a needle and a syringe advisedly held in a syringe holder enabling single handed suction to be exercised. This technique depends on the suction and occasionally can cause hematoma as well as yield hemorrhagic material.
In more recent times a modified technique called Fine needle non aspiration cytology (FNNAC) pioneered in France came into vague in 1981. It eliminates active aspiration by syringe , replacing it by the principle of capillary suction of fluid or semifluid material into a thin channel (fine
PAGE: 1 OF 107
Match Overview
1 www.genomeintegrity.c... Internet source 1%
2 Eu Chang Hwang. "Us... Publication 1%
3 www.thyroidmanager.org Internet source 1%
4 Kenneth D. Hopper. "C... Publication 1%
5 Jeong Won Kim. "Utility... Publication 1%
6 Rossi, E.D.. "Thin-layer... Publication 1%
7 David D Pothier. "Shou... Publication 1%
8 Federica Romitelli. "A ... Publication <1%
Text-Only Report
12:30
21-09-2014



INTRODUCTION

Fine needle cytology (FNC) has gained tremendous popularity in recent times among clinicians and pathologists. FNC technique is easy to perform , quick and has a high degree of specificity and sensitivity. Fine needle aspiration cytology (FNAC) and Fine needle non aspiration cytology (FNNAC) are two techniques of fine needle cytology.

The basic principle underlying the fine needle aspiration cytology is the aspiration of cellular material from the target masses or lesions often utilizing fairly high suction pressure. The procedure requires a needle and a syringe advisedly held in a syringe holder enabling single handed suction to be exercised. This technique depends on the suction and occasionally can cause hematoma as well as yield hemorrhagic material.

In more recent times a modified technique called Fine needle non aspiration cytology (FNNAC) pioneered in France came into vague in 1981. It eliminates active aspiration by syringe , replacing it by the principle of capillary suction of fluid or semifluid material into a thin channel (fine needle). It is less painful, less traumatic and patient friendly.

The FNAC initially used to confirm a clinically suspicious cases of malignancy and local recurrence of carcinomas without further surgical intervention. Clinical use of FNAC not only for neoplastic condition also

1

ABBREVIATIONS

FNC	:	Fine Needle Cytology
FNAC	:	Fine Needle Aspiration Cytology
FNNAC	:	Fine Needle Non Aspiration Cytology
LBP	:	Liquid Based Preparation
LBC	:	Liquid Based Cytology
CS	:	Conventional Smear
ICC	:	Immunocytochemistry
PAP	:	Papanicolaou stain
MGG	:	May Grunwald Giemsa stain
P	:	Probability

CONTENTS

CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNAECOLOGICAL SAMPLES

BACKGROUND AND OBJECTIVE

Fine needle cytology (FNC) has gained tremendous popularity in recent times among clinicians and pathologist. Liquid based cytology is a new technology for fine needle aspiration samples. It is used for mainly for cervical cancer screening, now also used for non gynaecolgical samples. E-PREP system is a Liquid based cytology processor with patent dual membrane filters. In this method able to collect large number of cells and make a monolayer preparation of cells with good cytological details .In LBP easier collection and transport of samples, standardized preparation, adequate cellularity, rapid fixation, even and monolayer distribution of cells , good preservation of cell morphology and increase clarity of nuclear feature, decreased obscuring background elements, decreased air drying artefacts.Disadvantages Of LBP are decreased and altered background material like necrosis, blood and inflammation, decreased and altered extracellular elements like mucin, colloid and stroma, disrupted cellular architecture like fragmentation of papillae, size of the cell smaller than conventional preparation. In this study thyroid, breast and lymphnode lesions(each 30 cases) are compared with both techniques of FNAC and Liquid based preparation (E-PREP) in non gynaecological samples.

MATERIALS AND METHODS

This study is a prospective study conducted at Goschen Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital Chennai, during the year 2014 (February 2014 to July 2014). This study included samples obtained from 90 patients who attended for cytology department for FNAC of thyroid, breast and lymphnode lesions. Collection of

clinical data of patients attending the cytology department for FNAC for thyroid, breast and lymphnode lesions and preparing CSs & LBP.

RESULTS

Smears were prepared by both methods. On analysing smears in thyroid lesion adequate smears more in CS method, superior quality smears more from LBP method and equal number diagnostically unsuitable smears from each methods of smear preparation, in breast lesions adequate smears more in LBP method, superior quality smears more from CS method and diagnostically unsuitable smears more from LBP method of smear preparation and in lymphnode lesion adequate smears more in LBP method, superior quality smears more from CS method and diagnostically unsuitable smears more from LBP method of smear preparation. On analysing and comparing average score obtained by both methods (CS & LBP) in thyroid, breast and lymphnode lesions and the P value calculated by Pearson Chi-Square test, the difference was found to be statistically insignificant $P > 0.05$.

CONCLUSION

The decision to make, use either Conventional method or LBP may be depends on basis of nature of the lesion (solid or cystic) and other ancillary tests to perform in the sample &each method has its own advantages and disadvantages and both methods can be combined to obtain a superior quality smears and lower the failure rates.

KEYWORDS:

FNAC (Fine needle aspiration cytology), CS (Conventional smear),LBP (Liquid based preparation), thyroid, breast, lymphnode.

INTRODUCTION

Fine needle cytology (FNC) has gained tremendous popularity in recent times among clinicians and pathologists. FNC technique is easy to perform , quick and has a high degree of specificity and sensitivity. Fine needle aspiration cytology (FNAC) and Fine needle non aspiration cytology (FNNAC) are two techniques of fine needle cytology.

The basic principle underlying the fine needle aspiration cytology is the aspiration of cellular material from the target masses or lesions often utilizing fairly high suction pressure. The procedure requires a needle and a syringe advisedly held in a syringe holder enabling single handed suction to be exercised. This technique depends on the suction and occasionally can cause hematoma as well as yield hemorrhagic material.

In more recent times a modified technique called Fine needle non aspiration cytology (FNNAC) pioneered in France came into vague in 1981. It eliminates active aspiration by syringe , replacing it by the principle of capillary suction of fluid or semifluid material into a thin channel (fine needle). It is less painful, less traumatic and patient friendly.

The FNAC initially used to confirm a clinically suspicious cases of malignancy and local recurrence of carcinomas without further surgical intervention. Clinical use of FNAC not only for neoplastic condition also

used for non neoplastic conditions like inflammatory and degenerative lesions.[1].

Liquid based cytology is a new technology for fine needle aspiration samples. It is used for mainly for cervical cancer screening , now also used for non gynecolgical samples. The basic principle of LBC is to collect specimen into the fixative solution and then make a monolayers of cells after staining . LBC preserveation of cells are excellent and reduces the bloody background**.** [2]

E-PREP system is a Liquid based cytology processor with patent dual membrane filters. In this method able to collect large number of cells and make a monolayer preparation of cells with good cytological details The quality of the smear is excellent due to application of both filtration & precipitation methods. Hence the method gives more accurate. E-PREP also facilitates preparation of more number of slides 150 slides / hr. [3]

In this prospective study, 90 cases were analysed, 30 cases each of thyroid, breast and lymphnode lesions and an attempt to made to compare both techniques FNAC and Liquid based preparation (E-PREP) with references to diagnostic adequacy and diagnostic accuracy.

AIMS AND OBJECTIVES

- To do both Conventional smears **(CS)** of Fine needle aspiration and Liquid based cytology **(LBC)** techniques for thyroid, breast and lymphnode lesions.
- To compare the efficacy of E-PREP for non gynaecological samples
- To compare the advantages and disadvantages of E-PREP for non gynaecological samples.
- To compare cytomorpholgical details of both methods.
- To compare the quantum of trauma by each methods.
- To compare quantum of yield in both methods.

Review of literature

REVIEW OF LITERATURE

During Medievel times, the Arabian physician Abul Casim (1013-1107AD) described a thyroid needle puncture to diagnosis of goitre.

First needle aspiration biopsy introduced by Kun in 1847. Pravaz in 1853 developed a metallic syringes for treatment of aneurysm, it also used for transthoracic needle aspiration for demonstration of organisms from pneumonia patients by Leyden.

Kronig was the first in 1884 to diagnose the lung cancer by introducing canula inserting through transthoracically and aspirating the tissues. Isolate the causative organisms of trypanosomiasis by aspiration of lymphnode by Greig and gray in 1904. He noticed that cells from nodal aspiration helpful for diagnosis. After that the development of FNAC remain dormant.

In the late 1920 and 1930s, cytologic scrap preparation of excised tissue used by Dudgon and Patrick from England. They also proposed that rapid diagnosis by needling of tumuors. In 1921, Guthrie by using needle aspiration technique successfully diagnosis the syphilis, lymphomas, tuberculosis and metastatic carcinomas.

During 1920s field of exfoliative cytopathology by Papanicalou, presented paper of “New cancer diagnosis”, later it was known as pap smear. Pap smear used for both diagnostic purpose and screening for cervical cancers.

In 1930, diagnosis of thyroid nodule by needle biopsy by Martin, Stewart and Ellis from united states. They used a 18 gauge needle (thicker needle) for aspiraion technique. Because of needle tract malignant implant and other complications, this technique was not acceptance widely.[4]

After World war II, reintroduced the special aspiration technique by Europeans particularly Scandinvanians for diagnose the lesions of thyroid. But they used a finer needle 22-25 gauge for performing aspiration. The FNAC technique described by Lowhagen et al from Institute of Karolinska is generally used now.[5]

Franzen et al [6], in 1955, introduced the special aspiration syringe holder described in detail in 1960-1967. In north America and India FNAC came into wide acceptance in 1980s. Since 1981, FNNAC a new modified technique of FNA pioneered in France by Zajdela et al [7]. The same technique was called by Brifford et al[8] as “cyto puncture” in 1982.

The FNAC technique is applicable to easily palpable superficial lesions like skin, subcutis, thyroid, superficial lymphnode, breast , salivary

gland . It is less demanding technique than biopsy and risk of complications are very low. FNAC procedure done in outpatient department, in radiology theatres and it is an office procedure. It is also suitable in debilitated patients and is readily repeatable. Modern imaging modalities like ultrasonography, computed tomography used for FNAC of deep structures by transthoracic and transperitoneal approach and safe.[9]

A preliminary tissue diagnosis or differential diagnosis provided within minutes and used for further investigation and management [10]. The complications reported in relation to different sites like hemorrhage, septicaemia, pneumothorax, acute pancreatitis and bile peritonitis [11]. The preoperative FNAC may cause tissue changes locally, like hematoma, infarction, pseudomalignant reparative reaction and capsular pseudoinvasion[12].

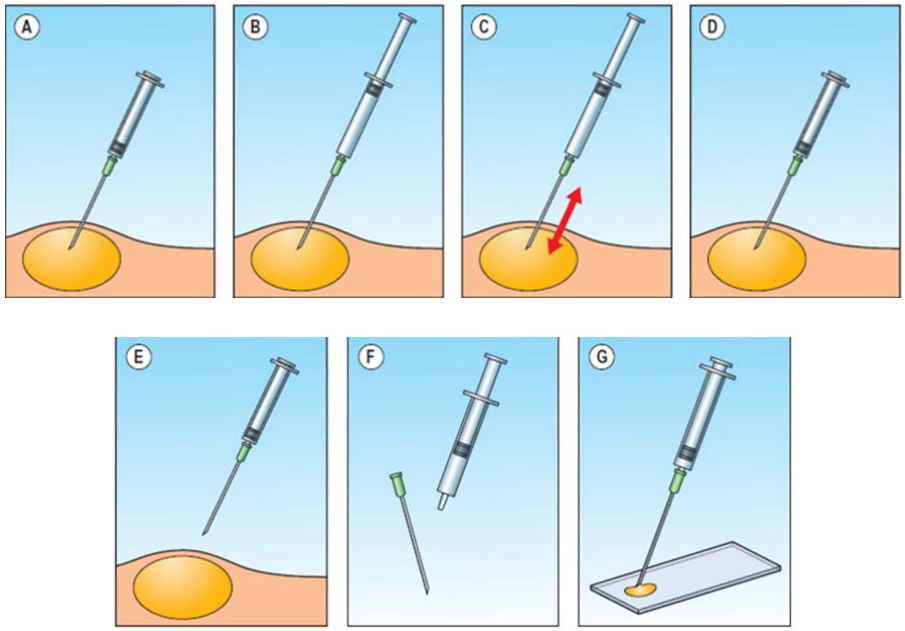
The success of FNA depends on following four fundamental requirements:

- Specimens must be representative of site of lesion
- Cells and other tissue components of the sample must be adequate
- Correctly smearing and processing of the sample must be done
- Biopsy also accompanied by adequate, correct clinical and radiological information.

Standard disposable 30-50mm long, 27-22gauge (0.4-0.7mm) needles are used for palpable, superficial lesions. 25 gauge needle used in many lesions but 27-gauge needles used for cell-rich and vascular tissues like lymphnode, thyroid, in sensitive sites like orbit, intra cutaneous lesions, eyelids and genitals. Although the yield is low, adequate samples and quality of smear due to less admixture of blood. The fibrotic lesions in breast and soft tissues yield is less predictable. 23-22 gauge needle often used. For sufficient material for ancillary tests larger-bore needles are used. Smears wet-fixed or air-dried, commonly wet fixation in 70-90% ethanol or spray fixative used. [5]

According to Thomson et al, use of negative pressure in FNAC the cells are not tear and hold the tissue against the sharp cutting needle edges. The needle advances into tissue which scrapes or cuts the tissue [13]. The needle moved back and forth within the lesion and negative pressure is maintained. Several passes needed for sufficient cell yields. Few rapid passes sufficient for highly cellular and vascular tissues like spleen ,thyroid, lymphnodes and liver.

FIGURE-1 FNA WITH ASPIRATION



In many centres FNAC performed by conventional smears with help of alcohol fixation and air-drying . Liquid based cytology is an alternative technique in which sample preserved in methanol based fixative and make smears on LBC slides. It is mostly used in gynaecological samples and non-gynaecological smears with diagnostic accuracy and sensitivity as superior as that of CS. The major usefulness of LBC is to perform immunohistochemical studies which needed for definitive diagnosis of some cases[14-17].

Fine needle aspiration cytology (FNAC) represents an invaluable diagnostic method for characterizing thyroid swelling with a worldwide consensus for its simplicity, safety, and considered as the most accurate and cost effective tool for the selection of surgical patients[18].

Santos and Leiman et al [19], in 1988 to compare the FNNAC and FNAC smear in thyroid nodules. They found that unsuitable samples in both techniques were not much different. They were graded the smears on the basis of certain criteria and compared the two techniques i.e.,

Unsuitable smears -If the smears contained mostly blood or absence of cellular material.

Diagnostically adequate - If smears are adequate to make diagnosis but suboptimal cellularity and degenerative changes or entrapment of samples in clots.

Diagnostically superior -If smears contained concentrated groups of cells or cells and well preserved cells , background unobscured by blood and retention of architectural structures.

According to Sharon Mair and Dunbar et al [20] in1989, compared FNAC and FNNAC , in their study the smears were compared with five objective parameters which are diagnostic adequacy, degree of trauma, retention of appropriate architecture, obscuring background material like blood or clot and degree of cellular degeneration.

The smears are classified as,

1. **Diagnostically Unsuitable** - with score of 0-2
2. **Diagnostically Adequate** - with score of 3-6
3. **Diagnostically Superior** - with score of 7-10

This study found that FNAC and FNNAC no statistical difference between two methods, but they found that FNNAC smears were diagnostically superior and smears were text book quality and it allows ease of sampling . FNAC smears were diagnostic for fibrous and cystic lesions and suggested that the method of sampling by fine needle employed for cytodiagnosis and to be left to the preference of the operator.

The Conventional smears are prepared by spreading the aspirating material on th glass slide and then smears are stained. But this technique needs a certain level of smear skill and also it carries risks with regarding to handling of the sample and needle. [21] Now a days, LBC is most commonly used method , mainly in the field of gynaecological specimen especially cervical cytological smears, and many reports in the literatures are pertaining to non-gynaecological LBC materials. [22-24] From the clinician's point of view , the LBC method is far easier, faster, and safer and also needs less skill to making smears and pathologist's point of view the advantages associated with cytologic diagnosis by applying the LBP. In LBP

fixation artefacts lesser than that of conventional smears like air drying artefacts, also number of slides needed for examination are less in number.

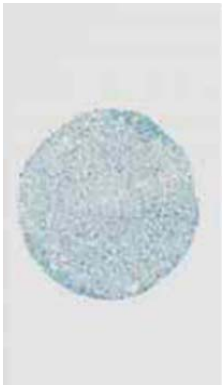
The Liquid based cytology (LBC) method, initially developed for gynaecological smear preparation like cervical cytology, then has progressively gained consensus for both non gynaecological and fine-needle aspiration cytological specimens [25-27].

Liquid-based cytology(LBC) developed on 1970s, automate the process of cervical cytology. LBC produce a sample which is fully representatives of material removed and easier to screen the slides. Two systems for LBC evaluated in English, Scottish and welsh pilots- SurePath and ThinPrep, these systems have different theory but produce same results[28&29].

Liquid based cytology now a popular for evaluating the non-gynaecological samples including FNA samples. LBC cellular contents confined to 20mm diameter circle, time needed for screen the slides may be reduced. LBC containing adequate diagnostic cells and superior to conventional smears(CS) due to presence of monolayers, absence of debris and blood in background and nuclear and cytoplasmic details . Preserved cell architecture , informative background cells as good as CSs and no statistically significant difference between LBC and CSs[30].

Liquid based cytology used mainly for cervical smears nowadays, surepath and thinprep methods very popular. Recently E-PREP system has been developed in Korea.[3]

The mechanism of Surepath is precipitation, ethanol based fixative used, and diameter of specimen is 11mm and time for preparation of slides12 cases/40min. Membrane filter is used in E-PREP, fixative is ethanol based and diameter of specimen 20mm and 15mm time for preparation of slides 4cases/min.[3]

	
E-PREP 20mm	E-PREP 15mm

Morphological changes in LBC : [30]

- background material is lost, reduced or altered
- cell clusters are more fragmented and small
- size of the cells are smaller
- well preserved nuclear details, prominent nucleoli
- well defined cytoplasm

In LBP smears are fixed rapidly, even distribution of cells over smaller area and obscuring background elements are decreased like blood, mucus and inflammation. Standardized LBP fixation used in centralized laboratories, when FNAC techniques are carried out without rapid assessment. CS artefacts hinder the diagnostic interpretation, but LBP lacks these artefacts.[30]

In LBPs automated slide preparation which eliminates artefacts due to mechanical spreading of material produces a monolayer preparation and evenly distribution of cells . Well preservation cell in fixative solution and also needs a smaller area to screen and decrease in unsatisfactory results[31]. LBC technique not only applied to gynaecological samples also to non-gynaecological samples.

LBC also to preserve specimens for sometimes and used for further investigation like immunocytochemistry or molecular studies.These advantages of LBC result in more objective diagnosis and also result in greater diagnostic accuracy. The application of LBC to aspirated samples make easy preparation of several slides for ancillary technique such as immunohistochemistry by using several antibodies.

The residual material in fixatives also used for ancillary studies ,like immunocytochemistry and higher cell dyshesion with high number of singly

dispersed cells and fragmentation of cells in LBC make a diagnosis difficult.[31]

FNAC- THYROID LESION

Fine needle cytology is used in diagnostic evaluation of goitre and single most effective technique for the preoperative assessment and diagnosis of solitary swellings of thyroid lesions. The main indications for FNAC of thyroid lesions are non toxic goitre, solitary or dominantly thyroid nodule and malignancies. FNAC can confirm benignity in 60% cases of thyroid lesion. FNAC provides distinction between colloid goitre and autoimmune thyroiditis because both conditions are diffuse non toxic goitre and the treatment is different for both conditions. TSH levels and antibodies levels are helpful to diagnosis.[32]

Thyroid swellings are common finding, even neoplastic conditions follow a favourable course [33]. Many thyroid lesions removed by surgically are histologically demonstrated by benign nature. To reduce the unnecessary surgery, an accurate preoperative evaluation must be done[34-38]. FNAC is the diagnostic tool with sensitivity and accuracy more than 95% in many series[39-42].

The main limitation of FNAC of thyroid is inability to differentiate between follicular adenoma and follicular carcinoma. This distinction

depends on tissue section by demonstrating capsular or vascular invasion. In papillary carcinoma of thyroid characteristic features of nuclear morphology is easily recognized in cytology smears.

The complications in FNAC of thyroid are hematoma, puncture of trachea causing coughing spasms, organisation of hematoma and necrosis which mimics the angiomatous tumour or a sarcoma and transient laryngeal nerve palsy. Damage to the capsule by needling may simulate invasion of capsule.[43]

Cytological features of normal thyroid gland:

Thyroid follicular epithelial cells have fragile cytoplasm, which are grey blue or pale blue cytoplasm and cell border is not distinct. Cytoplasmic granules appears as coarse blue like material. Bare nuclei are commonly seen which are size of the small lymphocytes. In non hemorrhagic aspirates thin colloid appear as blue, pink or violet in colour and thick colloid appears as dense round clumps of deep blue, magenta coloured acellular material.

According to Hamburger et al[44] suggests that in assessment of dominant nodule contain 6 clusters of benign follicular cells in at least 2 slides which are prepared from separate aspirates is reasonable minimum specimen for diagnosing of benign lesions.

Due to large proportion of inadequate smears (mechanical or air-drying artefacts or fibrin clots) which decrease the efficacy of FNA diagnosis. To minimize the inadequacy of smears to submit the all aspirated samples into liquid fixative. FNA diagnosis is generally accurate in thyroid lesions like thyroiditis, usual type of papillary carcinoma, anaplastic carcinoma, medullary carcinoma and high grade lymphoma. False negative result in cystic lesion harbouring malignancy, low grade or intermediate lymphomas in background showing Hashimoto's thyroiditis and in anaplastic carcinoma with necrosis and in focal involvement of thyroid gland by thyroiditis, in dual lesion where the non neoplastic lesion is dominant or obscures small carcinoma[45,47].

In difficult cases, use of CS then use of LBC and application of immunocytochemistry with use of these it modifies management of thyroid nodules by reducing number of surgery rate ranging from 23-50% in different series[33,48]. The incidence of thyroid carcinoma increased from 15%-30% in patients who had preoperative cytological diagnosis which requiring surgical treatment.[49,50]

In LBPs amount of colloid was decreased and colloid was dense, in droplets or fragmented and epithelial cells are crowded, clustered and nuclear overlapped. In conventional smears the epithelial cells are arranged in flat sheets or in a honeycomb arrangement. In LBPs loss of cellular preservation,

peripheral edge of smear is generally blurred and also associated artefact. In LBP, cytoplasm is more disturbed, naked nuclei also increased in number and have prominent nucleoli. In papillary carcinoma, nuclear grooves and pseudoinclusions were less apparent than CS.[30]

According to Cochand –Priollet et al., the diagnostic accuracy of LBP was better than CS. Oncocytic tumors and Lymphocytic thyroiditis were difficult to diagnose in their studies and lack of colloid background also important confounding factor.[51,52]

Despite the controversy regarding the efficacy of the use of ThinPrep alone [53-55] results achieved by many groups in different countries, mainly in the recent years. Since November 2003 to 2011 the majority of about 22,000 FNACs done in the "Agostino Gemelli" School of Medicine and Hospital of Rome have been processed by ThinPrep2000™ alone. This results has been reported in studies published since 2005 where the efficacy of theThinPrep2000™ method for a correct preoperative diagnosis of more than 500 malignant lesions are highlighted.

According to Rossietal et al.2009[27], three parameters of efficacy (inadequacy, indeterminacy, and malignancy rates) selected for determining the efficacy of ThinPrep2000™ in compared with CS alone and combine the ThinPrep2000™ and CS in more than 10,000 FNAC thyroid showing

that ThinPrep2000™ alone is as effective as CS in decreasing both inadequate and indeterminate diagnosis [56].

According to Geers and Bourgain 2011[57], SurePath method achieves controversial results in means of adequacy rate between LBC and SurePath.

According to Cochand-Priolle tet al 2003[53], Rosai et al [55] the ThinPrep2000™ materials were stored in the vial also be used for additional methods such as immunocytochemistry, flow cytometry, and molecular biology.

This method is based on a two-step procedure:

- fixation of the specimen in an methanol based solution
- automated processing of the sample to obtain monolayer preparation of cells.

Two most common technique for processing the cytologic specimens are thinPrep2000™ , the cells for the thinprep obtained from from a methanol-based material and then filtered , transferred the material on glass slide which are positively charged with a gentle positive pressure; and in the second method SurePath™ the cells are collected in an ethanol based samples, then samples centrifuged twicely, slowly sedimentated on a poly-l-lysinated slide and eventually stained with a hematoxylin and eosin

stain. Final result for is one slide for each lesion for both method and all cells concentrated in the central area of the slide and a sensitivity is77% and a specificityis 81%. [57]

The morphologic appearance of LBC differ from CS in two aspects:

a) cells in slide are arranged in monolayered sheets representative sample of the entire sample is collected in the container with a variable amount of cells and the cells are preserved in the preservative solution;
b) automated process result in changes in cellular and background morphology

One of the important morphological change, occurring in Liquid based preparation, is the appearance of colloid is fragmented and appear as small droplets in the background of a benign lesion with a quantitative detection. In CS the colloid does not need a quantization. LBC morphology of a thyroiditis is equal to CS with the exception of the of lymphocytes in the background and lymphocytes higher than normal because of the spinning of the sample before the automated process. When a thyroiditis is suspected, the identification of lymphoepithelial clusters of cells in an inflammatory background is the important clue for the diagnosis and simple follow up needed for the patient.[58]

LBC- Problems in thyroid cytology:

- **Colloid** – dense, fragmented, hard and droplets.
- **Papillary carcinoma** – fragmentation of papillae
- **Hashimoto's thyroiditis** – low sensitivity

Liquid-based preparation can give the possibility of immunocytochemical testing and evaluation and molecular testing for somatic mutations in thyroid FNAC as the nucleic acids are maintained in the preservative material for up to 6 months after the sampling. In this setting, possibility of a guideline composing the combined use of immunocytochemistry and molecular profiles for supplementing the morphologic diagnosis, can be the starting point for a complete evaluation preoperative assessment in a thyroid swelling.

Spreading out in thin monolayers which results from Liquid based preparation eliminates a many of the inflammatory cells, red blood cells and necrosis leading to "a cleaning" background. The LBC gives it possible and to eliminate the most of the artefacts of superposition on the conventional smear and the dispersion of the cellular elements removal also usual in visual reference marks. The cytopathlogists are used to evaluate the smears fixed in a preservative liquid for the urines, the serosa or the ovaries. It imposes an evaluate the element by element and a training needed at

least 6 months to adjust the morphological criteria. The cells are not flattened but deposited and modified by pictorial aspects . The nuclei are not hyperchromatic but take a vesicular aspect. The cytoplasm also used to differentiate the cellular origin. The performance was evaluated by several national agencies and conclusions are given as for the improve the quality of the smear. The unsatisfactory smears by means of the presence of inflammatory cells and red blood cells, these are statistically less value in LBC than with the conventional smear method. The absence of cellular material because of sampling of bad quality as frequent in LBC as in conventional smear preparation.[59]

FNAC- LYMPHNODE LESIONS

The most common cause of peripheral lymphadenopathy is due to reaction to symptomatic inflammatory conditions. Less than 1% Patients with peripheral lymphadenopathy have malignant lesion.[60] Intra-abdominal lymphadenopathy retroperitoneal lymphadenopathy is associated with sarcoidosis, tuberculosis and infectious mononucleosis in young patients. More than 40 years of patients with associated peripheral lymphadenopathy with more than 2cm size, matted firm nodes, non tender, painless nodes are most likelihood of malignant disease. [61]

Cytological features of normal lymphnode:

The cytoplasm of normal lymphoid cells is often fragile, the cells are appeared as naked nuclei or have scant amount of cytoplasm . Lympho glandular bodies which are small, spherical basophilic fragments, presence of this bodies is indicative of lymphocytes in the smear.

FNAC offers alternative to open biopsy, it is an immediate preliminary test, causing little trauma and cost although it is not always specific diagnosis but give more information for management.[62-66] Sufficient material is obtained by using 23-27 gauge needle except in presence if more fibrosis and necrosis. Non aspiration techniques result in less hemorrhagic material. Huge amount of blood adversely affect the fixation of cells and cause shrinkage and distortion of cells . Liquid based preparation minimally used in lymphnode aspiration. Interpretation of LBPs carefully because of alteration of cytomorphology of the cells[67] .

Although excision of palpable node is relatively simple method, FNAC gives an alternative technique for immediate and preliminary procedure with little trauma and cost. FNC of lymphnodes have been practised in Scandinavia and Central Europe for many years especially by haematologist in conjucation with bone marrow aspiration and spleen.[68]

Although the background of LBC is clear few numbers of red cells and debris are seen in necrotizing lymphadenitis cases. Reed- Sternberg cells and Hodgkin cells in Hodgkin's lymphoma is easily identified by means of monolayers. IHC performed in these cases and tumour cells were positive for EMA, CD30 and CD15. In another case of lymphoma cells are arranged in single or clustered in aggregates resembling oat cell carcinoma in LBC smears and IHC was useful in this case which are LCA and pan B cell markers positive. Carcinomas were difficult to diagnose in LBC because of lack of necrosis and presented as fragmentation of epithelial cell clusters. In CSs 12 cases reported as granulomatous lymphadenitis in LBC, 10 LBCs epitheloid cells were noted. [30]

The differing preparatory methods, the morphological differences between Conventional smears and LBP. LBC produces a single slide and representative cells and less amount of obscuring material like blood and inflammatory cells. But obscuring elements is sometimes considered as a diagnostic problem because of background element like tumour diathesis and necrosis which is useful in assessment of diagnosis. Especially lymphoma was difficult to assess in LBC method and in these method lymphoid cells aggregated together and appeared as smaller.

According to Wildi et al [69], found that Endoscopy ultrasound - FNA using Conventional smear is a good method for diagnosing

granulomatous lesions. In their study, 3 benign lesions diagnosed as granulomas which were identified on CS but not diagnosed on LBC. These diagnostic problems and unfamiliarity, application of the LBC technique on FNA cytology is limited. Nowadays, the LBC method can alternate method to CS but LBP cannot replace CS on FNA cytology[70] .

FNAC – BREAST LESIONS

Approach of palpable breast lumps are "triple test" by analysing clinical, radiological features combined with pathological features for diagnosing lesions and determine the further management.[71] Palpable breast lump preceded by ultrasonographic and mammographic investigation also FNAC performed as first line mode of investigation among symptomatic patients.[72&73] Use 23-27 gauge needle for aspiration. For benign and malignant lesions FNA without aspiration preferable.

The main purpose of FNAC of breast lesions are to confirm the carcinoma and to avoid the unnecessary surgeries in benign conditions. Subjective grading of cytological smears correlated with nuclear and histological grade and it shows association with prognosis of the lesions[74]. The sensitivity of FNC in breast cancer diagnosis is around

90-95%. But radiation induced atypia in benign glandular epithelium create risk of over diagnosis which mimics malignant lesions.[75]

Certain conditions well circumscribed lesions like simple cyst, fibroadenomas, lipomas, fat necrosis and intramammary lymphnodes which are diagnosed with confidence. But poorly circumscribed lesions of the breast lesions lik firocystic disease, hormonal mastopathy, fibroadenosis and mammary dysplasias which are cannot be diagnosed confidently by FNC.

Cytological features of normal breast:

The bimodal pattern of cells seen which are duct epithelial cells , these cells are arranged tightly in clusters or groups and have uniform round , small nuclei with granular chromatin, scant cytoplasm with indistinct borders. The myoepithelial cells are spindle shaped ,with dark homogenous bipolar nuclei.

According to Layfield et al [76] six clusters of benign epithelial cells are needed as threshold for diagnosis of satisfactory samples. In cases of fibrous mastopathy, sclerosed fibroadenoma and carcinoma with high desmoplastic stroma or hypertrophic adipose tissue conditions one cannot be expect cellular samples or to obtain many cells.

Complications are uncommon in FNAC breast lesions. Minor hemorrhage and pain may occur. Other rare complications are major

hematoma, pneumothorax and subpleural hematoma. [77] The impalpable breast lesions were detected by screening by mammography and can be investigated nowadays by image guided needle aspiration techniques.

The use of FNAC in assessment of breast lesions are, in suspected metastasis and recurrence, inoperable advanced cancer, preoperative confirmation , clinical management and obtain tumour cells for IHC, DNA analysis, molecular studies.

Use of LBC now used for non gynaecological cytological samples including FNAC. Diagnostic features of ductal carcinoma in LBC were the cells arranged in aggregates but stromal fragments were absent. In LBC diagnosis of ductal carcinoma was apparently easy due to presence of high cellularity, defined nuclear features and clear background but malignant cell clusters broken into small fragments.[30]

The LBPs were limited to residual material, in CSs adequacy was 84.2% and adequacy of 78.9% in LBC . Exellent cytoplasmic and nuclear (ER) antibodies in Liquid based preparation. It is alternative method for fixed and prepared slides from inexperienced aspirators.[78] In breast lesions sensitivity and specificity more than 95% in CSs.[79&80] FNAC specificity 100% and Core needle biopsy specificity 100% but FNAC more sensitivity (97%) than core needle biopsy (90%). [81]

According to Bedard YC et al, analysed breast FNAC of 7464 patients over a 4 years period and comparing both LBC and CSs, but in this study no significant diagnostic accuracy between these techniques.[82]

In LBP breast cytology problems are;

Fibroadenoma

- Fewer myoepithelial cells
- Less epithelial stromal relationship

Colloid carcinoma

- Scant mucin

Papillary neoplasm

- Fragmentation of papillae

FNAC OF OTHER LESIONS

Documentation of diagnostic accuracy of FNAC in salivary gland neoplasms of many types published from the Karolinska Hospital in the 1960s in Sweden.[83-87] More than 90% of salivary gland neoplasm recognized, over 90% were correctly typed as pleomorphic adenoma and most malignant tumours diagnosed as like. [88,89] A review of literature found that the diagnostic sensitivity of 81% and 100% and specificity of 94%-

100% and 61-80% of accuracy of tumour typing.[90] According to Klijanienko et al, sensitivity of 94% and accuracy of 95%.[91]

Generally salivary gland tumours not subjected to needle biopsy or incisional biopsy due to increased risk of resulting fistula seeding of tumour cells because of disruption of the capsule and leading to subsequent recurrence but no evidence of these complication in FNAC. According to Layfield et al,[92] potential cost savings due to preoperative evaluation of salivary gland tumours by FNAC. Liquid based preparation can be used as supplement to Conventional smears. [93]

There are most morphological differences between LBC and Conventional smears in the evaluation of salivary gland FNACs, mostly related to the quantity and stromal appearence . The diagnostic yield of CS higher than that of LBP yield in the diagnosis of pleomorphic adenoma, which is the most diagnosis in salivary gland. [93]

In mixed tumours of salivary gland lesions, epithelial and mesenchymal component s are present in cellular LBC but present in all CSs. Mesenchymal component is poor in LBP like poor myxoid background or droplets of myxoid areas. Epithelial cells arranged in small aggregates without specific information in LBP. So diagnosis was easier in CSs than LBCs because of myxoid background in CSs. Small cluster of

oncocytic cells on LBP in Warthin tumour but lymphoid cells on background was less which favours the oncocytic cells. Due to loss of necrotic background and fragmentation of epithelial clusters in diagnosis of carcinoma was difficult. Cytoplasmic details similar to that of CS, nuclear smudging and loss of nuclear details were present.[30]

Extracellular material is important for formulating a specific diagnosis in soft tissue lesion like endometriosis and it is a diagnostic problem. In LBP the extracellular material is diminished and quality also altered. Filamentous quality also indistinguishable from fibrin. Biphasic component stromal fragments and epithelial sheets of endometriosis seen in CSs . But stromal fragments not seen in LBC, it is difficult to diagnose.[30]

According to Lee KR et al,[94] outlined many differences between the two methodology and also emphasized the use of LBC for a correct interpretation and diagnosis. Also LBP should not primary method of diagnosis unless conditions are absolutely prohibitive.

According to Schmitt FC et al, in Portugal from the university of Porto, reported , the use of liquid based preparation for study of breast carcinoma cell lines and cells taken from tissue culture and prepared in easy way and uniform manner for research purposes[95].

Immunoperoxidase and in situ hybridization controls are prepared in the same techniques[96,97].

According to Rana DN et al,[98] a large study on respiratory cytology samples, this study no diagnostic accuracy between Liquid based cytology and CSs. But they favour LBC over CS due to a cleaner background, better cell preservation and evenly distribution of cells. Also LBC provides less obscuring blood, no air-drying or crush artefacts and good nuclear detail observed in LBC.

According to de Luna R et al,[99] study on pancreatic FNACs with comparing LBE and CSs. They reported diagnostic accuracy of Thinprep was inferior to conventional methods and they found that this result due to use of split specimen technique, less cellularity with use of LBC. In LBC technique important morphological difference was lack of background mucinous material which impedes the diagnostic accuracy of mucinous tumours of the pancreas. Thinprep was safe and acceptable technique for diagnosis of thyroid, lymphnode, breast, salivary gland and soft tissue lesions when combination of conventional FNAC method.[30]

Cystoscopy and cytology are commonly applied for the interpretation of diagnosis and follow up of superficial bladder carcinomas. Now a days cystoscopy is the most efficient method used for detecting primary or recurrent urothelial cancer of the bladder. But

cystoscopy is invasive method and it produces discomfort to the patient, and difficult to diagnose flat tumors. [100] Cytology is important test to use as a non-invasive adjunct to standard diagnostic technique and surveillance. [101] The cytology is clinically useful and easy to perform, have minimal needs for sample preparation and handling, and be reliable, [102] because of high sensitivity and specificity. Urine cytology is non-invasive method and the gold standard method for diagnosing high-grade urothelial lesions, with sensitivity was 95%and specificity near to 100%. But sensitivity is low in low grade tumors, which are the most common type of urothelial carcinomas.[100,103&104] The limitations of cytology and cystoscopy for the primary diagnosis and monitoring patients and early detection of transitional cell carcinoma.[100] LBP developed is an alternative to conventional cytological methods . Most comparative analysis shown that ThinPrep is better than conventional preparations and sensitivity and specificity more than 90% in non-gynaecological samples [103]. MonoPrep2 a newly developed liquid-based cytology method, It has a manual filtration methodology and it is simple technique and cost effective than CSs.

Liquid-based cytology as an alternative to conventional cytology. Many laboratories have applied LBP method to body fluids (e.g. urine, pleural effusions). Most of the studies reported good results with the

Thinprep system compared with conventional smear preparations , and the residual material within the container can be applied for immunohistochemical or other analyses. [105] In Korea, Thinprep was introduced in 1999, this method was required an expensive instrument and the sample preparation costs were higher. This is the main limitation factor for normal routine screening of public health system.

ADVANTAGES OF FINE NEEDLE CYTOLOGY

Fine needle cytology includes both FNAC and FNNAC. Fine needle cytology has clear advantages to both patients and clinicians. This method is simple, relatively painless ,OP procedure and produces an economical and speedy result.[106-108] The accuracy can approach to that of histopathology in providing an unequivocal diagnosis when applied by experienced, well trained practitioners. Though not a suitable for conventional surgical histopathology, it is an extremely valued complement to it.

FNC can be applied on easily palpable all superficial lesions like thyroid, breast, lymphnode, superficial nodules of skin, subcutaneous tissue. For deep seated lesions sampling under the radiologic guidance such as USG and CT scan and endoscopic USG guided FNC. [109]

In fact FNC, as the first step it can satisfy the avidity of clinicians for rapid diagnosis. The use of FNC in primary diagnosis of tumours has been enormous and successful. It is very useful and less demanding method than surgical biopsy. The low risk of complication is an important advantage of FNC, so this procedure performed as an outpatient procedure and in radiology rooms.

Ancillary techniques used in FNC

Due to more scientific technologies in recent times, many of the ancillary methods can be used in cytology for correct and rapid diagnosis.

Special stains

The commonly employed histopathological stains also used in cytology. Some of them are Alcian blue for mucins, PAS/ diastase for glycogen, Masson Fontana for melanin, Grimelius for argyrophilic granules, Ziehl Neilson for acid fast organisms, Prussian blue for iron, Congo red for amyloid. In air dried smears Oil red O for fat.[110]

Phase contrast microscopy

It is used in cytology for unstained smear tocheck the representativeness of smear and quality of smears can be used for

Electron microscopy or Immunoperoxidase staining. So that time and reagents were not wasted in unsatisfactory specimens.

Electron microscopy

This modality is mainly associated with the FNC at all site of lesions especially in deep seated lesions. Electron microscope mainly used in mediastinal and unusual lung lesions. The common method of fixation is to eject the aspirate material into the test tube which containing fixative like glutaraldehyde. Highly dry cellular aspirate, the sample to be ejected as semisolid droplet on a cleaned slide then it is immersed in a glutaraldehyde fixative. Lazzaro's method used for cell concentration, in this method separating tumour cells from contaminating red blood cells. In centrifugation, small pellet is produced which is carefully removed and used for subsequent processing. [111-113]

Immunocytochemistry (ICC)

It is recent modality in diagnostic cytology. The availability of monoclonal antibodies to proteins and cell products which are specific more or less to different cell lines and which are demonstrated by immunocytochemical techniques. Most commonly used technique is avidin-biotin complex with polyclonal and monoclonal primary antibodies. Marker dye use in this method is Diaminobenzidine. In cytological preparation, immune alkaline phosphatase staining method offer more

advantages. Due to the commercially produced kit have make immune cytochemistry a simple technique. To achieve the diagnostic accuracy appropriate controls must be present.[114&115]

The other new modalities that can be applied to cytologic specimens are image analysis which deals with different areas namely morphometry, object counting and cytometry. Molecular cytometry and flowcytometry also be employed.

Immunocytochemistry on LBC (According to dabbs et al)

- In LBP intensity of smear is high
- In LBP proper distribution and staining of cells
- Background is clear in LBP and interpretation of cells are easy.

LIMITATION OF FNC

FNC is still relatively a new discipline and experiences in this field is still insufficient.

- The diagnostic criteria necessary to be better defined in few less common conditions like soft tissue tumours, paediatric tumours etc., where specialised oncological expertise is necessary.
- The needle not reaching the lesions in deep seated lesions, so imaging technique guidance is needed in these situations.

- Minor complications like hematoma, pain and hemorrhage may occur.
- FNC can cause change in tissue which may make subsequent interpretation is difficult. Such changes are infarction, pseudo malignant changes, pseudo capsular invasion and reparative reactive changes. So FNC method must be done gently and carefully to minimize tissue damage.
- The possibility of carcinomatous cells disseminated along needle tract., but according to Roussel et al in 1989, and in 1996 Power et al confirmed that needle tract seeding was low in case of FNC when 22-25 gauge fine needle was used.[116] Multiple passes , absence of normal parenchyma covering the lesion and large needles increase the risk of seeding of tumour cells.
- Extreamly rare complications noted, when FNC applied to deeper organs. The complications are hemorrhage, bile peritonitis, acute pancreatitis, septicaemia and pneumothorax etc., where close follow up of the patients were needed.

ADVANTAGES OF LIQUID BASED PREPARATION

- Easier collection and transport of samples
- Standardized preparation
- Adequate cellularity
- Rapid fixation
- Even and monolayer distribution of cells over a small slide area
- Good preservation of cell morphology and increase clarity of nuclear feature
- Small screening area
- Decreased obscuring background elements
- Decreased air drying artefacts

DISADVANTAGES OF LIQUID BASED PREPARATION

- Decreased and altered background material like necrosis, blood and inflammation
- Decreased and altered extracellular elements like mucin, colloid and stroma
- Disrupted cellular architecture like fragmentation of papillae
- Size of the cell smaller than conventional preparation

LBC - ADVANTAGES IN CYTOMORPHOLOGY

- High diagnostic accuracy
- Preserved cell features
- Lesser fixation artefact
- Clean background
- Lesser unsatisfactory results

TABLE- 1 : DIFFERENCE BETWEEN CS AND LBC

PARAMETERS	CS	LBC
Cellularity	Cellularity is high	Low
Architecture	Well Preserved	Less preserved
Cell size	Size of the cells are preserved	Smaller
Background	Distracting	Cleaner
Diathesis	Diffuse	Clinging,Clumped
Nuclei	Preserved	Preserved
Ancillary tests	+/-	+

Materials and Methods

MATERIALS AND METHODS

This study is a prospective study conducted at Goschen Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the year 2014 (February2014 to July 2014). This study included samples obtained from 90 patients who attended for cytology department for FNAC of thyroid, breast and lymphnode lesions.

METHODS

Collection of clinical data of patients attending the cytology department for FNAC for thyroid, breast and lymphnode lesions and preparing

- Conventional smears (CS)
- Liquid based preparation (LBP)

INCLUSION CRITERIA:

- Palpable swelling of Breast, Thyroid, Lymphnode.

EXCLUSION CRITERIA:

- Swelling other than Breast, Thyoid and Lymphnode
- Gynaecological cases

CONVENTIONAL SMEAR PREPARATION

Equipments needed:

27-22 gauge needles, 5ml or 10 ml syringe, gloves, Fixatives, Coplin jars, cotton, skin disinfectant, glass slides, glass marking pencil, stains, sterile test tube or sterile container for collecting samples for liquid based preparation.

Patient preparation:

A clear explanation was given to the patient about procedure ,number of pricks that would be made and complications of procedure. A written consent was obtained first and the cooperation of patient to procedure is very essential. The procedure was normally carried out with the patient lying supine on the examination couch.

The swelling was located and palpated, then skin overlying was cleansed with alcohol. A 10ml plastic syringe attached with a needle (22gauge or 25 gauge) was held in the right hand. Two fingers of the left hand firmly grasp the swelling. Then the needle was inserted rapidly through the skin into the swelling.

Once the needle tip is in the swelling, gentle suction was applied while the needle is moved back and forth in the nodule vertically. This

manoeuvre allows the dislodging of the cellular material and easy suction into the needle. During the period of 5-10 passes, suction was maintained and as soon as fluid or aspirate appears in the hub of the needle, the suction was released and the needle was withdrawn.

The appearance of fluid suggests that nodule is cystic. The suction pressure is maintained to aspirate all the fluid material and then FNAC was to be done in the residual lesions or mass. Once the material is seen in the hub of the needle, the needle is taken out of the swelling and detached from the syringe.

5ml of air was drawn into the syringe and the needle was reattached to the syringe and with the level pointing down, drop of aspirated material was forced onto each of the several glass slides. It is important that all the slides are labelled and placed in order on a nearby table before the aspiration smears are prepared.

After the procedure is over, firm pressure is applied to the aspirated site with cotton. Once the bleeding has stopped, adhesive bandage is placed on it. The patients are observed for few minutes and if there are no problems, he/she is allowed to leave.

The aspirate contained in the needle was expelled on to a clean glass slide using air in syringe ,taking care to avoid splashing. The smears were

made by using a second glass slide exerting a light pressure to achieve a thin, even spread, in a manner similar to that of making blood smears. Too firm pressure produces a crush artefacts. Then smears are fixed with Isopropyl alcohol or Ethanol in coplin jars.

LIQUID BASED PREPARATION (LBP) PROCEDURE

Samples for LBP (E-PREP) and CS are taken from the patients attending cytology department for FNA.

For LBC (E-PREP) , samples are collected in 20 ml of cytosol solution, centrifuged at 1800 RPM for 5min. After centrifugation, supernatant solution is discarded and then the precipitated cells are resuspended in a 20 ml of cytopreservative solution .

The equipment then spreads the material on a clean glass slide to form a circle 20 mm in diameter. Hereby giving a monolayer preparation of cells with clean background and slides are routinely stained with Papanicolacou stain (PAP) and May Graunwald Giemsa stain (MGG).

STAINING PROCEDURE:

Both conventional smears and liquid based preparations stained with MGG stain and PAP stain.

MAY GRUNWALD GIEMSA STAINING

The smears are air dried and fixed with acetone free methanol for 30 minutes. Then smers are stained with May Grunwald working solution for 5 minutes and then stained with Giemsa working solution for 15 minutes and buffer water (pH-6.8) for 5minutes. Air dry the slides and mount with DPX.

PAPANICOLAOU STAINING

The smears fixed with Isopropyl alcohol for 20- 30 minutes; then 4-5 dips in each 80%, 70% and 50% alcohol then 4 dips in tap water and stained with Harri's haematoxylin for 4minutes the wash in tap water for 1-2 minutes then treated with 1% acid alcohol for 30 seconds followed by wash with tap water for 5 minutes . Then treated with 70%, 90%,90% alcohol for each 4-5 dips followed by stained OG-6 (Orange green-6) for 5 minutes then 4-5 dips in 95% alcohol and stained with EA-50 (Eosin-50) for 10 minutes. Two changes of 95% alcohol for each 4-5 dips followed by 4-5 dips in absolute alcohol then two changes of Xylene for each 4-5 dips then mount with DPX.

All needle sampling procedure were made by a single operator, bias was thus avoided in all stages of sampling from patient examination to slide fixation. The slides were studied and a cytological diagnosis was made.

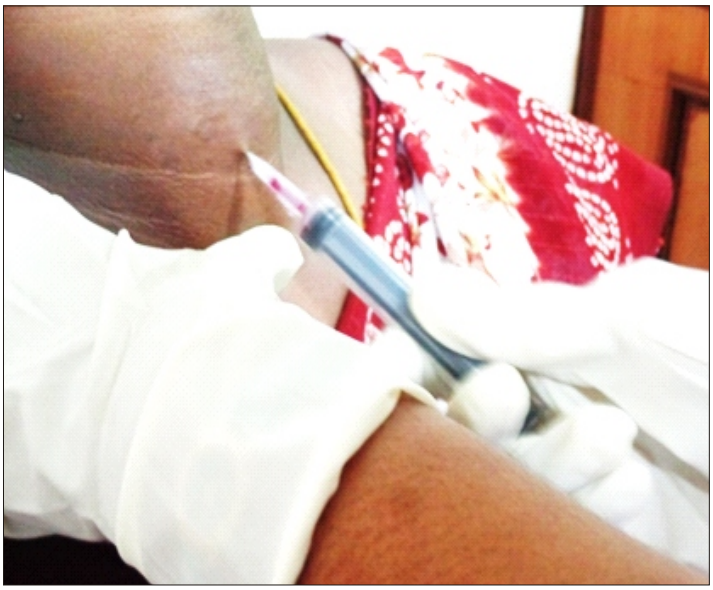
According to scoring system of **Mair et al** [20] quality of cytological aspirate samples analysed. The smears are compared with following parameters which are

- Cellularity
- Retention of appropriate architecture
- Blood or clot obscuring the background elements
- Degree of trauma
- Degree of cellular degeneration

On the basis of five parameters tabulated (annexure), for each cases a cumulative score was obtained then categorized as one of the 3 following categories,

- Unsuitable for cytological diagnosis – (0-2)
- Diagnostically Adequate – (3-6)
- Diagnostically Superior – (7-10)

Figure-2 Shows the procedure of FNAC



Figures-3&4 Equipments used in FNAC

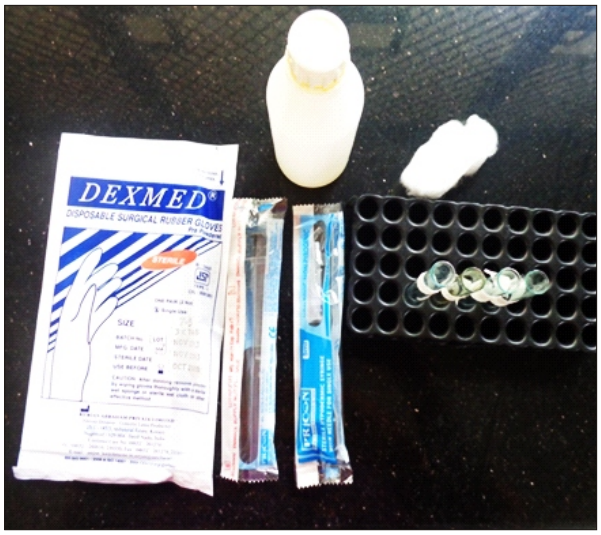


Figure-5 E-PREP PROCESSOR

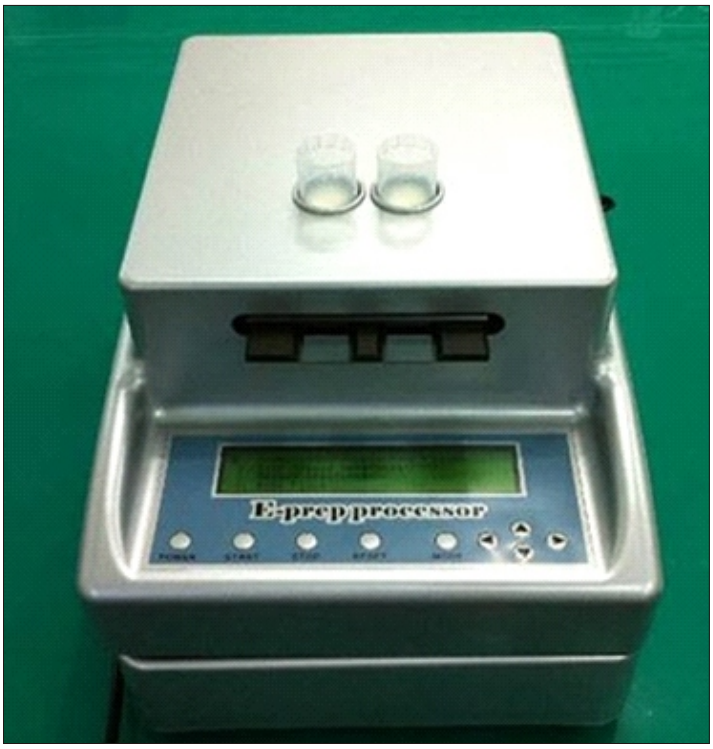


Figure- 6 Dual membrane filter

Figure-7 E-PREP solution

SMEAR PREPARATION

Figure -8 CS

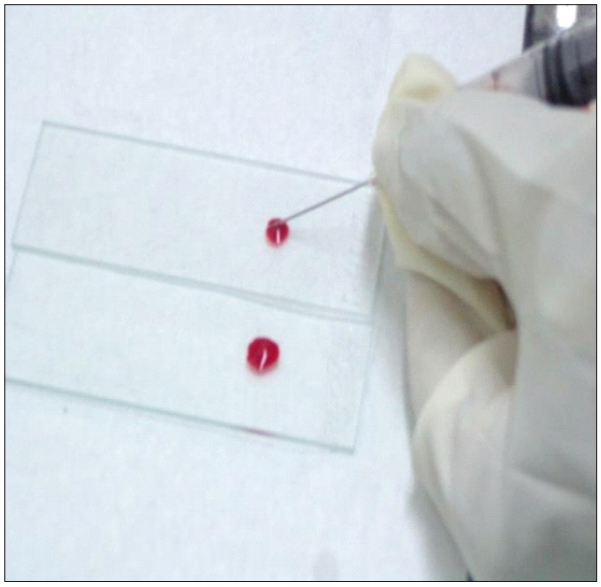
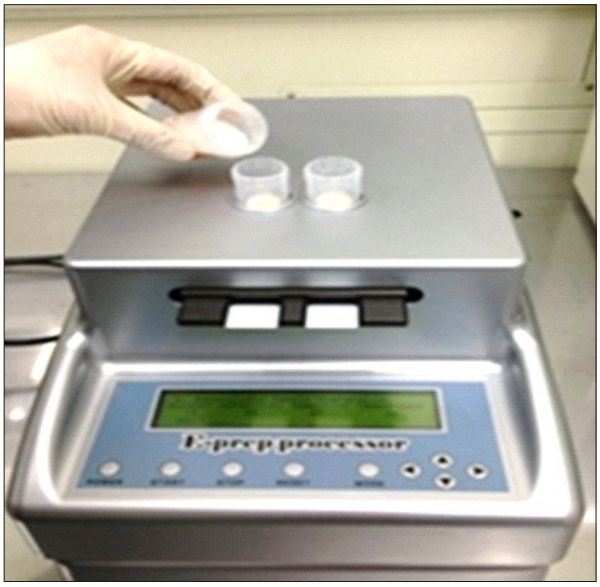
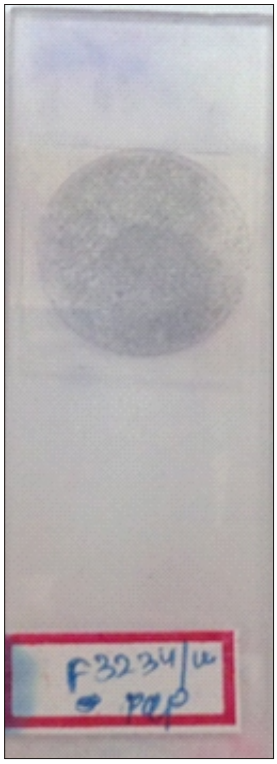


Figure-9 LBP



CS

LBP



Observation and Results

OBSERVATION AND RESULTS

This study is a prospective study. During the period of March 2014 to August 2014, total number of cases aspirated from thyroid, breast and lymphnode lesions are 90.Among 90 cases thyroid, breast and lymphnode lesions each 30.

TOTAL NUMBER OF CASES STUDIED - 90

THYROID SWELLING - 30

LYMPHNODE SWELLING - 30

BREAST LUMP - 30

In all cases conventional smear (CS) and Liquid based preparations (LBP) are made, then stained with MGG and PAP stain for each cases. The smears are graded according to scoring system developed by **Mair et al** [20] to classify quality of cytological aspirate. (Annexure-3)

RESULTS OF THYROID CYTOLOGICAL SMEARS:

In the 30 cases of thyroid swelling there were 6 males and 24 males out of which 19 cases of Nodular colloid goitre , 8 cases of Autoimmune thyroiditis (Hashimoto's thyroiditis and Lymphocytic thyroiditis), and Papillary carcinoma thyroid, Cystic colloid nodule and Colloid goitre with thyroiditis each 1 case . (Table 2&3 and Chart 1)

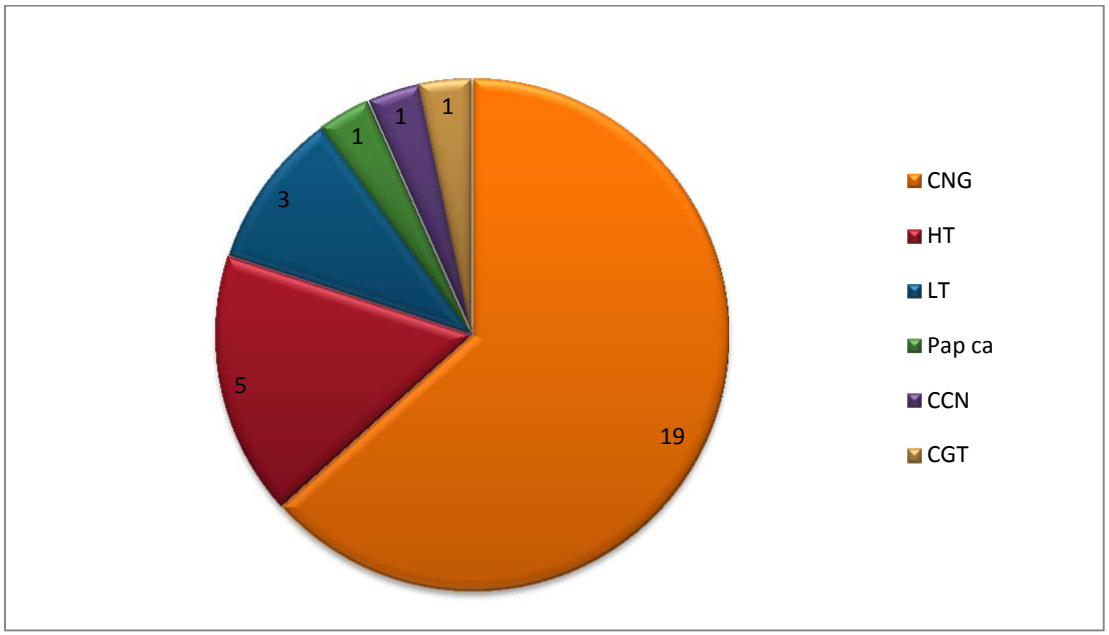
TABLE-2 SEX DISTRIBUTION

Total cases of thyroid	60
Male	6
Female	54

TABLE-3 DISTRIBUTION OF THYROID LESIONS

S.no	Diagnosis	Total
1	Colloid nodular goitre	19
2	Hashimoto's thyroiditis	5
3	Lymphocytic thyroiditis	3
4	Papillary carcinoma	1
5	Cystic colloid nodule	1
6	Colloid goitre with thyroiditis	1

CHART- 1 DISTRIBUTION OF THYROID LESIONS



The smears obtained by CS and LBC were scored and graded accordingly to background blood or clot, cellularity, degree of cellular trauma, degree of cellular degeneration, retention of appropriate cellular architecture.

In our study, it was found that number of diagnostically adequate smears were more from Conventional smear technique than LBC (CS- 25 cases, LBC-23) and diagnostically superior smears were from LBC more than Conventional method (CS-4 cases, LBC-6 cases) and diagnostically unsuitable smears were equal in both techniques(CS-1 case, LBC-1case). (Table-4 & Chart-2)

TABLE-4 GRADING OF SMEARS (THYROID)

S. NO	GRADING OF SMEARS	CS	LBC
1	Diagnostically unsuitable	1	1
2	Diagnostically adequate	25	23
3	Diagnostically superior	4	6
4	Total	30	30

CHART 2 - GRADING OF SMEARS IN THYROID LESIONS

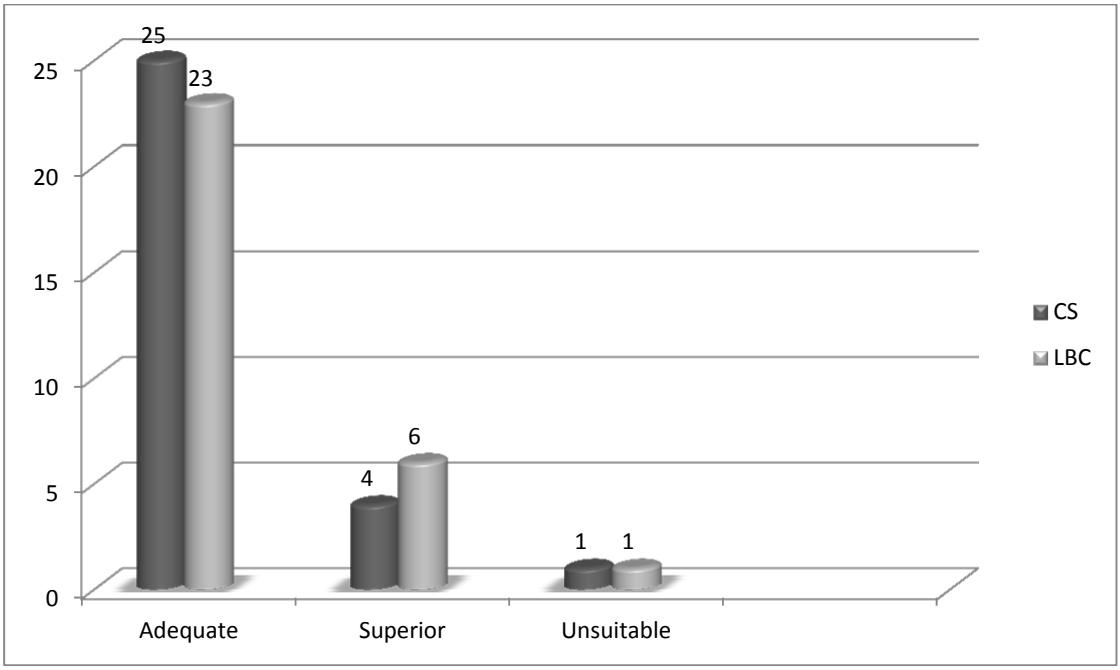


TABLE- 5 PERCENTAGE OF QUALITY OF SMEARS

GRADE		TECHNIQUES		TOTAL
		CS	LBC	
Adequate	Count	25	23	48
	% within grade	52.1%	47.9%	100%
Superior	Count	4	6	10
	% within grade	40%	60%	100%
Unsuitable	Count	1	1	2
	% within grade	50%	50%	100%
Total	Count	30	30	60
	% within grade	50	50	100%

On comparing the number of adequate smears, superior smears and unsuitable smears obtained by Conventional smears and LBP, it was found that CS produced more adequate smears(52.1%), LBP produced more superior smears (60%) and diagnostically unsuitable smears produced by both techniques were equal(50%).(Table5,Chart4)

CHART-4 PERCENTAGE OF SMEARS (THYROID)

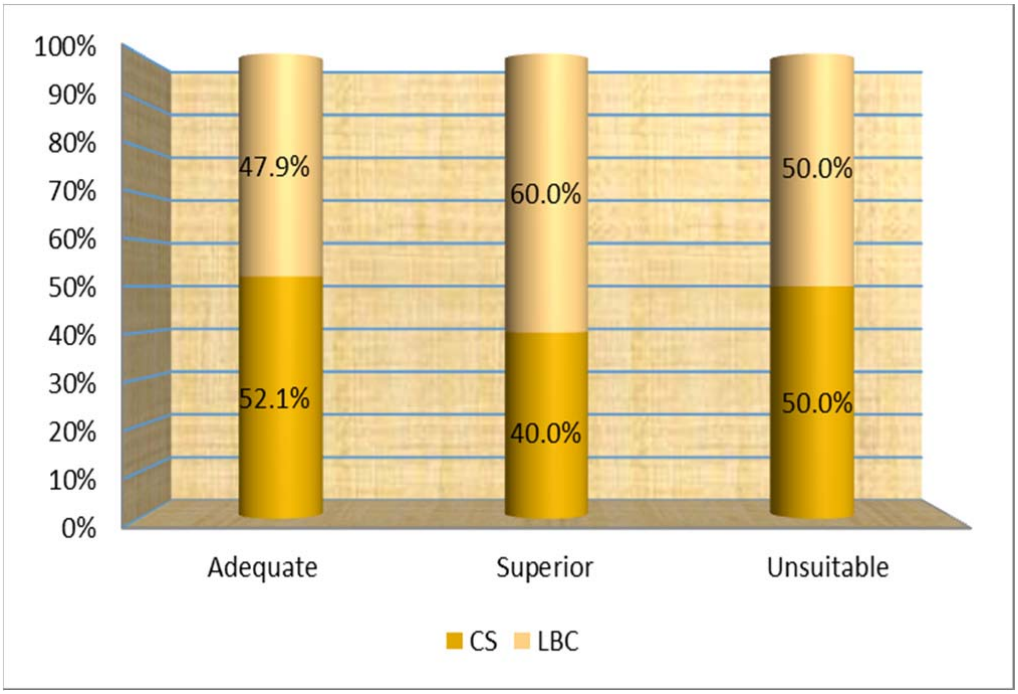


TABLE -6 AVERAGE SCORE OF EACH PARAMETERS

S.NO	PARAMETERS	CS	LBC
1	Background blood or clot	1.033	1.633
2	Cellularity	1.533	1.200
3	Degree of cellular trauma	1.066	1.266
4	Degree of cellular degeneration	0.933	0.866
5	Retention of architecture	1.133	0.866

The average score for each parameters in Conventional smear and LBP of thyroid lesion was calculated (Table-6 & Chart-5) and it was found that the average score of background blood numerically higher in LBP technique (Mean-1.633)

The mean value of cellularity numerically higher in Conventional smears. (Mean-1.533)

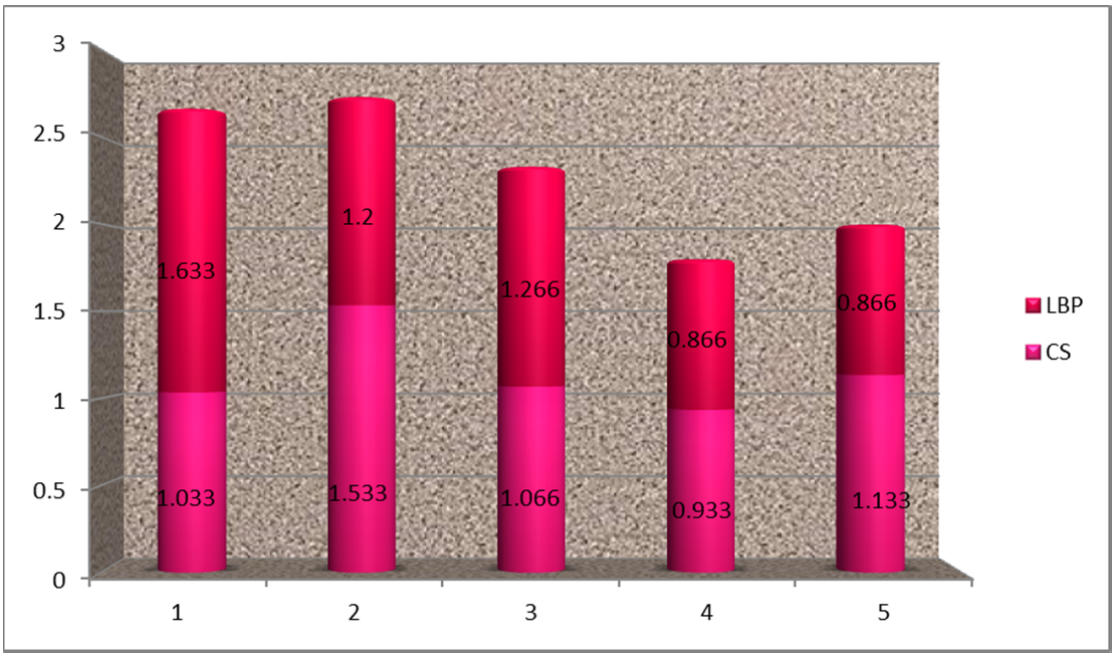
The mean value of degree of cellular trauma numerically higher in LBP technique. (Mean-1.266)

The mean value of degree of cellular degeneration numerically higher in Conventional method (Mean-0.933)

The mean value of retention of architecture numerically higher in Conventional method (Mean-1.133)

Total average score was analysed between two techniques it was found that average score was equal for both methods. (Mean-5.500)

CHART-5 MEAN SCORE (THYROID)



1.Background bloor or clot

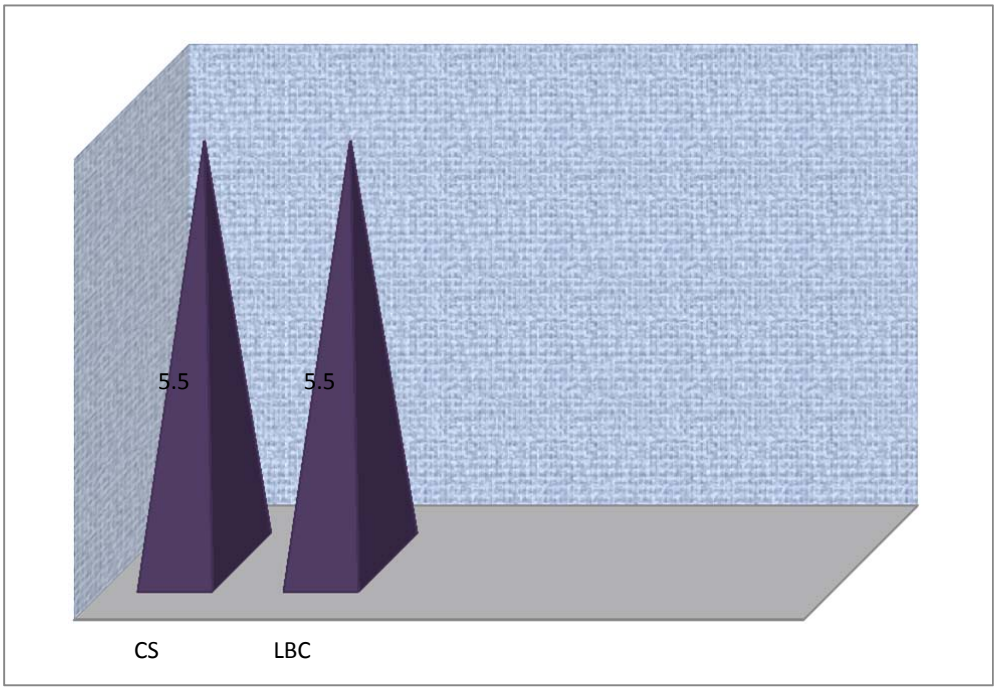
2.Cellularity

3.Degree of cellular trauma

4.Degree of cellular degeneration

5.Retention of appropriate architecture

CHART-6 MEAN SCORE (CS & LBP)



The average mean difference between both techniques (CS-5.500and LBP-5.500) calculated as 0.00001 . The P value of average scores of each techniques calculated by t- test and the P value =1. (Table7 & Chart6)

TABLE- 7 P VALUE OF BOTH METHODS

S.NO	METHODS	AVERAGE MEAN SCORE	MEAN DIFFERENCE	P VALUE
1	CS	5.500	0.00001	1
2	LBP	5.500		

TABLE-8 P VALUE OF PARAMETERS (THYROID)

S.NO	PARAMETERS	P VALUE
1	Background blood or clot	0.001
2	Cellularity	0.012
3	Degree of cellular trauma	0.565
4	Degree of cellular degeneration	0.471
5	Retention of architecture	0.033

The P value of each parameters were calculated by Pearson Chi-Square test.(Table-8)

- P value of background blood and clot score P= 0.01
- P value of parameter of cellularity score P=0.012
- P value of degree of cellular trauma score P=0.565
- P value of degree of cellular degeneration score P=0.471
- P value of retention of architecture score P=0.033

TABLE -9

COMPARISON OF BOTH TECHNIQUES (THYROID)				
Method	Total cases	Quality of smears		P value
CS	30	Adequate	25	0.785
		Superior	4	
		Unsuitable	1	
LBP	30	Adequate	23	
		Superior	6	
		Unsuitable	1	

Comparison of Conventional smears and LBP in thyroid lesions, the P value was calculated by using Pearson Chi-Square test and the P value =0.785. (Table 9)

RESULTS OF CYTOLOGY BREAST LESIONS

In the 30 cases of breast lumps there were 29 females and 1 male out of which 12 cases of Ductal carcinoma, 6 cases Fibroadenoma, 3 cases of each Fibroadenoma with fibroadenosis and Fibroadenoma with fibrocystic changes, 2cases of Fibrocystic disease and each one case of Proliferative breast disease with atypia, Benign phyllodes tumour, Abscess with granulomatous mastitis and one case show only hemorrhagic material(descriptive). (Table10&11, Chart 7)

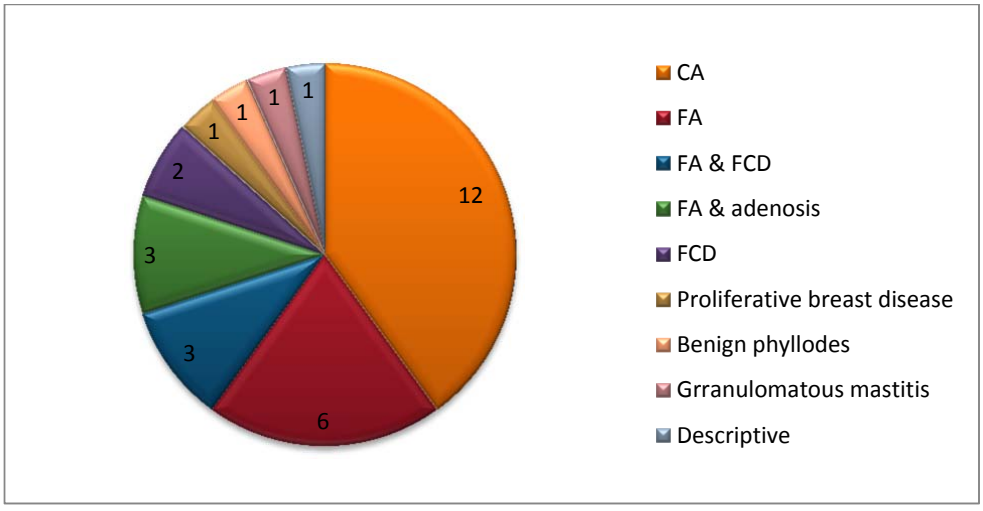
TABLE-10 SEX DISTRIBUTION

TOTAL CASES	FEMALE	MALE
30	29	1

TABLE-11 DISTRIBUTION OF BREAST LESIONS

S.NO	DIAGNOSIS	TOTAL
1	Ductal carcinoma	12
2	Fibroadenoma	6
3	Fibroadenoma with fibrocystic disease	3
4	Fibroadenoma with fibroadenosis	3
5	Proliferative breast disease with atypia	1
6	Fibrocystic disease	2
7	Benign phyllodes	1
8	Granulomatous mastitis	1
9	Descriptive (Haemorrhagic)	1

CHART – 7 : BREAST LESIONS



In our study the smears obtained by Conventional smears and LBC from breast lesions were scored and graded according to parameters of background blood, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of architecture (Table12 & Chart8). It was found that number of diagnostically adequate smears were more from LBP (LBP-19 cases,CS-18 cases)

The superior quality smears more from Conventional smears than LBP (LBP -7cases, CS-9cases)

The unsuitable smears less from Conventional smears than LBC preparation (LBP-4 cases, CS-3 cases)

TABLE -12 : GRADING OF SMEARS - BREAST

S.NO	GRADING OF SMEARS	CS	LBP
1	Adequate	18	19
2	Superior	9	7
3	Unsuitable	3	4
4	TOTAL	30	30

CHART-8 GRADING OF SMEARS

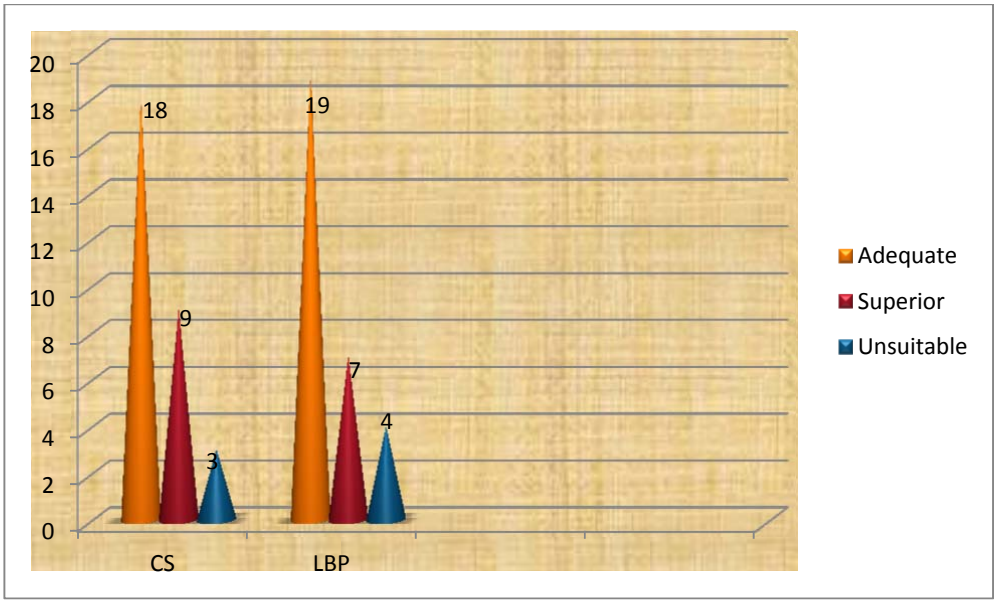
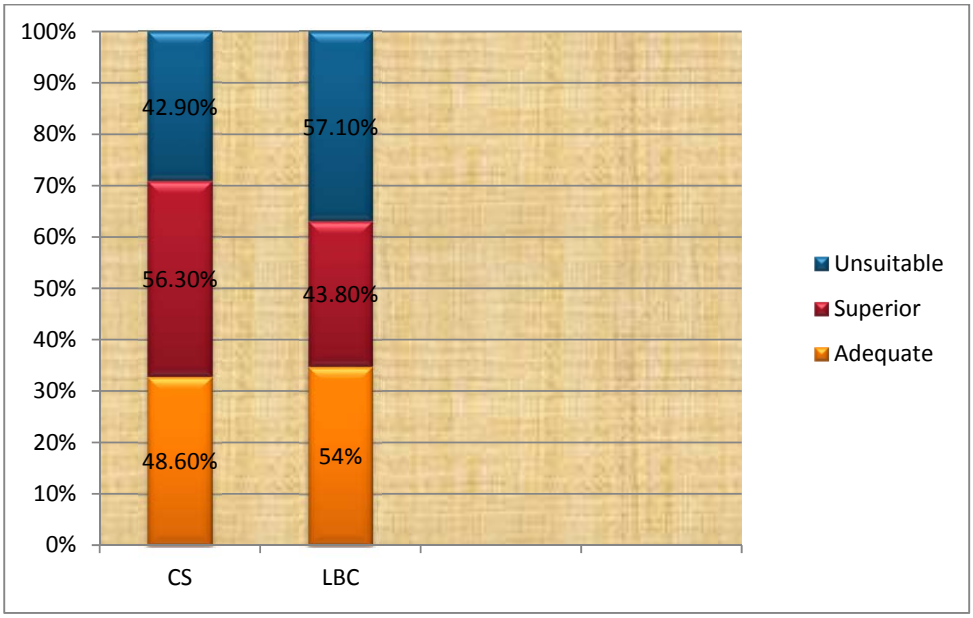


TABLE-13 PERCENTAGE OF QUALITY OF SMEARS

GRADE		TECHNIQUES		TOTAL
		CS	LBC	
Adequate	Count	18	19	37
	% within grade	48.6%	54%	100%
Superior	Count	9	7	16
	% within grade	56.3%	43.8%	100%
Unsuitable	Count	3	4	7
	% within grade	42.9%	57.1%	100%
Total	Count	30	30	60
	% within grade	50%	50%	100%

CHART-9 PERCENTAGE OF QUALITY OF SMEARS (BREAST)



On comparing percentage of quality of smears obtained by Conventional smears and Liquid based preparation the percentage of superior quality smears more from Conventional smears (56.3%) and percentage of adequate smears more from the Liquid based preparation(54%) and unsuitable smears more in Liquid based preparation (57.10%). (Table13 & Chart 9)

The average score (mean) for each parameters (Background blood or clot, Cellularity, Degree of cellular trauma , Degree of cellular degeneration and Retention of architecture) in Conventional smears and Liquid based preparation were calculated. (Table14 & Chart 10)

TABLE -14 AVERAGE SCORE OF PARAMETERS

S.NO.	PARAMETERS	AVERAGE SCORE	
		CS	LBP
1	Background blood or clot	1.100	1.7667
2	Cellularity	1.666	1.233
3	Degree of cellular trauma	1.133	0.8667
4	Degree of cellular degeneration	1.000	0.7667
5	Retention cellular architecture	1.066	0.766

The average score of parameter of background blood or clot found to be higher in LBP, average score of LBP was 1.7667, and average score of CS was 1.100.

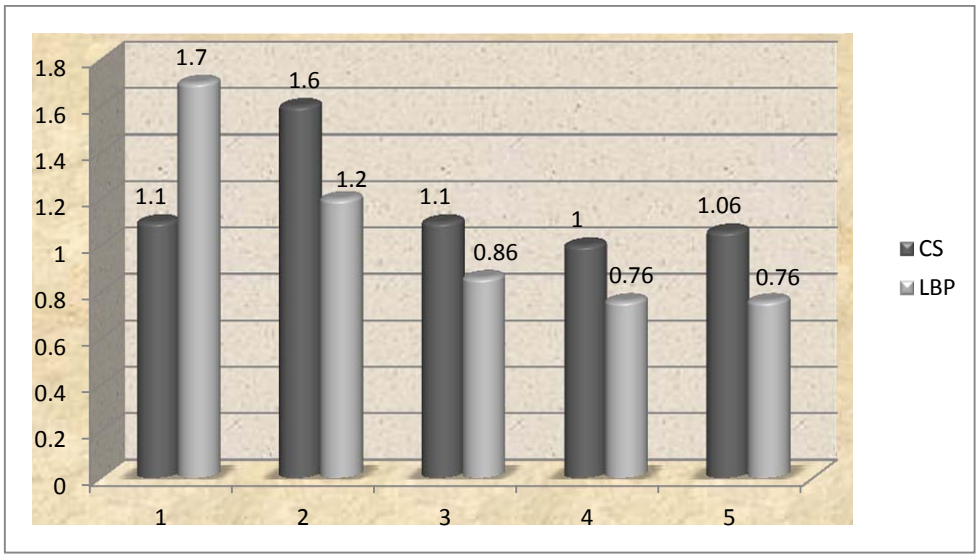
The average score of parameter of cellularity found to be higher in Conventional smears, average score of CS was 1.666 and average score of LBP was 1.233.

The average score of parameter of degree of cellular trauma found to be higher in Conventional smear, average score of CS was 1.133 and LBP was 0.8667.

The average score of parameter of degree of cellular degeneration found to be higher in Conventional smear , average score of CS was 1.000 and LBP was 0.7667.

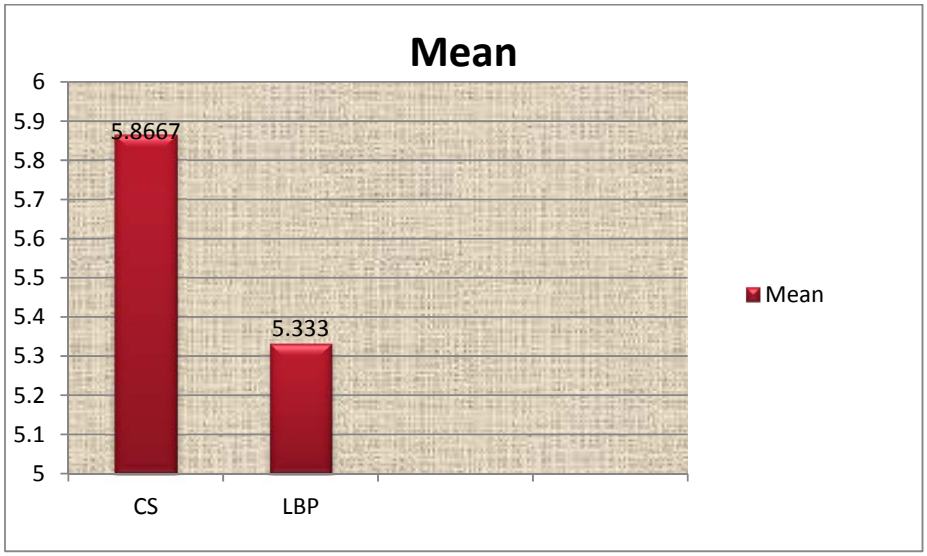
The average score of parameter of retention of cellular architecture found to be higher in Conventional smear, average score of LBP was 0.766 and CS was 1.066.

CHART-10 AVERAGE SCORE (BREAST)



1) Background blood or clot
2) Cellularity
3) Degree of cellular trauma
4) Degree of cellular degeneration
5) Retention of cellular architecture

CHART-11 MEAN SCORE (CS & LBP)



Total average score (Mean) of both techniques were compared and it found to be average mean value more in Conventional smears than LBP. (Mean value of CS-5.8667 and LBP- 5.333).(Table15 & Chart 11)

TABLE -15 P VALUE OF BOTH METHODS

S.NO	METHODS	AVERAGE MEAN SCORE	MEAN DIFFERENCE	P VALUE
1	CS	5.866	0.533	0.259
2	LBP	5.366		

The average mean difference between both techniques (CS-5.866and LBP-5.366) calculated as 0.533 . The P value of average scores of each techniques calculated by t- test and the P value = 0.259.(Table-15)

P VALUE OF PARAMETERS (BREAST)

The objective parameters of Background blood or clot, Cellularity, Degree of cellular trauma, Degree of cellular degeneration and Retention of cellular architecture were compared with Pearson Chi- Square test and P value calculated.(Table-16)

TABLE-16 P VALUE OF PARAMETERS (BREAST)

S.NO	PARAMETERS	P VALUE
1	Background blood or clot	0.001
2	Cellularity	0.004
3	Degree of cellular trauma	0.046
4	Degree of cellular degeneration	0.084
5	Retention of cellular architecture	0.018

In our study , the comparison of P value of each parameters of both techniques calculated by Pearson Chi- Square test.

The P value of background blood or clot score P=0.001

The P value of cellularity score P= 0.004

The P value of degree of cellular trauma score P=0.046

The P value of degree of cellular degeneration score P=0.084

The P value of retention of cellular architecture score P=0.018

TABLE-17 PEARSON CHI-SQUARE TEST

COMPARISON OF BOTH TECHNIQUES (BREAST)				
Method	Total cases	Quality of smears		P value
CS	30	Adequate	25	0.811
		Superior	4	
		Unsuitable	1	
LBP	30	Adequate	23	
		Superior	6	
		Unsuitable	1	

In our study , on comparing the Conventional smears and Liquid based preparation by all five parameters and the P value calculated by Pearson Chi-Square test and P value=0.811. (Table17)

RESULTS OF CYTOLOGY OF LYMPHNODE LESIONS

In the 30 cases of lymphnode swelling there were 7 females 23 males . The Conventional smear and Liquid based preparation were done for all 30 cases.(Table-18)

TABLE-18 SEX DISTRIBUTIONS

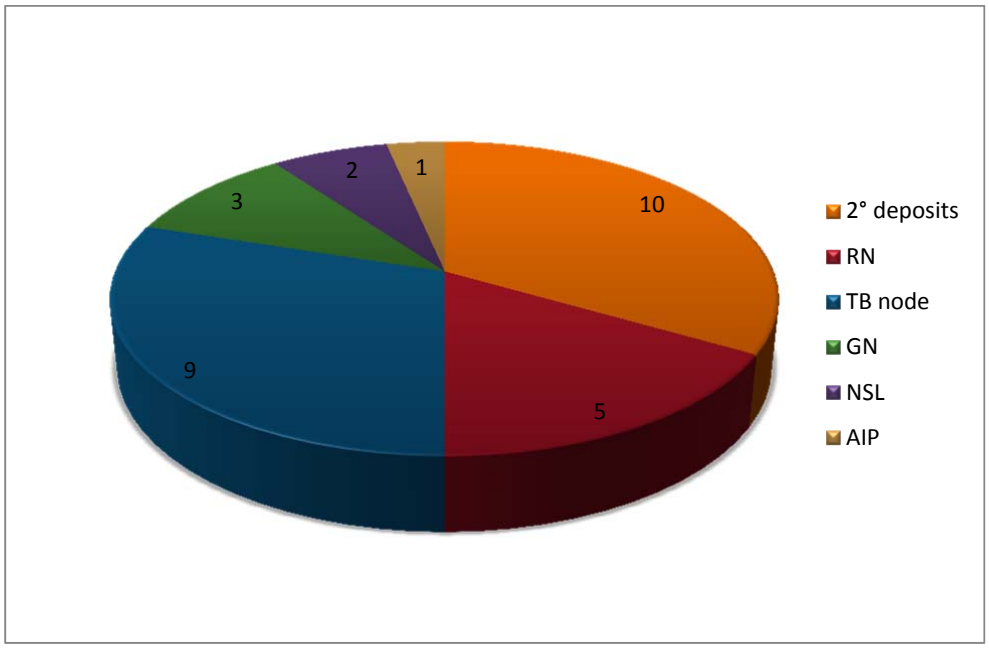
MALE	FEMALE	TOTAL
23	7	30

Out of 30 cases of lymphnode swelling , 10 cases were diagnosed as Secondary carcinomatous deposits (including squamous cell carcinomatous deposits and adenocarcinomatous deposits), 5 cases of Reactive node, 9 cases of caseating tuberculous lymphadenitis, 3 cases of Granulomatous lymphadenitis,2 cases of Nonspecific lymphadenitis and 1case of Acute inflammatory pathology. (Table19& Chart12)

TABLE -19 DISTRIBUTION OF LYMPHNODE LESIONS

S.NO	DIAGNOSIS	TOTAL
1	Secondary carcinomatous deposits	10
2	Reactive node	5
3	Caeseating Tuberculous lymphadenitis	9
4	Granulomatous lymphadenitis	3
5	Non specific lymphadenitis	2
6	Acute inflammatory pathology	1

CHART-12 DISTRIBUTION OF LYMPHNODE LESIONS



In our study, the smear obtained by Conventional and Liquid based preparation from lymphnode swellings were scored and graded according to background blood and clot, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of cellular architecture, (Table20&Chart 13) and it was found that number of diagnostically adequate smears more in LBP (LBP-24,CS-24).

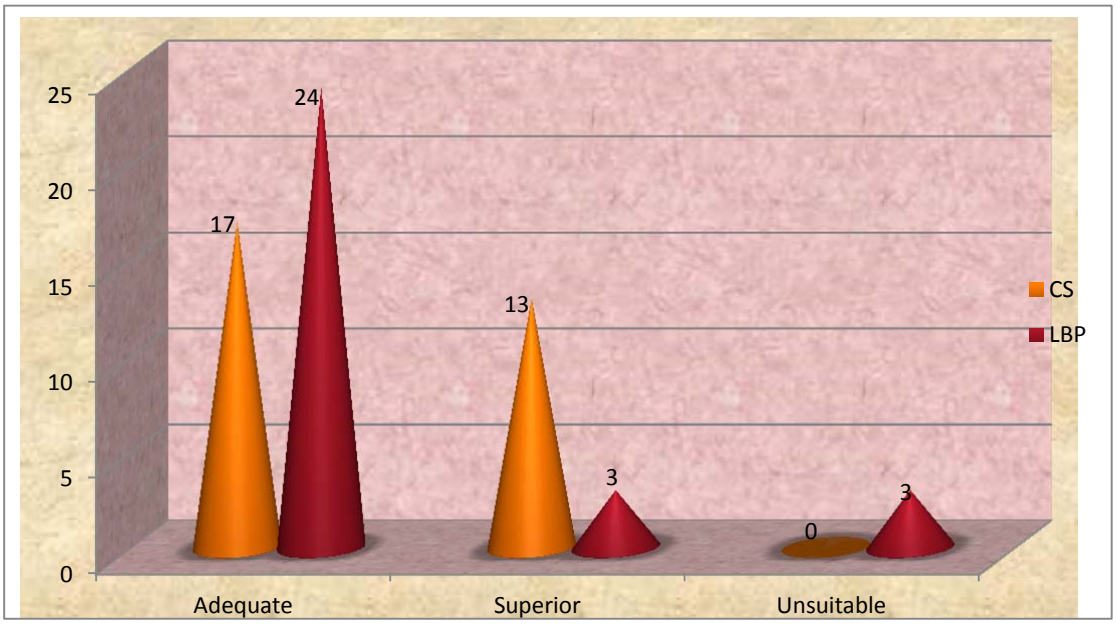
The diagnostically superior smears more from Conventional smears than LBP (CS-13, LBP-3).

The diagnostically unsuitable smears more from Liquid based preparation than Conventional smears (CS-0, LBP-3).

TABLE- 20 GRADING OF SMEARS (LYMPHNODE)

S.NO	GRADING OF SMEARS	CS	LBP
1	Adequate	17	24
2	Superior	13	3
3	Unsuitable	0	3
4	Total	30	30

CHART-13 GRADING OF SMEARS (LYMPHNODE)



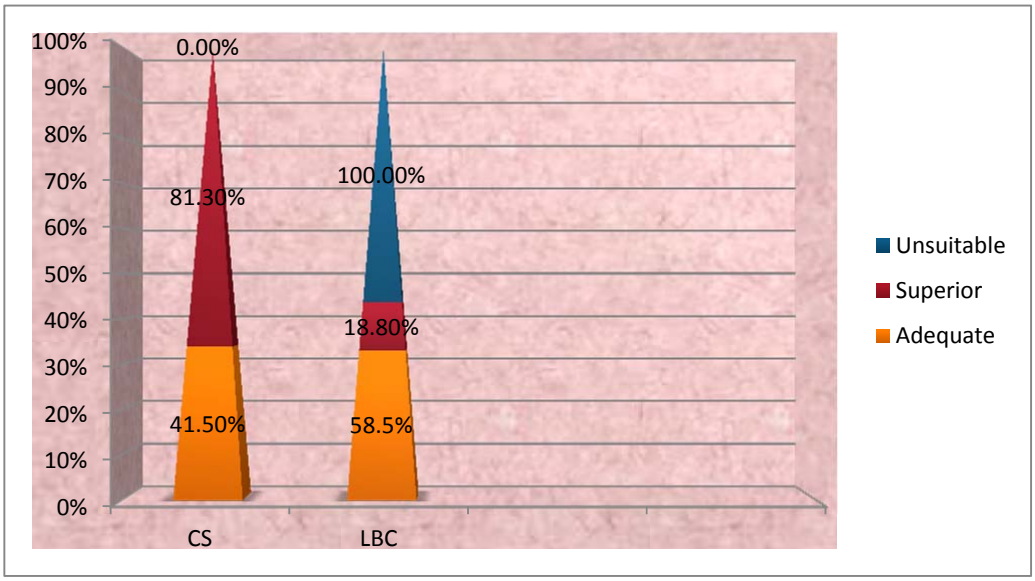
The average score (mean) for each parameters (Background blood or clot, Cellularity, Degree of cellular trauma , Degree of cellular degeneration and Retention of architecture) in Conventional smears and Liquid based preparation were calculated.

TABLE-21 : PERCENTAGE OF QUALITY OF SMEARS

(LYMPHNODE)

GRADE		TECHNIQUES		TOTAL
		CS	LBC	
Adequate	Count	17	24	41
	% within grade	41.5%	58.5%	100%
Superior	Count	13	3	16
	% within grade	81.3%	18.8%	100%
Unsuitable	Count	0	3	3
	% within grade	0%	100%	100%
Total	Count	30	30	60
	% within grade	50%	50%	100%

CHART-14 PERCENTAGE OF SMEARS



On comparing percentage of quality of smears obtained by Conventional smears and Liquid based preparation the percentage of superior quality smears more from Conventional smears (81.3%) and percentage of adequate smears more from the Liquid based preparation(58.5%) and unsuitable smears more in Liquid based preparation (100%). (Table21& Chart14)

The average score (mean) for each parameters (Background blood or clot, Cellularity, Degree of cellular trauma , Degree of cellular degeneration and Retention of architecture) in Conventional smears and Liquid based preparation were calculated.(Table22,Chart15)

TABLE- 22 : AVERAGE SCORE (LYMPHNODE)

S.NO	PARAMETERS	AVERAGE SCORE (MEAN)	
		CS	LBP
1	Background blood or clot	1.033	1.733
2	Cellularity	1.933	1.133
3	Degree of cellular trauma	1.166	0.900
4	Degree of cellular degeneration	1.166	0.866
5	Retention cellular architecture	1.266	0.800

The average score of parameter of background blood numerically higher in LBP, it was found that Mean score of LBP=1.733 and Conventional smear=1.033

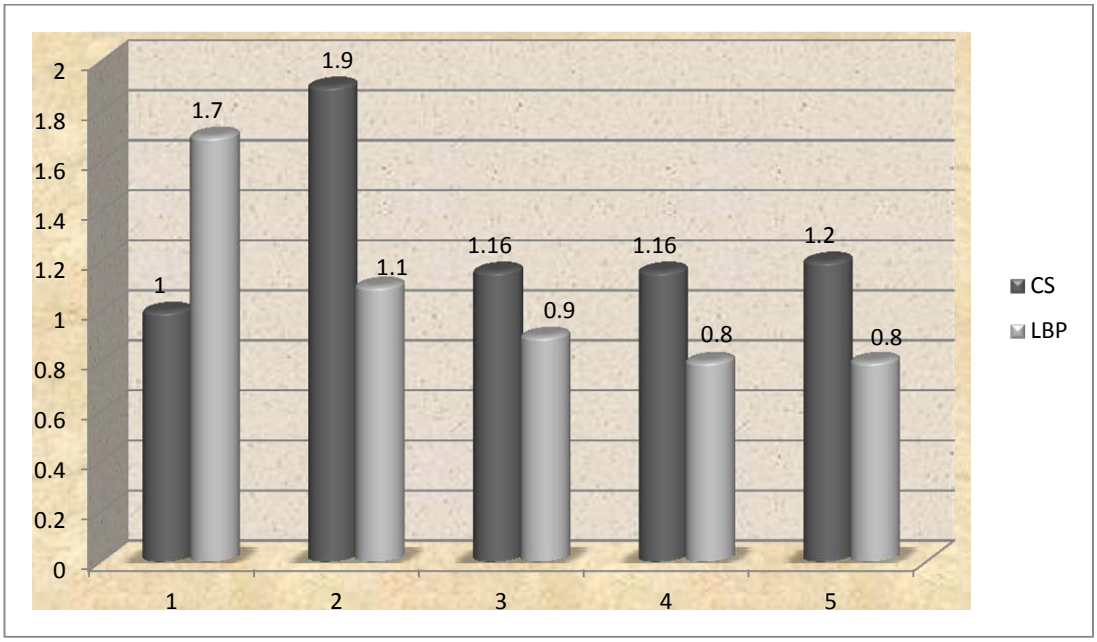
The average score of cellularity was more in Conventional smears , it was found that Mean score of LBC=1.133 and CS=1.933

The average score of degree of cellular trauma was higher in Conventional smear it was found that Mean score of CS=1.166 and LBP= 0.900

The average score of degree of cellular degeneration was higher in Conventional smears it was found that Mean score of CS=1.1667 and LBP=0.866

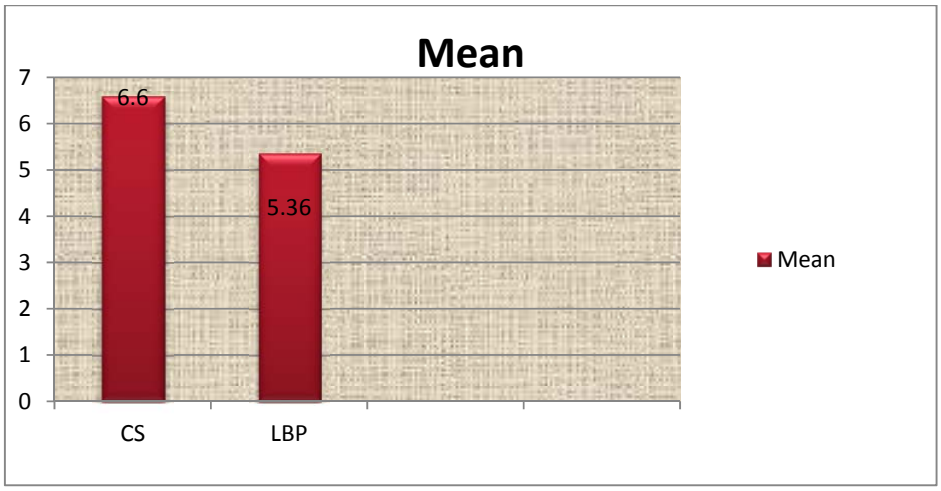
The average score of retention of cellular architecture numerically higher in Conventional smears it was found that Mean score of CS=1.266 and LBP= 0.800

CHART-15: MEAN SCORE OF PARAMETERS



1) Background blood or clot
2) Cellularity
3) Degree of cellular trauma
4) Degree of cellular degeneration
5) Retention of cellular architecture

CHART-16 MEAN SCORE OF LBP AND CS



Total average score (Mean) of both techniques were compared and it found to be average mean value more in Conventional smears than LBP. (Table23,Chart16)

Mean value of CS = 6.600

Mean value of LBP = 5.366

Mean difference = 1.233

TABLE-23 P VALUE OF BOTH METHODS

S.NO	METHODS	AVERAGE MEAN SCORE	MEAN DIFFERENCE	P VALUE
1	CS	6.600	1.233	0.259
2	LBP	5.366		

The average mean difference between both techniques (CS-5.866and LBP-5.366) calculated as 0.533 . The P value of average scores of each techniques calculated by t- test and the P value = 0.259 (Table23)

P VALUE OF PARAMETERS (LYMPHNODE)

The parameters of Background blood or clot, Cellularity, Degree of cellular trauma, Degree of cellular degeneration and Retention of cellular architecture were compared with Pearson Chi- Square test and P value calculated.

TABLE-24 P VALUE OF PARAMETERS (LYMPHNODE)

S.NO	PARAMETERS	P VALUE
1	Background blood or clot	0.001
2	Cellularity	0.001
3	Degree of cellular trauma	0.011
4	Degree of cellular degeneration	0.006
5	Retention of cellular architecture	0.001

In our study , the comparison of P value of each parameters of both techniques calculated by Pearson Chi- Square test. (Table24)

The P value of background blood or clot score P=0.001

The P value of parameter of cellularity score P=0.004

The P value of degree of cellular trauma score P=0.046

The P value of degree of cellular degeneration score P=0.084

The P value of retention of cellular architecture score P=0. 018

TABLE-25 : PEARSON CHI-SQUARE TEST

COMPARISON OF BOTH TECHNIQUES (LYMPHNODE)				
Method	**Total cases**	**Quality of smears**		**P value**
CS	30	Adequate	17	0.005
		Superior	13	
		Unsuitable	0	
LBP	30	Adequate	24	
		Superior	3	
		Unsuitable	3	

In our study , on comparing the Conventional smears and Liquid based preparation by all five parameters and the P value calculated by Pearson Chi-Square test and P value=0.005 (Table25)

TABLE - 26 : OVERALL GRADING & PERCENTAGE OF QUALITY OF SMEARS (THYROID, BREAST, LYMPHNODE)

GRADE		TECHNIQUES		TOTAL
		CS	LBC	
Adequate	Count	60	66	126
	% within grade	47.6%	52.4%	100%
Superior	Count	26	16	42
	% within grade	61.9%	38%	100%
Unsuitable	Count	4	8	12
	% within grade	33.3%	66.7%	100%
Total	Count	90	90	90
	% within grade	50%	50%	100%

In our study, on comparing grading of smears and percentage of quality of smears obtained by Conventional smears and Liquid based preparation for all thyroid, breast and lymphnode lesions.(Table 26 & Chart 17,18)

The total number of superior quality smears more from Conventional smears (CS-26, LBP-160)

The total number of adequate smears more from the Liquid based preparation(CS-60, LBP-66))

The total number of unsuitable smears more in Liquid based preparation(CS-4, LBP-8).

CHART-17 GRADING OF SMEARS

(THYROID, BREAST, LYMPHNODE)

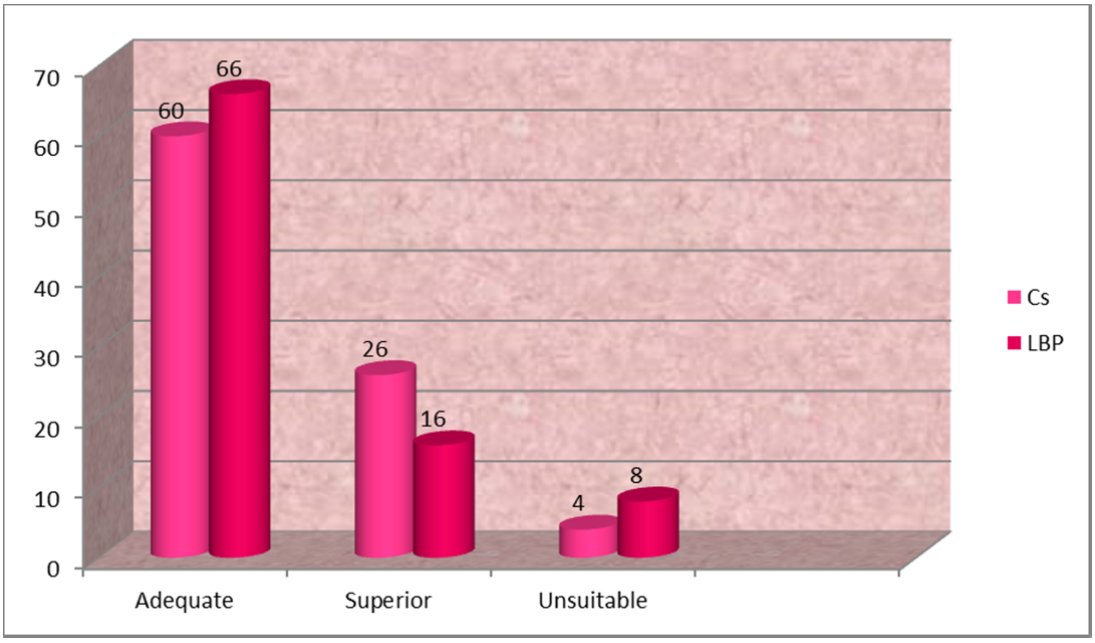
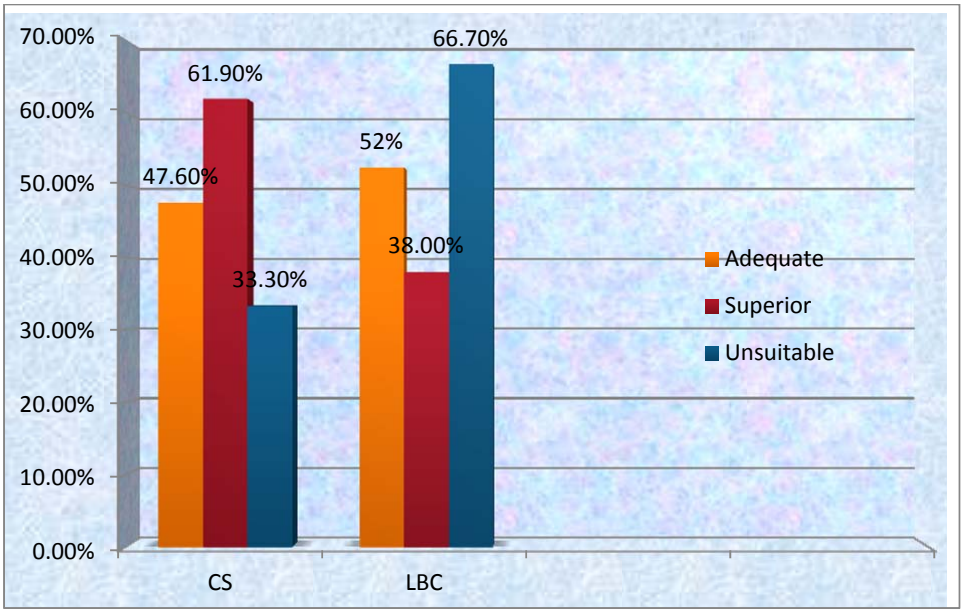


CHART-18 OVERALL PERCENTAGE OF QUALITY OF SMEARS (THYROID, BREAST, LYMPHNODE)



The percentage of superior quality smears more from Conventional smears (61.9%)

The percentage of adequate smears more from the Liquid based preparation(52.4%)

The percentage of unsuitable smears more in Liquid based preparation(66.7%).

TABLE -27 PEARSON CHI –SQUARE TEST

(Thyroid, breast and lymphnode)

COMPARISON OF BOTH TECHNIQUES				
Method	**Total cases**	**Quality of smears**		**P value**
CS	90	Adequate	60	0.135
		Superior	26	
		Unsuitable	4	
LBP	90	Adequate	66	
		Superior	16	
		Unsuitable	8	

In our study , on comparing the Conventional smears and Liquid based preparation b for thyroid, breast and lymphnode lesions by all five parameters like background blood or clot, cellularity, degree of cellular trauma,degree of cellular degeneration and retention of cellular architecture and the P value calculated by Pearson Chi-Square test and P value=0.135. (Table 27)

Colour Plates

THYROID SWELLING

NODULAR COLLOID GOITRE

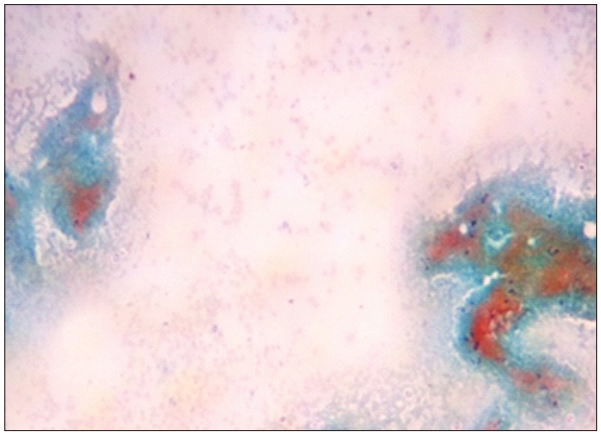


Figure -10 Colloid in the background of hemorrhage-pap stain 40x (CS)

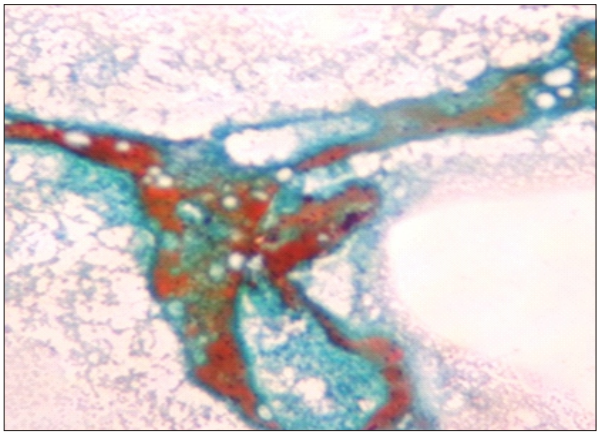


Figure -11 Colloid in the background of haemorrhage -Pap stain, 100x (CS)

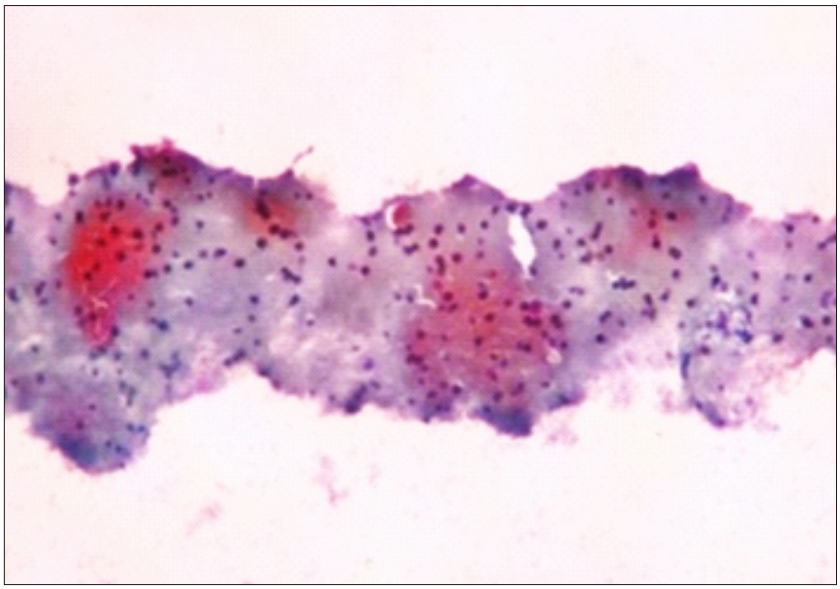


Figure-12 Scattered thyroid follicular cells & colloid in a clean background –Pap stain, 400x (LBP)

HASHIMOTO'S THYROIDITIS

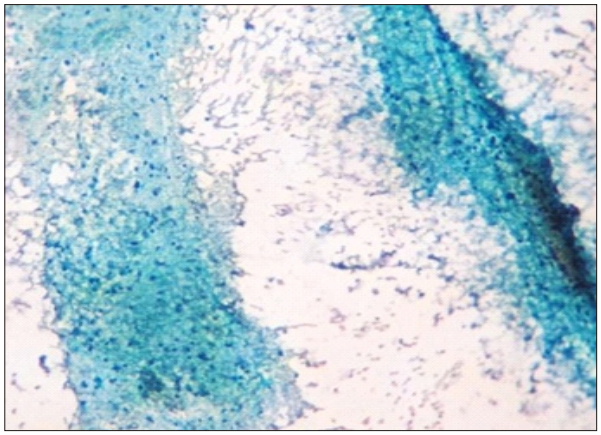


Figure-13 Thyroid follicular cells admixed with lymphocytes in a dirty background Pap satin ,100x (CS)

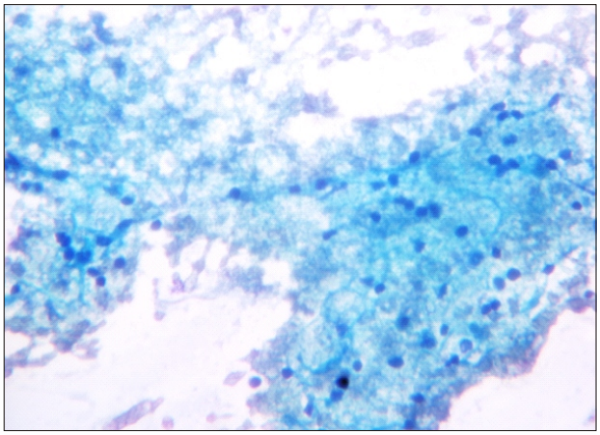


Figure-14 Thyroid follicular cells admixed with lymphocytes, Pap stain, 400x (CS)

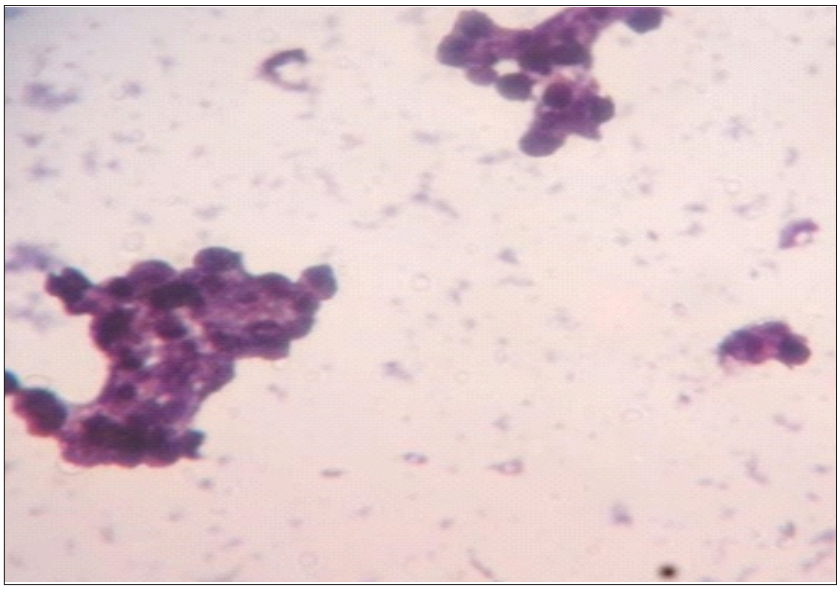


Figure-15 cluster of thyroid follicular cells admixed with lymphocytes in a clean background , Pap stain , 400x (LBP)

BREAST LESIONS

DUCTAL CARCINOMA

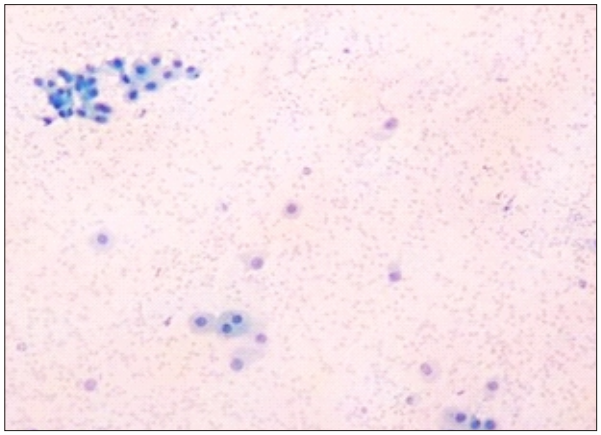


Figure-16 Dyscohesive clusters of duct epithelial cells in a hemorrhagic Background. Pap stain, 100x (CS)

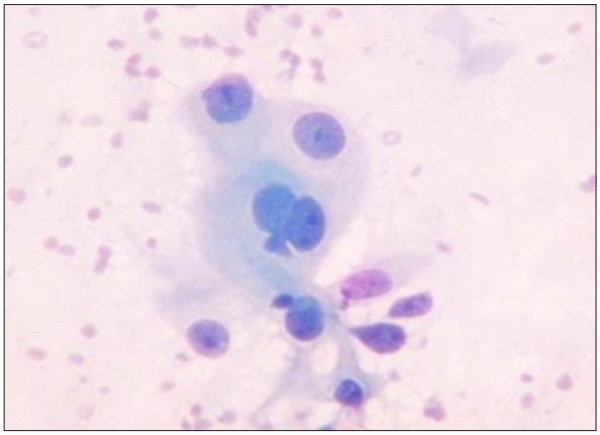


Figure-17 Malignant duct epithelial cells Pap stain, 400x (CS)

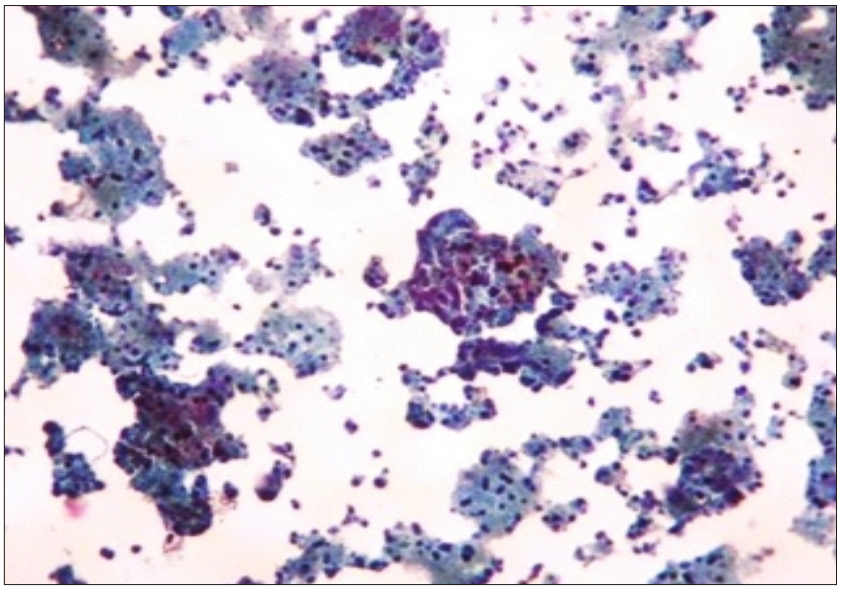


Figure-18 Cellular smear showing malignant duct epithelial cells , Pap stain, 100x (LBP smear)

DUCTAL CARCINOMA

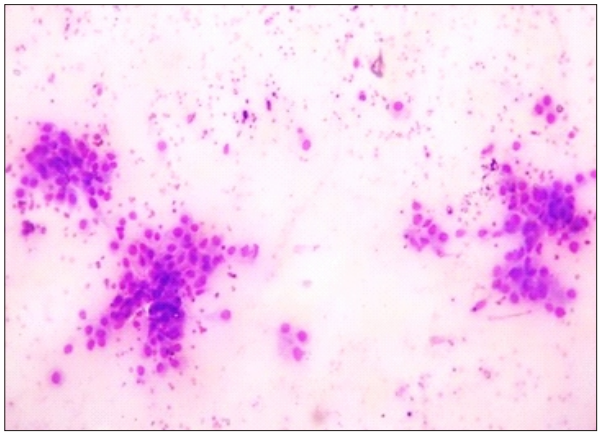


Figure-19 Dyscohesive duct epithelial cells in a hemorrhagic background. MGG stain, 100x (CS)

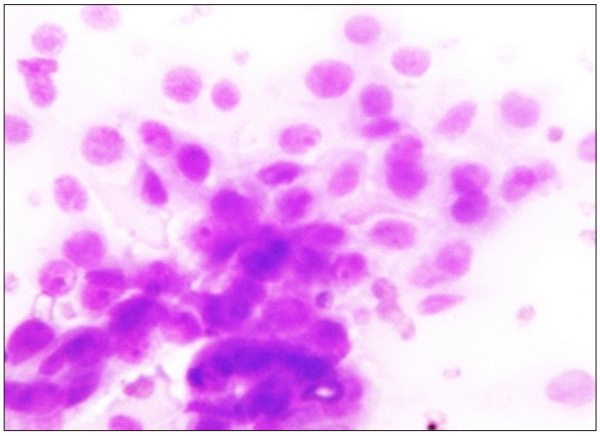


Figure-20 Malignant duct epithelial cells in MGG stain, 400x (CS)

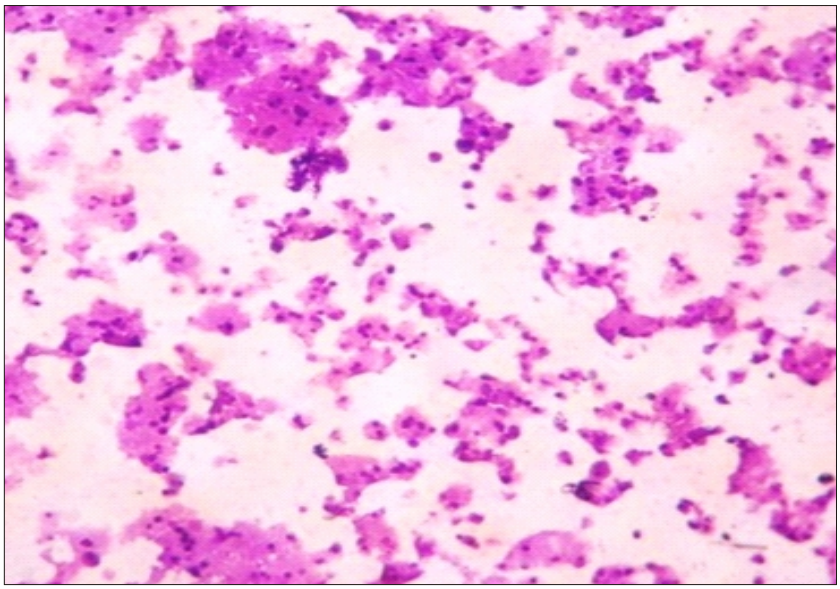


Figure-21 Malignant duct epithelial cells in MGG stain, 100x (LBP smear)

FIBROADENOMA

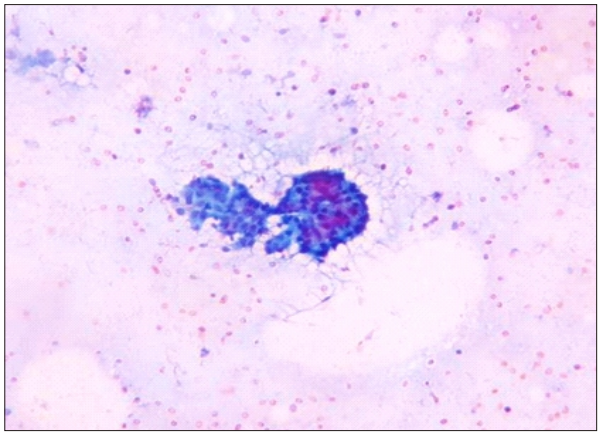


Figure-22 Cohesive cluster of benign duct epithelial cells in a hemorrhagic background. Pap stain 100x (CS)

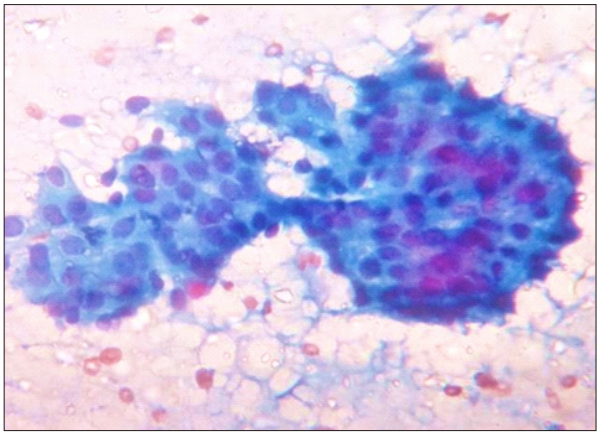


Figure-23 Benign duct epithelial cells in Pap stain, 400x (CS)

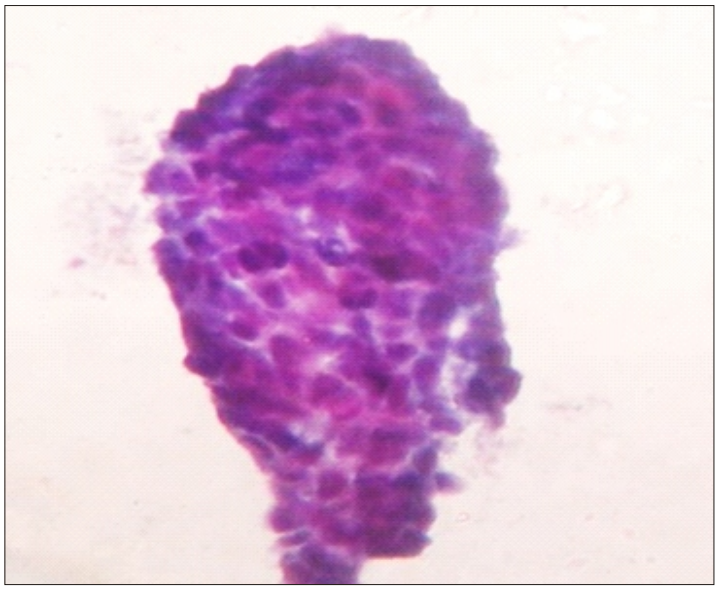


Figure-24 Cohesive cluster of benign duct epithelial cells in clean background. Pap stain, 400x (LBP smear)

LYMPHNODE LESIONS

GRANULOMATOUS LYMPHADENITIS

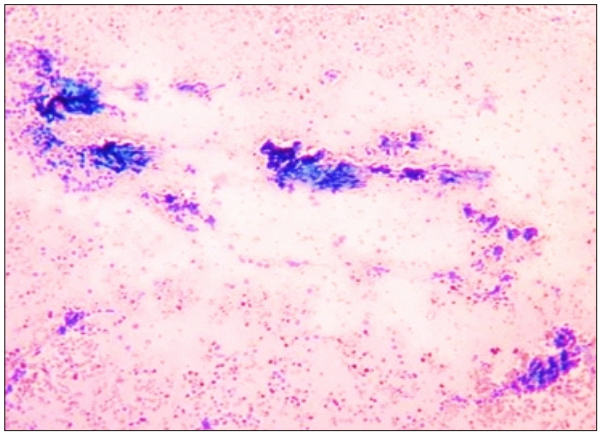


Figure-25 Epitheloid granulomata in a hemorrhagic background . Pap stain , 100x (CS)

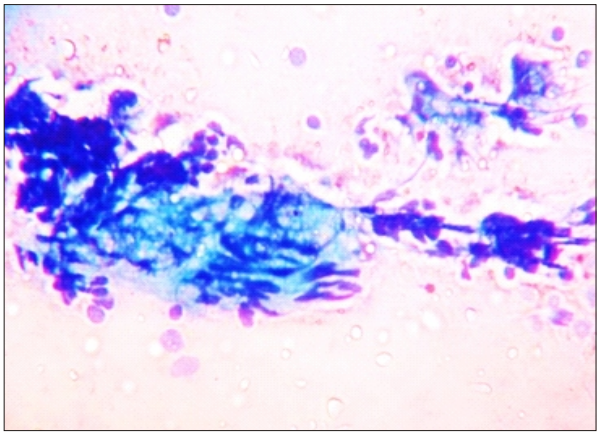


Figure-26 Epitheloid granulomata in Pap stain , 400x (CS)

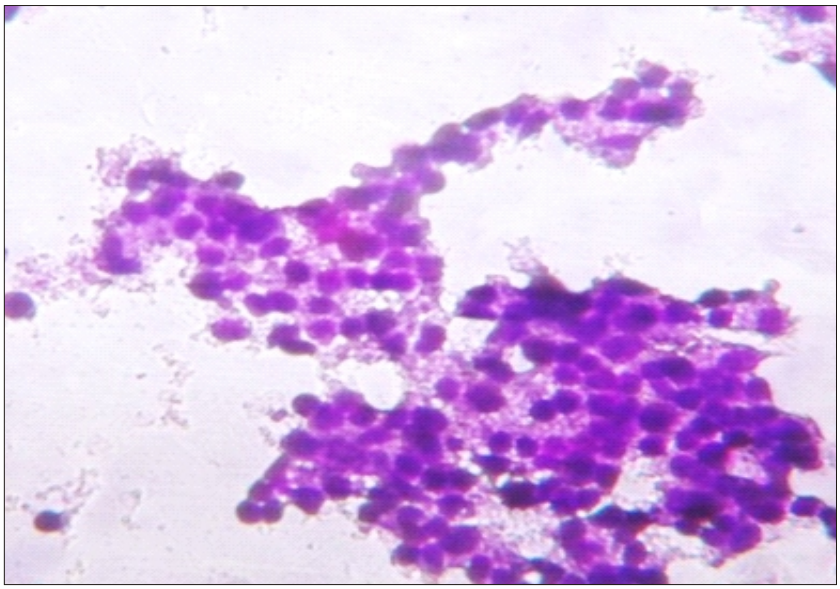


Figure-27 Epitheloid granulomata in Pap stain, 400x (LBP smear)

SECONDARY CARCINOMATOUS DEPOSITS

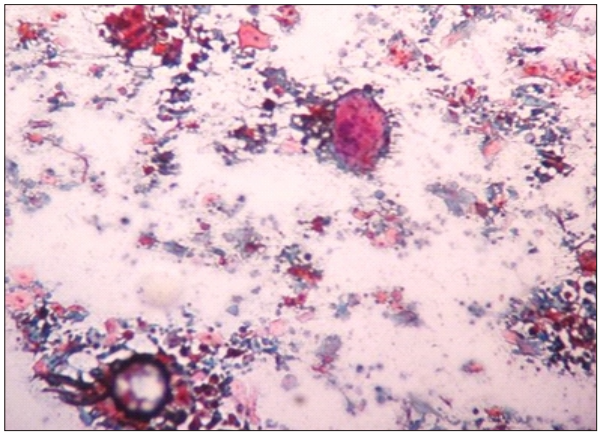


Figure -28 Pleomorphic cells in a hemorrhagic background. Pap statin, 100x (CS)

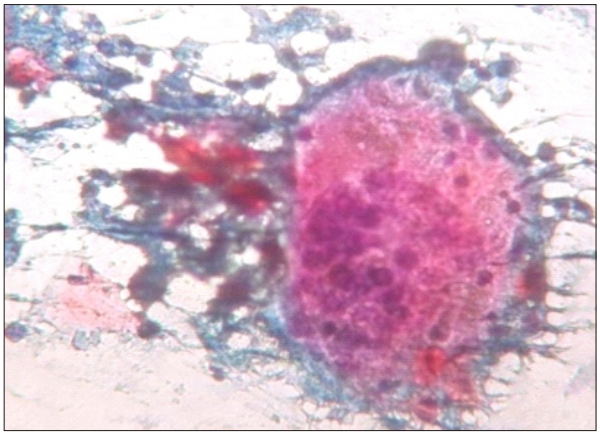


Figure-29 secondary carcinomatous deposits in lymphnode. Pap stain, 400x (CS)

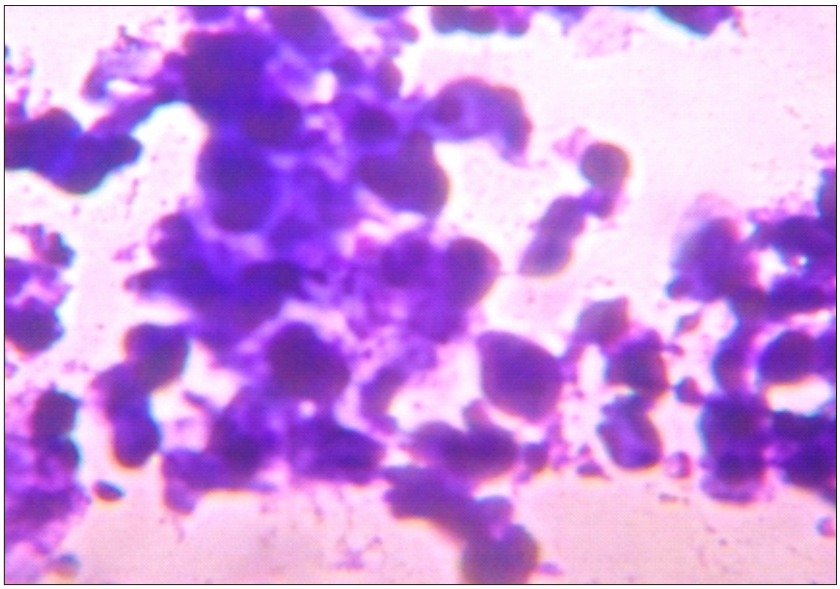


Figure-30 Plemorphic epithelial cells in a lymphnode. Pap stain 400x (LBP smear)

Discussion

DISCUSSION

Fine needle sampling (both aspiration and non aspiration technique) is most commonly used method in diagnosis of pathological conditions in cytology.

The basic principle underlying the fine needle aspiration cytology is the aspiratin cellular material from the target masses or lesions often using fairly high suction pressure. This technique needs a needle and a syringe advisedly held in a syringe holder enabling single handed suction to be exercised. This procedure depends on the suction and occasionally can cause hematoma as well as yield haemorrhagic material. The FNAC initially used to confirm a clinically suspicious cases of malignancy and local recurrence of carcinomas without further surgical intervention. Clinical use of FNAC not only for neoplastic condition also used for non neoplastic conditions like inflammatory and degenerative lesions.

The FNAC technique is applicable to easily palpable superficial lesions like skin, subcutis, thyroid, superficial lymphnode, breast, salivary gland . It is less demanding technique than biopsy and risk of complications are very low. FNAC procedure done in outpatient department, in radiology theatres and it is an office procedure. [9]

Liquid based cytology is a new technology for fine needle aspiration samples. It is used for mainly for cervical cancer screening , now also used for non gynaecolgical samples. The basic principle of LBC is to collect specimen into the fixative solution and then make a monolayers of cells after staining . LBC preservation of cells are excellent and reduces the bloody background. [2]

E-PREP system is liquid based cytology processor with patent dual membrane filters. In this method able to collect large number of cells and make a monolayer preparation of cells with good cytological details. The quality of the smear is excellent due to application of both filtration & precipitation methods. Hence the method gives more accurate results. E-PREP also facilitates preparation of more number of slides 150 slides / hr.[3]

In this prospective study , we included the samples obtained from 90 patients. The samples collected from the patients who were all attending the cytology department for fine needle sampling for thyroid, breast and lymphnode lesions and excluded swelling other than thyroid, breast and lymphnode and gynaecological cases.

Samples collected from 30 cases from thyroid swelling, 30 cases from breast lump and 30 cases from lymphnode swelling . The smears made by

both Conventional method and Liquid based preparation (E-PREP) for each cases.

Smears were obtained from both techniques were scored according to **Mair et al** in 1989,[20] on the basis of five objective parameters like background blood or clot, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of cellular architecture.

The number of adequate smears , superior quality smears and unsuitable smears from each techniques, total average score, mean score under each sub category and P value are compared for each techniques and analysed statistically Z test or student's 't ' test.

IN THYROID SWELLING:

In our study , on considering all observations and results of each technique (Conventional method and LBP) in thyroid swelling , number of diagnostically adequate smears, diagnostically superior smears and unsuitable smears, average score of each parameters, mean score, P value are calculated and analysed.

TABLE-28 P VALUE (THYROID)

S. NO	TECHNIQUE	QUALITY OF SMEARS	TOTAL	MEAN	P VALUE
1	CS	Adequate	25	5.500	0.785
		Superior	4		
		Unsuitable	1		
2	LBC	Adequate	23	5.500	
		Superior	6		
		Unsuitable	1		

In our study, it was found that number of diagnostically adequate smears were more from Conventional smear technique than LBC (CS- 25 cases, LBC-23) and diagnostically superior smears were from LBC more than Conventional method (CS-4 cases, LBC-6 cases) and diagnostically unsuitable smears were equal in both techniques (CS-1 case, LBC-1case). (Table4)

On comparing the number of adequate smears, superior smears and unsuitable smears obtained by Conventional smears and LBP, it was found that CS produced more adequate smears(52.1%), LBP produced more superior smears (60%) and diagnostically unsuitable smears produced by both techniques were equal(50%). (Table5)

The average score for each parameters in Conventional smear and LBP of thyroid lesion was calculated and it was found that the average score of background blood numerically higher in LBP technique (Mean-1.633)

The mean value of cellularity numerically higher in Conventional smears. (Mean-1.066)

The mean value of degree of cellular trauma numerically higher in LBP technique. (Mean-1.266)

The mean value of degree of cellular degeneration numerically higher in Conventional method (Mean-0.933)

The mean value of retention of architecture numerically higher in Conventional method (Mean-1.133)

Total average score analysed between two techniques it was found that average score was equal for both methods. (Mean-5.500) The average mean difference between both techniques (CS-5.500and LBP-5.500) calculated as 0.00001 . The P value of average scores of each techniques calculated by t- test and the P value = 1 . The difference was found to be statistically insignificant P>0.05. (Table 6-9)

The P value of each parameters were calculated by Pearson Chi-Square test. P value of background blood and clot score P= 0.01, the difference was found to be statistically significant $P<0.05$.

P value of parameter of cellularity score P=0.0012, the difference was found to be statistically significant $P<0.05$.

P value of degree of cellular trauma score P=0.565, the difference was found to be statistically insignificant $P>0.05$.

P value of degree of cellular degeneration score P=0.471 the difference was found to be statistically insignificant $P>0.05$.,

P value of retention of architecture score P=0.033 , the difference was found to be statistically significant $P<0.05$.

Comparison of Conventional smears and LBP in thyroid lesions , the P value was calculated by using Pearson Chi-Square test and the P value =0.785 the difference was found to be statistically insignificant $P>0.05$. (Table28)

Comparison with other studies:

The comparison of our study with other studies conducted in the past. (Table29. All observations and results were compared with following studies.

According to N.Mygdakos et al,[30] they compare the CS and LBP in nongynaecological samples. The samples were compared on basis of following parameters such as cellularity , background blood or cell debris, informative background (colloid, stromal fragments, mucus), presence of monolayer arrangement, cytoplasmic and nuclear details by a semi quantitative scoring system. By using Wilcoxon signed rank test on statistical analysis was made.

Adequate diagnostic cells in all smears were higher in LBPs than CS regarding in absence of background blood or clot, presence of monolayers and well defined nuclear and cytoplasmic details $P< 0.05$ and other parameters like cellularity , retention of cellular architecture and informative background equal to CSs and statistically insignificant differences $P>0.05$.

TABLE-29 COMPARISON WITH OTHER STUDIES

STUDY	PARAMETERS	P VALUE
N.Mygdakos et al[30]	Back ground blood or clot	0.057
	Cellularity	0.137
	Architecture	0.865
Present study	Back ground blood or clot	0.001
	Cellularity	0.012
	Architecture	0.03

Overall P value of in our study 0.785, the differences found to be statistically insignificant (P>0.05) . (Table28)

The diagnostically inadequate smears were obtained by our study compared with other studies. In our study diagnostically unsuitable smears obtained by both methods show equal percentage.(Table30)

TABLE-30 COMPARISON OF INADEQUATE SMEARS

STUDY	CS	LBC
Cochand-Priollet et al [53]	8%	22%
Garbar et al [117]	4.7%	17.7%
Present study	50%	50%

Although this study showed statistically insignificant difference between Conventional and Liquid based preparation with respect to average scores, retention of cellular architecture, degree of cellular trauma and degeneration, some practical consideration have emerged. In Colloid goitre, cystic degeneration in nodular colloid goitre and colloid nodule LBP preferred because of few thyroid follicular cells in colloid material are well preserved in these method.

IN BREAST LUMP:

In our study, on considering all observations and results of each technique (Conventional method and LBP) in breast swelling , number of diagnostically adequate smears, diagnostically superior smears and unsuitable smears, average score of each parameters, mean score, P value are calculated and analysed. (Table12-16)

TABLE-31 P VALUE (BREAST)

S.NO	TECHNIQUE	QUALITY OF SMEARS	TOTAL	MEAN	P VALUE
1	CS	Adequate	18	5.866	0.811
		Superior	9		
		Unsuitable	3		
2	LBC	Adequate	19	5.333	
		Superior	7		
		Unsuitable	4		

In our study the smears obtained by Conventional smears and LBC from breast lesions were scored and graded according to parameters of background blood, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of architecture. It was found that number of diagnostically adequate smears were more from LBP (LBP-19 cases, CS-18 cases)

The superior quality smears more from Conventional smears than LBP (LBP -7cases, CS-9cases)

The unsuitable smears less from Conventional smears than LBC preparation (LBP-4 cases, CS-3 cases)

On comparing percentage of quality of smears obtained by Conventional smears and Liquid based preparation the percentage of superior quality smears more from Conventional smears (56.3%) and percentage of adequate smears more from the Liquid based preparation(54%) and unsuitable smears more in Liquid based preparation (57.10%).

The average score (mean) for each parameters (Background blood or clot, Cellularity, Degree of cellular trauma , Degree of cellular degeneration and Retention of architecture) in Conventional smears and Liquid based preparation were calculated.

The mean score of parameter of background blood or clot found to be higher in LBP, mean score of LBP =1.7667

The mean score of cellularity found to be higher in Conventional smears, mean score of CS = 1.666

The mean score of degree of cellular trauma found to be higher in Conventional smear, mean score of CS = 1.133

The mean score of degree of cellular degeneration found to be higher in Conventional smear , mean score of CS = 1.

The mean score of retention of cellular architecture found to be higher in Convenional smear preparation, mean score of CS=1.066

The average mean difference between both techniques (CS-5.866and LBP-5.366) calculated as 0.533 . The P value of average scores of each techniques calculated by t- test and the P value = 0.259 .The difference was found to be statistically insignificant P>0.05.

In our study , the comparison of P value of each parameters of both techniques calculated by Pearson Chi- Square test.

The P value of background blood or clot score P=0.001 .The difference was found to be statistically significant P<0.05.

The P value of cellularity score P= 0.004. The difference was found to be statistically significant P<0.05.

The P value of degree of cellular trauma score P=0.046 The difference was found to be statistically significant P<0.05.

P<0.05.The P value of degree of cellular degeneration score P=0.084, the difference was found to be statistically insignificant P>0.05.

The P value of retention of cellular architecture score P=0.018. The difference was found to be statistically significant P<0.05.

Comparison of Conventional smears and LBP in breast lesions , the P value was calculated by using Pearson Chi-Square test and the P value =0.811the difference was found to be statistically insignificant P>0.05.(Table31)

COMPARISON WITH OTHER STUDIES:

The comparison of our study with other studies conducted in the past. All observations and results were compared with following studies.

According to N.Mygdakos et al,[30] they compare the CS and LBP in nongynaecological samples

TABLE-32 COMPARISON WITH OTHER STUDIES

STUDY	PARAMETERS	P VALUE
N.Mygdakos et al [30]	Back ground blood or clot	0.057
	Cellularity	0.137
	Architecture	0.865
Present study	Back ground blood or clot	0.001
	Cellularity	0.004
	Architecture	0.018

P value of in our study 0.785, the differences found to be statistically insignificant P>0.05

According to Dey P et al [(118)]

Adequate cellularity, informative background like stromal elements, retention of cellular architecture in LBPs in breast lesions (Fibroadenoma, Ductal carcinoma)

Present study shows P value of cellularity, retention of cellular architecture were P <0.05.

According to Bedard YC et al, [(82)]

In their study , comparing CS and LBP there was no statistically difference in diagnostic accuracy.

According to Aaron L Shibemba et al, [(78)]

Adequacy of CS and LBP was 82.4% and 73.7% respectively. CS was diagnostic in 82.4% cases and 71% in LBP method.

In our study, the breast lesions P value of 0.785, the differences found to be statistically insignificant P>0.05.

IN LYMPHNODE SWELLING:

In our study , on considering all observations and results of each technique (Conventional method and LBP) in lymphnode swelling , number of diagnostically adequate smears, diagnostically superior smears and unsuitable smears, average score of each parameters, mean score, P value are calculated and analysed. (Table20-24)

TABLE-33 P VALUE (LYMPHNODE)

S.NO	TECHNIQUE	QUALITY OF SMEARS	TOTAL	MEAN	P VALUE
1	CS	Adequate	17	6.600	0.005
		Superior	13		
		Unsuitable	0		
2	LBC	Adequate	24	5.366	
		Superior	3		
		Unsuitable	3		

In our study, the smear obtained by Conventional and Liquid based preparation from lymphnode swellings were scored and graded according to background blood and clot, cellularity, degree of cellular trauma,

degree of cellular degeneration and retention of cellular architecture and it was found that number of diagnostically adequate smears more in LBP (LBP-24, CS-24)

The diagnostically superior smears more from Conventional smears than LBP.(CS-13, LBP-3)

The diagnostically unsuitable smears more from Liquid based preparation than Conventional smears (CS-0, LBP-3)

The average score (mean) for each parameters (Background blood or clot, Cellularity, Degree of cellular trauma , Degree of cellular degeneration and Retention of architecture) in Conventional smears and Liquid based preparation were calculated.

The average score of parameter of background blood numerically higher in LBP, it was found that Mean score of LBP=1.733 and Conventional smear=1.033

The average score of cellularity was more in Conventional smears , it was found that Mean score of LBC=1.133 and CS=1.933

The average score of degree of cellular trauma was higher in Conventional smear it was found that Mean score of CS=1.166 and LBP= 0.900

The average score of degree of cellular degeneration was higher in Conventional smears it was found that Mean score of CS=1.1667 and LBP=0.866

The average score of retention of cellular architecture numerically higher in Conventional smears it was found that Mean score of CS=1.266 and LBP= 0.800

The average mean difference between both techniques (CS-6.600and LBP-5.366) calculated as 1.233 . The P value of average scores of each techniques calculated by t- test and the P value = 0.259, the difference was found to be statistically insignificant P> 0.05

In our study , the comparison of P value of each parameters of both techniques calculated by Pearson Chi- Square test.

The P value of background blood or clot score P=0.001, the difference was found to be statistically significant P<0.05

The P value of parameter of cellularity score P=0.004, the difference was found to be statistically significant P<0.05

The P value of degree of cellular trauma score P=0.046, the difference was found to be statistically significant P<0.05.

The P value of degree of cellular degeneration score P=0.084, the difference was found to be statistically significant P<0.05,

The P value of retention of cellular architecture score P=0. 018, the difference was found to be statistically significant P<0.05

In our study , on comparing the Conventional smears and Liquid based preparation by all five parameters and the P value calculated by Pearson Chi-Square test and P value=0.005, the difference was found to be statistically significant P<0.05. Comparison with other studies:

The comparison of our study with other studies conducted in the past.

All observations and results were compared with following studies.

According to N.Mygdakos et al,[30] they compare the CS and LBP in nongynaecological samples

TABLE-34 COMPARISON WITH OTHER STUDIES

(LYMPHNODE LESIONS)

STUDY	PARAMETERS	P VALUE
N.Mygdakos et al [30]	Back ground blood or clot	0.057
	Cellularity	0.137
	Architecture	0.865
Present study	Back ground blood or clot	0.001
	Cellularity	0.012
	Architecture	0.03

P value of in our study in lymphnode lesions 0.005 , the differences found to be statistically insignificant P>0.05.

In our study, total 90 cases number of adequate smears more from LBP method (adequate smears from LBP- 66 cases), superior smears more in CS method (superior smears from CS-26), Unsuitable smears more from LBP method (unsuitable smear from LBP-8).

Percentage of adequate smear high in LBP(52.4%), percentage of superior smear more from CS method (61.9%) and unsuitable smear more from LBP (66.7%)

In our study , on comparing the Conventional smears and Liquid based preparation for thyroid, breast and lymphnode lesions by all five parameters like background blood or clot, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of cellular architecture and the P value calculated by Pearson Chi-Square test and P value=0.135, (Table-34) the difference was found to be statistically insignificant P>0.05

Limitations:

The liquid based cytology (LBC) method, originally develope for gynaecological cervical smears, then has progressively gained consensus for both non gynaecological and fine-needle aspiration cytological specimens. [25-27]

Comparing Liquid based cytology on non gynaecological samples with the gynaecological samples, increased number of satisfactory results more in gynaecological samples than non gynaecological samples.

According to Diaz-Rosario and Kabawat[119], high number of premalignant lesions detected on LBP smear preparation than Conventional smears and in their study percentage of low grade squamous intraepithelial lesions increased from 1.6% to2.7% and percentage of hign grade squamous intraepithelial lesion increased from 0.3% to 0.5%.

According to Weintraub and Morabia [120], they reported an increased percentage of satisfactory cases (72.2% to 92%) in LBP than Conventional smears.

According to Bolick et al[121] , comparing pap spin and conventional pap smear , sensitivity (95.2%) and specificity (58%) more in liquid based cytology , on conventional smear sensitivity was 85% and specificity was 36%.

According to Baandrup U et al[122], Conventional smears had low sensitivity (70%-80%) because of sample collection, inadequacy and interpretation in cervical smears.[9]

According to McCrory DC et al, Weintraub J et al, Monosonego J et al, and Malle D et al,[123-126] Liquid based preparation had higher sensitivity (85%-95%) in cervical smears.

Bedard YC et al[82], analysed breast cytology samples with Conventional smear preparation and Liquid based cytology preparation in 7464 breast FNAC samples over a four years period and comparing both LBC and CSs, but this study no significant diagnostic accuracy between these techniques

According to Rana DN et al[98], they studied respiratory cytology samples and found that diagnostic accuracy not significant difference between LBP and Conventional method of smear preparations.

According to de Luna R et al[99], in pancreatic FNACs the diagnostic accuracy of thin prep less than Conventional smears.

In salivary gland cytology preparation, conventional smear preparation yield more than that of LBP.[93]

Liquid based preparation useful in gynaecological specimens cervical smears) than non gyneclogical samples.

According to N.Mygdakos et al [30], Liquid based preparation is acceptable in breast, thyroid, lymphnode, soft tissues and salivary gland lesions diagnosis when used with conventional smear preparation.

Summary

SUMMARY

This prospective study has been attempted at comparing efficacy of the two methods (Conventional and LBP). The FNAC technique is applicable to easily palpable superficial lesions like skin, subcutis, thyroid, superficial lymphnode, breast , salivary gland . It is less demanding technique than biopsy and risk of complications are very low.

Liquid based cytology is a new technology for fine needle aspiration samples. It is used for mainly for cervical cancer screening , now also used for non gynaecolgical samples.

E-PREP system is a Liquid based cytology processor with patent dual membrane filters. E-PREP also facilitates preparation of more numbers of slides 150 slides/hr.

LBP - advantages in cytomorphology

- High diagnostic accuracy
- Preserved cell features
- Lesser fixation artefact
- Clean background
- Less unsatisfactory results

Disadvantages Of Liquid Based Preparation

- Decreased and altered background material like necrosis, blood and inflammation Decreased and altered extracellular elements like mucin, colloid and stroma
- Disrupted cellular architecture like fragmentation of papillae
- Size of the cell smaller than conventional preparation

Present study

- ✓ Fine needle sampling of thyroid, breast and lymphnode swelling was done. Smears were prepared by both methods , and in two methods scores obtained by on basis of background blood, cellularity, degree of cellular trauma, degree of cellular degeneration and retention of cellular architecture.

- ✓ On analysing smears in thyroid lesions adequate smears more in Conventional method, superior quality smears more from LBP method and equal number diagnostically unsuitable smears from each methods of smear preparation.
- ✓ Analysing smears in thyroid lesions showed statistically insignificant difference between Conventional and Liquid based preparation with respect to average scores, retention of cellular architecture, degree of cellular trauma and degeneration,

some practical consideration have emerged. In Colloid goitre, cystic degeneration in nodular colloid goitre and colloid nodule LBP preferred because of few thyroid follicular cells in colloid material are well preserved in these method.

- ✓ On analysing smears in breast lesions adequate smears more in LBP method, superior quality smears more from CS method and diagnostically unsuitable smears more from LBP method of smear preparation.

- ✓ On analysing smears in lymphnode lesion adequate smears more in LBP method, superior quality smears more from CS method and diagnostically unsuitable smears more from LBP method of smear preparation.
- ✓ On analysing and comparing average score obtained by both methods (CS & LBP) in thyroid,breast and lymphnode lesions and the P value calculated by Pearson Chi-Square test, the difference was found to be statistically insignificant P> 0.05.

Comparing Liquid based cytology on non gynaecological samples with the gynaecological samples, increased number of satisfactory results

more in gynaecological samples than non gynaecological samples in many studies.

This study concludes that the results are statistically insignificant, it was found that:

- The diagnostically adequate smears without admixture background blood or clot, degree cellular trauma, degeneration and preserved cellular architecture more from LBP smear preparation method in lymphnode lesions.

- For the cystic lesions of thyroid, breast and lymphnode, the LBP method is preferred because of less background blood and cell debris, and preservation of cellular material.

- For fibrous lesion of breast like fibroadenoma and phyllodes tumour, Conventional method is preferable choice because of it produced more diagnostically superior smears than LBP.

Conclusion

CONCLUSION

- In Fine needle sampling, Conventional smear preparation with air drying and alcohol fixation is the standard method for processing samples.
- Liquid based preparation is an alternative method for processing of FNA samples in both gynaecological and non gynaecological samples.
- In LBP method aspirated samples are preserved in alcohol fixative, and then placed on LBC slides.
- E-PREP system is liquid based cytology processor with patent dual membrane filters. This method able to collect large number of cells and make a monolayer preparation of cells with good cytological details. E-PREP also facilitates preparation of more numbers of slides 150 slides/hr.
- Comparing Liquid based cytology on non gynaecological samples with the gynaecological samples, increased number of satisfactory results more in gynaecological samples than non gynaecological samples in many studies.

- In current study, on analysing and comparing average score obtained by both methods (Conventional smears and LBP) in nongynaecological samples thyroid, breast and lymphnode swellings and the P value calculated by Pearson Chi-Square test, the difference was found to be statistically insignificant P> 0.05.

- The decision to make, use either Conventional method or LBP may be depends on basis of nature of the lesion (solid or cystic) and other ancillary tests to perform in the sample.

- In conclusion each method has its own advantages and disadvantages and both methods can be combined to obtain a superior quality smears and lower the failure rates.

Bibliography

BIBLIOGRAPHY

1. Kün M. A new instrument for thediagnosis of tumours. Monthly J Med Sci1846;7:853.

2. Svante R. Orell andandphilipevielh, Orell&Sterrett's fine needle aspiration cytology, 5th edition; The techniques of FNA Cytology, chapter 2: 8-27.

3. HeejaeJoo, HyuneeYim, Taehui Lee, Sunglim Kim "Concordance rate between E-Prep and SurePathincervicalcytologic examination":Department ofpathology, Ajouuniv p pgy, j ersity hospital, Medplan pathology laboratory center.

4. Martin H E and Ellis E: Biopsy Needle puncture and aspiration. Ann Surg.92:169-181,1930.

5. Lowhagen T, WillemsJS,Lundell g, Sundblad R, GranbergP.AspirationBiopsy Cytology in Diagnosis of Thyroid Cancer. World J Surg 1981;5:61-73

6. Franzen S, GiertzG;Zajicek J:Cytological diagnosis of prostatic tumors by Trans rectal aspiration biopsy, a preliminary report. Br. J. Urol 32,193-196,1960.

7. ZajdelaA,Zillhardt P, Voillemot N:Cytological diagnosis by fine needle sampling without aspiration. Cancer 1987;59:1201-1205.

8. Briffod m, gentile A,HebertH.Cttopuncture in the follow- up of breast carcinoma. ActaCytol 1982;26:195-200.

9. Silverman JF, Geisinger KR. Fine needle aspiration cytology of the thorax and abdomen. Edinburgh: Churchill Livingstone; 1996.

10. Ballo MS, Guy CD. Percutaneous fine-needle aspiration of gastrointestinalwall lesions with image guidance. DiagnCytopathol 2001;24:16–20.

11. Smith EH: complications of percutaneous abdominal fine-needle biopsy. Review.Radiology 1991; 178:253–8.

12. Chan JKC, Tang SK, Tsang WYW, et al. Histologic changes induced by fine needle aspiration. Advances AnatPathol 1996;3:71–90.

13. Thomson: Thin needle aspiration Biopsy. ActaCytol 26:262-263,1982

14. Nasuti JF, Tam D and Gupta PK: Diagnostic value of liquidbased(Thin Prep) preparations in nongynecologic cases. DiagnCytopathol 24: 137-141, 2001.

15. Linder J: Recent advances in thin-layer cytology. DiagnCytopathol 18: 24-32, 1998.

16. Scurry JP and Duggan MA: Thin layer compared to direct smear inthyroid fine-needle aspiration. Cytopathology 11: 104-115, 2001.

17. Afify AM, Liu J and Al-Khafaji BM: Cytologic artefacts and pitfalls of thyroid fine-needle aspiration using Thin Prep. Cancer Cytopathol 93: 179- 186, 2001.

18. Galera-Davidson,H.,andGonzalez-Campora,R.(2008). Thyroidin:BibboMandWilburD:Comprehensive Cytopathology, 3rdEdn,Chap.23. Philadelphia:SaundersElsevier,633–670.

19. Santos JEC, LeimanG.Non aspiration Fine Needle Cytology.Application ofa new technique to nodular thyroid disease. ActaCytol 26:262-263,1982.

20. Mair S, Dumber F, Becker P, Plessis WD. Fine Needle Cytology – Is Aspiration Suction Necessary?A study of 100 masses in various sites. ActaCytol 1989;33:809-813.

21. Lieu D. Fine-needle aspiration: technique and smear preparation. Am FamPhysician 1997; 55: 839–46, 53–4.

22. Lee KR, Papillo JL, St. John T, Eyerer GJ. Evaluation of the ThinPrepprocessor for fine needle aspiration specimens. ActaCytol 1996; 40: 895–9.

23. Jung CK, Lee A, Jung ES, Choi YJ, Jung SL, Lee KY. Split samplecomparison of a liquid-based method and conventional smears inthyroid fine needle aspiration. ActaCytol 2008; 52: 313–9.

24. Biscotti CV, Hollow JA, Toddy SM, Easley KA. ThinPrep versusconventional smear cytologic preparations in the analysis of thyroid

25. Biscotti,C.V.,Hollow,J.A.,Toddy,S.M., and Easly,K.A.(1995).Thinprep Versus conventional smear cytological preparations in the analysis ofThyroid fine-needle aspiration specimens.Am.J.Clin.Pathol. 104, 150–153.

26. Rossi,E.D.,andFadda,G.(2008).Thin-layer liquid-based preparation ofExfoliative non-gynaecologic and fine-needle aspiration biopsy cytology.Diagn.Histopathol. 14,563–570.

27. Rossi,E.D.,Raffaelli,M.,Zannoni,G.F.,Pontecorvi,A.,Mule,A.,Calla,C., Lombardi,C.P.,andFadda,G.(2009). Diagnostic efficacy of Conventional as compared to liquid-based cytology in thyroid lesions.Evaluation of 10,360 fine needle aspiration cytology cases.ActaCytol.53, 659–666.

28. NICE. Guidance on the use of Liquid Based Cytology for Cervical screening. NICE Technology Appraisal 69.London:NICE,2003.

29. Moss S, Gray A, Legood R, Henstock E. Evaluation of HPV/LBC cervical screening pilot studies. First report to the Department of Health onevaluation of LBC. London: Department of Health,2002.

30. N.Mygdakos,Sylvia Nikolaidou,Anna TzilivakiD. Tamiolakis,"Liquid Based Preparation (LBP) cytology versus Conventional Cytology (CS) in FNA samples from breast,thyroid, salivary gland and soft tissues. Our experience in Crete (Greece): Romanian Journal of Morphology and Embryology 2009,50(2):245-250.

31. Fischler DF, Toddy SM. Nongynaecological cytology Utilising the ThinPrepProcessor.ActaCytol 1996;40:669–675.

32. Layfield LJ, Fin needle aspiration cytology of cystic parathyroid lesions, Actacytol 35:445-447, 1991.

33. Hamming JF, Gosling BM, van Steenis GJ, vanRavenswaayClaasen H, J. Hermans J and van de Velde CJH: The value of fine-needle aspiration biopsy in patients with nodular thyroid disease divided into groups of suspicion of malignant neoplasms on clinical grounds. Arch Int Med 150: 13-116, 1990.

34. Fadda G, Balsamo G, Fiorino MC, Mulé A, Lombardi CP, Raffaelli M and Bellantone R: Follicular thyroid lesions and risk of malignancy: a new diagnostic classification on fine needle aspiration cytology. J ExpClinCancer Res 17(1): 103-107, 1998.

35. Baloch ZW, Fleisher S, LiVolsi VA and Gupta PK: Diagnosis offollicular neoplasm: a gray zone in thyroid fine-needle aspiration cytology.DiagnCytopathol 26(1): 41-44, 2002.

36. Baloch ZW and LiVolsi VA: Follicular-patterned lesions of thethyroid: the bane of the pathologist. Am J ClinPathol 117(1):143-150, 2002.

37. Lowhagen T, Willems JS, Lundell GR, Sundblad and Granberg P-O: Aspiration biopsy cytology in diagnosis of thyroid cancer.World J Surg 5(1): 61-73, 1981.

38. Fadda G and LiVolsi VA: Histology and aspiration cytology of benign thyroid diseases. Rays 24(2): 182-196, 1999.

39. Buley ID: The thyroid gland. In: Diagnostic Cytopathology. Gray W. London: Churchill Livingstone, pp. 557-582, 1995.

40. Ogawa Y, Kato Y and Ikeda K: The value of ultrasound-guided fine-needle aspiration cytology for thyroid nodules: an assessment of its diagnostic potential and pitfalls. Surg Today 31: 97-101, 2001.

41. Bennedbaek FN, Perrild H and Hegedus L: Diagnosis and treatment of the solitary thyroid nodule. Results of a European survey.ClinEndocrinol 50: 357-363, 1999.

42. Gharib H and Goellner JR: Fine-needle aspiration biopsy of the thyroid: an appraisal. Ann Intern Med 118: 282-289, 1993.

43. MC Henry, walfish PG, Rosen IB., Non diagnostic fine needle aspirationbiopsy, a dilemma in management of nodular thyroid disease. Ann Surg 59, 415-419, 1993.

44. Hamburger JI, Hamburger SW: Fine needle biopsy of thyroid nodules avoiding the pitfall NY state J med 86: 241-249, 1986.

45. La Rosa GL, Belfiore A, GiuffridaD,et al. Evaluation of the fine needleaspiration biopsy in the preoperative selection of cold thyroid nodules. Cancer 1991;67:2137–41.

46. Hsu C, Boey J. Diagnostic pitfalls in the fine needle aspiration of thyroidnodules. A study of 555 cases in Chinese patients.ActaCytol 1987;31:699–704.

47. Sarda AK, Bal S, Dutta Gupta S, et al. Diagnosis and treatment of cysticdisease of the thyroid by aspiration. Surgery 1988;103:593–6.

48. Silverman JF, West L and Larkin EW: The role of fine-needle aspiration biopsy in the rapid diagnosis and management of thyroid neoplasm. Cancer 57: 1164, 1986.

49. Hamburger JI, Miller M and Kini SR: The impact of fine-needle biopsy on the diagnosis of the thyroid nodule. In: Diagnosis and Management of Endocrine-Related Tumors. Santen RJ and Manni A (eds.). Boston: MartinusNijhoff 99: 123-149, 1984.

50. Charles MA and Heller K: Cytologic determinants of well differentiatedthyroid cancer. Am J Surg 174: 545-547, 1997.

51. Saleh H. A., Hammoud J., Zakaria R., Khan A. Z.,Comparison ofThin-Prep and cell block preparation for theevaluation of thyroid epitheliallesions on fine needleaspiration biopsy, Cytojournal, 2008, 5:3.

52. Stamataki M., Anninos D., Brountzos E., Georgoulakis J., Panayiotides J., Christoni Z., Peros G., Karakitsos P., The role of liquid-based cytology in the investigation of thyroid lesions, cytopathology, 2008, 19(1):11–18.

53. Cochand-Priollet,B.,Prat,J.J.,Polivka,M., Thienpont,L.,Dahan,H., Wassef,M.,andGuillauseau,P.J.(2003).Thyroid fineneedleaspiration:the morphological featuresonThinPrep slidepreparations.Eighty cases with histological control. Cytopathology 14,343–349.

54. Fadda,G.,Rossi,E.D.,Raffaelli,M.,Mule,A.,Pontecorvi,A.,Miraglia,A., Lombardi,C.P.,and and Vecchio,F.M.(2006). Fine-Needle Aspiration biopsy of thyroid lesions processedbythin-layercytology:one-yearinstitutionalexperiencewithhistologiccorrelation. Thyroid16, 975–981.

55. Rossi,E.D.,Raffaelli,M.,Mule,A.,Miraglia,A.,Lombardi,C.P.,Vecchio,F. M,andFadda,G.(2006). Simultaneous immunohistochemicalexpression of HBME-1 and Galectine 3 differentiates papillary carcinomas from hyperfunctioning lesions ofthethyroid. Histopathology 48, 795–800.

56. Fadda,G.,Rossi,E.D.,Martini,M.,Zannoni, G.F.,Vellone,V.G.,Pontecorvi, A., Lombardi,C.P.,Larocca,L.M., and Rindi,G.(2011b).Brafmutation (V600E)inpapillarycarcinoma identified on LBC processe thyroid aspirationbiopsies. Mod.Pathol. 24, 89A.

57. Geers, A.J.,andBourgain,C.(2011).Li quid basedFNACofthyroid: a4 yearsurveywithSurePath.CancerCytopathol. 119, 58–67.

58. Das, D.K.(2006).Marginal vacuoles (fire-flare appearance) in fineneedle Aspiration smears of thyroid lesions: does it represent diffusing out of Thyroid hormones at the base of follicular cells?Diagn.Cytopathol. 34,277–283.

59. Scottish Cervical Screening Programme : Steering group report on thefeasibility of introducing liquid-based cytology, January 2002

60. Australian Cancer Network Diagnosis and Management of LymphomaGuidelines Working Party. Guidelines for the Diagnosis and Management ofLymphoma. Sydney: The Cancer Council Australia and Australian CancerNetwork; 2005.p. 136.

61. Ferrer R. Lymphadenopathy: differential diagnosis and evaluation. Am FamPhysician 1998;58:1313–20.

62. Gupta AK, Nayar M, Chandra M. Reliability and limitations of fineneedleaspiration cytology of lymphadenopathies: an analysis of 1,261 cases. ActaCytol 1991;35:777–83.

63. Perkins SL, Segal GH, Kjoldsberg CR. Work-up of lymphadenopathy inchildren. SemDiagn Pathology 1995;12:284.

64. Leong A SY, Stevens M. Fine-needle aspiration biopsy for the diagnosis oflymphoma: a perspective. DiagnCytopathol 1996;15:352–7.

65. Daskalopoulou D, Harhalakis N, Maouni N, et al. Institution fine needleaspiration cytology of non-Hodgkin lymphomas: a morphologic andimmunophenotypic study. ActaCytol 1994;39:180–6. Lymph nodes 113

66. Geisinger KR, Rainer RO, Field AS.Lymph nodes. In: Geisinger KR, Silverman JF, editors. Fine needleFineneedleaspiration cytology of superficial organsand body sites. New York: Churchill-Livingstone; 1999. p. 1–49.

67. Michael CW, Hunter B. Interpretation of fine-needle aspirates processed byThinPrep techniques: cytologicartifacts and diagnostic pitfalls. DiagnCytopathol 2000;23:6–13.

68. Lopescardazo P: The cytologic diagnosis of lymphnode punctures, actacytol 8: 194-198,1964.

69. Wildi SM, Judson MA, Fraig M, et al. Is endosonography guided fineneedle aspiration (EUS-FNA) for sarcoidosis as good as we think?Thorax 2004; 59: 794–9.

70. Jung CK, Lee A, Jung ES, Choi YJ, Jung SL, Lee KY. Split samplecomparison of a liquid-based method and conventional smears inthyroid fine needle aspiration. ActaCytol 2008; 52: 313–9.

71. Kocjan G, Bourgain C, Fassina A, etal.The role of breast FNAC inDiagnosis and clinical management: a survey of current practice.Cytopathology2008;19:271–8.

72. Ahmed I, Nazir R, Chaudhary M Y, Kundi S. Triple assessment of breastlump. J Coll Physicians Surg Pak 2007;17:535–8.

73. NHSBSP. Non-operative diagnosis subgroup of the national coordinatinggroup for breast screening pathology 2001, Publication 50.

74. Robinson IA, MC KeeG,Kissin M: Typing and grading of breast carcinomain fine needle aspiration cytology, is this clinically useful information? Diagcytopathol 13; 309-310, 1987.

75. Bondesan L:Aspiration cytology of radiation induced changes in the normal breast epithelium. Actacytol 31:309-310, 1987.

76. Layfield LJ, Glasgow B, Coogan A,: What constitutes an adequate smear in fine needle aspiration cytology of breast? Cancer 81: 16-21,1997.

77. Lee KC, Chan JK, HoI :Histologic changes in the breast after fine needleaspiration. Am J Surgpathol 18: 1039-1047,1994.

78. Comparison Of Conventional Cytology Smears And Liquid Based (SynermedGlucyte®) Smears In Breast Aspirates – Morphology And Immunocytochemistry Aaron L. Shibemba, Pawel T. Schubert, Colleen A. Wright. Division of Anatomical Pathology, Department of

Pathology, University of Stellenbosch and National Health Laboratory Service,Tygerberg Hospital, South Africa

79. Ariga R, Bloom K, Reddy VB, et al. Fine-needle aspiration of clinicallysuspicious palpable breast masses with histopathologic correlation. Am JSurg 2002; 184(5): 410-3.

80. Sneige N. Fine-needle aspiration of the breast: a review of 1,995 cases with emphasis on diagnostic pitfalls. DiagnCytopathol 1993; 9(1): 106-12.

81. Ballo MS, Sneige N. Can core needle biopsy replace fine-needle aspirationcytology in the diagnosis of palpable breast carcinoma. A comparative study of 124 women. Cancer 1996; 78(4): 773-7.

82. BédardY. C., PollettA. F., Breast fine-needle aspiration. A comparison of ThinPrep and conventional smears, Am J ClinPathol, 1999,111(4):523–527.

83. Eneroth CM, Zajicek J. Aspirationbiopsy of salivary gland tumours. II. Morphologic studies on smears and histologic sections from oncocytictumours. ActaCytol 1965;9:355–61.

84. Eneroth CM, Zajicek J. Aspiration biopsy of salivary gland tumours. III. Morphologic studies on smears and histologic sections from 368 mixed tumours. ActaCytol 1966;10:440–54.

85. Eneroth CM, Zajicek J. Aspiration biopsy of salivary gland tumours. IV. Morphologic studies on smears and histologic sections from 45 cases ofadenoid cystic carcinoma. ActaCytol 1969;13:59–63.

86. Zajicek J, Eneroth CM. Cytological diagnosis of salivary gland carcinomatafrom aspiration biopsy smears. ActaOtolaryng 1970;263(Suppl.):183–5.

87. Zajicek J., Eneroth CM, JakobsonP.Aspiration biopsy of salivary glandtumors. VI. Morphologic studies on smears and histologic sections frommucoepidermoid carcinoma. ActaCytol. 1976;20:35–41.

88. Atuta T, Grenman R, LaippalaP,Klemi P-J. Fine-needle aspiration biopsyin the diagnosis of parotid gland lesions: evaluation of 438 biopsies.DiagnCytopathol 1996;15:185–90.

89. Al-Khafaji B, Nestok BR, Katz RL. Fine-needle aspiration of 154 parotidmasses with histologic correlation. Ten-year experience at the University ofTexas M.D.Anderson Cancer Center. Cancer (Cancer Cytopathol).

90. Young JA. Diagnostic problems in fine needle aspiration cytopathology ofThe salivary glands. J ClinPathol 1994;47:193–8.

91. Klijanienko J, Vielh P, Batsakis JD, etal.Monographs in Clinical Cytology.Volume 15.Salivary Gland Tumours. Basel, Switzerland: Karger; 2000.

92. Layfield L, Gopez E, HirschowitzS.Cost efficiency analysis for fine-needleaspiration in the workup of parotid and submandibular gland nodules. DiagnCytopathol 2006;34:734–8.

93. Parfitt JR, McLachlin M, Weir MM. Comparison of Thin Prep andconventional smears in salivary gland fine-needle aspiration biopsies. Cancer2007;111:123–9.

94. Lee K. R., PapilloJ. L., St. John T., EyererG. J. A., Evaluation oftheThinPrep processor for fine needle aspiration specimens, ActaCytol, 1996, 40(5):895–899.

95. Schmitt F. C., Dias A. J., Wagner R. A., Use of ThinPrep processingfor studying breast cancer cell lines, DiagnCytopathol, 2000, 23(1):27–28.

96. KonofaosP., KontzoglouK., GeorgoulakisJ.,MegalopoulouT., ZoumpouliC., Christoni Papadopoulos O., KouraklisG., KarakitsosP.,The role of ThinPrep cytology in the evaluation of estrogenand progesterone receptor content of breasttumors,SurgOncol, 2006, 15(4):257–266.

97. VocaturoA., NovelliF., BenevoloM., PipernoG.,MarandinoF., CianciulliA. M., MerolaR., PerroneDonnorsoR., SperdutiI., BuglioniS., MottoleseM.,Chromogenic in situ hybridization to detect HER-2/neugene amplification in histological and ThinPrep®-processedbreast cancer fine-needle aspirates: a sensitive andpractical method in the trastuzumab era, TheOncologist,2006, 11(8):878–886.

98. Rana D. N., O'donnell M., Malkin A., Griffin M., A comparative study: conventional preparation and ThinPrep 2000 in respiratory cytology, Cytopathology, 2001, 12(6):390–398.

99. De Luna R., Eloubeidi M. A., Sheffield M. V., Eltoum I., Jhala N., Jhala D., Chen V. K., Chhieng D. C., ComparisonofThinPrep and conventional preparations in pancreatic fine-needle aspiration biopsy, DiagnCytopathol, 2004, 30(2):71–76.

100. Wiener HG, Mian CH, Hiatel A et al. Can urine bound diagnostic testsreplace cystoscopy in the management of bladder cancer? J. Urol. 1998; 159: 1876–80.

101. Ozen H, Hall CM. Bladder cancer. Curr.Opin.Oncol.2000; 12: 255–9.

102. Lokeshwar VB, Soloway MS. Current bladder tumor tests: does theirprojected utility fulfill clinical necessity? J. Urol. 2001; 165: 1067–77.

103. Nasuti JF, Tam D, Gupta PK. Diagnostic value of liquid based(ThinPrep) preparation in non-gynecologic cases.Diagn.Cytopathol.2001; 24: 137–41.

104. Linder J. Recent advances in thin-layer cytology. Diagn.Cytopathol.1998; 18: 24–32.

105. Skacel M, Pettay JD, Tsiftsakis EK et al. Validation of a multicolourinterphase fluorescence in situ hybridisation assay for detection oftransitional cell carcinoma on fresh and archival thin-layer, liquid-basedcytology slides. Anal. Quant. Cytol. Histol.2001; 23: 381–7. 107.

106. Dudgeon Ls Patrick Cr: A new method for the microscopical diagnosis of tumour, Br J Surg 15: 250-261, 1927.

107. Frable WJ: Needle Aspiration Biopsy : Past,present and Future , Human Pathol 20.504-517, 1989.

108. Frable WJ: Needle Aspiration Biopsy: Philadelphia,1983.

109. OrellSr, Slerrett GF, Walters MHH, Whilaker D: Manual and Atlas of Fine Needle Aspiration Cytology, 2nd Edition, Churchill Livingston.

110. Sachdeva R kline TS: Aspiration Biopsy Cytology and special stains.,Actacytol 25:678-683/1981.

111. Dabbs DJ, Silverman JF: Selective use of EM in needle AspirationCytology,ActaCytl 32:880-884,1988.

112. Davidson DD, Gohen MD: Preparation of fine needle aspirations biopsiesfor electron microscopy, In Scmidit WA, ed, Cytopathology Manual - 225-264, 1983.

113. Kurtz SM : Rapid Ultrastructural examination of FNAC on diagnosis ofintra thoracic tumours, Diagcytopathol 8: 289-292, 1992.

114. Osborn SR, Domagal W: Immunocytochemistry, In Bibbo M. ed,Comprehensive Cytopathology 2nd Edition, Saunders, Philadelphia 1997.

115. Polak J van Noorden S,: Introduction to Immunocytochemistry . 2ndEdition springer merkg berlin 1997.

116. Roussel F, Dalion J, Benozio H: The risk of tumoural seeding in needlebiopsies, ActaCytol 33: 936-939, 1989.

117. Garbar C, Remmelink M, Mascaux C. Fine needle aspiration cytology oflymph node: experience of 2 university hospitals with conventional smears and liquid-based cytology. ActaCytol 2008; 52: 418–23.

118. Dey P., Luthra U. K., George J., Zuhairy F., George S. S., Haji B. I., Comparison of ThinPrep and conventional preparations on fine needle aspiration cytology material, ActaCytol, 2000, 44(1):46–50.

119. Diaz Rosario LA, Kabawat SE. Performance of a fluid basedthin layer Papanicolaou smear method in the clinical setting ofan independent lab and outpatient screening of population inNew England. Arch Path Lab Med 1999, 123: 817-21.

120. Wintraub J, Morabia A. Efficacy of a liquid based-thin layermethod of cervical cancer screening in a population with a lowincidence of cervical cancer. DiagnCytopathol 2000; 22: 52-9.

121. Bolick DR, Hellman DT. Laboratory implementation and efficacyassessment of the thin prep cervical cancer screening system.ActaCytol 1998, 42: 209-13.

122. Baandrup U, Bishop JW, Bonfiglio TA, Branca M, HutchinsonML, Laverty CR et al. Sampling, sampling errors and specimenpreparation. ActaCytol 2000; 44: 944–8.

123. McCrory DC, Matchar DB, Bastian L, Datta S, Hasselblad V,Hickey J et al. Evidence report: evaluation of cervical cytology.Rockville (MD): Agency for Health Care Policy and Research;1999.

124. Weintraub J, Morabia A. Efficacy of a liquid-based thin layermethod for cervical cancer screening in a population with alow incidence of cervical cancer. DiagnCytopathol 2000; 22:52–9.

125. Monsonego J, Autillo-Touati A, Bergeron C, Dachez R, LiarasJ, Saurel J et al. Liquid-based cytology for primary cervicalcancerscreening: a multi-centre study. Br J Cancer 2001; 84:360–6.

126. Malle D, Pateinakis P, Chakka E, Destouni C. Experience witha thin layer, liquid-based cervical cytologicscreening method.ActaCytol 2003; 47: 129–34.

Annexures

ANNEXURE:1

PROFORMA

Name:

Age:

Sex:

Cytology No. :

Presenting complaints:

H/O swelling-Thyroid/Breast/Lymphnode-

H/O duration of swelling-

H/O pain/fever associated with swelling-Yes/No

H/O difficulty in swallowing-Yes/No

H/O loss of weight& appetite-Yes/No

H/O any other specific complaints-Yes/No

Past history:

Previous H/O treatment/ surgery

O/E:

Site of swelling-Thyroid/Breast/ Lymphnode

Size of swelling-

Consistency of swelling-Soft/firm/hard

Mobility of swelling- Freely mobile/restricted/fixed

ANNEXURE:2
INFORMED CONSENT FORM

Title of the study : **"CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNAECOLOGICAL SAMPLES"**

Name of the Participant :
Name of the Principal (Co-Investigator) :
Name of the Institution : Madras Medical College
Name and address of the sponsor / agency (ies) (if any) :
Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"CONVENTIONAL CYTOLOGICAL SMEAR VERSUS LIQUID BASED PREPARATION (E-PREP) IN NON GYNAECOLOGICAL SAMPLES".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the aspirated samples from breast/thyroid/lymph node will be subjected to conventional smear preparation and Liquid based preparation (E-PREP).
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant
incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ANNEXURE- 3

Scoring system developed by Mair et al to classify quality ofcytological aspirate

Criterion	Qualitative description	Point score
Backgroundblood or clot	Large amount; great compromise to diagnosis	0
	Moderate amount; diagnosis possible	1
	Minimal diagnosis easy; specimen of "text book" quality	2
Amount of cellular material	Minimal to absent; diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant; diagnosis simple	2
Degree of cellular degeneration	Marked; diagnosis impossible	0
	Moderate; diagnosis possible	1
	Minimal; good preservation; diagnosis easy	2
Degree of cellular trauma	Marked; diagnosis impossible	0
	Moderate; diagnosis possible	1
	Minimal; diagnosis obvious	2
Retention of appropriate architecture	Minimal to absent; non-diagnostic	0
	Moderate; some preservation of, for example follicles	1
	Excellent architectural display closely reflecting histology; diagnosis obvious	2

On the basis of five criteria tabulated , a cumulative score was obtained for each case which was then categorized accordingly to one of the 3 categories;

1. Unsuitable for cytological diagnosis - score of 0-2
2. Diagnostically Adequate - score of 3-6
3. Diagnostically superior - score of 7-10

Master chart

MASTER CHART - THYROID

S.No	FNAC. No	Age	Sex	FNAC							LBC							Diagnosis
				A	B	C	D	E	Total	Grade	A	B	C	D	E	Total	Grade	
1	1241/14	55	M	1	1	1	1	0	4	Adequate	1	1	1	1	0	4	Adequate	NCG
2	1257/14	47	F	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	NCG
3	1270/14	48	M	1	1	1	1	1	5	Adequate	1	1	1	1	0	4	Adequate	HT
4	1561/14	55	M	1	1	1	0	1	4	Adequate	1	0	11	0	1	3	Adequate	CG
5	1569/14	40	M	1	1	1	1	1	5	Adequate	1	1	0	0	1	3	Adequate	HT
6	1640/14	40	M	1	1	1	1	1	5	Adequate	1	1	1	1	0	4	Adequate	CG
7	1647/14	45	M	1	2	1	1	1	6	Adequate	1	1	1	1	1	5	Adequate	NCG
8	1655/14	35	F	1	1	1	1	1	5	Adequate	1	2	1	1	1	6	Adequate	NCG
9	1847/14	26	F	1	1	1	1	1	5	Adequate	1	2	1	1	1	6	Adequate	HT
10	1992/14	39	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	NCG
11	2602/14	40	F	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	NCG
12	2606/14	25	F	1	1	0	0	0	2	Unsuitable	1	1	0	0	0	2	Unsuitable	NCG
13	2982/14	60	F	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	CCN
14	3032/14	13	F	2	1	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	LT
15	3231/14	24	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CG
16	3428/14	28	F	1	2	1	0	1	5	Adequate	2	1	1	0	0	4	Adequate	Pap ca
17	3433/14	48	F	1	2	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	CNG
18	3345/14	57	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	NCG
19	3369/14	40	M	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	CNG
20	2913/14	65	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CG
21	3790/14	27	F	1	2	1	1	2	2	Superior	2	1	1	1	1	6	Adequate	CG
22	3794/14	28	F	1	2	2	2	2	9	Superior	1	1	1	1	1	5	Adequate	CG
23	3804/14	14	F	1	2	1	1	2	7	Adequate	2	2	1	1	1	7	Superior	CG & thyroiditis
24	3809/14	46	F	1	2	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	CG
25	3811/14	40	F	1	2	1	1	1	6	Adequate	2	1	1	1	2	7	Superior	HT
26	3816/14	19	F	1	2	2	1	2	8	Superior	2	1	1	1	1	6	Adequate	LT
27	3817/14	35	F	1	2	2	1	2	8	Superior	2	2	1	1	1	7	Superior	HT
28	3821/14	50	F	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	LT
29	3839/14	65	F	1	2	1	1	1	6	Adequate	1	1	1	1	1	5	Adequate	CG
30	3800/14	29	F	1	2	1	1	2	6	Adequate	2	1	1	1	1	6	Adequate	CG

BREAST

S.No	FNAC. No	Age	Sex	FNAC							LBC							Diagnosis
				A	B	C	D	E	Total	Grade	A	B	C	D	E	Total	Grade	
1	1258/14	32	F	1	2	1	1	2	7	Superior	2	1	1	1	1	6	Adequate	CA
2	1558/14	54	F	1	2	2	1	2	8	Superior	2	2	1	1	1	7	Superior	FCD
3	1856/14	40	F	1	1	0	0	0	2	Unsuitable	1	1	0	0	0	2	Unsuitable	FA
4	1913/14	45	F	1	1	1	1	1	5	Adequate	1	1	0	0	0	0	Unsuitable	PBD
5	2017/14	62	F	1	1	1	1	1	5	Adequate	2	1	1	1	1	6	Adequate	CA
6	2850/14	65	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA
7	2884/14	48	F	1	2	1	0	1	5	Adequate	2	2	1	1	1	6	Adequate	CA
8	2907/14	70	M	1	0	1	0	0	2	Unsuitable	1	0	0	0	0	1	Unsuitable	Descriptive
9	2942/14	52	F	2	0	0	0	0	2	Unsuitable	2	1	0	0	0	3	Adequate	CA
10	2949/14	38	F	1	2	1	1	1	6	Adequate	2	2	2	1	1	7	Superior	FA
11	2977/14	50	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA
12	3227/14	34	F	1	2	1	1	1	6	Adequate	2	1	1	1	0	6	Adequate	BPT
13	3234/14	65	F	1	2	1	1	1	6	Adequate	2	2	2	1	1	8	Superior	CA
14	3314/14	65	F	2	1	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	CA
15	3430/14	26	F	1	2	2	2	2	6	Adequate	2	1	1	1	1	7	Superior	GM
16	2845/14	35	F	1	2	1	0	1	5	Adequate	2	2	1	1	1	7	Superior	FA
17	3563/14	38	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	FCD
18	3709/14	59	F	1	2	1	2	1	7	Superior	2	1	1	1	1	6	Adequate	CA
19	3707/14	29	F	1	1	1	1	1	5	Adequate	1	1	0	0	0	2	Unsuitable	FA with adenosis
20	3797/14	21	F	1	2	2	1	1	7	Superior	2	2	1	0	1	6	Adequate	FA
21	3789/14	75	F	1	2	1	2	1	7	Superior	2	1	1	1	1	6	Adequate	FA
22	3819/14	38	F	1	2	2	1	2	8	Superior	2	1	1	1	1	6	Adequate	FA with adenosis
23	3832/14	53	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA
24	3844/14	39	F	1	1	1	1	1	5	Adequate	1	1	1	1	1	5	Adequate	FA with adenosis
25	3846/14	26	F	1	2	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	FA
26	3851/14	50	F	2	2	1	1	1	7	Superior	2	1	1	1	1	6	Adequate	FA with FCD
27	3853/14	37	F	1	2	1	1	1	6	Adequate	1	1	1	1	1	5	Adequate	CA
28	3858/14	37	F	1	2	1	1	1	6	Adequate	2	1	0	0	0	3	Adequate	FA with adenosis
29	3864/14	32	F	1	2	2	2	1	8	Superior	1	1	1	1	1	5	Adequate	CA
30	3886/14	17	F	1	2	2	2	2	9	Superior	2	1	1	1	1	6	Adequate	FA

LYMPHNODE

S.No	FNAC. No	Age	Sex	FNAC							LBC							Diagnosis
				A	B	C	D	E	Total	Grade	A	B	C	D	E	Total	Grade	
1	1271/14	65	F	1	2	1	1	2	7	Superior	2	1	1	1	1	6	Adequate	CA deposits
2	1547/14	60	F	1	2	1	1	2	7	Superior	2	1	1	1	1	6	Adequate	RN
3	1854/14	51	M	1	2	1	1	1	6	Adequate	2	1	1	1		6	Adequate	CA deposits
4	1903/14	19	F	1	2	1	1	1	6	Adequate	1	1	0	0	0	2	Unsuitable	RN
5	2000/14	49	M	1	1	2	2	1	7	Superior	2	1	0	0	0	2	Unsuitable	RN
6	2008/14	28	F	1	2	1	1	1	6	Superior	2	1	1	1	1	6	Adequate	TB LN
7	2013/14	56	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	GL
8	2663/14	67	F	1	2	1	1	1	6	Adequate	1	1	0	0	0	2	Unsuitable	CA deposits
9	2682/14	32	M	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	TB LN
10	2801/14	70	M	1	2	2	1	1	7	Superior	2	1	1	0	1	5	Adequate	RN
11	2830/14	62	M	1	1	1	1	1	6	Adequate	1	2	1	1	1	6	Adequate	CA deposits
12	2866/14	55	M	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA deposits
13	2929/14	50	M	1	2	1	1	1	6	Adequate	2	2	1	1	1	7	Superior	CA deposits
14	2946/14	25	m	1	2	1	1	1	6	Adequate	2	1	1	1	0	5	Adequate	AIP
15	2985/14	40	M	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA deposits
16	3028/14	20	M	1	2	1	2	1	7	Superior	2	2	1	1	1	7	Superior	GL
17	3063/14	44	F	1	2	1	1	1	6	Adequate	1	1	1	1	0	4	Adequate	RN
18	3226/14	40	M	1	2	1	1	1	6	Adequate	2	2	1	1	1	6	Adequate	GL
19	3316/14	35	M	1	2	2	2	1	8	Superior	2	1	2	2	1	8	Superior	CA deposits
20	3320/14	61	M	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	CA deposits
21	3793/14	56	M	1	2	1	1	2	7	Superior	2	1	1	1	1	6	Adequate	TB LN
22	3808/14	23	M	1	2	1	1	2	7	Superior	2	1	1	1	1	6	Adequate	TB LN
23	3815/14	11	M	2	2	1	2	2	9	Superior	2	1	1	1	1	6	Adequate	LA
24	3829/14	24	M	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	TB LN
25	3840/14	68	F	1	2	1	1	1	6	Adequate	2	1	1	1	1	6	Adequate	TB LN
26	3881/14	27	M	1	2	2	2	2	9	Superior	1	1	1	1	1	5	Adequate	TB LN
27	3882/14	26	M	1	2	2	1	2	8	Superior	2	1	1	0	0	4	Adequate	TB LN
28	3893/14	61	M	1	2	1	1	2	7	Superior	1	1	0	1	1	4	Adequate	CA deposits
29	3913/14	20	F	1	2	1	1	1	6	Adequate	1	1	1	1	1	5	Adequate	LA
30	3955/14	15	F	1	2	1	1	1	6	Adequate	1	1	1	1	1	5	Adequate	TB LN

KEY TO MASTER CHART

FNAC	-	Fine needle aspiration cytology
LBC	-	Liquid based cytology
F	-	Female
M	-	Male
A	-	Amount of background blood or clot
B	-	Cellularity
C	-	Degree of cellular trauma
D	-	Degree of cellular degeneration
E	-	Retention of appropriate architecture
NCG	-	Nodular colloid goitre
CG	-	Colloid goitre
CCN	-	Cystic colloid nodule
CNG	-	Colloid nodular goitre
HT	-	Hashimoto’s thyroiditis
LT	-	Lymphocytic thyroiditis
Pap ca	-	Papillary carcinoma
CG & thyroiditis	-	Colloid goitre with thyroiditis
CA	-	Ductal carcinoma
FCD	-	Fibro cystic disease
FA	-	Fibroadenoma
PBD	-	Proliferative breast disease
BPT	-	Benign phyllodes tumour
GM	-	Granulomatous mastitis
CA deposits	-	Secondary carcinomatous deposits
RN	-	Reactive node
TB LN	-	Caseating tuberculous lymphadenitis
GL	-	Granulomatous lymphadenitis
AIP	-	Acute inflammatory pathology
LA	-	Non specific lymphadenitis