COMPARATIVE STUDY OF CONVENTIONAL SMEARS, CYTOSPIN SMEARS AND CELL BLOCK ON BODY FLUID CYTOLOGY

By

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Under the Guidance of

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LIST OF ABBREVATIONS USED

CB - Cell Block

CS - Centrifuge smear/ Conventional smear

Fig - Figure

FNAC - Fine Needle Aspiration Cytology

GIT - Gastro Intestinal Tract

H&E - Hematoxylin& Eosin

IHC – Immunohistochemistry

CSF- Cerebrospinal fluid

ABSTRACT

BACKGROUND

Cytological study of body fluid is a complete diagnostic modality.The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears.Distinguishing benign from malignant cellular changes requires meticulous screening and understanding of the range of reactive changes.

Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity, cytocentrifuge and cell block are useful methods. Also the morphology of the cells is well appreciated by cytocentrifuge and cell block as compared to routine centrifuge.

OBJECTIVE

To compare conventional smear, cytospin smear and cell block technique in analyzing cytology of body fluids.

MATERIALS AND METHODS

A prospective hospital based study will be carried out on patients fulfilling the inclusion criteria, referred to the Department of Pathology in BLDEU'S ShriB.M.Patil Medical College, Hospital and Research centre, Vijayapur.

Study period:1st December 2014 to 30th June 2016

RESULTS

Inthisstudyminimal amount of background blood and proteinaceous material in 65% of cytospin smears 5% of conventional smears and 46.2% of cell blocks. Abundant cellularity was seen in 47.5% of cytospin smears 23.8% of conventional smears and 5% of cell blocks. Minimal cell degeneration was noted in 82.5% of cytospin smears 53.8% of conventional smears and 45% of cell blocks. Even distribution of cells was seen in 76.2% of cytospin smears 8.8% of conventional smears and 17.5% of cell blocks.

CONCLUSION

Cytospin smears showed clear background, high cellularity, better nuclear features and even distribution of cells.Hence cytospin smears are better when compared to centrifuge and cell block in analysing body fluids.

KEY WORDS:

Cytospin, cell block, centrifuge, body fluids

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INTRODUCTION

Excessive accumulation of fluid in a serious cavity more than normal amount is referredto as an effusion.Benign and malignant causes of effusion can be identified by the relatively non-invasive technique of fluid cytology.[1] Cytological study of body fluid effusions is a complete diagnostic modality as it aims at pointing out the etiology of effusion as well as in certain cases a means of prognosis of the disease process.[2] It is the best way to detect the presence of malignancy in body cavity fluids, identify the immediate precipitating factors causing the effusion and results in a specific diagnosis.[1]

There is no standard technique for processing of body fluids which is reliable and cost effective. The technique most commonly used in many cytology laboratory of India is the centrifugation and sedimentation smear preparation technique. Thus resulting in effusion being reported as negative without definitive diagnosis and increase in false negative cases.[2]

A common diagnostic problem in conventional cytological smears is accurate identification of cells as either malignant or reactive mesothelial cells. Distinguishing benign from malignant cellular changes requires meticulous screening and understanding of the wide range of reactive changes.[3]

The diagnostic performance of the cytologic study of the fluid may be attributable to the fact that the cell population present in sediment is representative of a much larger surface area than that obtained by needle biopsy.Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity, cytospin

and cell block are useful methods. Also the morphology of the cells is well appreciated by cytocentrifuge and cell block as compared to routine centrifuge.[2]

The cell block method provides high cellularity, better architectural patterns, morphological features and increases the sensitivity of the cytodiagnosis when compared with the conventional method.[4] Cytospinpreparations preserves the cellular details and reduce the overlapping of cells than conventional smears thus enabling precise interpretation.[1]

In this study, a comparison was made among various techniques in the study of body fluids which includes conventional smears, cytospin smears and cell block technique.

AIMS AND OBJECTIVES

To compare conventional smear, cytospin smear and cell block technique in analyzing cytology of body fluids.

REVIEW OF LITERATURE

Serous effusions are accumulation of fluids other than the blood in excess of normal small amount in three body cavities- The Pleural, the Peritoneal and Pericardial. Effusions are designated according to their location as pleural, peritoneal and pericardial.[5]

The examination of effusions to determine the presence of malignant cells has been done for at least 130 years. Bahrenburg and Mandlebaum (1917), before world-war II used the technique of cell block for effusion sediments. After world-war II, many began using the Papanicolaou's stain on smears of effusion fluids. Additional techniques have included Millipore filters and Giemsa staining on air dried smears of sediments. The cytologic examination of body fluids is of distinct value in confirming or disapproving malignant metastatic tumors to the cavities. The method is more of prognostic value rather than for the early detection or prevention of further tumor growth.[6]

Cytology is more sensitive than blind biopsy for detecting serosal malignancy (71% versus 45%),presumably because fluid provides a more representative sample. Estimates of the sensitivity of cytology for diagnosing serosal malignancy ranges from 58% to 71%.The cancer detection rate by cytology is increased by 2% to 38% when multiple specimens are examined.[7]

Cytologic examination of a serous effusion is of paramount importance because the finding of cancer cells in such a specimen denotes that the patient has cancer that is not only advanced but also almost always incurable. With the exception of cerebrospinal fluid, in no other type of cytologic specimen does the finding of exfoliated cancer cells have such ominous prognostic significance. Apart from the finding of cancer cells,

cytologic examination of pleural, peritoneal, and pericardial effusions may also reveal information about inflammatory conditions of the serous membranes, parasitic infestations, and infection with bacteria, fungi, or viruses. It can also supply evidence of the presence of a fistulous connection with a serous cavity.[8]

Sampling Technique

Although a serous effusion may be removed at the time of surgical exploration, it is usually sampled by the relatively simple procedure of inserting a wide-bore needle (under local anesthesia) through the body wall into the fluid-containing cavity. Specimens are obtained by inserting a needle into the pleural space (thoracentesis), pericardial space (pericardiocentesis), and peritoneal cavity (paracentesis). No more than 1500 mL of pleural fluid should be removed at any given time because larger evacuations can cause postexpansion pulmonary edema.[7]

PROCESSING OF FLUID SPECIMENS

The fluid is collected into a clean, dry container, which need not be sterile, and sent to the laboratory as soon as possible. If the fluid cannot be sent immediately, it should be stored in a refrigerator at 4ºC and not allowed to freeze. Anticoagulant or fixative is not required to be added to the fluid.[8]

Formalin, alcohol, or any other kind of cellular preservative must not be added to specimens of serous fluid sent to laboratory. Formalin not only prevents cells from adhering well to a slide but also interferes with the quality of staining by the Papanicolaou method. Adding alcohol causes some precipitation of protein in virtually all specimens, thereby interfering with adherence of the cells to the slide.[9]

Gross Appearance of Serous Effusions

The appearance to the naked eye of a serous effusion sometimes reveals clues about the cause of the effusion and the nature of its cellular contents. Many serous effusions are noticeably bloodstained. A serous effusion occasionally contains so many cancer cells that if allowed to stand and sediment spontaneously, the cells form a thick, whitish-yellow layer at the bottom of the container. Spontaneously occurring sediment of similar appearance may develop in fluids containing numerous neutrophilic leukocytes.[8]

ANATOMY, HISTOLOGY, CYTOLOGY OF SEROUS EFFUSIONS:

The major serous cavities include the two pleural cavities, the peritoneal cavity and the pericardial sac. These cavities are lined by parietal and visceral mesothelium. The visceral mesothelium is reflected over the organs therein. The serous cavity histology and serous cavity cytology of individual cavities are significantly identical without any site-specific differences.[7,10]

Mesothelial cells are derived from mesoderm. Underlying these mesothelial cells is a thin layer of fibrous connective tissue with varying amount of adipose tissue, small blood vessels and lymphatics. The lymphatic vessels open on to the surface lining of the serous cavities through stoma between the mesothelial cells, which provides continuity between lymphatic system and serous cavities.[10]

In effusions, the mesothelial cells desquamate from the surface of the lining of the body cavity and accumulate or even proliferate in the body fluids. Free-floating mesothelial cells in fluids appear singly, in doublets, or in clusters of variable sizes and configuration. Single mesothelial cells in body fluids are usually spherical or oval and measure between 15 and 20 µm in diameter. The cyanophilic or faintly

eosinophiliccytoplasm is sharply demarcated. Under higher magnification, two cytoplasmic zones can be recognized: a perinuclear, denser zone, and a peripheral, clear zone. The difference is caused by an accumulation of cell organelles in the perinuclear area. A characteristic feature of mesothelial cells in doublets or small linear clusters is the flattening of the opposite cell membranes with formation of a clear gap or "window". The "windows" are most likely due to microvilli separating the cells.[10]

The serous cavities may be affected by variety of processes including inflammation, hepatic cirrhosis, congestive heart failure and metastatic neoplasms. These processes stimulate reactive changes in the mesothelial cells. The reactive mesothelial cells are hypertrophied and appear somewhat cuboidal with enlarged nuclei and conspicuous nucleoli.[10]After exfoliation, mesothelial cells roundup and appear polyhedral due to surface tension of the surrounding fluid. They are usually 15-30 micro meters in diameter, but they may vary and range up to 550 micro meters.[11]

Mesothelial cells form clusters of various configurations in effusions. Some of the clusters may be circular, linear or have a rosette like configuration. The outer edges of such clusters are usually composed of rows of cells showing smooth borders or scalloping.[9] Mitoses in effusions can be observed in benign conditions. Mitotic figures, small signet ring forms, loose clusters and occasionally papillary clusters without malignant characteristics and also multinucleated giant cells with fused nuclei with low N: C ratios are not of diagnostic value. These cells often appear in long standing effusions and pleurisy.[11]

Atypical and Reactive Mesothelial Cells

We commonly come across reports from other laboratories of “atypical” or “reactive” mesothelial cells, overused and inappropriate designations given to mesothelial cells that are merely hypertrophic and often hyperplastic.

Reactive mesothelial cells show large hyperchromatic nuclei, high N: C ratio, enlargement in size with prominent nucleoli. Proliferating mesothelial cells often show normal mitotic figures and are common in hepatic cirrhosis, allergic pleurisy, polyarteritis, pulmonary infarcts and long standing effusions due to cardiovascular disease. The most reliable and useful criteria for diagnosis of malignancy in fluids are clusters of malignant cells with organoid arrangement, individual malignant cells and cells staining positive for mucin. Type of preservation and state of preservation are important while categorizing the malignant cells.[11]

It is possible to determine the primary site by observing the morphological features of tumor cells present in the effusions. Each tumor has distinct tumor cells and arrangements. The cells of mammary carcinoma are usually not numerous, are large, spheroidal and distinctly outlined. Their cytoplasm is either pale and vacuolated or homogenous and hyaline.The cells of bronchogenic carcinoma are not numerous, are large spheroidal, with distinct outline. Their cytoplasm may be pale and vacuolated and sometimes slightly keratinized. Sausage shaped nuclei and multinucleation is noted. The cells are arranged in proliferation spheres.The cells of gastric carcinoma are very sparsely distributed and hard to find. The cells are small round with pale vacuolated cytoplasm and distinct cellular outline.[12]

The narrow gap of 5- 10 micron that separates the parietal and visceral layers of the serosa under physiological conditions is widened in the presence of effusion of fluid into the serous cavity. Irrespective of their cellular composition, all effusions are pathological.Effusions may be of two types, according to the pathogenesis of their occurrence. They may be either transudates (a passive process resulting in protein and cell poor fluid) or exudates (an active process leading to exudation of protein and cells in to the fluid). Effusions can also be classified according to the etiological process, which may be due to reactive benign (infective/inflammatory) conditions or due to malignant deposits into the cavity. Peritoneal cavity can accumulate up to 15 – 20L while the pleural cavities can hold up to 3 L of fluid in each cavity however the pericardial cavity cannot hold > 0.6 L without leading to tamponade.[5]

Transudates are clear straw colored fluids often characterized by low specific gravity, often below 1.015 and low protein content (usually less than 3.0 gm/dl). The accumulation of transudates within the body cavities is caused by filtration of serum across physically intact vascular wall, either by reduced intravascular osmotic pressure, as in hypoproteinemia or by increased filtration pressure, as in heart failure. There is no alteration in the endothelial permeability. It is mainly seen in non inflammatory edema.Cellular components of transudates are scanty and are limited to few mesothelial cells and leucocytes. The glucose content is same as plasma and PH is more than 7.3. LDH content is low and the effusion to serum LDH ratio is less than 0.6.[10]

The exudates result from accumulation of fluid within the body cavities, associated with damage to the walls of the capillaries. Exudates are mainly associated with inflammatory edema. The exudates are cloudy and opaque fluids of various colours.

The presence of blood tinged reddish fluid is an important diagnostic sign that should be recorded because it may indicate the presence of primary or metastatic tumor or tuberculosis.[10]

The exudates are characterized by a relatively high protein content (usually more than 3.0 gm/dl) and therefore high specific gravity (usually more than 1.015).The exudates are rich in fibrin and may coagulate on standing and contain variable, but often significant population of cells that are the targets of cytologic investigations.The glucose content is low (less than 60mg/dl). The PH is usually less than 7.3. The LDH level is high and serum to effusion LDH ratio is more than 0.6. It usually grossly seen as purulent material such as pus.[10]

The customary methods of examination of serous fluids are cytologic examination of the fluid by both conventional smears and cellblock sections, biopsy of the pleura, peritoneum and pericardium and supplemented by immunohistochemistry, biochemical, bacteriological and cytogenetic investigations.[8,9]

Non-neoplastic Effusions

Inflammation of a serous cavity is usually a complication of an underlying lesion, although not necessarily an inflammatory lesion. For example, pulmonary neoplasms often induce inflammation of the surrounding pulmonary parenchyma (pneumonia), which frequently extends to the pleura which may be accompanied by effusion showing a cytologic picture of inflammation with or without neoplastic cells. Another frequently occurring situation is development of a sterile, noninflammatory effusion, as, for example, in congestive heart failure.[8]

1) Acute Serositis

Acute pleuritis, pericarditis, and peritonitis are usually the result of a bacterial infection. Cytologic preparations are highly cellular and composed almost exclusively of polymorphonuclear leukocytes. It is important to screen such cases carefully for malignant cells because acute infection can be a complication of metastatic malignancy.[7]

2)Eosinophilic effusions

A pleural effusion is considered “eosinophilic” when eosinophils account for 10% or more of the nucleated cells present. The most common causes are pneumothorax and hemothorax. Less common causes include drug reactions, parasitic infections, pulmonary infarction, and the Churg-Strauss syndrome. Charcot leyden crystal are spindle-shaped and are derived from eosinophils and may occur in eosinophilic pleural effusion.[7,10]

3)Lymphocytic Effusions

A pleural effusion consisting mostly of small lymphocytes is a relatively common but nonspecific finding. Cytologic preparations are often highly cellular and composed almost exclusively of dispersed, small lymphocytes.Mesothelial cells and histiocytes are either conspicuously absent or present in small numbers. It is seen in malignancy, tuberculosis and post coronary artery bypass.[7]

4) Rheumatoid Disease

The pathognomonic of rheumatoid serositis consists of three elements: elongated spindle-shaped macrophages, multinucleated giant histiocytes, and necrotic granular background material. Cytologic preparations are sparsely or moderately cellular. An abundant granular material dominates the picture.[7,10]

Neoplastic Effusions

Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions. The diagnosis of cancer in a pleural, pericardial, or peritoneal fluid is of capital importance for the patient and the attending physician or surgeon. Although, in many such instances, a fatal outcome of the disease may be anticipated, some tumors offer a much better prognosis than others.[13]

Most patients with a malignant effusion have a previously documented primary neoplasm. In some cases,however, a malignant effusion is the first manifestation of an occult malignancy. The most common occult primary in women and men who present with a malignant pleural effusion is lung cancer. It is extremely uncommon for breast cancer to manifest itself initially as a malignant effusion.The most common occult sources of a malignant peritoneal effusion are intestinal and pancreatic cancer in men and ovarian cancer in women.Other tumors that can present as a malignant effusion include lymphoma, melanoma, and mesothelioma.[7]

Primary tumors of the serosal surfaces are uncommon,being far outnumbered by secondary involvement bytumors from other locations. The two primary serosalmalignancies considered here are malignant mesotheliomaand primary effusion lymphoma.

Adenocarcinomas

Adenocarcinomas of various origins are by far the most common type of tumors encountered in effusionsare more common than squamous and small cell cancers.In most cases the carcinoma cells are easily recognizedbecause, in one way or another, they are

morphologicallydistinct from mesothelial cells. The common features of adenocarcinomas in fluids are:

- The tumor cells form gland-like or tubular structures with a central lumen
- Tumor cells forming multilayered, spherical or oval cell clusters, suggestive of papillary growth, also known as “spheroids” or “hollow spheres”. In paraffin-embedded cell blocks, cross-sections of such clusters usually reveal glandular features of adenocarcinoma
- Single cells of adenocarcinoma may be recognized if they are of columnar configuration because such cell types practically never occur in other tumor types
- Signet ring cancer cells, with an abnormal nucleus displaced to the periphery by a large mucus vacuole.[7,13]

Squamous cell carcinoma

It which rarely metastasize tothe pleura, pericardium, or peritoneum. Those that doare most commonly carcinomas of the lung, larynx, andfemale genital tract. The primary tumor is known in virtuallyall cases before an effusion develops. The cells of squamous cell carcinoma are arrangedeither in large clusters or as isolatedcells. The cytologic appearance differs dependingon the degree of keratinization of the tumor.The cytoplasmis usually dense and occasionallyorangeophilic.Cells with a tadpoleor spindle shape areuncommon. Nuclei are enlarged, hyperchromatic, andcoarsely granular; nucleoli are usually not prominent.[7]

Small cell tumors

Tumors composed of small cells may be of epithelial origin such as oat cell carcinoma and related tumors and small cell tumors of the breast or of other derivation,

such as malignant small cell tumors of childhood, or malignant lymphomas. The principal difficulty in the recognition of this group of malignant tumors in effusions is the small size of cancer cells that may be overlooked or mistaken for inflammatory cells.[13]

Melanoma

Most metastatic malignant melanomas arise in the skin, but extracutaneous tumors, such as ocular melanomas, do occur. Contrary to most other malignant tumors where clinical evidence of metastatic disease usually precedes the accumulation of fluid, effusions in malignant melanoma may occur as the primary evidence of metastases, sometimes many years after the treatment of the primary tumor. Thus, the identification of this tumor type may be of considerable diagnostic importance. This diagnosis should always be considered in effusions with a population of malignant cells of carcinomatous or unusual configuration in the absence of a known primary tumor. The presence of melanin pigment in tumor cells is characteristic of malignant melanoma.[7,13]

Involvement of serous cavities by cancer cells correlates with poor prognosis even in the absence of obvious effusion. Cases positive for malignant cells in serous cavity samples are upstaged clinically.[14,15]

Cytological examination of serous effusions has been done for nearly a century in the diagnosis of malignancy and eventually the detection of primary lesion. It helped in the staging and prognosis of malignant tumors and also gave information regarding various inflammatory lesions of the serous membrane. It gained increased acceptance to such an extent that an effusion positive for malignancy was often considered as a definitive diagnosis.[11,16]

Presently the examination of body fluids for the presence of malignant cells is accepted as a routine laboratory procedure, not only for the detection of unsuspected cancers, but also for detection of metastasis of unknown primary.[11]

A study of 183 serous effusions by Takagi F *et al*[11]concluded that the most reliable anduseful criteria for diagnosis of malignancy in fluids were:

1. Clusters of malignant cells with an organoid arrangement,
2. Individually lying malignant cells and
3. Cells staining positive for mucin.

Fluids in which the medium small round cells were found representing undifferentiated carcinoma arising in glands (stomach, breast, ovary) were extremely difficult to diagnose. They also suggested that if the number of tumor cells were small, it was impossible to differentiate the tumor cells from reactive mesothelial cells.[11]

Foot NC *et al*[12]studied 219 effusions of proven malignancy in an attempt to identify the types and primary sites of metastatic tumor from exfoliated cells in serous fluids. They found that cells from malignant tumors could be readily recognized as such in smear preparations of serous fluids and proposed that it was possible to diagnose the type and source of malignant tumor cells in serous effusions with an overall accuracy of 50%.

Foot NC *et al*[17] while analyzing smears from 2029 persons in the identification of neoplastic cells in serous effusions found that examination of smears were of distinct value in confirming or ruling out suspicion of a tumor. He proposed smear examination might not assist in early detection of malignant growth, since these are already far advanced when cells are exfoliated into these effusions.

Malignant cells in pleural or ascitic fluids were almost always indicative of metastatic tumors as primary malignancy arising from mesothelial cell lining these spaces were rare. When mesothelial primary was present, tumor cells were usually numerous and seen in clusters.[18]

Murphy WM *et al*[19]retrospectively studied 117 malignant effusions from various sites in an attempt to determine the primary site of malignancy. Their study comprised of well-documented cases of pleural and peritoneal serous effusions containing malignant tumor cells derived from the carcinoma of the breast, lung, ovary, endometrium and stomach. Sufficient cytologic similarities occurred to permit classification into three distinct patterns. According to him malignant tumor cells could be identified in approximately 90% of the effusions related to malignant neoplasm. They found that cytoplasmic vacuoles are uncharacteristic. In fact, the occurrence of vacuoles in more than 5% of the cells was a major factor in differentiating mammary from ovarian neoplasm. Uniformity of the cell size and shape was found to be a hallmark of mammary cancer in fluids. Major characteristics of pulmonary tumors in effusions were irregularity of cells with vacuoles and macro nucleoli, large percentage of isolated cells and the prominence of multinucleated cells.

Most of the authors were in agreement to the fact that the examination of cell patterns (for example, proliferation spheres, Indian file pattern in cases of mammary cancers, prominence of vacuolated cells in ovarian cancers, etc.) aided in prompt identification of the primary site.[12,19,20]

Foot NC *et al*[21,22] found that the most frequent and disturbing obstacle in the accurate cytological diagnosis of cancer in sediments of serous effusions was the fact that

histiocytes and mesothelial cells may undergo misleading metaplastic changes under certain circumstances and were then readily mistaken for cancerous elements.

Luse SA *etal*[20]did a detailed morphological study of cellular forms in 920 effusions from pleural, peritoneal and pericardial cavities. They studied the cellular changes not associated with malignant neoplasm and in particular those that might be misinterpreted as evidence of cancer and changes observed in the presence of malignant tumors.

According to Kumar *et al*[23] the Bull's eye inclusions, which consists of a body mass at the centre of cytoplasmic vacuole, which were positively stained with PAS and Alcian blue stain was useful finding for corroborating the diagnosis of metastatic adenocarcinoma, although the exact site could not be identified.

Sujathan K *et al*[24] studied significance of AgNOR count in differentiating malignant cells from reactive mesothelial cells in 100 samples of serous effusions and concluded that AgNOR studies were clinically useful as an additional diagnostic tool for use in ascitic and pleural fluid samples where cytologic diagnosis was difficult.

Booth S.N. *et al*[25]studied 56 cases of serous effusions (ascites and pleural effusions) and demonstrated that CEA levels in cancerous effusions were frequently several times higher than corresponding serum levels in contrast to the fluid levels of pregnancy associated α2 glycoprotein (PAG). Thus, the measurement of carcinoembryonic antigen (CEA) and pregnancy associated α2 glycoprotein (PAG) in serum and effusion fluids would increase the diagnostic accuracy in patients with effusions.

Singh G *etal*[26] studied immunodiagnosis of mesothelioma with the use of anti-mesothelial cell serum in an indirect immunofluorescence assay. They found that the antimesothelial cell serum could be used as a diagnostic reagent in confirming or refuting the diagnosis of mesothelioma, and established the nature of cells cultured from serous effusions. (singh G)

Bramwell ME *et al*[27] studied Ca2 and Ca3 monoclonal antibodies evaluated as tumor markers in serous effusions. The Ca2 monoclonal antibodies appeared to have a high specificity for the identification of carcinoma and mesothelioma cells in pleural and peritoneal fluids. Ca2 was found to label mesothelioma, carcinomas of the ovary and endometrium however oat cell carcinomas of the lung and colon remained unlabeled. Anti CEA labeled carcinomas of lung and colon but did not stain carcinomas of endometrium and ovary. Hence they suggested that Ca2 would be particularly useful in combination with anti CEA. A low degree of specificity was found with Ca3.

In conventional smears, the accurate identification of cells as either malignant or benign reactive mesothelial cells is a diagnostic problem. The reactive mesothelial cells which are common in hepatic cirrhosis, allergic pleurisy, polyarteritis, pulmonary infarcts and in long standing effusions due to cardiovascular diseases may show reactive changes such as cytomegaly, nucleomegaly, multinucleation, mitotic figures and high N/C ratio.[11]

CYTOSPIN

A cytocentrifuge is a device that spins cells in a fluid suspension directly onto a glass slide. Since the introduction of the Cytospin I by Thermo Electron Corporation, other instruments have been developed with slightly different features. Following the

guidelines and procedures recommended by the manufacturer of the instrument usually results in excellent cytologic material.[9]

The principle of cytospin is based on the fact that because the cell is denser than the suspending fluid, under an applied force the cell will have greater momentum than the fluid. This means that after passing through the sample chamber the cells will be projected towards the microscope slide with sufficient momentum. The suspended fluid is absorbed by the filter card by capillary action and the cells are deposited on the microscope slide.[6]

Types of Cytocentrifuges

1) Shandon Cytospin II and III

Newer Cytospin models have features that increase cell recovery. The Cytospin II and III form an air bubble between the sample and the slide which increased cell recovery rates when compared to the Cytospin I.

Also available is a Megafunnel for use with the Cytospin II or III, which allows the processing of up to 12 times the sample volume (6ml) and deposits the cells over an area 10 times larger than the cell deposition area of Cytofunnel. The Megafunnel is designed for highly cellular samples such as effusions, bronchial washings and sputums.

2) WescorCytopro

WescorCytopro is an economical, easy to use, 8-chamber, stand-alone cytocentrifuge. Wescor also has an automated cytology slide stainer that can deliver 160 stained slides per hour. Since each slide is stained individually, there is no possibility of cross contamination and no filtering of reagents is necessary. This stainer can be converted to a cytocentrifuge by adding the Cytopro rotor.

3) HettichCytocentrifuge

The HettichCytocentrifuge has 8 ml chambers that resemble a flat bottom test tube. It deposits cells on the slide in a circle 17.5 mm in diameter. Because of its comparatively low cost, ease of operation and reusable chambers, some workers recommend this instrument for liquid-based gynecologic cytology as well as for nongynecologic material.

4) Leif's Centrifugal Cytology Buckets

In this method cells are suspended in small amounts of fluid, for example, in cerebrospinal fluid, could be spun directly onto glass slides. The 8-chamber bucket, which can be adapted with almost any laboratory centrifuge, allows simultaneous processing of three samples. The slides prepared in this manner exhibited excellent cytologic detail with uniform cell distribution. The cells are wet-fixed during centrifugation, thereby avoiding air-drying artifacts.[9]

Cytospin is a cytology method that is specifically designed to concentrate cells that are found in small numbers, i.e., they can be used to separate cancer cells from non cancer cells. After the cytospin is completed, other cytology methods such as immunocytochemistry can be preformed to evaluate the cells. The cytospin process is a simple procedure. The cytospin technique can be used on any single cell suspensions of any source such as peripheral blood mononuclear cells (PBMCs), effusions, cerebral spinal fluid (CSF), bronchial lavages, fine-needle aspirates, culture cells, etc.[9]

In cytospins, single cell suspensions are spun onto a microscope slide by use of a cytocentrifuge. A cytocentrifuge spins cells at an angle, at low speeds, and accelerates and decelerates gradually. The fluid from the suspension is absorbed onto filter paper

while the centrifuge is spinning. This allows the cells to adhere to the slide in a monolayer format. Major objections to the use of the cytocentrifuge include distortion of cellular morphology due to air-drying artifact and loss of cells by absorption of fluid into the filter card. Both of these difficulties have been overcome. The rare drying artifact of polymorphonuclear leukocytes rarely affects epithelial cells; hence, the diagnostic value of the preparation is not reduced.[9]

Applications:

1) To retain the morphology of cells after centrifugation with ordinary centrifuges, morphology of cells is usually lost.
2) To prepare monolayer of cells on a given area of slide. No overlapping of cells.
3) Helps in identifying malignant cells.
4) No breakage of cells
5) Retrieval of cells from very less and rare cellular material like CSF, urine etc.
6) Even if there is less volume of body fluids, cells can be recovered.[9]

Dai L *et al*[28] studiedthirty-nine joint and bursa effusions. They observed tissue fragments were not seen in Wright-stained cell smears and only rarely in wet drop preparations. In contrast,variously sized fragments with the histological appearance of hyperplastic synovial lining were detected in ethanol-fixed,haematoxylin/eosin-stained cytospins from bursitis and all arthropathies studied. Immunostaining revealed CD68 expression in a subset of cells in a pattern characteristic ofhyperplastic synovial lining. Synovial lining fragments can be detected in effusions from diverse arthropathies and bursitis. They maintainimportant properties of the synovial lining and can be analysed by

immunohistochemistry. They may afford the opportunityto study a relatively pure preparation of synovial lining cells without the need for cell culture, and to evaluate their possible rolein augmenting or perpetuating synovitis or joint damage.

CELL BLOCK (CB)

Most of the fluids received in the cytology laboratory contain blood clots or smallbits of tissue from the lesion while preparing the slides. These tissue bits remain in bottleand will not be available for microscopy.[9,29]

Cellblocks prepared from residual tissue and fluids can be particularly useful foridentification of tumors that cause diagnostic difficulties on smears. This technique issimple, reproducible and safe. Further the effectiveness of cellblock lies in theavailability of diagnostic material for further histological examination, histochemistryand IHC studies for better classification of the tumor and identification of infectiouscauses with microbiologic stain.[30]

The advantages of the CB are summarized as:

1. Recognition of histological patterns of diseases that sometimes cannot beidentified reliably in conventional smears.[29]

2. Possible to study multiple sections by routine staining, special staining andimmunocytological procedures.[4]

3. Less cellular dispersal, which permits easier microscopic observation than dotraditional smears.

4. Less difficulty in spite of background showing excess blood on microscopicobservation.

5. Possibility of storing slides for retrospective studies. Storage of the CS is a practical problem.[4]

The disadvantages with cellblock technique are:

1. Delay in the diagnosis when compared to conventional smears.

2. Sometimes, risk of losing material during processing.[9]

3. Some mesothelial cells because of centrifugation artifacts may form rosettes orpseudoacini that can be a source of misdiagnosis.

4. The histologic preparation time and increased cost are additional drawbacks.[8]

Cellblock from serous effusions can be prepared by various methods.

1. FIXED SEDIMENT METHOD: the fluid specimen is fixed in a suitable fixative followed by centrifugation leading to the formation of sediment. This cell button is processed like histopathological specimen. Or the clot present in the fluid is processed. The main disadvantage with this technique is the risk of losing material.[9]

2. PLASMA-THROMBOPLASTIN METHOD: Adding a few drops of plasma andthrombin solution to the centrifuged button and fixing it in 95% alcohol and 5%formalin. The main drawback of this technique is that if the specimen has beenfixed in formalin priorly, it has to be washed many times as plasma- thrombin do not form a clot.[9]

3. BACTERIAL AGAR METHOD: Fixative such as 2% agar with 10% formalincan also be used for cellblock preparation. Problem with this technique is that theagar has to be kept in a molten state.[9]

4. SIMPLIFIED CELL BLOCK TECHNIQUE: introduced by Krogerus andAnderson in 1988, a unique technique in that the procedure is carried out in thesample tube, ensuring

minimal cell loss where molten paraffin is added to the tubeand solidified. No transfer of cells to a cassette is necessary. The bottom of thetube is cut-off and processed entirely.[9]

Usefulness of Cell-Block Preparations

Cell blocks are especially useful asadjuncts to the "cytologic" preparations previouslylisted. To prepare a cell block, the remainder of thesediment is wrapped in filter paper, placed in a cassette,embedded in paraffin, and cut and stained inthe manner of histologic sections. Before placing it ina cassette, however, it is helpful to coagulate the sedimentby adding a few drops of plasma and several drops of a thrombin solution.[7]

A discrepancy between positive smears and negative cellblocks can be explained by a slowly forming spontaneousclot, thereby allowing cancer cells to sink to the bottom ofthe container.In the cell block the spontaneously formed clotcomposedof dense magenta fibrin would contain very few, ifany, cancer cells, whereas the induced clot prepared from thesediment obtained by centrifugation of the unclotted portionof the specimen would contain many. The reversesituation can also take place.

Cell-block preparations may also reveal certain histologicaspects of a neoplasm such as papillary, acinar, or duct-likeformations.Also extremely well demonstrated in cell blocksare psammoma bodies, which may be difficult or impossibleto detect in the permanent smears.Cell blocks reveal other interesting histologic or cytologicentities, some mere curiosities but others of importanceillustrates a fragment of liver in the cell block of peritonealfluid obtained when the paracentesis needle traversed the liveron its way to the peritoneal cavity; retrospective analysis of thesmears revealed only an occasional hepatocyte.[9]

Cell blocks have also revealed granulation tissue a manifestation of pleural inflammation, cholesterol clefts infibrin, skin, squamous epithelial cells, skeletal muscle, cartilage,colonies of microorganisms, accessory skin structuresin subcutaneous adipose tissue, fragments ofhyperplastic mesothelium with collagenous stroma,fibroblastic tissue, vegetable cells, and pulmonary parenchyma.Many of these entities were either not present or were not recognizedin the smear preparations.The routine use of cell block by agar or plasma thrombin methods are not costeffective as it requires additional materials and consumption of extra time compared toearlier conventional methods.[8]

Joshi A *et al*[2]conducted a prospective study on 150 effusion samples, which included peritoneal fluid(53.33%), pleural fluid(44.67%) and pericardial fluid(2%). These were subjected to routine centrifuge, cytospin and cell block. In the present study, of 34 cases of malignant effusion, 14 were reported as positive for malignancy, 15 showed atypical cells suspicious of malignancy on routine centrifuge. Out of the 14 malignant effusions, 4 were reported as epithelial malignancy.

Thus 5 malignant effusions were missed on routine centrifuge. Also the 4 cases reported as epithelial malignancy on centrifuge, showed better architectural pattern on cytocentrifuge and cell block and a specific diagnosis of adenocarcinoma was made. Thus adenocarcinoma was diagnosed in 28 cases. 2cases on NHL, 2 cases of Mesothelioma, 1 case of SCC and 1 case of CLL/SLL were seen.

It was found that there was significant difference between the results obtained by routine centrifuge as compared to cytocentrifuge and cell block.However cytocentrifuge and cell block method were positive for malignant cells for all 34 cases. The smears obtained by each of the above technique were evaluated for features such as background,

cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale according to the Maire*t al*[31] scoring system.Nuclear and cytoplasmic preservations were as good as cytospin.In evaluating the cytological details brought out by each technique, cytospin was superior in demonstrating cellularity, cell retrival , less cellular crowding, better cytoplasmic and nuclear preservation than routine method.Sections prepared from cell block showed good architectural pattern (cell balls, acinar pattern, papillary structures). Nuclear and cytoplasmic preservations were as good as compared to cytospin.

A study on 23 synovial fluids for differential counts by usual smear or by adding two drops of hyaluronidase to 0.25cc of synovial fluid and cytocentrifuging at 800rpm for 10 minutes was done by Mario J *et al*.[32] They concluded that cytospin preparations gave better morphology and also the differential counts on cytospin preparations showed higher percentage of monocytes, suggesting that these cells were undetected and misinterpreted as lymphocytes on routine smears.

ThaparM *et al*[29]conducted a study on 190 cases of serous effusion to compare cell block technique with conventional smear method. They inferred that cell block technique not only increased the positive results but also helped to demonstrate better architectural pattern which aid in making diagnosis of primary site.

Liu K *et al*[33]conducted a study to compare the effectiveness of obtaining a diagnosis by smear, cytospin, or cell block preparations. The average costs involved were also evaluated. Three hundred sixty-one cases with both smears and cytospins and 483 other cases with both smears and cell blocks collected over a 2-yr period were reviewed. They concluded that it is notcost-effective to obtain either cytospins or cell blocks in

addition tosmears on all cases. However, it is cost-effective to obtain cellblocks when the immediate smear evaluation is nondiagnostic.

In study done by Deshpande AK*et al*[6]on 150 samples of ascitic and pleural fluids were studied which were aspirated from admitted patients of medical, surgical and gynecologic wards for a period of one year and two months. The comparative study between the Cytospin II and ordinary centrifuge revealed that the Cytospin II preparation gives a better yield of cells specially when they are scanty, morphology of cells is very well preserved and much time is saved in screening the slide. The Cytospin II is better in picking up malignant cells than the ordinary centrifuge.

Singh M*et al*[1]did a comparative study on different techniques in serous fluid cytology. Ninety eight samples were subjected to diagnostic evaluation. Along with conventional smear, fluids were subjected to cytocentrifuge and cell block technique. Cell blocks were prepared using 10%formalin as a fixative agent. Smears obtained by each of techniques were scored for different parameters according to the Mair*et al*[31]scoring system. Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hencefor fluids with scant cellularity,cytocentrifuge and cell block are useful methods. Also the morphologyof the cells were well appreciated by cytocentrifuge and cell block as compared to routine centrifuge,thus aids in accurate diagnosis. In this study the diagnoses which were missed or incompletely diagnosed on routinecentrifuge were diagnosed accurately by the other two techniques. Also there was statisticaldifference between the results obtained by the three techniques. Thus cytocentrifuge and cell blockproved to be superior method for the study of effusion as compared to routine centrifuge.

Bhanvadia VM*et al*[3]examined 150 fluid specimens were examined for conventional cytological smear (CS) and cell block method. Each fluid specimen was divided in two equal parts: one part was subjected to conventional smear technique, while the other part was subjected to 10% alcohol-acetic acid-formalin cell block technique. Overall morphological details, cellularity, architecture, nuclear and cytoplasmic details were studied in both CS and CB techniques. In this study, the utility of the CB method in the cytodiagnosis of malignant effusions wasfound to be highly significant as compared to the CS method. The additional yield of malignancy was10% more as was obtained by the CB method. They concluded that CB is superior to CS method.

Nathan NA*et al*[16] prepared cell blocks from residual tissue fluids and fine-needle aspirations as useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. They used a modified cell block technique using an improvised ethanol formalin fixative (Nathan alcohol formalin substitute which is consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde) followed by a simple paraffin processing schedule is described. This improved preparation offers excellent cytomorphologic features corresponding closely to cells in Papanicolaou-stained smears and ensures optimal preservation of histochemical and immunocytochemical properties.

DekkarA *et al*[30]did study on usefulness of cell blocks versus smears. Approximatelyhalf of 351 body-cavity effusions from 263 patients wereexamined prospectively in paraffin-embedded cell blocks and insmears, while the other half were examined in smears alone. It is recommendedthat both cell blocks and smears be used in evaluatingall fluids submitted to the cytology laboratory.

Kulkarni MB *et al*[34] assessed seventy cell blocks which were prepared by thromboplastin-plasmatechnique. They concluded thatcell block serves as a useful adjunct to traditional cytologicalsmears. TP method is simple, cost effective, and reproducible. Itis easy when compared with agar-embedding technique. Ancillarytechniques like ICC can be performed successfully.

Bista P[35] did a study on 37 serous effusion and concluded that cell blocks provides better architectural display, good yieldof diagnostic material from serous effusions with minimal degenerative changes. Findings of both the cell blocks andsmears will complement each other to come to a definitediagnosis.

Jalal R *et al*[36] studied 600 cases of serous fluids from pleural, pericardial and peritoneal effusions and concluded that clot preparation from body cavity fluids on the second day can be used as an adjunct to smear androutine cell block preparation to improve the accuracy and yield of the cytological diagnosis and may also be ofgreat help for special studies such as IHC staining.

Murugan P *et al*[37] studied the combined diagnosticefficacy of epithelial membrane antigen (EMA), carcinoembryonic antigen(CEA), E-cadherin (EC), calretinin (CAL), desmin (DES) and vimentin (VIM) in distinguishing reactive mesothelial(RM) from adenocarcinoma(AC) cells in serous effusions. EMA was the best single marker for AC. For the mesothelial cells, CAL is best marker. DES was more specific than CAL. Among the combinations, twopanels - EMA+ AND (CAL- OR DES-) for ACs and CAL+ AND (EMA- OR CEA-)for RM had 100% specificities and sensitivities.

In a study done by Shobha SN *et al*[38] on 100 pleural fluids which were studied by conventional smear and modified cell block technique containing ethanol, acetic acid and

formalin. Additional yield for malignancy was 46.15 % more by modified cell block method when compared to conventional smears. In conclusion a combined approach by both conventional smears and MCB helps in diagnosing the cause of pleural effusion suspected of malignancy.

In a study done by Koksal D *et al*[39]on forty patients with lung cancer andexudative pleural effusions were included. This study confirms that the cell block method combinedwith conventional smear increases the diagnostic yield in exudativepleural effusions accompanying lung cancer.

PLEURAL EFFUSIONS

Kushwaha R *et al*[40]conducted a study on one hundred samples of pleural fluid were examined for total cell count, cell type and cellularfeatures. They were also subjected to biochemical study to find out the level of protein, glucose and chloride. The study showed that the most useful test in establishing the diagnosis of pleural effusion is pleural fluid cytology and pleural fluidcell count. Cytologic study of pleural fluid is a complete diagnostic modality which aims at pointing out the etiology of effusionas well as, in certain cases, a means of prognostication of disease process.

UdasimathS *et al*[4] studied 60 pleural fluid samples with combined conventionalsmears and cell block technique, reported 15% increased yield for malignancy bycellblock method. They concluded the CB method provided high cellularity, betterarchitectural patterns, morphological features and additional yield of malignant cellshence thereby increasing the sensitivity of cytodiagnosis.

Koksal D *et al*[39] studied 40 patients with lung cancer and exudative pleuraleffusions by both CS and CB and found increased ratio of diagnosis of malignancy

by10%. They could also subtype lung cancer as adenocarcinoma in 35% of cases. Thus, CBcombined with CS increased the diagnostic yield in exudative pleural effusionsaccompanying lung cancer in their study.

Khan S *et al*[41]studied 47 FNA smears and cell blocks from lung and liver were stained with immunocytochemistry stains consisting of CK7, CK20, AE1/3,Hepar 1, Synaptophysin, TTF1. They concluded that direct FNA smears and cell blocks complement each other and their results indicate that both are needed in the diagnostic workup of patients. The cost implications of performing both techniques on all FNA material warrants further evaluation. The Papanicolaoustainedconventional FNA smears fared better than the cell block for the evaluation of nuclear andmorphologic characteristics. The ICC stains worked better on the cell block samples due to lack ofbackground and aberrant staining.

PERITONEAL EFFUSIONS

Cytologic sampling of fluid from the peritoneal cavity or, more specifically, from the pelvic culde-sac (pouch of Douglas)

The principal applications of pelvic peritoneal lavage are:

- Securing evidence of persisting or recurring cancer during the second-look surgical procedures
- Occasional discovery of occult cancer during exploratory laparotomies or laparoscopies for benign disease
- Incidental discovery of metastatic cancer from non-gynecologic sites

The principal benign cellular components of peritoneal fluids are mesothelial cells, macrophages, leukocytes, and epithelial cells or cell fragments derived from the peritoneal lining and various benign cysts and other structures. The fluids may also contain collagen balls, calcified debris and, occasionally, psammoma bodies.[8,13,42]

PERICARDIAL EFFUSIONS

Pericardial tamponade is a life-threatening disorder caused by varying medical conditions.[43]Malignancy and complications of its treatment are a common cause of pericardial effusion.Malignant pericardial effusions occur less frequently than Malignant pleural effusions; however,they often are acutely life threatening. The pathophysiologic condition usually involves metastasis to the parietal/fibrous pericardium, although visceral or epicardial involvement with or without frank myocardial invasion isalso seen.[44]

Pericardial effusionsthat are exudates are likely to be caused by viral or bacterialinflammation of the pericardium or uremic pericarditis.The aspiration of pericardial fluid is not as simple a procedure as aspiration of the pleural or ascitic fluid. Because of the danger of myocardial perforation, the procedure is not undertaken lightly and only in patients in whom the diagnosis of pericardial effusion is a major dilemma or patients threatened with cardiac tamponade for whom a pericardial “window” must be established. The differential diagnosis often comprises a primary tumor, such as a mesothelioma or involvement by a mediastinal tumor a metastatic tumor, or a chronic inflammatory process, such as a rheumatic pericarditis or postinfarction pericarditis. All of the benign disorders cause atypias of mesothelial cells which deserve a special note.[8]

Kind DT *et al*[45] studied pericardial effusions from 27 patients were examined cytologically. Malignant cells were found in eightcases (30 percent). In three of these patients malignancy was unsuspectedclinically, and this was the first time the cancer was diagnosed.

In a study done by Gornik HL*et al*[43] two hundred nineteen patients underwent pericardiocentesis during the study period. The effusion was cancer-related in 43.8% of cases. They concluded that cancer-related pericardial effusion is associated with adverse outcomes, and abnormalcytology further worsens prognosis. The poor survival among cancer patients with pericardialeffusion and abnormal fluid cytology may have important implications for management.

Zipf RE*et al*[46]did a study to show that the cytologic examination of pericardial fluids may play a significant role in the differential diagnosis of pericardial effusions. 70 pericardial fluid specimens were examined.In 47 of these patients sufficient histologic or clinical information was available to leave no doubt about the absence or presence of a neoplasm involving the pericardium. Fifteen of the 47 cases had histopathologic confirmation (by open pericardial biopsy or autopsy examination) of a neoplasm involving the pericardium. The absence of pericardial neoplasm in the other 32 cases included in the study was confirmed by histopathologic examination or by adequate clinical followup.

CEREBROSPINAL FLUID

The cerebrospinal fluid (CSF) is formed from circulating blood that is filtered though a complex epithelial organ, the choroid plexus, located within the ventricular system of the brain. The CSF is formed by the choroid plexus at a constant rate and is

drained by the leptomeninges, resulting in constant volume of fluid, at 10 to 60 ml in children, depending on age, and 90 to 150 ml in adults.Normal CSF is a crystal clear liquid containing only very few mononucleated blood cells and lower levels of glucose and proteins than the blood serum.[47]

Increased numbers of inflammatory cells in a sample of CSF,particularly if a component of polymorphonuclear leukocytesor enlarged and atypical lymphocytes is observed; suggestthat an infectious process may be present.[8]

Cytologic examination of the CSF is an important part of a complete neurologic evaluation of cancer patients and patients with the acquired immunodeficiency syndrome (AIDS), particularly if there is clinical evidence or suspicion of central nervous system (CNS) involvement. Also, patients with space-occupying lesions of the CNS of unknown nature should have the benefit of this examination. Cytologic evaluation of CSF has become an essential step in the follow-up of patients with lymphoma and leukemia and some small-cell tumors, such as oat cell carcinoma of pulmonary origin, because the therapy of these tumors has been revolutionized by this procedure. An increasingly important application of cytology of the CSF is the diagnosis of infectious processes, particularly in patients with AIDS.[47]

Nathan *et al*[16] studied 544 washings and exfoliative specimens, of which only 4were from CSF. They concluded that contribution of cellblocks to final cytologicdiagnosis supports the view that cellblocks should be considered. In their study cellblockswere possible only in cases in which some material or blood was visible to the naked eyein the specimen container.

Glantz MJ *et al*[48] studied 39 patients with leptomeningealcarcinomatosisfor physician-dependent variables (CSF sample volume, site of CSF,sampling, processing time, and frequency of CSF sampling) were identified and their contributions to the false-negative rate of CSF cytology were evaluated. They concluded that false-negative CSF cytology results are common, but can be minimized by: 1) withdrawing at least 10.5 ml of CSF for cytologic analysis; 2) processing ofCSF specimen immediately; 3) obtaining CSF from a site of knownleptomeningeal disease; and 4) repeating this procedure once if the initial cytologyis negative.

Dunbar SA *et al*[49]reviewed the results of microscopic Gram stain examination and routine culture for 2,635 cerebrospinalfluid (CSF) samples processed in an adult hospital microbiology laboratory during 55 months. CSF specimens were prepared for microscopicexamination by cytocentrifugation.In conclusion, microscopic examination of gram-stained,concentrated CSF, a rapid and inexpensive test, is 92% sensitiveand 99% specific in diagnosing untreated bacterial or fungal meningitis.

In a study conducted by Cheng P *et al*[50] on twenty-nine patients whose CSFspecimens were culture positive for *Mycobacterium* by the MGIT 960mycobacteria culture system were diagnosed with TBM and enrolledin this study. They developed a modified Ziehl-Neelsenstain, involving cytospin slides with Triton processing, in which only 0.5 ml of cerebrospinal fluid specimens was required. Thismethod not only improved the detection rate of extracellular *M. tuberculosis* significantly but also identified intracellular *M.tuberculosis* in the neutrophils, monocytes, and lymphocytes clearly. They concluded that modified method is more effective and sensitivethan the

conventional Ziehl-Neelsen stain, providing clinicians a convenient yet powerful tool for rapidly diagnosing tuberculousmeningitis.

MATERIALS AND METHODS

SOURCE OF DATA

A prospective hospital based study was carried out on patients fulfilling the inclusion criteria, referred to the Department of Pathology in BLDEU'S ShriB.M.Patil Medical College, Hospital and Research centre, Vijayapura.

Study period:1st December 2014 to 30th June 2016

METHODS OF COLLECTION OF DATA:

- Fresh body fluid samples received were examined by naked eye for physical characteristics and then divided into three parts.
- First part was subjected to routine centrifugation at 3000 rpm for 5minutes and smear was prepared with the sediment and was stained with hematoxylin and eosin after fixation with 95%ethanol.

CENTRIFUGED SMEAR:

- For conventional smear, the fluid was centrifuged at 2500 rpm for 10 minutes

HAEMATOXYLIN AND EOSIN STAIN

Reagents required:

1. Harris Hematoxylin

2. 1% Eosin.

3. 1% Acid alcohol.

Technique:

1. Fix smear in 95% alcohol – 15 min

2. Wash with water.

3. Stain with Harris Hematoxylin – 5 minutes.

4. Wash with water.

5. Dip in 1% Acid alcohol.

6. Wash in running tap water until bluing.

7. Counter stain with 1% Eosin.

Dehydration:

1. Rinse in 70% Alcohol for 30 seconds.

2. Rinse in 90% Alcohol for 90 seconds.

3. Rinse in Absolute Alcohol for 30 seconds.

4. Rinse in two changes of Xylene.

5. Clear and mount with D.P.X.

CYTOSPIN

Second part was subjected to cytospin i.e. 200 microlitre of fluid was placed in cytospin funnel with the filter paper placed between the slide and the funnel, then spun in cytospin[MedSpin4] at 800rpm for 5 minutes and the slide was stained with hematoxylin and eosin after fixation with 95%ethanol.(Figure 4.1)

CELL BLOCK

Third part of the fluid was fixed in 10%formal alcohol in the ratio 1:1 and was kept for 1 hour. After fixation it was centrifuged at 2000rpm for 5 minutes. The supernatant was poured off and the cell button was obtained on whatman filter paper number 1, wrapped inthe same and was processed in tissue processor and embedded in paraffin. Sections prepared were stained with hematoxylin and eosin.The sediment was then wrapped in the same filter paper and processed inhistokinette as routine histopathological specimen. Multiple thin sections of 4-5 micron thickness from paraffin blocks wereobtained, stained with Haematoxylin and Eosin stain and examinedmicroscopically.

Statistical analysis:

Study design:

A Prospective cross sectional study

Statistical Methods:

All characteristics were summarized descriptively. For continuous variables, the summary statistics of N, mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries.

For continuous data, the differences of the analysis variables were tested with the t-test. ANOVA had been used for multi-group comparison. If the p-value is > 0.05, then the results was considered to be not significant.

Statistical software:

Data were analyzed using SPSS software v.20.0 and Microsoft word and Excel have been used for DTP work.

INCLUSION CRITERIA: 1) Pleural fluid

2) Peritoneal fluid

3) Cerebrospinal fluid

INTERPRETATION OF CONVENTIONAL SMEARS, CYTOSPIN SMEARS AND CELLBLOCKS

The smears obtained by each of the above technique were evaluated for features such as background, cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale according to the Mair*et al*[31] scoring system. Based on cytomorphological features & available clinical data fluids were categorizedas **Non neoplastic, Suspicious** and **Neoplastic** lesions. (Table 4.1)

Table 4.1: Mair*et al*[31]Scoring System

	PARAMETER	QUANTITATIVE DESCRIPTION	POINT SCORE
1.	Background blood or proteinaceous material	1.Large amount, great compromise in diagnosis. 2.Modearteamount,diagnosis possible. 3.Minimal,diagnosis easy.	0 1 2
2.	Amount of cellular material	1.Minimal to absent,diagnosis not possible. 2.Sufficient for cytodiagnosis. 3.Abundant,diagnosis simple.	0 1 2
3.	Cell morphology,cellular degeneration and trauma	1.Marked cellular degeneration,diagnosis not possible. 2.Moderate cellular degeneration,diagnosis possible. 3.Minimal cellular degeration,diagnosis easy.	0 1 2
4.	Distribution of cells	1.Totally in the periphery or sparsely distributed. 2.Combination. 3.Evenly distributed.	0 1 2

Figure 4.1: Cytospin(Med Spin 4) and Cytofunnel

RESULTS

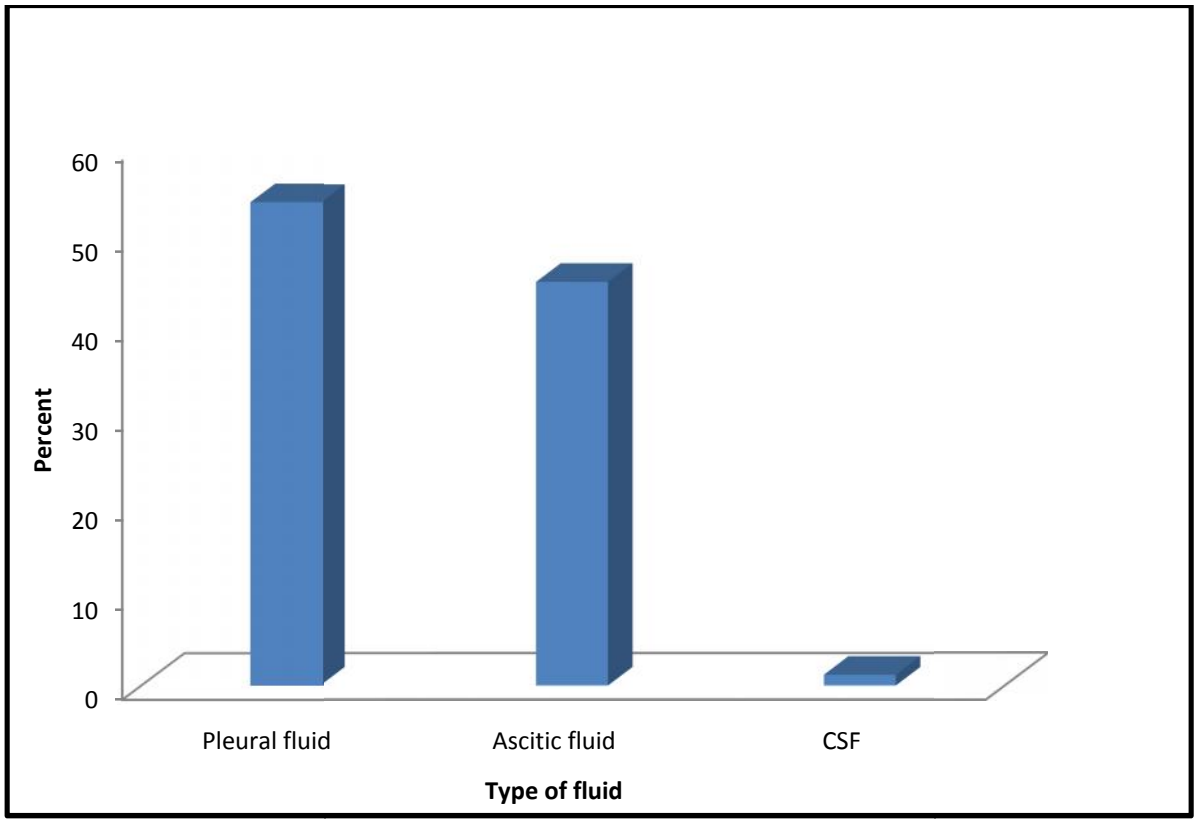
DISTRIBUTION OF TYPE OF BODY FLUIDS

A total of 80 body cavity fluid samples were studied. Out of which 43 (53.8%) were pleural, 36 (45%) were peritoneal and 1 (1.2%) were CSF fluids. (Table5.1 & Figure 5.1)

Table 5.1: Percent Distribution of Type of Fluids

FLUID	N	PERCENT
Pleural fluid	43	53.8
Ascitic fluid	36	45
CSF	1	1.2
Total	80	100

Figure 5.1: Percent Distribution of Type of Fluids



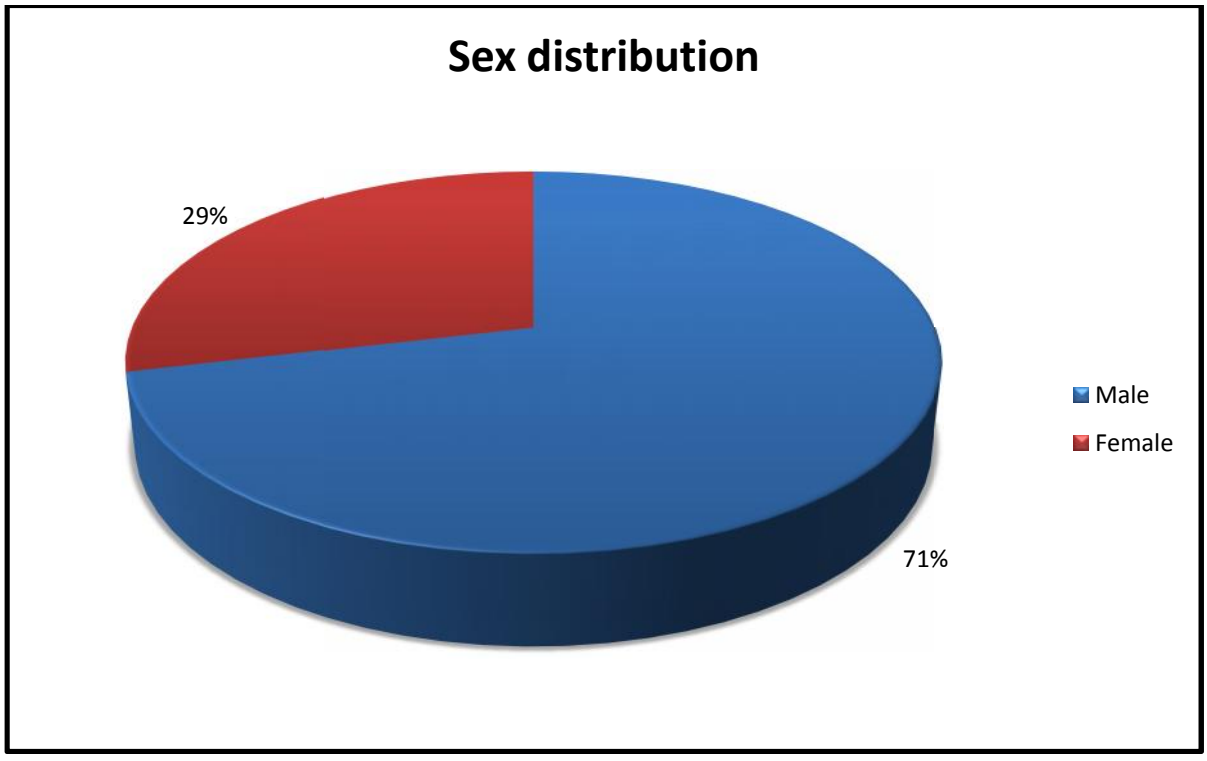
DISTRIBUTION OF CASES ACCORDING TO SEX

Out of 80 cases studied, 57 cases (71.2%) were from male and 23 cases (28.8%) were from female. (Table5.2 & Figure5.2)

Table 5.2: Sex wise distribution of cases

SEX	N	PERCENT
Male	57	71.2
Female	23	28.8
Total	80	100

Figure5.2: Sex wise distribution of cases



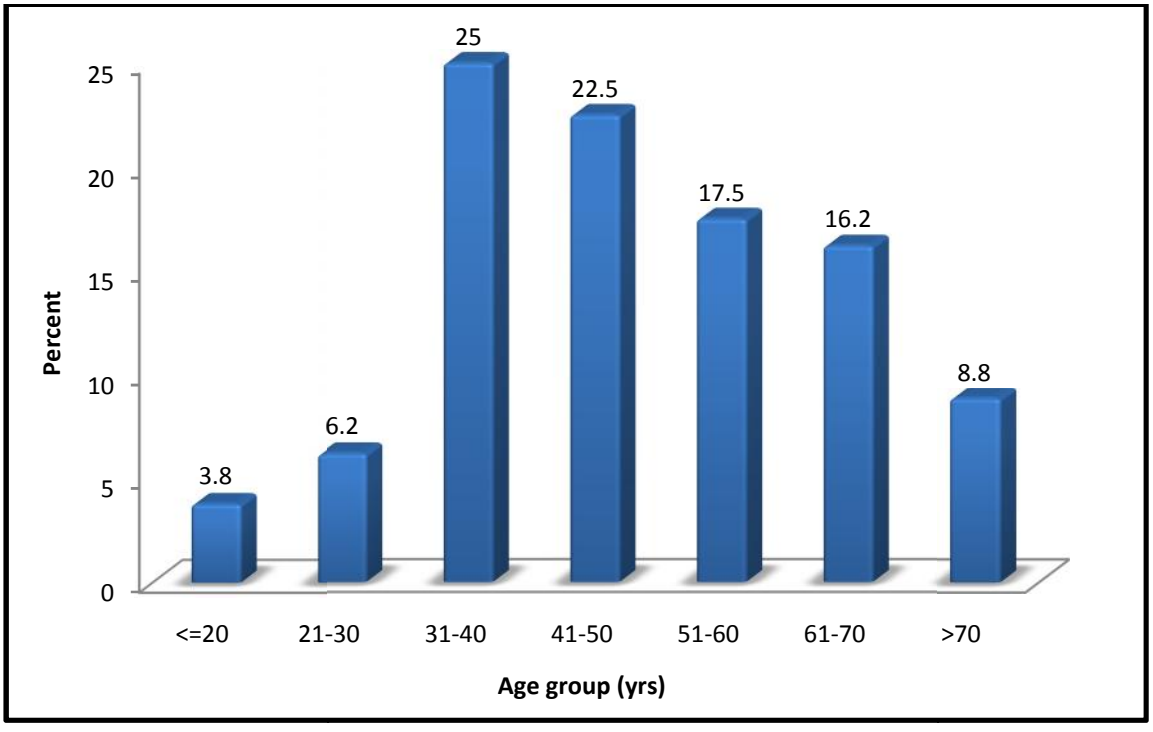
DISTRIBUTION OF CASES ACCORDING TO AGE

Out of 80 cases, 20cases (25%) were in the age group of 31-40 years and 18 cases (22.5%) were in age group 41-50. (Table 5.3& Figure5.3)

Table 5.3: Age wise distribution of cases

AGE GROUPS (YRS)	N	PERCENT
<=20	3	3.8
21-30	5	6.2
31-40	20	25
41-50	18	22.5
51-60	14	17.5
61-70	13	16.2
>70	7	8.8
Total	80	100

Figure 5.3: Age wise distribution of cases



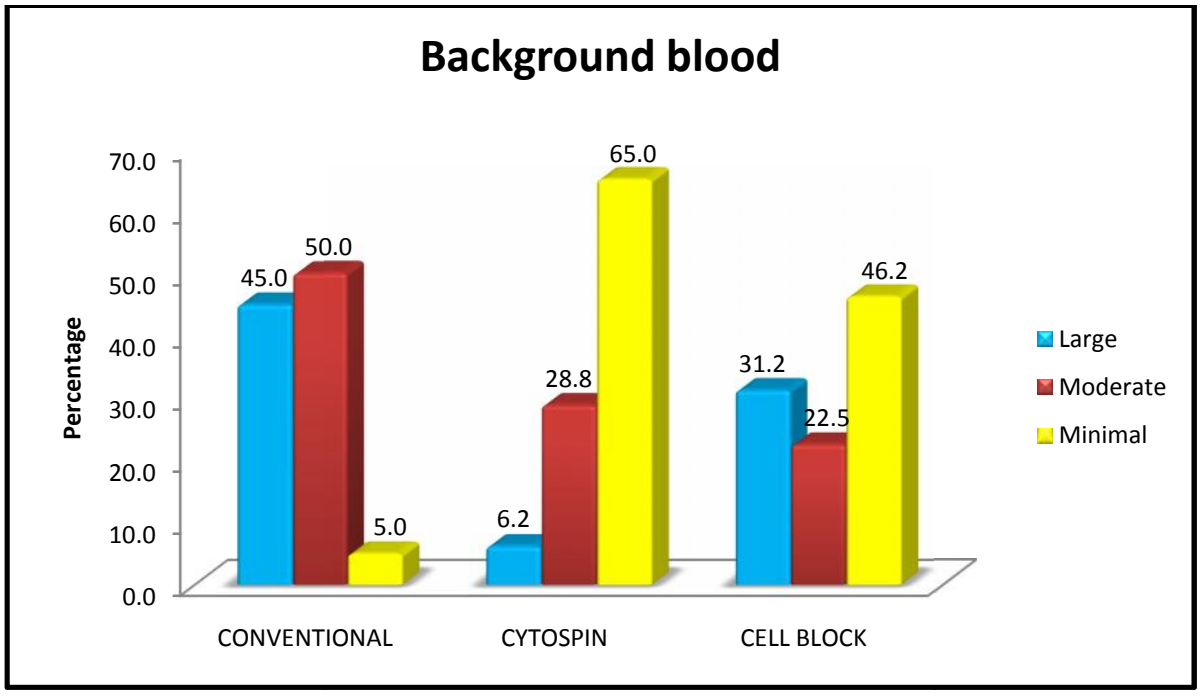
COMPARISON OF BACKGROUND BLOOD/PROTEINACEOUS MATERIAL BY VARIOUS METHODS

The background blood/proteinaceous material was minimal in 4(5%) of conventional smear, 52(65%) of cytospin smears and 37(46.2%) of cell blocks. (Table5.4&Figure5.4)

Table 5.4: Comparison of background blood/proteinaceous material by various methods

Background blood	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	N	%	N	%	N	%
Large	36	45.0	5	6.2	25	31.2
Moderate	40	50.0	23	28.8	18	22.5
Minimal	4	5.0	52	65.0	37	46.2
Total	80	100.0	80	100.0	80	100.0

Figure5.4: Comparison of background blood/proteinaceous material by various methods



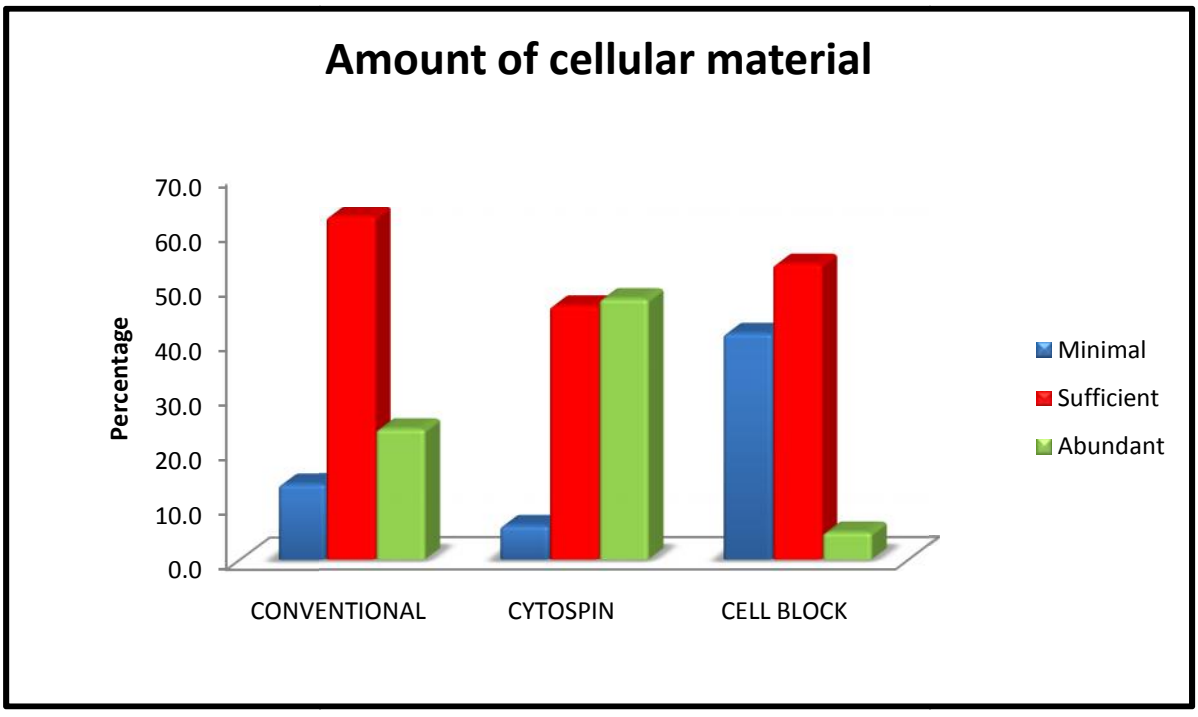
COMPARISON OF AMOUNT OF CELLULAR MATERIAL BY VARIOUS METHODS

The amount of cellular material was abundant in 19(23.8%) of conventional smear, 38(47.5%) of cytospin smears and 4(5%) of cell blocks. (Table5.5&Figure5.5)

Table 5.5: Comparison of amount of cellular material by various methods

Amount of cellular material	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	N	%	N	%	N	%
Minimal	11	13.8	5	6.2	33	41.2
Sufficient	50	62.5	37	46.2	43	53.8
Abundant	19	23.8	38	47.5	4	5.0
Total	80	100.0	80	100.0	80	100.0

Figure 5.5: Comparison of amount of cellular material by various methods



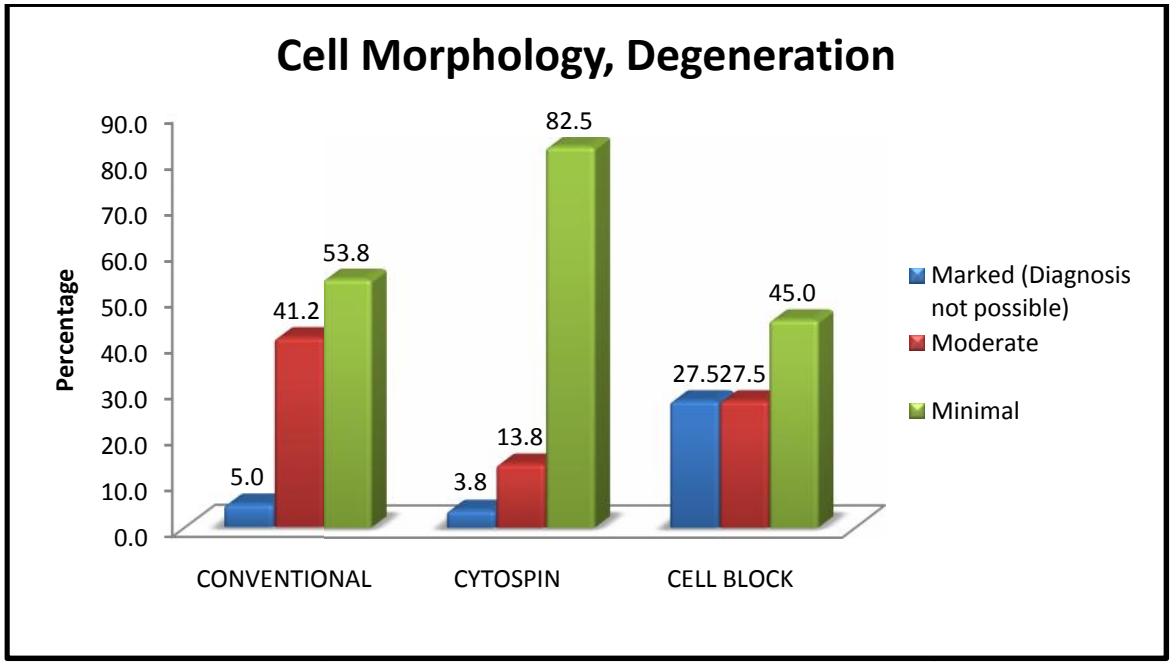
COMPARISON OFCELL MORPHOLOGY AND DEGENERATIONBY VARIOUS METHODS

The degeneration of cells was minimal in 43(53.8%) of conventional smear, 66(82.5%) of cytospin smears and 36(45%) of cell blocks. (Table5.6&Figure5.6)

Table 5.6: Comparison ofCell morphology and Degenerationby various methods

Cell Morphology, Degeneration	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	N	%	N	%	N	%
Marked (Diagnosis not possible)	4	5.0	3	3.8	22	27.5
Moderate	33	41.2	11	13.8	22	27.5
Minimal	43	53.8	66	82.5	36	45.0
Total	80	100.0	80	100.0	80	100.0

Figure 5.6: Comparison ofCell morphology and Degenerationby various methods



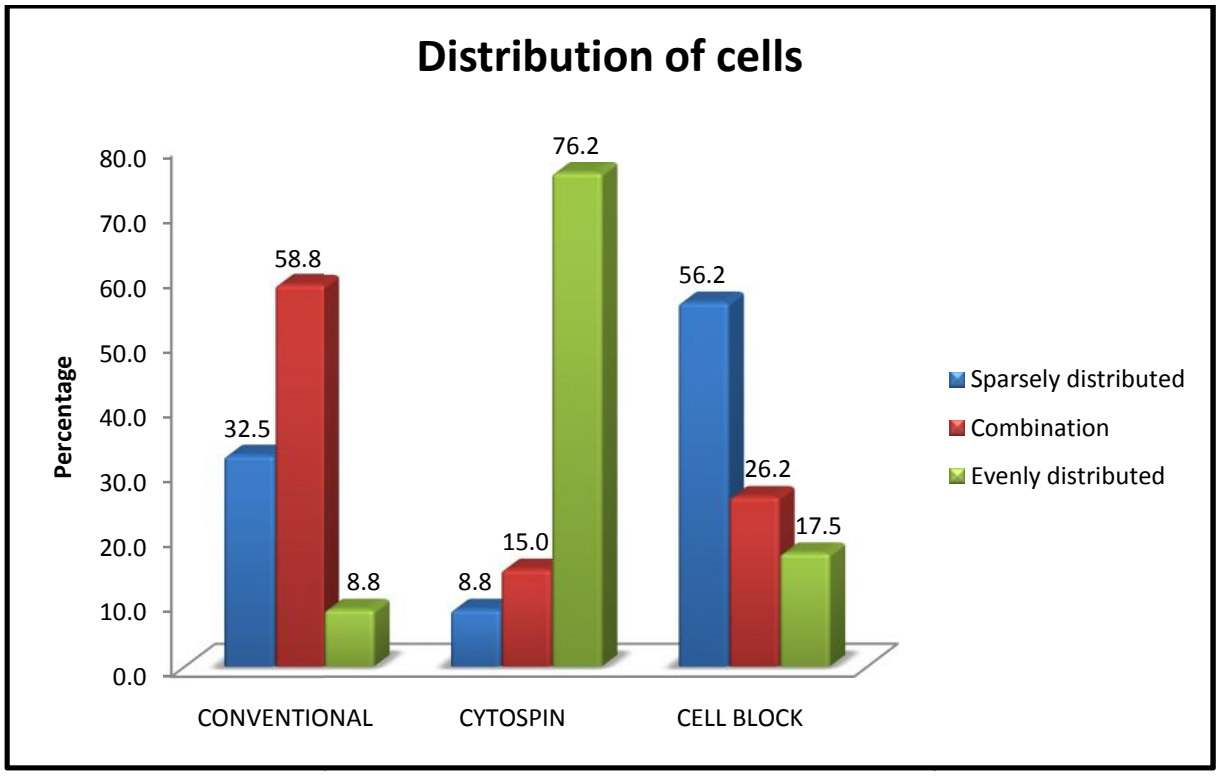
COMPARISON OF DISTRIBUTION OF CELLS BY VARIOUS METHODS

The cells were evenly distributed in 7(8.8%) of conventional smear, 61(76.2%) of cytospin smears and 14(17.5%) of cell blocks. (Table5.7&Figure5.7)

Table 5.7: Comparison of Distribution of cells by various methods

Distribution of cells	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	N	%	N	%	N	%
Sparsely distributed	26	32.5	7	8.8	45	56.2
Combination	47	58.8	12	15.0	21	26.2
Evenly distributed	7	8.8	61	76.2	14	17.5
Total	80	100.0	80	100.0	80	100.0

Figure 5.7: Comparison of Distribution of cells by various methods



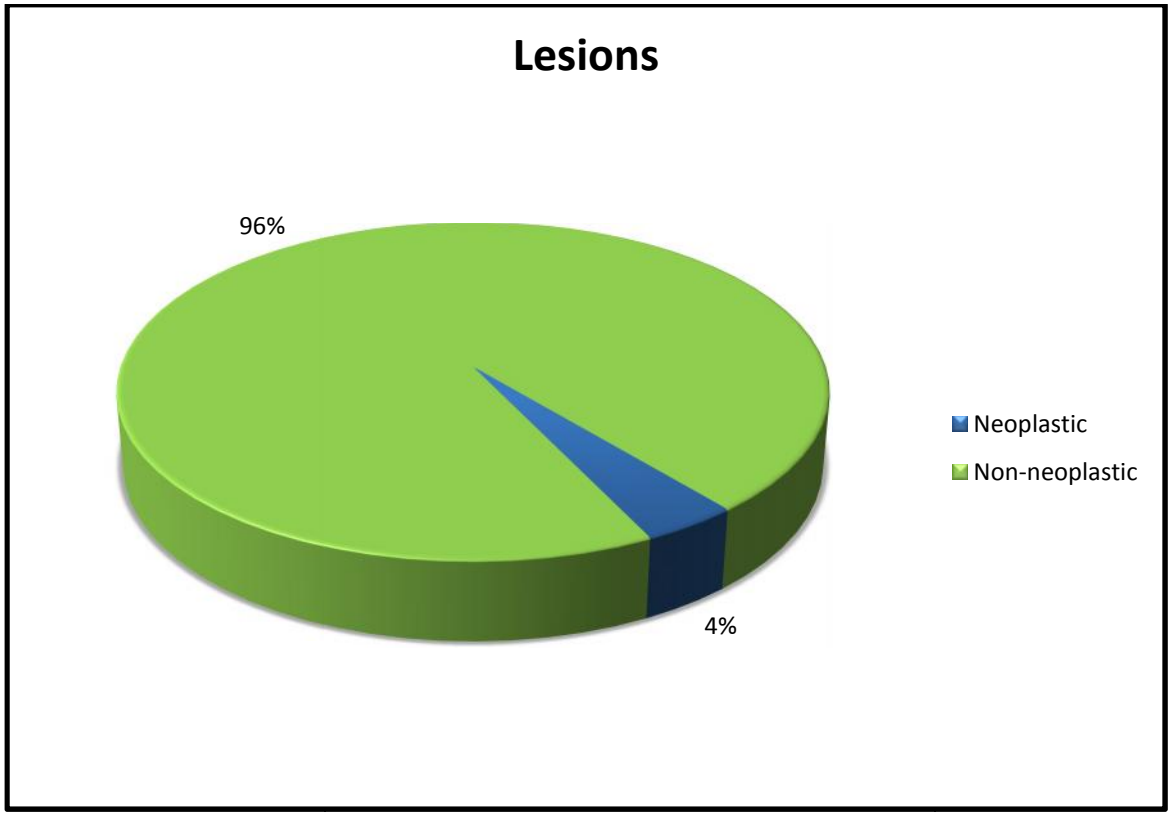
DISTRIBUTION OF TYPE OF LESIONS BY CONVENTIONAL SMEAR

Out of 77 diagnosed by conventional smear 74 cases were non neoplastic and 3 cases were neoplastic. (Table5.8& Figure5.8)

Table 5.8: Percent Distribution of Type of Lesions by Conventional smear

LESIONS	N	PERCENT
Neoplastic	3	3.9
Non-neoplastic	74	96.1
Total	77	100.0

Figure 5.8: Percent Distribution of Type of Lesionsby Conventional smear



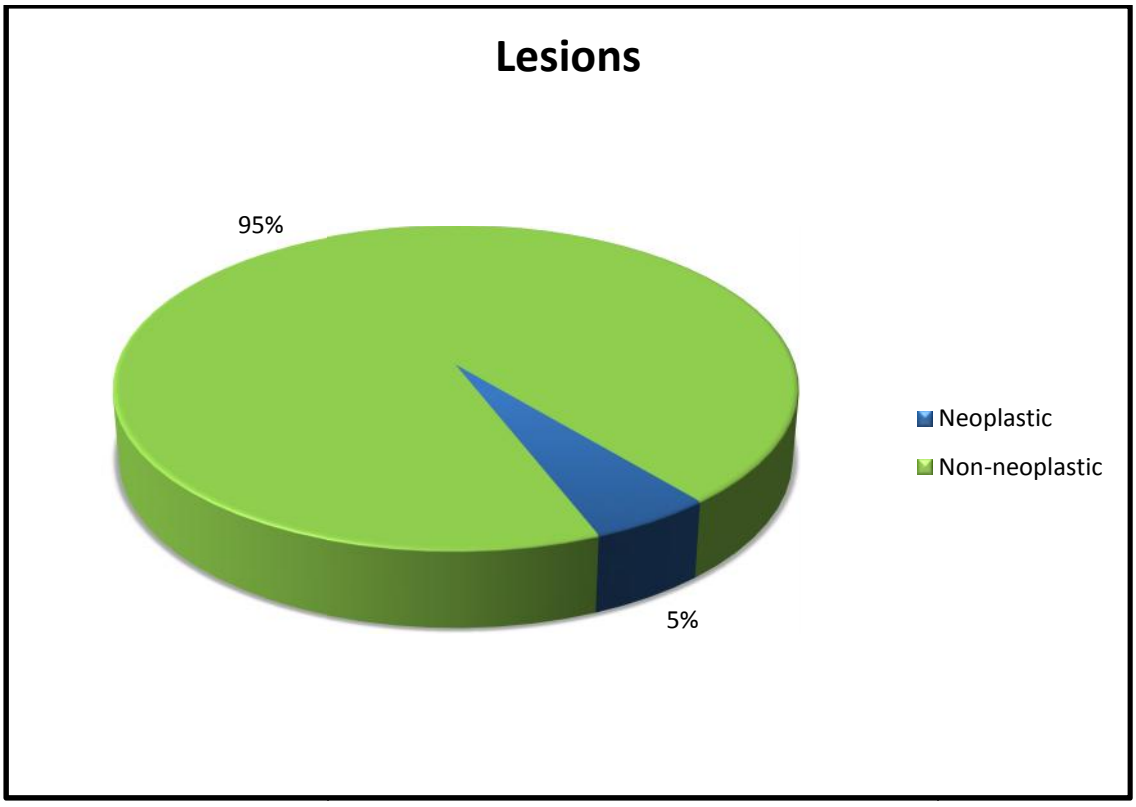
DISTRIBUTION OF TYPE OF LESIONS BY CYTOSPIN SMEAR

Out of 78 diagnosed by cytospin smear 74 cases were non neoplastic and 4 cases were neoplastic. (Table5.9& Figure5.9)

Table 5.9: Percent Distribution of Type of Lesions by Cytospin smear

LESIONS	N	PERCENT
Neoplastic	4	5.1
Non-neoplastic	74	94.9
Total	78	100.0

Figure 5.9: Percent Distribution of Type of Lesionsby Cytospin smear



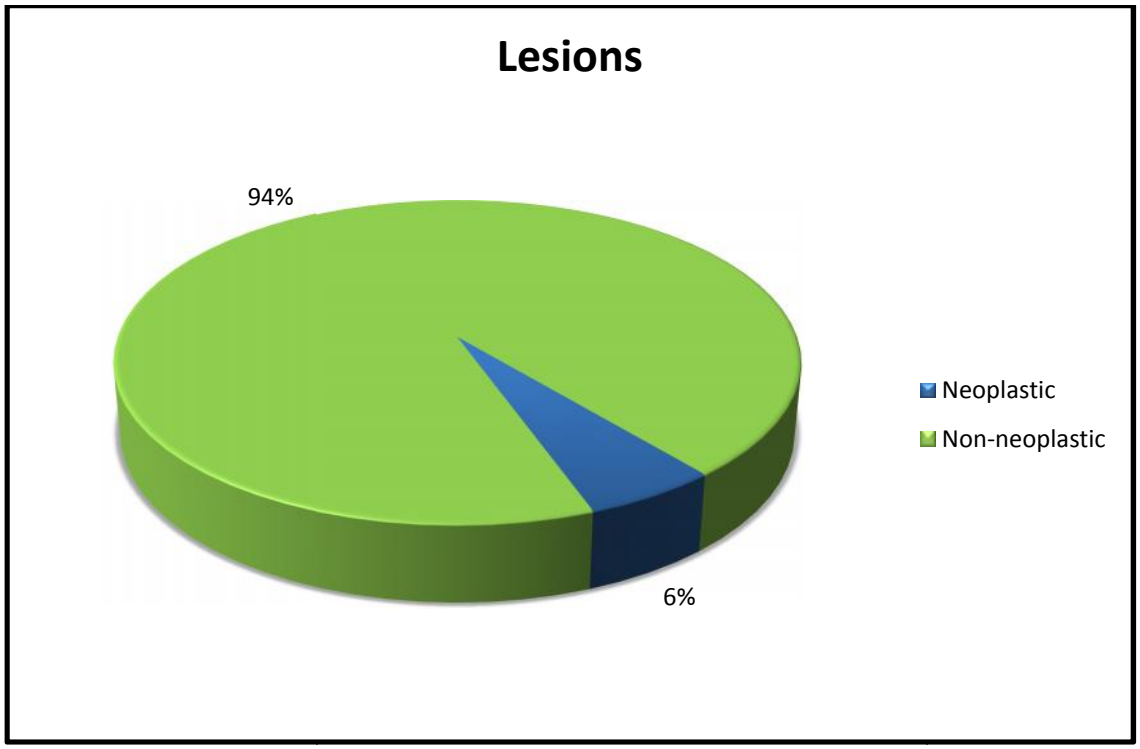
DISTRIBUTION OF TYPE OF LESIONS BY CELL BLOCK

Out of 54 diagnosed by cell block 51 cases were non neoplastic and 3 cases were neoplastic. (Table5.10&Figure5.10)

Table 5.10: Percent Distribution of Type of Lesions by Cell Block

LESIONS	N	PERCENT
Neoplastic	3	5.6
Non-neoplastic	51	94.4
Total	54	100.0

Figure 5.10: Percent Distribution of Type of Lesionsby Cell Block



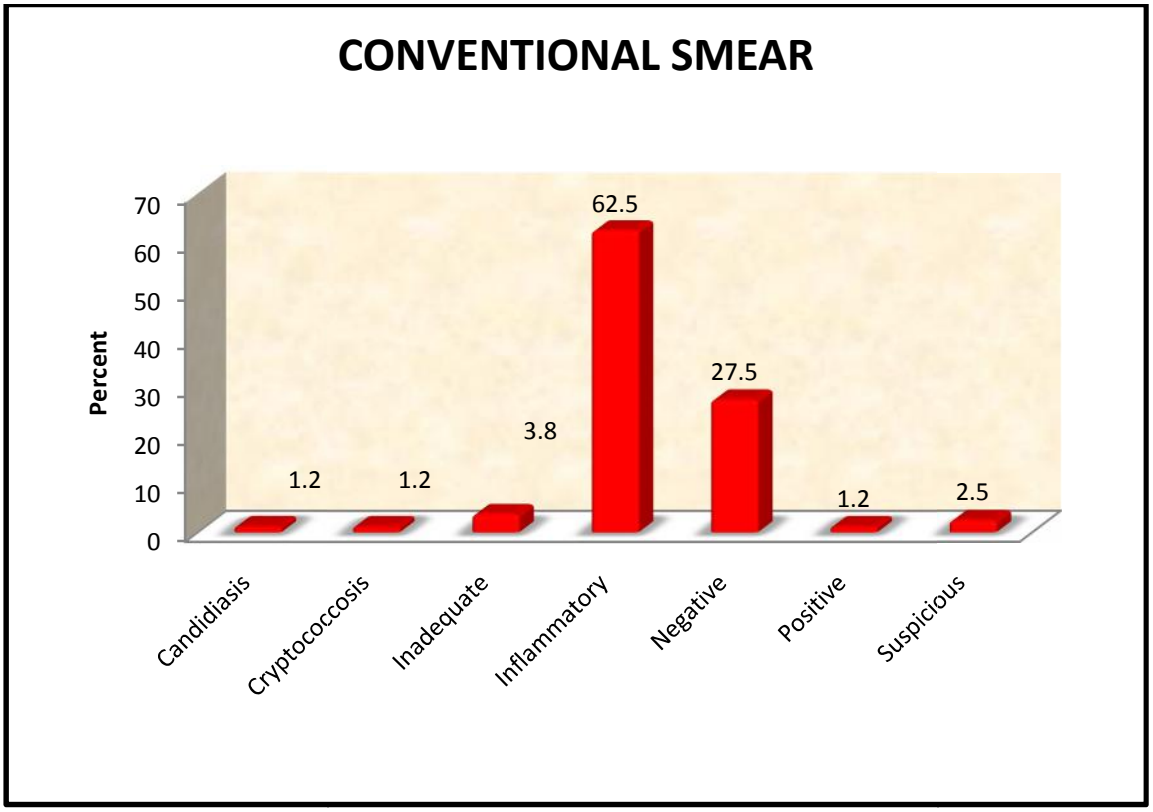
DISTRIBUTION OF NEOPLASTIC AND NON NEOPLASTIC LESIONS BY CONVENTIONAL SMEAR

Out of 80 cases 50 (62.5%) cases were of inflammatory smears, 1 (1.2%) case was diagnosed to be Crypotococcosis, 1 (1%) case was of fungal etiology caused by Candida, 22 (27.5%) cases were negative for malignancy, 1 (1.2%) case was positive for malignancy, 2 (2.5%) cases were suspicious for malignancy and 3 (3.8%) cases were inadequate for opinion.(Table5.11& Figure5.11)

Table 5.11: Percent Distribution of Neoplastic and Non neoplastic lesions by Conventional smear

IMPRESSION	N	PERCENT
Candidiasis	1	1.2
Cryptococcosis	1	1.2
Inadequate	3	3.8
Inflammatory	50	62.5
Negative	22	27.5
Positive	1	1.2
Suspicious	2	2.5
Total	80	100

Figure 5.11: Percent Distribution of Neoplastic and Non neoplastic lesions by Conventional smear



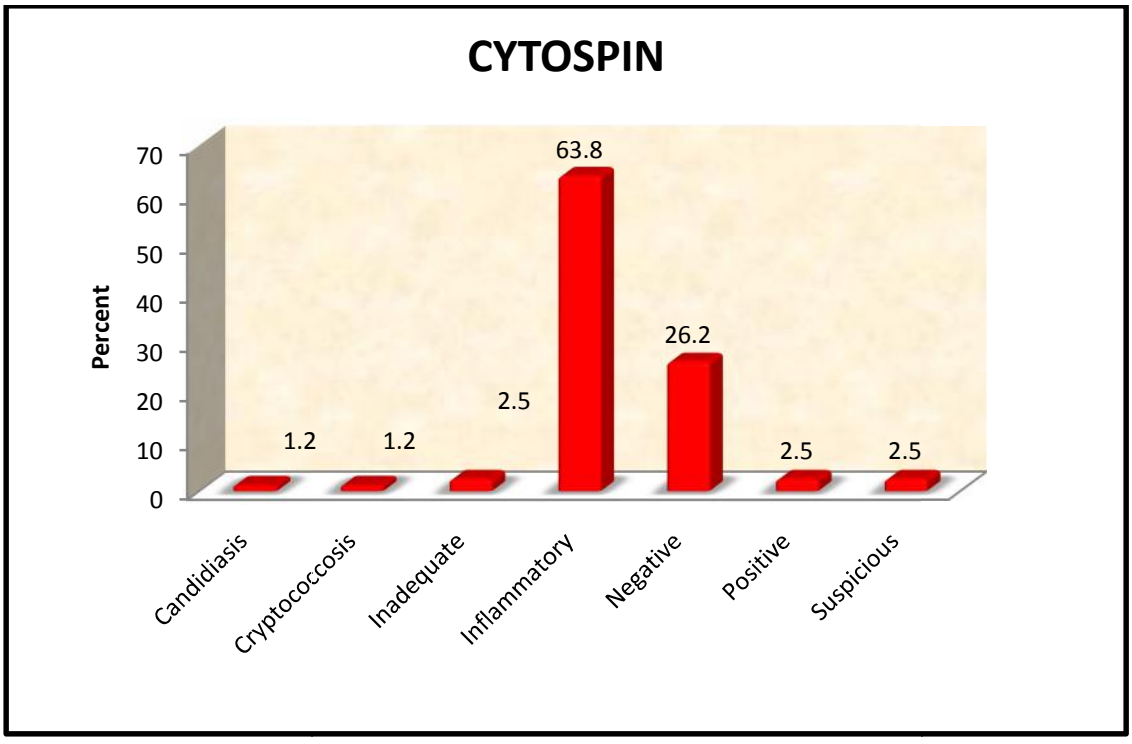
DISTRIBUTION OF NEOPLASTIC AND NON NEOPLASTIC LESIONS BY CYTOSPIN SMEAR

Out of 80 cases 51 (63.8%) cases were of inflammatory smears, 1 (1.2%) case was diagnosed to be Crypotococcosis, 1 (1.2%) case was of fungal etiology caused by Candida, 21 (26.2%) cases were negative for malignancy, 2 (2.5%) cases were positive for malignancy, 2 (2.5%) cases were suspicious for malignancy and 2 (2.5%) cases were inadequate for opinion. (Table 5.12& Figure 5.12)

Table 5.12: Percent Distribution of Neoplastic and Non neoplastic lesions by Cytospin smear

IMPRESSION	N	PERCENT
Candidiasis	1	1.2
Cryptococosis	1	1.2
Inadequate	2	2.5
Inflammatory	51	63.8
Negative	21	26.2
Positive	2	2.5
Suspicious	2	2.5
Total	80	100

Figure 5.12: Percent Distribution of Neoplastic and Non neoplastic lesions by Cytospin smear



DISTRIBUTION OF NEOPLASTIC AND NON NEOPLASTIC LESIONS BY CELL BLOCK

Out of 80 cases 45 (56.2%) cases were of inflammatory smears, 1 (1.2%) case was diagnosed to be Crypotococcosis, 5 (6.2%) cases were negative for malignancy, 3 (3.8%) cases were positive for malignancy and 26 (32.5%) cases were inadequate for opinion. (Table 5.13& Figure 5.13)

Table 5.13: Percent Distribution of Neoplastic and Non neoplastic lesions by Cell Block smear

IMPRESSION	N	PERCENT
Cryptococcosis	1	1.2
Inadequate	26	32.5
Inflammatory	45	56.2
Negative	5	6.2
Positive	3	3.8
Total	80	100

Figure 5.13: Percent Distribution of Neoplastic and Non neoplastic lesions by Cell Block smear

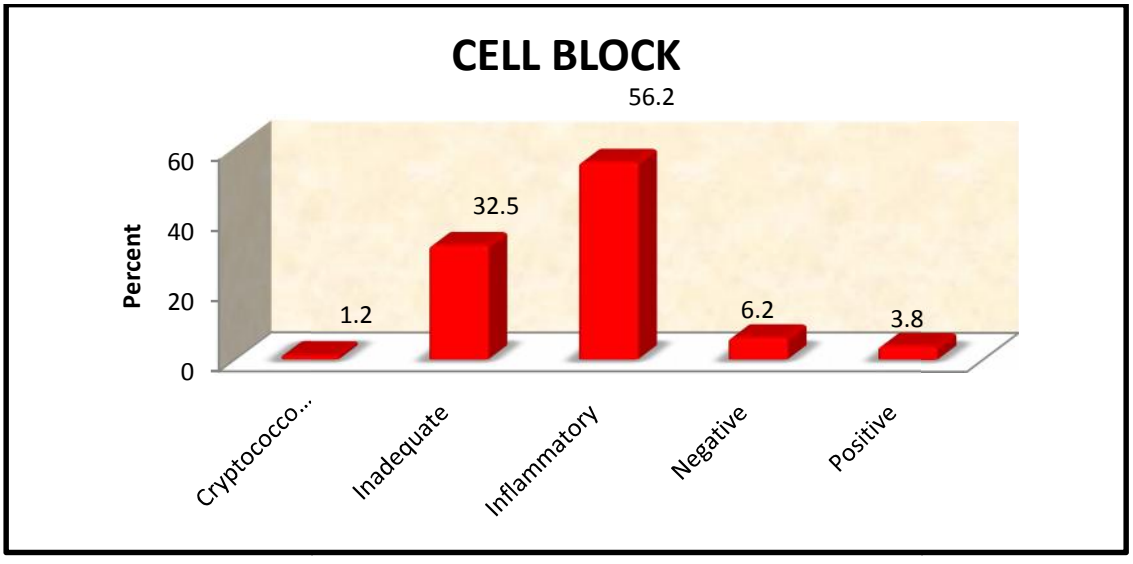


Table 5.14: Comparison of means of morphological features of Conventional, Cytospinand Cell Block

Morphological features	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	Mean	SD	Mean	SD	Mean	SD
Background	0.60	0.59	1.59	0.61	1.15	0.87
Cellularity	1.10	0.61	1.41	0.61	0.64	0.58
Morphology	1.49	0.60	1.79	0.50	1.18	0.84
Distribution	0.76	0.60	1.68	0.63	0.61	0.77

Table 5.15: Level of Significance difference of means of morphological features of Conventional smear, Cytospin smear and Cell Block

Morphological features	CONVENTIONAL & CELL BLOCK & CYTOSPIN (ANOVA)	CONVENTIONAL & CELL BLOCK	CYTOSPIN & CELL BLOCK
Background	<0.001*	<0.001*	<0.001*
Cellularity	<0.001*	<0.001*	<0.001*
Morphology	<0.001*	0.004*	<0.001*
Distribution	<0.001*	0.164	<0.001*

Note: *Difference is statistically significant at 5% level of significance

The mean score of amount of background blood, cellular material, Cell morphology, degeneration and distribution of cells is significantly different in all methods. The mean score of Distribution of cells is not significantly different between conventional and Cell block. (Table 5.14&5.15)

IQV (Index of Qualitative Variation) ranges from 0 (No variation) to 1(Maximum variation). IQV was found minimum for parameters of Cytospin smears (Table 5.16)

Table 5.16: Index of qualitative variation of morphological features of Conventional, Cytospin and Cell Block methods

Method	Morphological features	IQV
CONVENTIONAL	Background	0.818
	Cellularity	0.801
	Morphology	0.808
	Distribution	0.812
CYTOSPIN	Background	0.736
	Cellularity	0.835
	Morphology	0.449
	Distribution	0.583
CELL BLOCK	Background	0.957
	Cellularity	0.808
	Morphology	0.969
	Distribution	0.876

DISTRIBUTION OF PLEURAL FLUID ANALYSIS

Out of the 43 cases of pleural fluid, by conventional smear cytology 41 cases (95.3%) were benign (Inflammatory), 1 case(2.3%) was suspicious for malignancy and 1 case (2.3%) was positive for malignancy. On cytospin smear cytology 41 cases (95.3%) were benign (Inflammatory), 2 cases (4.6%) were positive for malignancy. By cellblock preparation, 28 cases 26 cases (92.8%) were benign (inflammatory), 2 cases (7.1%) were positive for malignancy. (Table 5.17)

Table 5.17: Distribution of Pleural fluid analysis

FEATURE	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	No	%	No	%	No	%
Benign	41	95.3	40	93	26	92.8
Suspicious	1	2.3	-	-	-	-
Malignant	1	2.3	3	6.9	2	7.1

DISTRIBUTION OF ASCITIC FLUID ANALYSIS

Out of the 36 cases of pleural fluid, by conventional smear cytology 35 cases (97.2%) were benign (Inflammatory) and 1 case (2.7%) was positive for malignancy. On cytospin smear cytology 35 cases (97.2%) were benign (Inflammatory), 1 case (2.7%) were positive for malignancy. By cellblock preparation of 26 cases 25 cases (96.1%) were benign (inflammatory), 1 case (3.8%) was positive for malignancy. (Table 5.18)

Table 5.18: Distribution of Ascitic fluid analysis

FEATURE	CONVENTIONAL		CYTOSPIN		CELL BLOCK	
	No	%	No	%	No	%
Benign	35	97.2	35	97.2	25	96.1
Suspicious	-	-	-	-	-	-
Malignant	1	2.7	1	2.7	1	3.8

DISTRIBUTION OF CSF ANALYSIS

One case of cerebrospinal fluid was analysed and was diagnosed to be cryptococcosis by conventional smear, cytospin and cell block methods.

DISTRIBUTION OF ETIOLOGY OF BENIGN CASES IN PLEURAL EFFUSION

Out of 41 cases of benign pleural effusions and based on available clinical data &cytomorphologic features the diagnosis of Anemia with hypoproteinemia and Pulmonary Tuberculosis were commonest etiological factor. (Table 5.19)

Table 5.19: Distribution of etiology of benign cases in Pleural effusion

SL NO	CLINICAL DIAGNOSIS	TOTAL
1	Anemia with hypoproteinemia	10
2	Pulmonary Tuberculosis	11
3	Pneumonia	08
4	Congestive cardiac failure	05
5	Candidiasis	01
6	Non specific inflammation	06
	Total no of cases	41

DISTRIBUTION OF ETIOLOGY OF BENIGN CASES IN ASCITIC FLUID EFFUSION

Out of 35 cases of benign Ascitic fluid effusions and based on available clinical data &cytomorphologic features the Non specific inflammation and Cirrhosis were commonest etiological factor. (Table 5.20)

Table 5.20:Distribution of etiology of benign cases in Ascitic fluid effusion

Sl no	Clinical diagnosis	Total
1	Anemia with hypoproteinemia	05
2	Congestive cardiac failure	03
3	Cirrhosis	09
4	Non specific inflammation	18
	Total no of cases	35

MALIGNANT EFFUSIONS DIAGNOSED BY CELL BLOCK METHOD

The cause of metastatic pleural effusion for one case was found to be lung and the other case was unknown. The cause of metastatic ascetic fluid effusion was found to be ovary. (Table 5.21)

Table 5.21:Malignant effusions diagnosed by cell block method

SL NO	PRIMARY LESIONS	PLEURAL FLUID	ASCITIC FLUID
1	Primary identified	01	01
2	Primary unknown	01	00

NEGATIVE FOR MALIGNANCY- ASCITIC FLUID CYTOLOGY

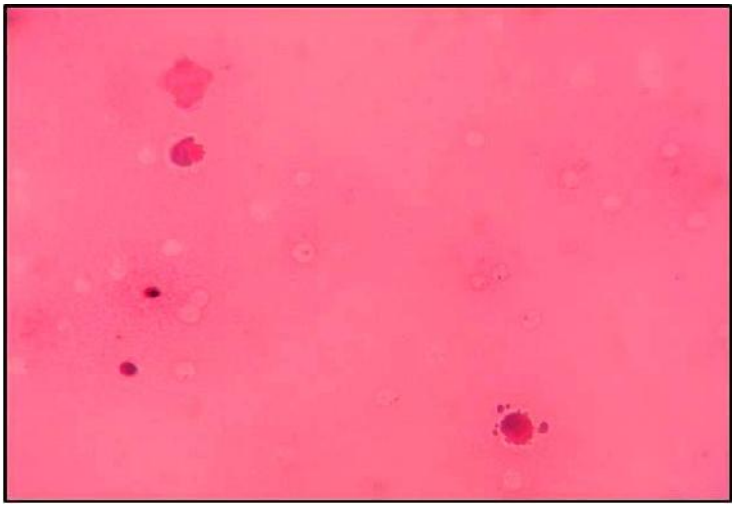


FIGURE 5.14.A

Conventional smear showing scattered mesothelial cells in proteinaceous background. H&E X100

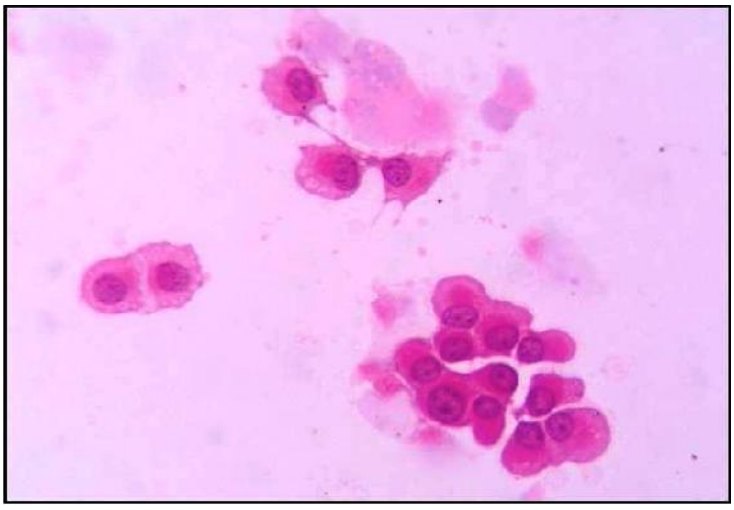


FIGURE5.14.B

Cytospin smear showing windowing of mesothelial cells. H&E X400

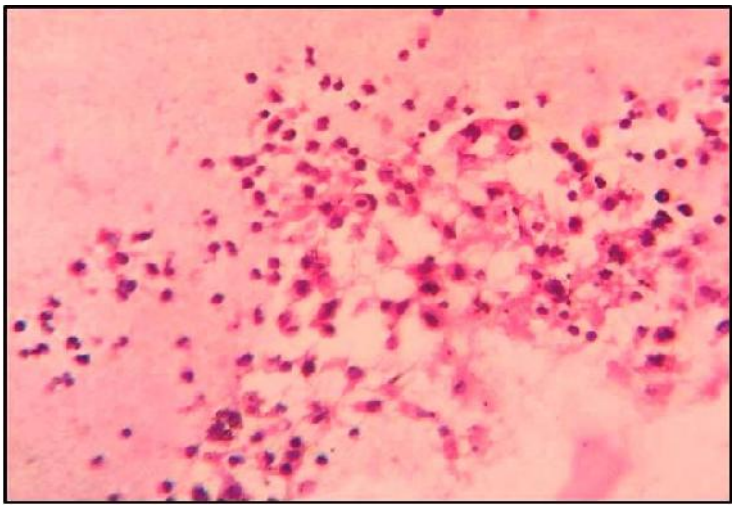


FIGURE5.14.C

Cell block showing mesothelial cells and lymphocytes. H&E X100

POSITIVE FOR MALIGNANCY-PLEURAL FLUID CYTOLOGY

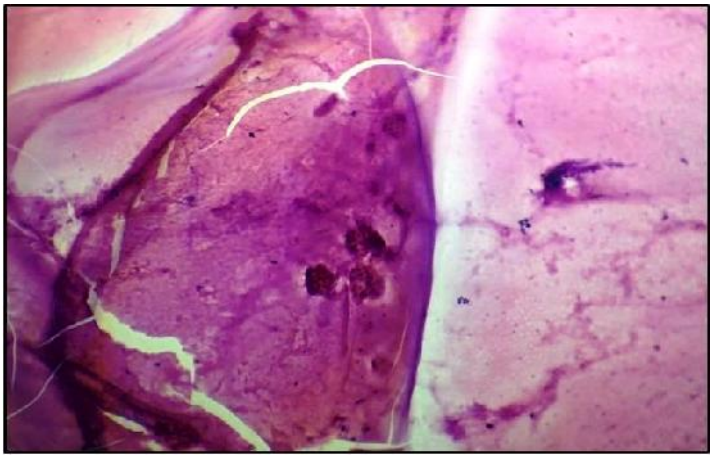


FIGURE 5.15.A

Conventional smear showing tumor cells in clusters and proteinaceous background. H&E X100

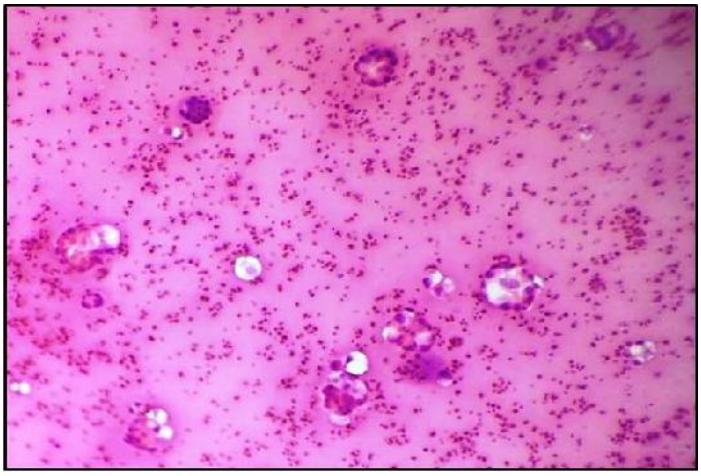


FIGURE 5.15.B

Cytospin smear showing tumor cells in clusters, acinar pattern and singly scattered in a proteinaceous background. H&E X100

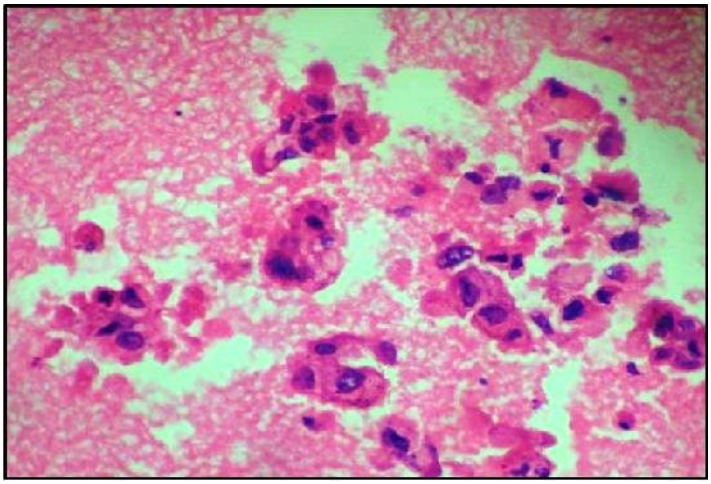


FIGURE 5.15.C

Cell block showing tumor cells in clusters and proteinaceous background. H&E X400

CRYPTOCOCCOSIS- CEREBRO SPINAL FLUID

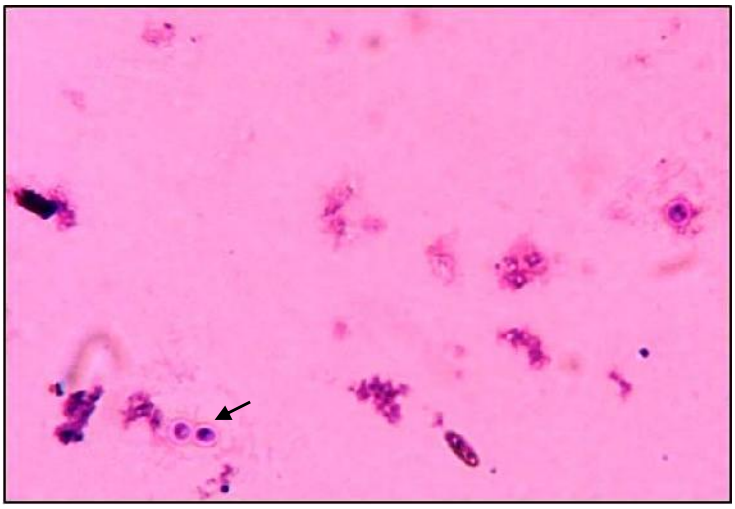
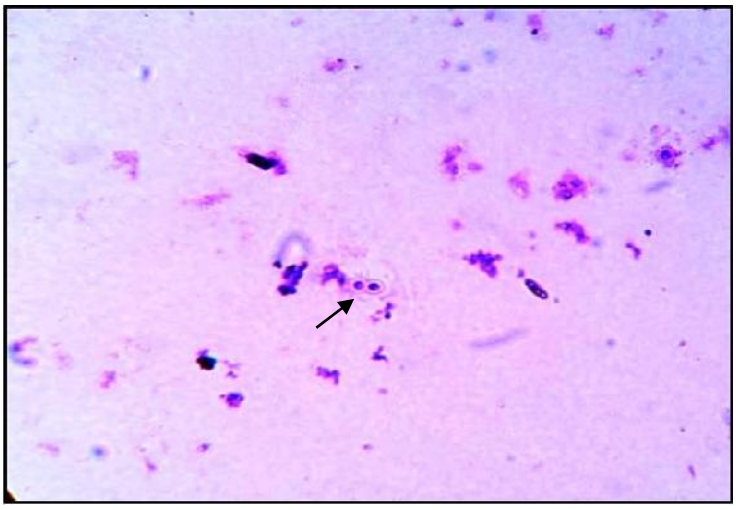


FIGURE 5.16.A

Conventional smear showing cryptococci and proteinaceous background. H&E X1000

FIGURE 5.16.B

Cytospin smear shows scattered cryptococci. H&E X1000



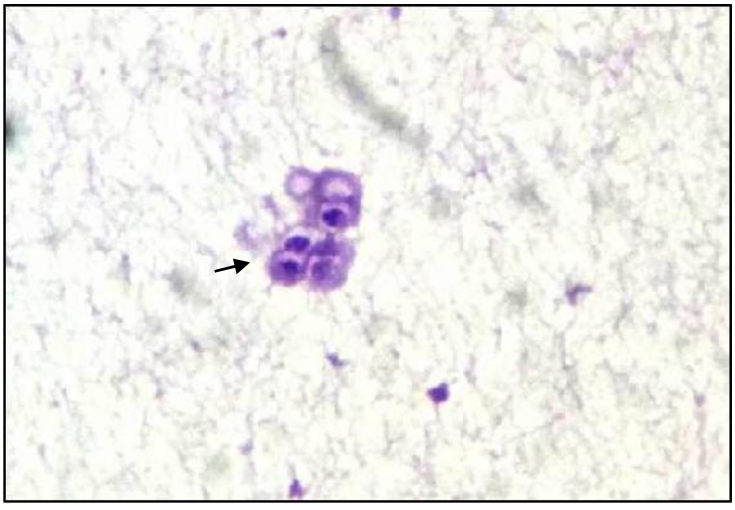


FIGURE 5.16.C

Cell block showing Crytococcocci in clusters . H&E X1000

METASTATIC ADENOCARCINOMA-PLEURAL FLUID

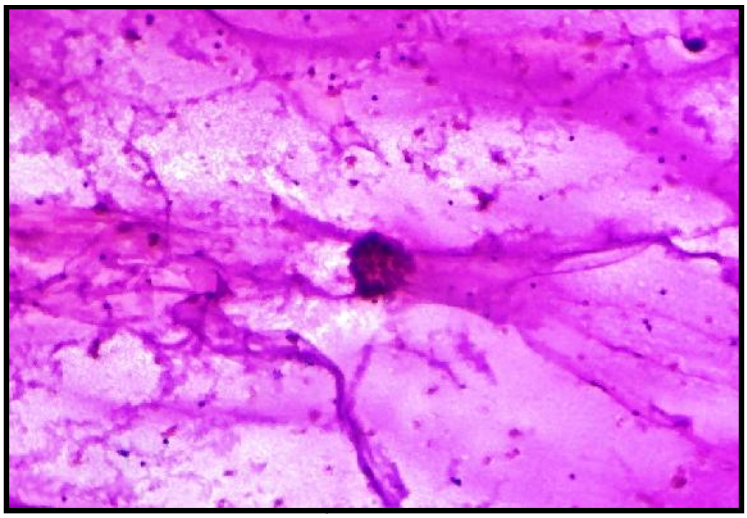


FIGURE 5.17.A

Conventional smear showing tumor cells in clusters obscured by proteinaceous background. H&E X100

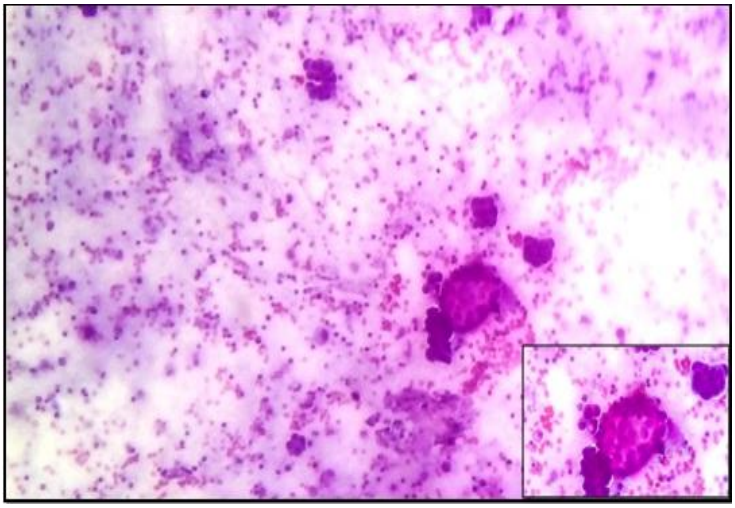


FIGURE 5.17.B

Cytospin smear shows tumor cells in clusters and scattered singly in a clear background. H&E X100. Inset: H&E X400

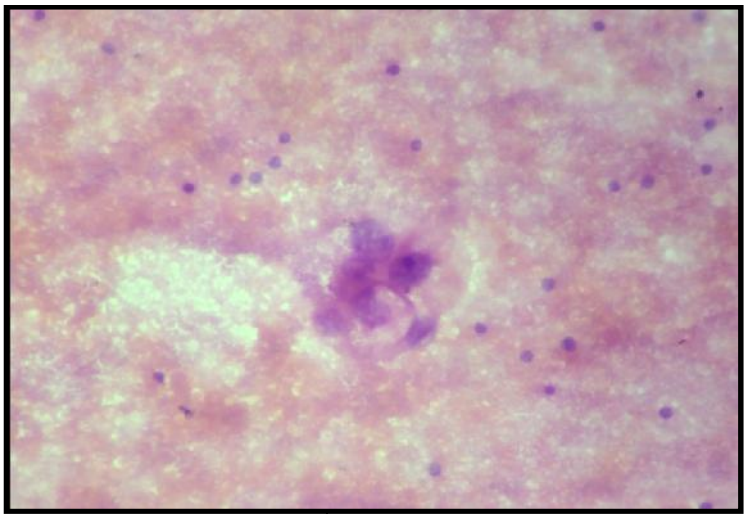


FIGURE 5.17.C

Cell block showing tumor cells with prominent nucleoli and abundand cytoplasm in clusters . H&E X400

DISCUSSION

Cytological examination of serous fluids has increasingly gained acceptance in clinical practice to such an extent that a positive diagnosis often is considered the definitive test there by avoids unnecessary exploratory surgery. The cytological examination of serous effusions has increasingly gained acceptance in clinical medicine,to such an extent that a positive diagnosis is often considered the definitive test and obviates explorative surgery.[2,4]It is important not only in the diagnosis of malignant lesions, but also help in staging and prognosis. In most of the cytology laboratories, cytologistsprefer direct smear prepared from centrifuged deposits of effusion.The cell block technique which is amongst the oldest method of processing cytological material for microscopy has been abandoned by many laboratories as several alternative excellent cell preparation methods have been developed. However several authors have reported the advantages of cell blocks in cytology which includes valuable diagnostic evidence that can be observed in smears.[2]

For this reason, in the present study an attempt was made to prepare and analyze both by conventional smears, cytospin smears and cellblock preparation from the same specimen. Due consideration was given to age, sex, site of effusion and clinical findings to arriveat final diagnosis.

In cell blocks, one could very often observe glandular formations and acinar groupings thatare less beautifully manifested in smears.[4] Present study is in concordance with Singh M *et al*[1] and Joshi A *et al*[2] which showed cytospin preparations preserve the cellulardetails and reduce the overlapping of cells thus enabling precise interpretation than conventionalsmears. The use of cytospin and cell block not only increases the

cellularity as compared toroutine centrifuge, but also the cells were evenly distributed. The cellular morphology, nuclear andcytoplasmic details, were better appreciated on cytospin smears and cell block technique.

Table 6.1: Comparison of type of body fluids in various studies

STUDIES BY	PLEURAL FLUID	PERITONEAL FLUID	CSF
Present study	53.8%	45%	1.2%
Joshi A et al	44.6%	53.3%	-
Singh M et al	74%	26%	-
Viral BM et al	53%	46%	-
Thapar M et al	48.3%	45%	-

In the present study majority of the fluids were pleural fluid(53.8%) followed by ascitic fluid (45%) similar to studies done by Joshi A *et al*,[2] Singh M *et al*,[1] Viral BM *et al*[3] and ThaparM *et al*[29](Table 6.1)

Table 6.2: Comparison of cases according to sex in various studies

STUDIES BY	MALE:FEMALE
Present study	2.44:1
Joshi A et al	1.13:1
Singh M et al	1.55:1
Viral BM et al	3.38:1

In the present study of 80 cases, 57 cases (71%) were male and 23 cases (29%) were female. The age of patients in our study ranged from 20 to 70 years. Male to Female ratio was 2.44:1. Similar findings were noted in the studies by Joshi A *et al*[2], Singh M *et al*[1]and Viral BM *et al*[3] (Table 6.2)

In the present study most common benign cause of pleural effusion was Pulmonary tuberculosis and most common cause of peritoneal effusion was Non specific cause followed by cirrhosis. Similar findings were seen in study done by Singh M *et al,*[1]Joshi A *et al*[2]and Udasimath S *et al.*[4]

Table 6.3: Comparison of impression by Conventional, Cytospin and cell block with other study

Feature	**Present study**			**Joshi et al**		
	Conventional	**Cytospin**	**Cell block**	**Conventional**	**Cytospin**	**Cell block**
Benign	74(96.1%)	74(94.8%)	51(94.4%)	121(80.6%)	116(66.6%)	116(66.6%)
Suspicious	2(2.5%)	2(2.5%)	-	15(10%)	-	-
Malignant	1(1.4%)	2(2.7%)	3(5.6%)	14(9.3%)	34(22.6%)	34(22.6%)

In the present study of the three cases confirmed as malignant by cell block 2 cases were found to be malignant on cytospin smears when compared to one case diagnosed as malignant by conventional smear. In this study done by Joshi A *et al*[2] 34 cases were found to be malignant by both cytospin smears and cell block when compared to 14 cases diagnosed as malignant by conventional smears.The less number of malignant effusions in the present study was due to less sample size when compared to other study.(Table 6.3)

Table 6.4: Comparison of impression by Conventional and Cell block with other study

Feature	Present study		Viral BM et al	
	Conventional	**Cell block**	**Conventional**	**Cell block**
Benign	74(96.1%)	51(94.4%)	116(77%)	117(78%)
Suspicious	2(2.5%)	-	16(11%)	-
Malignant	1(1.4%)	3(5.6%)	18(12%)	33(22%)

In the present study 96.1% of effusions were benign, 2.5% were suspicious for malignancy and 1.4% cases were malignant effusions on conventional smears when compared to cell block which showed 94.4% of effusions were benign and 5.6% cases were malignant. The two cases which were suspicious on conventional smears were found to be malignant on cell block. Two cases causes were found to be metastasis from lung and ovary. One case primary was unknown.Our study is in concordance with Viral BM *et al*.[3]Due to less number of malignant effusions the present study was possibly due to less sample size. The splitting of the sample for Conventional smear, Cytospin and Cell block yielded less cellularity in cell blocks. (Table 6.4)

In our study, we noted better morphological details on CB such aspreservation of architectural patterns like three dimensional clusters, presence of cellballs, aciniand better cytoplasmic features. Nuclear morphology wasbetter appreciated in CB when compared to CS. Similar findings were noted in variousstudies.[4,29,30,35,38]

Table 6.5: Comparison of IQV with other study

Present study			Joshi et al
Method	**Morphological features**	**IQV**	**IQV**
CONVENTIONAL	Background	0.818	0.7879
	Cellularity	0.801	0.4439
	Morphology	0.808	0.7879
	Distribution	0.812	0.7583
CYTOSPIN	Background	0.736	0.0395
	Cellularity	0.835	0.0395
	Morphology	0.449	0.0199
	Distribution	0.583	0.0588
CELL BLOCK	Background	0.957	0.0000
	Cellularity	0.808	0.0199
	Morphology	0.969	0.0000
	Distribution	0.876	0.0000

IQV (Index of Qualitative Variation) ranges from 0 (No variation) to 1 (Maximum variation) IQV was found minimum for all parameters in cell block in study done by Joshi A *et al*[2] and in present study was found minimum for morphology and distribution in Cytospin.(Table 6.5)

Table 6.6: Comparison of level of significance difference of means of morphological features of conventional smear, cytospin smear and cell block with other study

	Present study	**Joshi et al**
Morphological features	**CONVENTIONAL & CELL BLOCK & CYTOSPIN**	**CONVENTIONAL & CELL BLOCK & CYTOSPIN**
Background	<0.001*	<0.001*
Cellularity	<0.001*	<0.001*
Morphology	<0.001*	<0.001*
Distribution	<0.001*	<0.001*

Present study showed difference of median scores for Conventional&Cytospin as well as Conventional & Cell block on each parameter were statistically significant ($p<0.0001$) this is in accordance with study done by Joshi A *et al.*[2](Table 6.6)

Table 6.7: Comparison of level of significance difference of means of morphological features of Conventional, Cytospin smears of various studies.

	Present study	**Joshi et al**	**Singh et al**
Morphological features	**CONVENTIONAL & CYTOSPIN**	**CONVENTIONAL & CYTOSPIN**	**CONVENTIONAL & CYTOSPIN**
Background	<0.001*	<0.001*	<0.001*
Cellularity	<0.001*	<0.001*	<0.001*
Morphology	<0.001*	<0.001*	<0.001*
Distribution	<0.001*	<0.001*	<0.001*

Present study showed difference of median scores for Conventional &Cytospin on background, cellularity, morphology and distribution were statistically significant ($p<0.0001$) this is in concordance with study done by Joshi A *et al* and Singh M *et al*.[1]

Table 6.8: Comparison of level of significance difference of means of morphological features of Conventional smears and Cell block of various studies.

	Present study	**Joshi et al**	**Singh et al**
Morphological features	**CONVENTIONAL & CELL BLOCK**	**CONVENTIONAL & CELL BLOCK**	**CONVENTIONAL & CELL BLOCK**
Background	<0.001*	<0.001*	<0.001*
Cellularity	<0.001*	<0.001*	<0.001*
Morphology	0.004*	<0.001*	<0.001*
Distribution	0.164*	<0.001*	<0.001*

Present study showed difference of median scores for conventional & cell blocks on background, cellularity, morphology were statistically significant ($p<0.0001$) this is in accordance with study done by Joshi A *et al*.[2] The distribution of cells was statistically insignificant. (Table 6.8)

Table 6.9: Comparison of level of significance difference of means of morphological features of Cytospin smears and Cell block of various studies.

	Present study	**Joshi et al**	**Singh et al**
Morphological features	**CYTOSPIN & CELL BLOCK**	**CYTOSPIN & CELL BLOCK**	**CYTOSPIN & CELL BLOCK**
Background	<0.001*	0.158	0.141
Cellularity	<0.001*	0.565	0.341
Morphology	<0.001*	0.321	0.113
Distribution	<0.001*	0.082	0.061

Present study showed difference of median scores for Cytospin& Cell blocks on each parameter were statistically significant ($p<0.0001$) this is in discordance with study done by JoshiM*et al*[2] and SinghM*et al*[1] were the difference was found to be statistically insignificant. This is due to the limitation of this study which was for each case the quantity of fluid received was divided and Conventional smears, Cytospin and Cell block were prepared. The cell blocks showed inadequate material because of insufficient sample. (Table 6.9)

In present study is in concordance with study done by Singh M *et al*[1] showed in evaluating the cytological details brought out by each technique, Cytospin smears was superior in demonstrating cellularity, cell retrival, less cellular crowding, better cytoplasmic and nuclear preservation than routine method.

According to various studies additional diagnostic yield for malignancy was noted if conventional smear technique is supplemented by Cytospin and Cellblock method. The present study also concludes cellblock serves as a useful adjunct to traditional Conventional smears. A major disadvantage of the cellblock is more turnaround time as compared to conventional smears. Lack of cellular material in cellblock maybe observed due totechnical errors such as inadequate sampling or degenerated sample.

Study done by Sumi M G *et al*[51]on CSF-cytospin smears from TBM patients showed the technical aspects of this immunocytological methodfor the demonstration of mycobacterial antigens are simple, rapid, and reproducible, as well as specific, andtherefore can be applied for the early diagnosis of TBM, particularly in patients in whom bacteriologicalmethods did not demonstrate the presence of *M. tuberculosis* in the CSF. Similar morphological features were found in our study.

The present study showed Cytospin smears are cost effective, and less amount of sample is sufficient for cytodiagnosis. Approximately 100-200µl of fluid is sufficient for diagnosis. The screening time is less and malignancy can be easily diagnosed because of monolayer of cells, clear background, less cellular degeneration and even distribution of cells. Similar findings were noted by other studies.[1,2] Immunocytochemistry can be done on cytospin smears and thus the need for cell block is not required.

SUMMARY

In the present study, 80 body cavity fluid samples were evaluated by conventional smear, cytospin smear and cellblock from 1st December 2014 to 30th June 2016 in Department of Pathology in BLDEU'S ShriB.M.Patil Medical College, Hospital and Research centre, Vijayapura.

1. Out of 80 body cavity fluids, 43(58%) were pleural, 36(45%) were peritoneal & 1 (1.2%) was CSF.

2. Out of 80 studied, 57 cases (71.2%) were male & 23 cases (28.8%) were female, Male: Female ratio was 2.44:1.

3. Minimal amount of background blood and proteinaceous material in 52(65%) of cytospin smears 4 (5%) of conventional smears and 37 (46.2%) of cell blocks.

4. Abundant cellularity was seen in 38(47.5%) of cytospin smears 19(23.8%) of conventional smears and 4 (5%) of cell blocks.

5. Minimal cell degeneration was noted in 66 (82.5%) of cytospin smears 43(53.8%) of conventional smears and 36 (45%) of cell blocks.

6. Even distribution of cells was seen in 61(76.2%) of cytospin smears 7(8.8%) of conventional smears and 14 (17.5%) of cell blocks.

7. Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hencefor fluids with scant cellularity, cytospin is a useful method.

8. The morphology of the cells were well appreciated by cytospin as well as cell block as compared to conventional smears, thus aids in accurate diagnosis.

9. The difference of median scoresfor each parameter for conventional, cytospin and cell blocks were statistically significant ($p<0.0001$)

CONCLUSION

- The present study concludes that cytospin smears shows clear background, high cellularity, better nuclear features and even distribution of cells thereby decreasing screening time. Hence cytospin smears are better when compared to Conventional smears and Cell block in analysing body fluids.
- Conventional smears showed cellular overlapping and diffuse arrangement of cells thereby increasing the screening time.
- Cell block showed less cellularity as thesame sample was divided for conventional and cytospin smears.
- Though CBs were complementary to Conventional smears in the overall categorization ofbenign and malignant groups, they appeared to be more useful indiagnosis of malignancy by better preserved architectural patterns, as seenin corresponding histopathology sections.
- Cytospin and CBs are an excellent resource material for ancillary techniques like immunocytochemistry/immunohistochemistry and also useful in predicting the primary site of malignancy.

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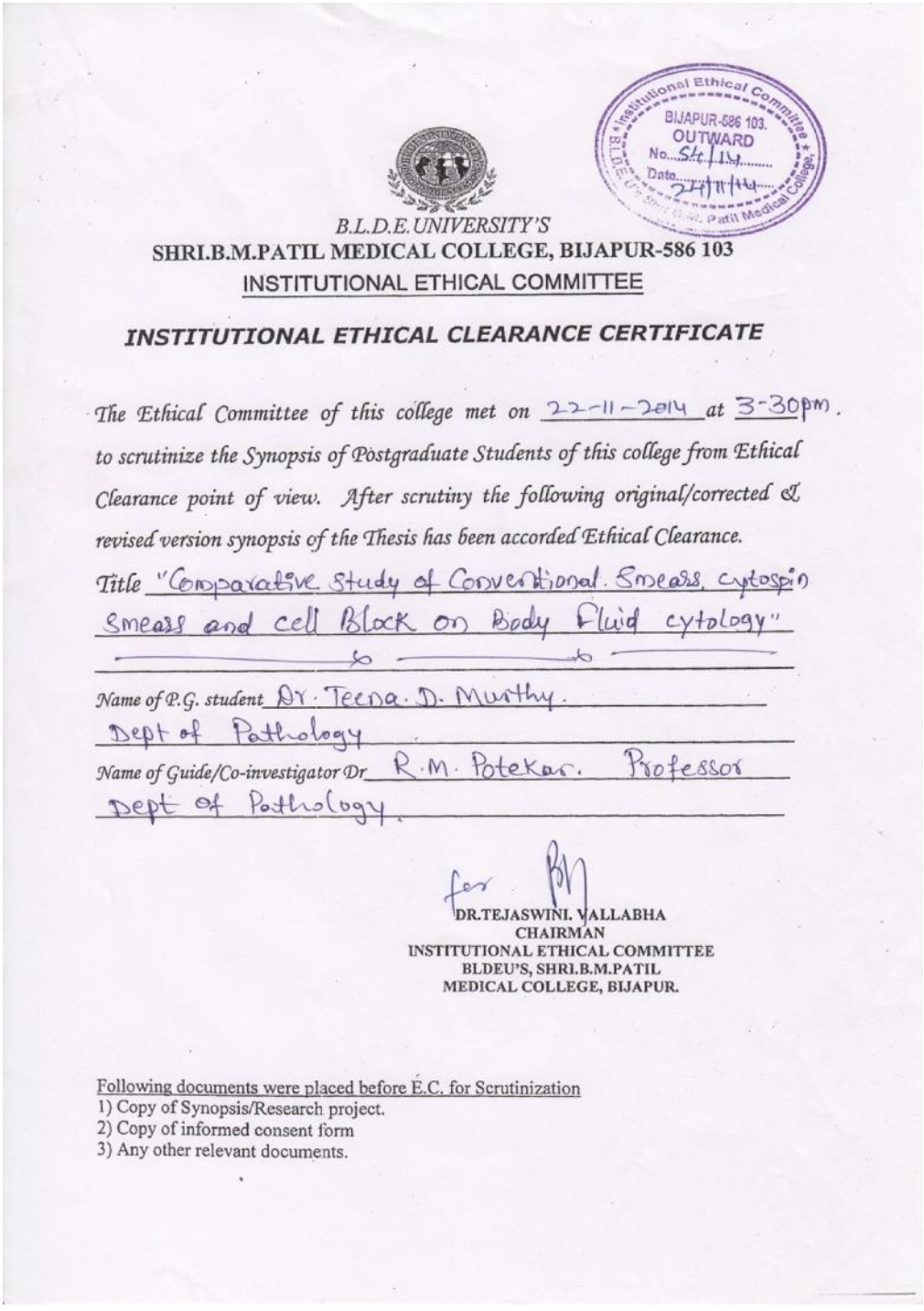
ANNEXURES

ETHICAL CLEARANCE



Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 54/14
Date 27/11/14
B.L.D.E. Shri B.M. Patil Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 22-11-2014 at 3-30pm. to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Comparative Study of Conventional Smears, cytospin Smears and cell Block on Body Fluid cytology"

Name of P.G. student Dr. Teena. D. Murthy.
Dept of Pathology

Name of Guide/Co-investigator Dr R.M. Potekar. Professor
Dept of Pathology.

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

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

BLDE University's

Shri B M Patil Medical College, Hospital & R.C

Vijayapur, Karnataka

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, ,S/O D/O W/O, _____ aged years, ordinarily resident of do hereby state/declare that DrTeena D Murthy of Shri B M Patil Medical College, Hosptital has examined me thoroughly on at (place) and it has been explained to me in my own language thatI am suffering from _____disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled Comparative study of Conventional smears, Cytospin smears and Cell block on Body Fluid cytology under the guidance of DrR M Potekar requesting my participation in the study. Apart from routine treatment procedure the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor Teena D Murthy has also informed me that during conduct of this procedure _____like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further doctor has informed me that my participation in this study help in evaluation of the results of the study which is

useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

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

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

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

PROFORMA

CASE NO:

NAME : OP/IP No. :

AGE :

SEX : D.O.A :

RELIGION : D.O.D :

OCCUPATION :

RESIDENCE :

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

General physical examination:

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphedenopathy present/absent

Edema present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

Haematological investigations:

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
LYMPHOCYTES(%)	

MIXED (%)	
NEUTROPHILS(%)	
RDW	
PDW	
MPV	
P-LCR	

Urine routine:

Sugar:

Albumin:

Microscopy:

BODY FLUID ANALYSIS

Physical examination:

Type of fluid:

Quantitiy:

Appearance:

Microscopic examination:

Cell counts:

Differential leukocyte count:

Chemical Analysis:

pH:

Protein:

Glucose:

	CENTRIFUGE	**CYTOSPIN**	**CELL BLOCK**
Background			
Amount of cellular material			
Cell morphology, Cellular degeneration			
Distribution of cells			
Inference			

KEY TO MASTER CHART

- Sex: 1- Male, 2- Female
- Fluid: 1- Pleural, 2- Ascitic, 3-CSF
- Background blood/Proteinaceous material

  0- Large amount

  1- Moderate amount

  2- Minimal amount
- Amount of cellular material

  0- Minimal

  1- Sufficient

  2- Abundant
- Cell morphology/degeneration

  0- Marked degeneration

  1- Moderate degeneration

  2- Minimal degeneration
- Distribution of cells

  0- Periphery/sparse

  1- Combination

  2- Evenly distributed

MASTER CHART

NAME	AGE	SEX	LAB NO	FLUID	CONVENTIONAL					CYTOSPIN					CELL BLOCK				
					Background	Cellularity	Morphology	Distribution	Impression	Background	Cellularity	Morphology	Distribution	Impression	Background	Cellularity	Morphology	Distribution	Impression
Dundawwa	62	2	5370/15	2	1	1	1	0	negative	2	1	2	2	neg	0	0	0	0	inadequate
Suresh	38	1	16588/15	3	2	1	2	0	cryptococosis	2	1	2	1	cryptococosis	2	1	2	0	cryptococosis
Shridevi	30	2	1375/15	1	1	1	2	1	candidiasis	2	2	2	2	candidiasis	0	0	0	0	inadequate
Nagappa	62	1	4096/15	1	0	1	2	1	negative	0	0	0	0	inadequate	1	0	0	0	inadequate
Maiboob	19	1	11907/15	1	1	1	2	1	inflamm	1	1	2	1	inflamm	0	0	0	0	inadequate
Mallappa	46	1	15455/15	1	0	1	1	1	inflamm	1	2	1	2	inflamm	0	0	0	0	inadequate
Samuel	47	1	6346/15	1	1	1	2	1	inflamm	2	2	2	2	inflamm	2	0	0	0	inadequate
Somaling	70	1	11326/15	1	1	1	2	2	inflamm	2	1	2	2	inflamm	0	0	0	0	inadequate
Gouramma	55	2	20559/15	1	1	1	2	1	inflamm	2	2	2	2	inflamm	0	0	1	0	inadequate
Dundawwa	50	2	22412/15	1	0	2	2	1	inflamm	2	2	2	2	inflamm	0	0	0	0	inadequate
Danpal	53	1	20847/15	1	0	0	1	0	inflamm	2	2	2	2	inflamm	2	0	1	0	inadequate
Basavanthray	62	1	23780/15	1	1	1	1	1	inflamm	2	2	2	2	inflamm	0	0	0	0	inadequate
Yankappa	61	1	23878/15	2	1	1	1	0	inflamm	2	1	2	2	inflamm	2	0	0	0	inadequate

Somanna	40	1	26568/15	1	0	2	2	1	inflamm	2	2	2	2	inflamm	0	0	0	0	inadequate
Irappa	50	1	379/14	2	1	1	2	1	inflamm	2	2	2	2	inflamm	0	0	0	0	inadequate
Pramila	30	2	22379/15	2	0	0	0	0	inadequate	0	1	2	2	inflamm	2	0	0	0	inadequate
Durgawwa	40	2	232251/15	2	0	0	2	0	inadequate	2	2	2	2	inflamm	0	0	0	0	inadequate
Radha	39	2	16281/15	1	0	0	0	0	inadequate	1	1	2	2	inflamm	0	0	0	0	inadequate
Shankaremma	70	2	1492/15	1	0	1	1	1	neg	1	2	2	2	neg	1	0	0	0	inadequate
Shantabai	60	2	4059/15	2	2	1	1	0	neg	2	1	1	2	neg	0	0	0	0	inadequate
Madiwalamma	24	2	4928/15	2	1	0	1	1	neg	0	0	1	1	neg	0	0	0	0	inadequate
Shivanand	25	1	5544/15	2	1	1	1	0	neg	1	1	1	1	neg	0	0	0	0	inadequate
Sayabana	75	1	5634/15	1	1	2	1	2	neg	2	2	2	2	neg	0	0	0	0	inadequate
Ameerbee	82	2	5940/15	1	0	1	2	1	neg	1	0	2	0	neg	2	0	2	0	inadequate
Shanarao	45	1	5758/15	1	0	1	2	1	neg	1	2	2	2	neg	0	1	1	0	inadequate
Manjunath	31	1	10944/15	2	1	1	1	1	neg	2	1	2	2	neg	0	0	0	0	inadequate
Shantawwa	60	2	13980/15	2	1	0	1	0	neg	2	1	2	2	neg	0	0	0	0	inadequate
Basangouda	38	1	22395/15	2	1	1	1	0	inflamm	2	0	0	0	inadequate	2	1	2	1	inflamm
Kasturbai	50	2	5935/15	1	1	2	2	1	inflamm	2	1	2	0	inflamm	2	1	2	1	inflamm
Krishna	42	1	6448/15	1	0	1	2	0	inflamm	1	1	1	2	inflamm	1	1	1	2	inflamm
Basappa	45	1	9038/15	2	0	2	2	1	inflamm	1	2	2	1	inflamm	2	0	1	0	inflamm
Vittal	52	1	14040/15	1	1	1	1	0	inflamm	2	1	2	2	inflamm	2	0	1	0	inflamm
Nagaraj	40	1	10093/15	2	1	1	2	0	inflamm	2	1	2	2	inflamm	2	1	2	1	inflamm
Ningappa	65	1	14196/15	1	0	0	1	0	inflamm	2	1	2	2	inflamm	1	1	2	2	inflamm
Irappa	74	1	16394/15	1	1	1	1	1	inflamm	2	1	2	2	inflamm	2	1	2	2	inflamm
Chandrashekar	38	1	16809/15	1	1	1	1	1	inflamm	2	1	2	2	inflamm	2	0	1	0	inflamm
Kumar	28	1	18808/15	1	1	2	2	1	inflamm	2	2	2	2	inflamm	2	1	2	1	inflamm
Shrishail	35	1	11962/15	1	0	1	2	1	inflamm	1	1	2	1	inflamm	2	1	2	2	inflamm
Shivaling	42	1	9443/15	2	1	0	2	0	inflamm	2	1	2	2	inflamm	2	0	2	1	inflamm

Ramling	72	1	8688/15	1	1	1	1	1	inflamm	2	1	2	2	inflamm	2	1	2	1	inflamm
Subadar	35	1	19537/15	2	1	1	1	2	inflamm	2	2	2	2	inflamm	1	1	2	1	inflamm
Siddu	38	1	19233/15	2	0	2	2	1	inflamm	2	2	2	2	inflamm	2	1	2	1	inflamm
Mallappa	44	1	19850/15	2	0	1	1	1	inflamm	2	2	2	2	inflamm	1	1	1	0	inflamm
Siddalingawwa	70	2	19524/15	2	0	2	2	2	inflamm	2	2	2	2	inflamm	2	2	2	1	inflamm
Sachin	10	1	20420/15	2	0	2	1	1	inflamm	2	2	2	2	inflamm	2	1	2	0	inflamm
Guramma	55	2	20487/15	2	1	1	2	1	inflamm	2	2	2	2	inflamm	2	1	1	1	inflamm
Shivappa	36	1	22200/15	1	0	2	2	1	inflamm	1	2	2	1	inflamm	2	1	2	2	inflamm
Shivappa	60	1	22678/15	1	0	1	1	1	inflamm	2	2	2	2	inflamm	2	1	2	2	inflamm
Bhimaray	50	1	23620/15	2	2	1	1	0	inflamm	2	1	1	2	inflamm	2	1	2	2	inflamm
Madiwallappa	70	1	22272/15	2	1	1	2	0	inflamm	2	2	2	2	inflamm	2	1	1	0	inflamm
Rulidas	47	1	24192/15	2	1	1	1	1	inflamm	2	2	2	2	inflamm	1	1	1	1	inflamm
Mahadevi	55	2	22892/15	2	0	1	2	2	inflamm	1	1	2	2	inflamm	2	1	2	0	inflamm
Chandrashekar	38	1	16809/15	1	0	1	2	1	inflamm	2	2	2	2	inflamm	1	1	2	1	inflamm
Mallikarjun	34	1	23454/15	2	1	1	2	1	inflamm	2	1	1	2	inflamm	2	1	1	0	inflamm
Somappa	75	1	22909/15	2	0	2	2	1	inflamm	1	2	2	2	inflamm	1	1	2	1	inflamm
Basappa	45	1	23212/15	1	1	1	1	1	inflamm	1	1	1	0	inflamm	2	1	1	0	inflamm
Horiba babu	45	1	19502/15	2	2	2	2	2	inflamm	2	1	2	1	inflamm	0	1	1	1	inflamm
Babugouda	65	1	18907/15	2	1	0	0	0	inflamm	2	1	0	0	inflamm	1	0	1	0	inflamm
Chandrakanth	36	1	21906/15	1	0	2	1	1	inflamm	1	2	2	2	inflamm	2	2	2	2	inflamm
Mahadevi	55	2	22892/15	2	0	1	2	1	inflamm	2	1	2	2	inflamm	2	1	2	2	inflamm
Shankar	53	1	23642/15	2	0	1	1	1	inflamm	1	1	1	1	inflamm	0	1	1	1	inflamm
Babugouda	70	1	22369/15	2	1	1	1	0	inflamm	2	2	2	2	inflamm	2	1	1	0	inflamm
Bouramma	55	2	26382/15	1	0	2	2	1	inflamm	2	2	2	2	inflamm	0	1	1	1	inflamm
Sarojini	75	1	19753/15	1	0	0	0	0	neg	1	1	1	2	inflamm	1	2	2	2	inflamm
Bapuraya	65	1	5532/15	1	1	2	2	1	inflamm	1	0	2	0	neg	1	1	2	1	inflamm

Rekha	15	2	109/15	1	0	2	1	1	neg	1	2	2	1	neg	1	1	2	2	inflamm
Mahadevi	38	2	439/15	1	0	1	2	1	neg	0	2	2	2	neg	1	1	2	0	inflamm
Mahadevi	38	2	440/15	1	0	2	2	1	neg	0	2	1	2	neg	1	1	2	0	inflamm
Monesh	48	1	1991/15	2	1	1	2	0	neg	2	1	2	2	neg	0	1	1	0	inflamm
Dhasarat	80	1	3595/15	1	1	1	2	1	neg	2	1	2	2	neg	1	1	1	0	inflamm
Siddu	38	1	19233/15	2	1	2	1	1	neg	2	1	2	2	neg	2	1	2	0	inflamm
Uma	45	2	622/15	1	1	0	1	0	neg	1	1	2	2	suspicious	1	0	2	0	inlamm
Dapal	53	1	20845/15	1	0	1	2	0	inflamm	1	2	2	2	inflamm	0	0	2	2	neg
Shivappa	60	1	22678/15	1	1	1	2	1	inflamm	2	2	2	2	neg	2	1	2	2	neg
Annapagouda	58	1	4254/15	2	1	1	1	0	neg	2	2	2	2	neg	1	1	1	1	neg
Anand	40	1	5798/15	2	1	1	2	1	neg	2	1	2	2	neg	0	1	1	1	neg
Raju	38	1	6860/15	1	0	1	2	1	neg	1	2	2	1	neg	2	0	2	0	neg
Mallappa	65	1	27008/15	1	0	1	2	0	positive	1	1	2	1	positive	2	1	2	1	positive
Jagadev	50	1	38442/15	1	1	1	2	2	suspicious	2	2	2	2	positive	2	2	2	2	positive
Sulochana	48	2	76655/15	2	0	2	1	1	suspicious	2	1	2	2	suspicious	2	1	2	1	positive