CHAPTER 2

LUNG CANCER AND SPUTUM CYTOLOGY

2.1 INTRODUCTION

Human body is made up of trillions of individual units called cells, in the usual case most cells make new cells to substitute older cells that age or become damaged. Sometimes this ordered process, of only making new cells while a person is growing, breaks down. When this happens cells can grow out of control and they form a lump or mass called a tumor (Cancer).

In this system, classification of the lung glandular cells as benign and malignant (Cancer Cells) are dealt. After getting the necessary information regarding the details of lung cancer, methods available for the diagnosis of lung cancer, screening methods for lung cancer, different type of Lung Cancer, details of sputum cytology and how image is captured from sputum cytology slides, an automated system is developed. In this work, detection of adenocarcinoma lung cancer which causes the highest mortality was considered.

2.2 DIAGNOSIS OF LUNG CANCER

Patients with symptoms of cough, hemoptysis or chest pain and radiologic abnormalities, whether or not indicative of lung cancer, there are several methods of further investigation. Conventional and high-resolution Computerized Tomography (CT) may help clarify the nature of a suspicious
Cytological examination of sputum, bronchial secretions, and aspirates are the major techniques for diagnosis. Of which sputum cytology is the oldest and simplest of these diagnostic procedures and is readily available to every medical practitioner. It can provide a diagnosis in most of primary lung carcinomas, depending on tumor type and stage. Sputum cytology also may afford a substrate to search for diagnostic molecular markers of lung cancer.

Samples obtained by the fiber optic bronchoscope also are very effective in the diagnosis and differential diagnosis of cancer. Brush specimens, obtained directly from the suspect lesion, often provide an excellent sample and exact information on the location of the disease. Bronchoalveolar Lavage (BAL) is also very successful in the diagnosis of lymphangitic carcinomatosis caused by metastatic cancer. Percutaneous Fine-Needle Aspiration (FNA) biopsy is used with increasing frequency to investigate pulmonary infiltrates as well as more discrete masses in the lung. Transbronchial FNA (TBNA) biopsy is performed during fiber optic bronchoscopy for diagnosis of tumor masses located outside the bronchial lumen. It is also of potential value in the recognition and diagnosis of inflammatory disease and certain benign mediastinal or parabronchial masses. CT-Guided Core Biopsies utilizes an automatic gun provided with an 18 or 20 gauge cutting needle to obtain core biopsies of lung lesions.
2.3 SCREENING FOR LUNG CANCER

To curb the mortality rate of lung cancer it is utmost important to detect the cancer at an early stage. This is possible only by a mass screening of all men with a higher than average risk for developing lung cancer. Chest X-ray along with sputum examination has been tried as a screening method to detect the presence of lung cancer. But it was not found useful. Imaging techniques combined with sputum cytology will be of great value to detect early lung cancers and its precursor lesions. This may need huge man power and resources to screen all eligible people in the community. The microscopic evaluation of sputum samples may necessitate large number of trained cytologists. If a low cost technology for the microscopic evaluation of sputum cytology smears using computer is developed it will be of great help to implement mass screening programme for lung cancer. Moreover computer based screening methods will be more reliable and objective.

2.4 SPUTUM CYTOLOGY

The human respiratory system serves the twofold purpose of supplying oxygen to the blood stream and removing carbondioxide from it. The respiratory tract is separated into three parts- cranial, intermediate and lung has been pointed out by Koss & Melamed (2006). The cranial part...
bronchioles which ultimately reaches the blood rich alveoli. The gas exchange takes place at the wall of the alveoli.

The surface of internal organs are typically enclosed by two types of cells (epithelium) - squamous and columnar (glandular cells). The squamous cells are for giving guard to the organs while columnar or glandular cells are associated with secretions. The rate of adenocarcinoma is the highest among all lung cancers has been pointed out by Yu et al (2010) and is mainly attributed due to smoking. The glandular (respiratory) epithelium covers the key portion of bronchial tree. This respiratory epithelium forms a pseudostratified columnar formation. The pseudostratified form is due to the positioning of nuclei at diverse levels. The respiratory epithelium is poised of ciliated columnar cells along with mucus secreting goblet cells. The mucus secreted by the goblet cells is distributed throughout the respiratory epithelium which traps dust particles which happen to be inhaled. The basic functional unit of lung, alveoli, is lined with two types of cells - pneumocytes type 1 and pneumocytes type 2. The type 1 covers majority of alveoli and are metabolically inactive. The type 2 produces a detergent like protein which prevents the collapsing of alveolar region and reduces surface tension. The foreign particles entering the body are engulfed by a certain type of cells called phagocytes. The presence of phagocytic cells is typical of sputum cytology. These cells are known as alveolar macrophages or pneumocytes type 3 or dust cells. For considering the obtained slide to be adequate, the presence of these macrophages is highly required. Figure 2.1 shows the categorization of epithelium cells.
Figure 2.1 Epithelial cell classification

Figure 2.2 shows sample sputum cytology Image of Benign glandular cells.

Figure 2.2 Sputum cytology image of benign glandular cells
Figure 2.3 shows a sample sputum cytology image of malignant glandular cells.

![Sputum cytology image of malignant glandular cells](image)

**Figure 2.3 Sputum cytology image of malignant glandular cells**

### 2.5 TYPES OF LUNG CANCER

Tumor classification is vital for steadiness in patient treatment and because it provides a foundation for epidemiologic and biological studies. The cancer affecting lung is of various types. Each varies highly in the type of cells affected and so the morphological features are of varied kinds. They can be classified into the following categories:

1. Squamous carcinoma
2. Adenocarcinoma
2.5.1  **Squamous Carcinoma**

Cytologic examination of sputum and/or aspirated bronchial secretions can yield a rapid and accurate diagnosis of squamous lung cancer, regardless whether the tumor is visualized bronchoscopically or not. Squamous cancer cells are reminiscent of normal squamous epithelial cells, but differ from normal in several important features.

Cells of squamous carcinoma vary considerably in shape and size, are typically found in a background of inflammation and necrosis, and often assume a most bizarre appearance has been pointed out by Koss & Melamed (2006). Spindly cancer cells and tadpole cells are quite common and their presence is characteristic of these neoplasms. Very large squamous cells may appear next to very small cells. The cytoplasm produces keratin and assumes a brilliant orange or yellow color in Papanicolaou stain, with a certain quality of thickness and refractility that may be brought out when the condenser of the microscope is lowered. The keratin also confers a very sharp cell outline. In some degenerating highly keratinized cancer cells, the densely yellow or orange cytoplasm drowns out a fading nucleus that is undergoing karyolysis. The resulting abnormally shaped yellow or orange ghost cells have only faint outlines of a nucleus, or no nucleus at all.

In the absence of nucleated cancer cells, the ghost cells in sputum or bronchial specimens are strongly indicative, although not fully diagnostic of squamous carcinoma. Only in tracheitis sicca associated with tracheostomy are there likely to be benign squamous ghost cells with nuclear atypia that mimics malignant cells; otherwise the ghost cell nuclei are bland, if at all visible. Most carcinomas of the lung similar to cancer at other sites arise by a step wise accumulation of genetic abnormalities that transform benign bronical epithelium to neoplastic tissue has been pointed out by Koss & Melamed (2006).
2.5.2 Adenocarcinoma

This is a malignant epithelial tumor with glandular differentiation or mucin production by the tumor cells. Adenocarcinoma grows in various patterns, including acinar, papillary, bronchioloalveolar, and solid with mucin formation. In this work adenocarcinoma of lung was dealt.

Adenocarcinoma of the lung is associated with cigarette smoking, and has been increasing in frequency both in male and female cigarette smokers. The tumor is usually more peripherally located, and tends to be smaller as compared with squamous cell cancers. They differ histologically from well-differentiated tumors with obvious glandular elements. Adenocarcinoma grows more slowly than squamous cell carcinoma but tend to metastasize widely and earlier. Peripheral adenocarcinomas with a small central invasive component associated with scarring and a predominantly peripheral bronchioloalveolar growth pattern may have a better outcome than invasive carcinomas of the same size. Two forms of pulmonary adenocarcinoma may be differentiated on histologic and clinical grounds: adenocarcinomas of so-called central bronchial origin and peripheral bronchioloalveolar or terminal bronchiolar carcinomas.

Adenocarcinomas of central bronchial origin were sub classified into subtypes such as acinar, papillary, and solid. The exfoliated cells in sputum and bronchial secretions are large, usually round or polygonal, occasionally columnar, and are found in clusters or singly in sputum and bronchial wash specimens. The cell clusters have a three-dimensional papillary or approximately spherical pattern with tumor cells superimposed upon each other. Cytoplasm of the cancer cells may be scanty or stripped away. In the case of well-preserved cells, it is moderate in amount, often finely vacuolated and faintly staining and has been pointed out by Koss & Melamed (2006).
Single vacuolated tumor cells may be misguided for macrophages, and on rare occasions are phagocytic, but they have the nuclear features of cancer cells. Such cells are seen in the lumens of adenocarcinoma in histologic sections and represent desquamated, degenerating, mucin secreting tumor cells. The larger mucin vacuoles frequently seem to displace the nucleus to one side, sometimes causing it to bulge out of the cell. In the case of histiocytes this is not possible. Sporadically it is observed, lipid-containing vacuolated macrophages accompanying the tumor cells of adenocarcinoma, consistent with an endogenous lipid pneumonia.

The nuclei of pulmonary adenocarcinomas are paramount studied in single cancer cells. They are large for the size of the cells, with finely granular chromatin and usually slight to moderate hyperchromasia, often with prominent, single or multiple nucleoli. There may be serration of the nuclear membrane and sometimes a study of cytoplasm into the nucleus, forming the so-called nuclear holes.

Primary adenocarcinoma of the lung may be hard to distinguish from anaplastic carcinoma of large-cell type, particularly if the tumor is represented by single cells without cell clusters. The presence of papillary clusters or columnar cancer cells clearly favors the diagnosis of adenocarcinoma. Figure 2.4 shows a sample Benign and Malignant Glandular cells.

These tumors occur in bronchiolar or alveolar epithelium of peripheral lung tissue and could present as a localized mass or masses in lung parenchyma similar to central adenocarcinoma or as a diffuse pneumonic type of infiltrating carcinoma that represents intrapulmonary spread. Sputum is by far the best diagnostic medium for this group of tumors.
In nonmucus-producing type II tumors, the sputum contains variable numbers of well-demarcated, rounded, or papillary clusters of tumor cells. Such clusters are composed of overlapping small, round, or roughly cuboidal cancer cells with scanty clear or lightly stained cytoplasm and moderately hyperchromatic nuclei with one or two small nucleoli. The cell groups of adenocarcinoma are tightly clustered, and the component cells lack the molding. Some of the papillary clusters of cancer cells may resemble and are distinguished from the Creola bodies. Isolated single cancer cells are few, and may be difficult to identify in this tumor type. And finally the analysis rests on identifying tumor cell clusters.

In the mucus-producing bronchioloalveolar carcinoma, of type I are characterized by tall columnar mucus-secreting cells. In this case the cytologic presentation is unusual. The sputum has single identifiable malignant cells as well as cell clusters. The tumor cells are bigger than those carcinoma of type II and have copious mucus-producing apparent cytoplasm. They have one or two finely textured nuclei with sharply defined nuclear membranes and visible or sometimes prominent nucleoli have been pointed out by Koss & Melamed (2006).
Tumor cell clusters may be composed of overlapping cells or of flat coherent groups of cells with a glandular or canard configuration. Columnar cancer cells are uncommon. Some may have a flat free cell border and mimic benign bronchial cells, but they do not have cilia or a terminal bar has been pointed out by Koss & Melamed (2006).

2.6 RESPIRATORY REGION – CYTOLOGY

There are different types of epithelium such as Squamous and respiratory epithelium. They are as follows:

2.6.1 Squamous Epithelium

The exfoliated superficial squamous cells, which control in specimens of saliva as they do in graze smears of other squamous mucosal surfaces, are identical in all respects to the superficial and midway squamous cells. They are available as a single cell, but are often in plaques and encountered more commonly in inflammatory disorders of the oral cavity. They are alleged to symbolize unfinished maturation of regenerating epithelium. Onion-like arrangements of benign squamous cells and occasionally small spindly squamous cells also may be observed. Anucleated squamous cells are few, if present at all, but may exfoliate from the normal mucosa overlying and fixed to bone, or from sites of chronic irritation as occur with poor dentition.

2.6.2 Respiratory Epithelium

Contrary to squamous epithelium, which desquamates with no trouble and is well represented in all exfoliated samples. But, the normal respiratory epithelium does not desquamate liberally. Hence, cells derived from this epithelium are rare in sputum. If they are present at all in a sputum
specimen, it is a suggestion of prior instrumentation, trauma, or rigorous cough. However, respiratory epithelial cells may also originate in the nasal cavity or nasopharynx. So their presence in a specimen is not absolute insurance of origin from the lower respiratory tract.

### 2.6.3 Ciliated Cells

Respiratory epithelium is voluntarily familiar in cytologic material by the incident of ciliated columnar cells. Columnar cells may appear individually or in groups or clusters of cells. In brush specimens, large numbers of bronchial cells are usually observed, sometimes forming clusters of considerable complexity, and sometimes also with fanatic reserve or basal epithelial cells. At the periphery of such clusters, normal ciliated cells may appear at a right angle to the main axis of the cluster, giving the intuition of feathering, palpably an artifact induced by brushing.

The individual cells, resulting from larger bronchi, are classically cilia bearing and columnar in pattern. Much smaller, roughly square bronchial cells with scanty cytoplasm and a flat surface, with or without cilia, derived from terminal bronchioles, are occasionally observed. There is a prominent linear thickening or flat terminal plate at the luminal end of the columnar cell. On close inspection, under very high magnification by light microscopy, the terminal plate is composed of a series of confluent dots representing roots of the cilia or basal corpuscles. In a well-executed Papanicolaou stain, the cilia stain a distinct pink color. While cilia may be spoiled or lost, the terminal plate is usually conserved.

Clusters or sheets of dislodged respiratory cells lying flat on the slide and viewed from the luminal surface have a honeycomb appearance. These clusters are formed by the cytoplasmic borders of adjacent cells. The cytoplasm of the ciliated epithelial cell seen in profile is harmonized and
lightly basophilic or less commonly eosinophilic. Infrequently, small mucus vacuoles may be observed.

The nuclei are frequently very gracefully textured and oval in shape, with their long axis comparable to the long axis of the cell. Sometimes, the nucleus appears to be bigger than the oblique diameter of the slender cell, ensuing in a slight bulge at the level of the nucleus. However, electron micrographs show that the nucleus is always enclosed by a rim of cytoplasm. Within most bronchial cell nuclei, there are usually one or two small, but distinct, chromatin granules and sometimes a tiny nucleolus.

The spot of the nucleus comparative to the ciliated cell surface is variable, usually halfway stuck between the ciliated or luminal end of the cell and the tapered basal end. The normal nuclei may also show folds or creases and occasionally, intra nuclear cytoplasmic inclusions, or clear intra nuclear “holes”.

2.7 LUNG TUMORS - CONVENTIONAL CYTOLOGY

The following section details the various types of Carcinoma and their characteristics which will help to classify the cells.

2.7.1 Carcinoma

Carcinoma is a general form of main lung cancer. Afflicting primarily cigarette-smoking men and women older than fifty years of age, these neoplasms begin mainly in the epithelium of secondary or tertiary bronchi, and may cause bronchial obstruction. They are twice as frequent in upper lobes as middle or lower lobes; those that arise in the lower lobes are almost always in an upper segment.
Very large squamous cells may emerge next to very small cells. The cytoplasm produces keratin and assumes a brilliant orange or yellow color in Papanicolaou stain, with a certain quality of thickness and refractility that may be brought out when the condenser of the microscope is lowered. The keratin also confers a very sharp cell outline.

Although nuclear hyperchromasia is characteristic and typical, it does not apply to all squamous cancer cells. Peculiar staining characteristics of the nuclei are evident in tumor cells that are undergoing degeneration. They may sometimes have a smudgy or remarkably homogeneous water color appearance. More often, the nuclei are deeply and evenly hyperchromatic. On closer examination, chromatin structure is generally visible in such nuclei. Significant aberrations of nuclear shape are common. Many nuclei are angular or irregular in configuration and commonly variable in size as well as shape. The nuclear/cytoplasmic ratio varies considerably in this type of tumor and, although nuclei are on the whole quite large for cell size, there may be some very small pyknotic nuclei as well.

2.7.2 Large-Cell Undifferentiated Carcinoma

The large-cell undifferentiated bronchogenic carcinomas are poised of extensive, diffusely sensitive sheets of usually moderate size tumor cells with modest- to abundant cytoplasm. Many of these tumors are peripheral in origin and/or unrelated to major bronchi, and it is believed that they most apt represent undifferentiated adenocarcinomas. They are derived from the same basal epithelial cells that give rise to squamous and adenocarcinomas. As all non-small-cell lung cancers have the same prognosis and are treated in the same way.
2.7.3 Small Cell Carcinoma (SCC)

SCC consists of two type’s oat cell carcinoma, and an intermediate cell type. There are dissimilar cytologic differences between the small and intermediate cell types of SSC, based on cell size and other morphologic characteristics that are of significance in cytological analysis.

Oat cell carcinoma may be complicated to analyze because, at low magnification, the small cancer cells can be misinterpreted as lymphocytes. Sometimes they are entirely escaped from the attention of an inexperienced observer. However, the cytological presentation of this tumor is very characteristic and, once the hurdle of initial recognition has been overcome. Then the analysis is rather simple and exact. Sputum processed by the “pick-and-smear” method is higher to Saccomanno's technique in the diagnosis of this tumor type and has been pointed out by (Saccomano 1979).

The cells desquamating from intermediate type SSC are alike to those of oat cell carcinoma, but are rather larger, with more cytoplasm, and larger nuclei with finer chromatin structure. There are fewer pyknotic nuclei and less necrosis of tumor cells than in classical oat cell carcinoma. They may form cohesive sheets or structures suggesting adenocarcinoma; in the Papanicolaou stain, the eosinophilic cytoplasm of some cells may be retained, suggesting kinship to epidermoid carcinoma.

2.7.4 Lung Adenocarcinoma

Adenocarcinoma of the lung is associated with cigarette smoking. Two forms of pulmonary adenocarcinoma may be differentiated on histologic and clinical grounds. Lung adenocarcinomas are central bronchial origin and peripheral bronchioloalveolar or terminal bronchiolar carcinomas. The nuclei of lung adenocarcinomas are best studied in single cancer cells. They are
large for the size of the cells, with finely granular chromatin and usually slight to reasonable hyperchromasia, frequently with well-known, single or multiple nucleoli. Prime adenocarcinoma of the lung may be difficult to differentiate from anaplastic carcinoma of large-cell type, mainly if the tumor is represented by single cells without cell clusters.

2.8 PREPARATION OF SPUTUM CYTOLOGY SLIDES

Cytology is obviously ‘the study of cells’ and is correlated to anatomical pathology. Sputum is collected by impulsive coughing. Cytology uses various cell preparation technologies. Specimens are obtained involuntarily, by scrapings or needle aspirates, or by gathering of exfoliated material from various body fluids.

Sputum cytology is an analytic test not a screening test. A sequence of three profound cough morning sputum specimens has a sixty six percentage, concern for confirming lung carcinoma. Post-bronchoscopy sputum samples from time to time give diagnoses not achieved by other means. Critical to sputum specimens is ‘deep cough’. Many specimens are oral and contain saliva, oral squamous cells and micro-organisms.

Cytologists referee sample adequacy by the presence of pigmented pulmonary macrophages. Cytology can only look at a small measure of substance so technologists subsample by picking blood or streaked areas for slide production. Sputum is mostly mucus which interferes with the creation of a homogeneous representative subsample and limits the diagnostic yield. On the other hand, with some manipulation monolayer and similar technologies can be used. Sputum is more likely to be diagnostic with large and central lung lesions and less likely with small and peripheral lesions. Cytology can usually make the therapeutically vital characteristic between small cell and non-small cell carcinomas. There is greater difficulty in reliably
distinguishing adenocarcinoma, squamous cell carcinoma and large cell carcinoma, particularly when poorly differentiated. The difference has become key with oncologists wishing to screen adenocarcinomas for activating mutations of epidermal growth factor receptor to determine eligibility for specific inhibitors such as gefitinib. Currently it requires a tissue sample or a good cell block preparation. Normally, samples are taken by fine needle aspiration or core biopsy.

Prior to sample collection patients are educated to clear their nasal cavity and rinsing the mouth. For sputum cytology two or three days continuous repeated sampling is done. Those patients having difficulty, coughing is induced by inhaling heated aerosol twenty percentage of polypropylene glycol in hypertonic saline.

The sputum produced is fixed using Saccomannos's method has been pointed out by (Saccomano 1979). After fixation pap staining is done by using EA-65 and OG-6. The cell samples collected are placed under a light microscope and digitized using a customized digital camera. The images are properly labeled and stored. The images are so chosen such that the target region contains glandular cells.

### 2.9 SUMMARY

In this chapter, discussion was made about the cancer, types of lung cancers, different characteristics to identify and classify the cells as benign (non-cancerous cells) and malignant (cancer cells). Also discussed about the preparation of sputum cytology slides images which are the input for the proposed system.