DISSERTATION ON ANALYSIS OF FIBREOPTIC LUNG BIOPSY SPECIMENS REGARDING DEMOGRAPHIC CHARACTERISTICS, HISTOLOGIC TYPES AND WITH REFERENCE TO IMMUNOHISTOCHEMISTRY (p63)

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CERTIFICATE

This is to certify that this dissertation titled "**ANALYSIS OF FIBREOPTIC LUNG BIOPSY SPECIMENS REGARDING DEMOGRAPHIC CHARACTERISTICS, HISTOLOGIC TYPES AND WITH REFERENCE TO IMMUNOHISTOCHEMISTRY (p63)**" is the original and bonafide work done by **Dr. K. Barani** under my guidance and supervision at the Government Stanely Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2008 to April-2011 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

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CONTENTS

ABBREVIATIONS

HPE	-	Histopathological examination
FOB	-	Fibreoptic bronchoscopy
IHC	-	Immunohistochemistry
HRP	-	Horse raddish peroxidase
CIN	-	Cervical intraepithelial neoplasia

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INTRODUCTION

Lung cancer ranks among the most common and most lethal malignancies worldwide. It is rapidly emerging as a major cause of mortality in the Middle East, Africa, and Asia as well; and is increasingly being recognized in India[2]. Death rates attributable to this disease are expected to increase substantially over the next several decades[3].

Procedures used to diagnose bronchopulmonary neoplasms should be as accurate as possible and should provide optimum characterization of the tumor type. Fibreoptic bronchoscopic biopsy refers to the bronchoscopic technique of obtaining pulmonary parenchymal tissue for histological analysis. It is one of the common modality used to diagnose lung cancer and is the best technique for obtaining specimens to diagnose endobronchial lung carcinoma. The lesion can be visualized and the location of the specimen can be mapped precisely with a fibreoptic bronchoscope.

The histopathologic distinction of small cell from non–small cell carcinoma of lung is important therapeutically. However, such distinction may not always be straightforward based on morphologic findings alone, especially in cytologic specimens, but in tissue specimens as well.

p63 is a recently discovered member of the p53 family that has been shown to be important in the development of epithelial tissues. p63 may also play a role in squamous cell carcinomas of the lung, head and neck, and cervix,

and its expression is increased in these tumors[1]. The purpose of this study was to analyse the demographics, risk factors, distribution of lung carcinomas and to investigate the expression of p63 in various histologic types of lung tumors and correlate with histological gradings.

AIMS AND OBJECTIVES

1. To estimate the incidence of carcinomas in patients attending chest clinic.

2. To analyse the demographics, risk factors and distribution of lung carcinomas.

3. To study the expression of p63 in different histologic types of lung carcinomas.

4. To correlate histological grade with expression of p63 in fibreoptic bronchoscopic biopsy specimens.

REVIEW OF LITERATURE

Lung cancer is the most frequent and one of the most deadly cancer types, with more than 1.1 million deaths annually worldwide. In men, 85-90% of cases can be attributed to tobacco smoking. Despite innovations in diagnostic testing, surgical technique, and the development of new therapeutic agents, the five-year survival rate has remained about 13–15% throughout the past three decades[4].

Factors contributing to the low lung cancer survival rate include the small proportion of patients presenting with resectable disease and chemotherapy response rates ranging from 13–42% in patients with advanced stage disease[5].

Although the lung is an organ in which many histological types of malignant epithelial tumor can develop, about 95% of cancers occurring there are of four major histological types: squamous cell carcinoma, small cell carcinoma, adenocarcinoma and large cell carcinoma. The distinction between these four major types of lung carcinoma on routine bronchoscopic biopsies sometimes presents diagnostic problem. Materials obtained under fiberoptic bronchoscopic guidance are often very small and are sometimes crushed, particularly in the case of small cell carcinoma. Also, in small tissue fragments, differentiated tumor features (such as a glandular structure or keratinization) may not be presented. Histological diagnosis of large cell carcinoma is made

after the exclusion of squamous cell carcinoma, small cell carcinoma, adenocarcinoma, and other lung cancers of a specific type (especially main groups like large cell neuroendocrine carcinoma and carcinoids)[6]

NORMAL LUNG

The lung consists of airways, blood vessels, the connective tissue framework and the pleura[7].

Airways

Airways include the trachea, bronchi, bronchioli, and acini. The main bronchi branch dichotomously and give rise to gradually smaller and smaller bronchi. On an average there are 16 generations of bronchi and bronchioles before the first respiratory bronchioles are reached, but the number of generations varies approximately from 8 to 23 in different regions of lung.

The walls of the trachea and main bronchi are reinforced by C shaped rings of cartilage anteriorly and laterally with a sheet of transverse smooth muscle filling the gap posteriorly. As the successive generations of bronchi become smaller, they are reinforced by progressively smaller and fewer islands of cartilage. The smooth muscle continues as bundles that wind in a spiral down the intrapulmonary bronchi, which extend into gradually narrower and narrower airways.

The mucosa of the trachea and bronchi are lined by a pseudostratified ciliated columnar epithelium. By electron microscopy, four cell types can be recognized in the bronchial epithelium – ciliated cells, mucous cells, neuroendocrine cells and basal cells. Ciliated cells account for more than 90% of all bronchial cells. Mucous cells are filled with mucin rich granules. Their cytoplasm extends to the luminal surface. Neuroendocrine cells contain round neuroendocrine cytoplasmic granules.

The basal cells are small, triangular cells with relatively few organelles, which are attached to the basal lamina with hemidesmosomes and to the neighboring columnar cells by desmosomes.

The bronchial glands are located in the lamina propria of the bronchi. These glands are compound tubuloacinar structures composed of three cell types.

1) Mucous cells, the cytoplasm of which is filled with weakly basophilic secretory vacuoles;

2) Serous cells, the apical cytoplasm of which contains eosinophilic granules 1 to 2 micrometre in diameter.

3) Myoepithelial cells, which form a contractile network enclosing the glands. The myoepithelial cells resemble smooth muscle ultrastructurally and contain smooth muscle actin isoforms.

Bronchi extend into bronchioles. The walls of bronchioles are devoid of both cartilage and glands and consist of only smooth muscle and connective tissue. The simple columnar epithelium lining the bronchioli is composed mainly of ciliated epithelial cells interspersed with non – ciliated secretory cells called clara cells. These bronchioles divide and give rise to respiratory bronchioles. The epithelium of respiratory bronchiole is continuous with that of alveoli.

The alveoli are airspaces lined by thin walls composed of capillaries covered by epithelium and supported by a delicate mesh of connective tissue. The alveolar lining consists predominantly of type 1 epithelial cells, which cover 98% of alveolar surface. Interspersed between these cells are small cuboidal epithelial cells, type 2 cells. Type 2 cells are characterized by a microvillous surface and cytoplasmic lamellar bodies, which contain alveolar surfactant.

Pulmonary vasculature

The lungs have a dual blood supply: functional circulatory system comprising of pulmonary artery and its branches and the nutritional system originating from bronchial arteries. The pulmonary artery extends into branches that enter the lung accompanying the bronchi and run with them in the lung enclosed by a common sheath of adventitial connective tissue. The smallest branches of the pulmonary artery enter the acinus with the respiratory

bronchioles giving rise to precapillary arterioles, which accompany the alveolar ducts. The pulmonary veins lie at the periphery of the acini, often with connective tissue septa that divide the parenchyma into lobules. One vein drains two or more acini. The bronchial arteries originate from the thoracic aorta. The branches of the bronchial arteries run along the bronchial tree down to the level of respiratory bronchioles, whereupon they form capillaries that anastomose with the branches of the pulmonary artery.

Bronchus associated lymphoid tissue

Immune system of lung is represented by lymphoid nodules associated with the bronchioles, lymphnodes at the bifurcations of the bronchi and by lymphocytes, plasma cells and mast cells diffusely scattered through out the mucosa of the bronchi[8].

Pleura

The pleural investment of lung is composed of alternating layers of collagen and elastic fibers covered by a single layer of epithelium called mesothelium because of its mesodermal origin.

CLASSIFICATION OF LUNG TUMOURS

Tumors of the lungs can be classified clinically, histogenetically, or pathologically into several categories. Clinically, the tumors may be symptomatic or asymptomatic, benign or malignant. Histogenetically, the tumors may be classified according to their provenience, as originating from the bronchi, bronchioles, pulmonary connective tissue and blood vessels, or pleura. Pathologic classification may be based on microscopic features. On the basis of location, the tumors may be classified as central or peripheral, localized or diffuse, solitary or multiple.

WHO CLASSIFICATION OF PRIMARY LUNG TUMORS[9]

Epithelial tumors	Non- Epithelial tumors
A. Benign tumors 1. Papillomas 2. Adenomas a. Pleomorphic adenoma b. Monomorphic adenoma B. Dysplasia/Carcinoma in situ C. Malignant tumors 1. Squamous cell carcinoma (epidermoid carcinoma) 2. Small cell carcinoma a. Oat cell carcinoma b. Intermediate cell type c. Combined oat cell carcinoma 3. Adenocarcinomas a. Acinar adenocarcinoma b. Papillary adenocarcinoma c. Bronchioloalveolarcarcinoma d. Solid carcinoma with mucus formation 4. Large cell carcinoma variants a. Giant cell carcinoma b. Clear cell carcinoma 5. Adenosquamous carcinoma 6. Carcinoid tumor 7. Bronchial gland carcinomas a. Adenoid cystic carcinoma b. Mucoepidermoid carcinoma 8. Others	• Soft tissue tumors primary in lung • Pleural tumors A. Benign mesothelioma B. Malignant mesothelioma • Miscellaneous tumors A. Benign tumors B. Malignant tumors 1. Carcinosarcoma 2. Pulmonary blastoma 3. Malignant melanoma 4. Malignant lymphoma 5. Others • Unclassified tumors • Tumor like lesions

Most of the malignant tumors of the lower respiratory tract are called bronchogenic carcinomas because they originate from the epithelium of bronchi. They result from the malignant transformation of bronchial stem cells or their immediate descendents and are therefore composed of cells that are normally found in the bronchial epithelium. Tumors composed of ciliated or mucous cells are classified as adenocarcinoma; those composed of neuroendocrine cells, as small cell carcinomas; whereas those that are not committed or do not show such differentiation are classified as large cell carcinomas. Squamous cell carcinomas originate from foci of squamous metaplasia that are common in smokers.

SQUAMOUS CELL CARCINOMA

20% to 35% of all lung tumors are squamous cell carcinomas. Two thirds of squamous cell carcinomas are central tumors involving main and lobar bronchi. Gross appearance is not distinctive. The expectoration of keratinous or necrotic material may produce cavities within the tumor. Microscopic diagnosis depends on the identification of either intercellular bridges or keratinization. Keratinized cells are recognized by their brightly eosinophilic cytoplasm and pyknotic nuclei. The cells are arranged in nests that palisade at periphery of the lobules and become enlarged and flattened centrally. Whorls or eddies of cells may be keratinized (epidermoid pearls)[10].

ADENOCARCINOMA

Adenocarcinomas account for one third of all lung tumors. They appear as discrete masses, usually at the periphery. Microscopically, they are highly variable in appearance. According to the WHO classification, four subtypes are recognized. 1) acinic or glandular, 2) papillary, 3) bronchioloalveolar and 4) solid adenocarcinoma. Bronchioloalveolar carcinoma is the most common. It has a tendency to spread through the lungs along the existing air space walls, which serve as stroma for the tumor. The walls of alveoli, alveolar ducts are lined by malignant epithelial cells that vary in shape from cuboidal to columnar[11].

LARGE CELL CARCINOMA

Large cell carcinomas comprise 7% to 15% of lung carcinomas. They are composed of relatively large cells that lack specific features by which they could be assigned to either the squamous or adenocarcinoma group. There are two variants.

Giant cell carcinoma is a highly malignant form of undifferentiated carcinoma composed of huge, poorly cohesive cells with eosinophilic cytoplasm and one or several large convoluted nuclei. Clear cell carcinomas are composed of cells with clear cytoplasm that is rich in glycogen[12].

ADENOSQUAMOUS CARCINOMA

1% to 3% of lung carcinomas have clear evidence of both keratinization and glandular or secretory differentiation. Most are peripheral tumors associated with scars.

SMALL CELL CARCINOMA

Small cell carcinomas account for 15% to 20% of lung carcinomas. Grossly, they appear as fleshy encephalad tumors forming nodules or infiltrating lesions destroying the wall of a major bronchus. Microscopically, several patterns are recognized. The oat cell type consists of round or elongated poorly cohesive cells slightly larger than lymphocytes. The tumor cells have dark clumped chromatin and little cytoplasm. Necrosis is usually present. The intermediate cell type of small cell carcinoma is composed of cells slightly larger than those of the oat cell type and with somewhat better intercellular cohesion and organization into lobules[13].

CARCINOIDS

Bronchial carcinoids are tumors of low grade malignancy with neuroendocrine differentiation. The majority of carcinoids arise in central bronchi where they form a smooth endobronchial polypoid growth. Microscopically carcinoids are formed of uniform cells with a fairly abundant

finely granular cytoplasm and oval, centrally located nuclei with clumped chromatin[14].

MESENCHYMAL TUMORS

Mesenchymal tumors of the lung are rare. These tumors have the same histological features as their counterpart in the soft tissues.

MIXED EPITHELIAL – MESENCHYMAL TUMORS

According to WHO classification, carcinosarcoma is defined as a malignant tumor with an admixture of carcinoma and sarcoma.

LYMPHOMA

Primary pulmonary lymphoma accounts for less than 0.5% of all lymphomas. Most primary lymphomas of the lung originate from the mucosa associated lymphoid tissue (MALT) and present as slow growing solitary lesions.

MESOTHELIOMA

Mesotheliomas are tumors of pleura. They are subdivided into epithelial, sarcomatoid and biphasic tumors. Epithelial tumors may be indistinguishable from adenocarcinoma. Sarcomatous tumors have usually features of

fibrosarcoma or malignant fibrous histiocytoma. Biphasic tumors resemble synovial sarcoma.

MISCELLANEOUS RARE TUMORS AND TUMOR LIKE LESIONS

Rare tumors and tumor like lesions of the lung include pulmonary blastula, pulmonary endodermal tumor resembling fetal lung, primary malignant melanoma, malignant ependymoma, meningioma, malignant melanotic schwannoma, and many others.

METASTATIC TUMORS

Lungs are commonly involved by metastatic tumors, which originate from any site. Both carcinomas and sarcomas metastasize to lungs. Histologically many of these tumors are indistinguishable from primary pulmonary neoplasms.

FIBREOPTIC BRONCHOSCOPY

In the late 1890's Gustav Killian used a rigid tube to remove an impacted piece of bone from the right mainstem bronchus of an awake 63 year old man. Twenty years later, Chevalier Jackson popularized extensive examination and therapeutic interventions using rigid bronchoscopy. Jackson developed a rigid bronchoscope with a small light at its tip to illuminate the airways. His techniques were very effective; however they required specialized

training, and only a few physicians obtained the skills required to safely perform the procedures.

The advent of the flexible fibreoptic bronchoscope in 1970s revolutionized the field of brochoscopy. It was first introduced by Ikeda in 1967. Various models of fibreoptic bronchoscopes are produced by several manufacturers, and each model has particular advantages and limitations. These models differ in outer diameter from less than 4 mm to more than 6 mm and also in arc of bending from 60 to 180 degrees. Viewing angles vary from 66 to 100 degrees, and side channel diameters range from1.2 to 3.2 mm. Ultrathin fibreoptic bronchoscopes (1.8 to 2.2 mm) are also available, but their usefulness is still under evaluation[15].

INDICATIONS OF FLEXIBLE BRONCHOSCOPY

- Examination of airway to the subsegmental level
- Aspiration of secretions
- Mucosal brushings
- Biopsy of endobronchial lesions
- Development of expandable airway stents
- Removal of small foreign bodies
- Transbronchial needle biopsy

PROCEDURE

General considerations

- Complete review of medical and surgical history of the patient
- Identification of medical issues such as bleeding dyscrasias or significant allergy to an anesthetic agent
- All recent radiographic studies should be reviewed
- Patient must be informed about risks and benefits of the procedure

Awake fibreoptic bronchoscopy

Oxygen is provided either via a nasal canula or by face mask with an opening to allow for the passage of bronchoscope. Monitoring should include pulse oximetry and heart at a minimum. Intravenous line must be started. Bronchoscopy carts are maintained. These carts are stocked with a flexible bronchoscope, a light source, suction tubing, bite blocks, oxygen masks, local anesthetics, pulse oximetry and emergency airway equipment. A standard adult bronchoscope with an external diameter of 5.9 mm used for these procedures. Adequate topical anesthesia is essential.

Flexible bronchoscopy under general anesthesia

It is performed in operating room in conjunction with an anesthesiologist. Monitoring includes pulse oximetry, non – invasive blood pressure monitoring and three lead electrocardiogram monitoring. The

endotracheal tube used is of 8 mm in diameter. This tube allows ventilation via bronchoscope during use of a standard 5.9 mm diameter bronchoscope.

Operation

Awake fibreoptic bronchoscopy

The patient is placed in a bed and back elevated to 60 degrees. Pulse oximetry and heart rate monitoring is begun. Supplemental oxygen is provided by a face mask with a hole cut in it to allow passage of the bronchoscope. Topical anesthesia is provided. Once the oral pharynx, vocal cords and airway have been completely anesthetized, a bite block is placed in the mouth and the bronchoscope is introduced into the oral pharynx. The patient is asked to take deep breath opening the vocal cords and the bronchoscope is passed into the proximal trachea. The observer is oriented by noting the posterior longitudinal muscle along the membranous portion of the trachea. The carina is located and a systematic examination of the airway is carried out down to the subsegmental level. Samples are obtained from suspected lesions.

Fibreoptic bronchoscopy under general anesthesia

The patient is brought to the operating room and general anesthesia is induced. Direct laryngoscopy is performed and an 8 mm endotracheal tube is placed. The endotracheal tube is connected to the ventilator through a bronchoscopy adapter. The surgeon stands at the head end of the table. Flexible bronchoscopy is carried out. Samples are collected[16].

Complications

Complications are low. Bleeding dyscrasias should be addressed prior to the procedure, especially if biopsy is planned. A high percentage of complications surrounding awake flexible bronchoscopy is related to preprocedural intravenous sedation. This can be avoided by proper application of local anesthesia. Significant hypoxia must be avoided[17].

Histopathological grading:

Squamous cell carcinoma:

Grading of squamous cell carcinoma into well, moderately and poorly differentiated types will depend on the degree of squamous differentiation within the tumor such as the presence of intercellular bridges and keratinization. Well differentiated tumors are characterized by sheets of cells which adapt a pavement like architecture and contain ample eosinophilic cytoplasm, round to oval nuclei and prominent nucleoli. The cell borders are well defined and show well formed intercellular bridges. In the less differentiated tumor, the above features may be focally observed and the lesions are characterized by more pronounced cytologic atypia, increased mitotic activity and frequent areas of necrosis and hemorrhage.

Broder's Classification of Squamous cell carcinoma:

1	Well differentiated (Grade I)	< 25% undifferentiated cells
2	Moderately differentiated (Grade II)	< 50% undifferentiated cells
3	Poorly differentiated (Grade III)	<75% undifferentiated cells
4	Anaplastic/pleomorphic (Grade IV)	>75% undifferentiated cells

Adenocarcinoma:

Grading of adenocarcinoma into well, moderately and poorly differentiated tumors depend on the degree and extent of glandular differentiation. Well differentiated neoplasms are characterized by proliferation of well formed glands lined by atypical cells that infiltrate the surrounding stroma whereas poorly differentiated tumors grow as solid sheets of tumor cells with scant or poorly formed glandular structures and are recognized as adenocarcinomas on the basis of demonstration of intra cellular mucin production.

p63

p63 gene is structurally similar to p53 gene, consisting of 5’ region that codes for a protein moiety that activates transcription of same genes activated by p53, a central sequence coding for a DNA binding region, and a3’ sequence coding for an oligomerization – promoting region.

The p63 gene codes for 6 protein isoforms based on alternate splicing and the existence of 2 promoters, one conventional, the other a biologically active internal promoter that generates truncated p63 proteins that fail to activate transcription and act as dominant negative blockers of p53 protein actions.

p63 has been postulated to have a critical role in maintaining the balance between basaloid stem cell commitments to undergo amniotic differentiation versus retention of a dividing undifferentiated stem cell phenotype.

To date p63 expression has been demonstrated in basal layers of squamous epithelia, urothelium, basal layers of prostate gland epithelia, myoepithelial cells of, submucosal gland epithelia and in the basal reserve cells of ciliated bronchial epithelia.

p63 is hypothesized to play an important role in maintaining the epidermal stem cell population. Immunohistochemical analyses show p63 protein localization and expression in basal/progenitor cells of several epithelial tissues such as epidermis mammary glands, prostate and urogenital tract. p63 expression is lost as these cells migrate from the basal layer and become terminally differentiated cells.

As with **Pierre P. Massion et.al,** in invasive carcinomas, p63 staining was scored 0–4 based on intensity[19]. In preinvasive lesions, p63 was scored based as follows:

S. No	Score	Characterized by
1	0	No staining
2	1	Basal layer staining
3	2	Basal and parabasal layer staining
4	3	Full thickness staining
5	4	Invasion of basement membrane

Charles J. Di como et al, (feb, 2002) in their study examined the expression pattern of p63 in human normal and tumor tissues by immunohistochemistry using a monoclonal antibody that recognizes all p63 spilce variants, and by reverse transcription-PCR using isoform-specific primers. They observed that the expression was restricted to epithelial cells of stratified epithelia, such as skin, esophagus, exocervix, tonsil, and bladder, and to certain subpopulations of basal cells in glandular structures of prostate and breast, as well as in bronchi. They found that p63 is expressed predominantly in basal cell and squamous cell carcinomas, as well as transitional cell carcinomas, but not in adenocarcinomas, including those of breast and prostate. Thymomas and a subset of Non-hodgkin’lymphomas were also found to

express p63. p63 was not found to be expressed in endocrine tumors, germ cell neoplasms, or melanomas. Soft tissue sarcomas were found to have undetectable p63 levels[18].

Pierre P. Massion et al, (nov, 2003) analysed p63 gene copy number by fluorescence in situ hybridization and expression by immunohistochemistry in tissue microarrays of 217 non-small cell lung carcinomas and correlated them with survival. They also analysed p63 copy number and protein expression in 41 preinvasive squamous lesions. The p63 genomic sequence was amplified in 88% of squamous carcinomas, in 42% of large cell carcinomas, and in 11% of adenocarcinomas of the lung. They also found p63 genomic amplification and protein staining intensity associated with better survival. They found a significant increase in p63 in preinvasive lesions graded severe dysplasia or higher. They demonstrated that there is early and frequent genomic amplification of p63 in the development of squamous cell carcinoma of the lung and that patients with non small cell lung carcinoma showing amplification and over expression of p 63 have prolonged survival. These observations suggest that p63 genomic amplification has an early role in lung tumorigenesis[19].

Claudia Auw-Haedrich et al, (2006) investigated the expression of p63 in conjunctival intraepithelial neoplasia of different grades and conjunctival squamous cell carcinoma and its correlation to the proliferation marker MIB-1. They took seventeen conjunctival specimens excised with a suspicion of either

conjunctival intraepithelial neoplasia or squamous cell carcinoma and diagnosed histologically as: squamous cell carcinomas of conjunctiva, CIN grade 1, CIN grade 2, CIN grade 3, normal conjunctiva with no dysplasia. Sixteen microscopically normal post mortem conjunctival specimens were also also taken. All the specimens were stained immunohistochemically with antibodies against p63 and MIB-1. atleast 500 cells per specimen were counted and the percentage of positively stained cells of each antibody was calculated. Their results showed that a mean of 80% of the dysplastic cells from the CIN specimens stained positively with antibodies against p63, especially in the lower two thirds of the epithelium, statistically significantly more when compared with the normal specimens. They did not find a correlation between the percentage of p63 positive cells and the differentiation grade of the malignant specimens. MIB-1 positivity was seen in 0-1% of cells in the normal postmortem controls, 3-30% cells in the basal and occasionally in the middle layer of the CIN specimens and 16-61% in the carcinoma specimens. They concluded that p63 was preferentially expressed in the immature dysplastic epithelial cells. Its staining does not correlate with MIB-1 expression and therefore does not appear to be linked to cell proliferation[20].

P Taniere et al, (2001), studied the amplication of p63 in squamous cell carcinoma of esophagus from a low incidence area in Western Europe and compared with p63 amplification in areas with high incidence of squamous cell carcinomas of esophagus. Results indicated that squamous cell carcinomas of

esophagus from areas of high and low incidence present with similar pattern of p63 amplification but differ by the type of TP53 mutations[21].

Hina A Sheikh et al, (2004) studied, 33 cases of adenocarcinoma and 43 cases of benign lungs with fibrosis and metaplasia for nuclear p63 expression by immunohistochemistry. Five additional cases each of atypical adenomatous hyperplasia and adenosquamous carcinoma and three cases of squamous cell carcinomas were also stained. The diagnostic categories of benign lung conditions were usual interstitial pneumonia, parenchymal scar, cryptogenic organizing pneumonia and diffuse alveolar damage. In neoplastic cases, p63 positivity was calculated as percentage of all tumor cells examined. In areas of normal lung, p63 decorated the reserve cells of large and small airways and occasional cells of the distal lobular unit. In fibrotic reactive processes, an interrupted but distinct pattern of nuclear staining was present in all cases, with staining of basal cells of the airways as well as bronchiolar- and squamous-metaplastic epithelium (43/43, 100%). p63 immunoreactivity was less uniform in areas of acute lung injury within these cases. One adenocarcinoma and two cases of atypical adenomatous hyperplasia showed strong immunoreactivity (>80%), while three adenocarcinomas highlighted only rare tumor nuclei (<5% of tumor cells). Morphologic areas where p63 immunostaining was not helpful included the junction of normal lung and lepidic growth of adenocarcinoma, and retrograde spread of adenocarcinoma into small airways. Their results highlight the differential expression of p63

across various bronchioloalveolar lesions. They concluded that p63 may be helpful in distinguishing reactive from neoplastic glandular proliferations in the lung[22].

Hu NH et al (2004), investigated the expression of p63 in a broad spectrum of histologic types of lung tumors. A total of 441 cases of primary lung tumors with follow-up data were identified, and the paraffin-embedded tissue blocks were used to construct a duplicate core tissue microarray. After review of the tissue cores, 408 cases, consisting of 123 squamous cell carcinomas, 93 adenocarcinomas, 68 large cell carcinomas, 68 classic carcinoids, 31 atypical carcinoids, 11 large cell neuroendocrine carcinomas, and 14 small cell carcinomas, were adequate for analysis. Immunohistochemistry was performed to detect the expression of p63, using different staining protocols. p53 expression was also studied with immunohistochemistry. A large proportion of squamous cell carcinomas expressed p63 (96.9%), most showing strong positive nuclear immunoreactivity. Expression in other non small cell lung cancers was also present. 30% of adenocarcinomas and 37% of large cell carcinomas showed p63 expression. In the neuroendocrine tumors, an increasing proportion of tumors stained for p63 as tumor grade increased; 1.9% of classic carcinoids, 30.8% of atypical carcinoids, 50% of large cell neuroendocrine carcinomas, and 76.9% of small cell carcinomas were positive. Approximately half of the positively staining neuroendocrine cases showed strong staining. Expression of

p63 was of prognostic significance in neuroendocrine tumors, with higher-grade tumors more likely to express p63. Correlation between p63 and p53 expression was not observed in non small cell lung cancers; however, a significant correlation between the 2 markers was found in neuroendocrine tumors. p63 staining was repeated with a different staining protocol, yielding similar results overall but a lower percentage of positive cases (34.2% vs. 48.4% of tumors positive). They concluded that p63 expression is consistently expressed in squamous cell carcinoma in the lung, but is also expressed in a subset of adenocarcinomas and large cell carcinomas. Pulmonary neuroendocrine tumors also show p63 staining in some instances, particularly in higher-grade tumors, and the majority of small cell carcinomas are p63-positive[23].

Jorda M et al, (2005) studied the value of p63 immunocytochemical analysis in classification of nonsmall cell carcinomas into squamous and non squamous subtypes. They considered 51 consecutive pulmonary specimens (16 fine needle aspiration samples, 15 washes, 12 brushes, and 8 lavages) with the diagnosis of nonsmall cell lung carcinoma (9 carcinomas with squamous differentiation and 42 carcinomas without squamous differentiation). Histologically, they all proved to be nonsmall cell carcinomas, 26 with squamous differentiation and 25 without squamous differentiation. p63 immunocytochemical stain was performed. 23 (88 %) of the 26 histologically proven squamous cell carcinomas were positive for p63 on cytologic smears.

By using p63 immunocytochemistry, they detected 14 carcinomas with squamous differentiation not identified by cytomorphology. Smears from all histologically proven carcinomas with squamous differentiation were positive for p63. Sensitivity of cytology for the detection of non small cell carcinoma of lung with squamous differentiation increased from 35% to 88% using p63 immunocytochemistry. The squamous component in 4 carcinomas was detected only in cytologic and not in corresponding histologic samples when subsequent p63 immunostaining was performed. They concluded that p63 is a useful marker for the detection of non small cell carcinomas of lung with squamous differentiation when used in cytologic pulmonary samples. p63 immunocytochemistry significantly increases the sensitivity for the identification of lung neoplasms with squamous differentiation from 35% to 88%. Therefore, p63 immunocytochemistry may be used in pulmonary cytologic samples of non small cell carcinomas to identify squamous differentiation and to improve therapeutic selection of patients with lung cancer[24].

Viktor Shtilbans Ph.D, et al, (2005) attempted to detect p63 in destained slides from a spectrum of pulmonary malignancies (small biopsies and cytological cell blocks). 60 cases of cytologically diagnosed malignancies in bronchoscopically or fine-needle aspiration-obtained specimens were immunostained with p63. Normal ciliated and goblet cells were p63 negative, but reserve cells were p63 positive. All cases of squamous-cell carcinoma were

positive for p63. Of 10 tumor samples originally diagnosed as Squamous cell carcinomas, only 6 samples were p63 negative and 4 samples exhibited positive staining. However, proper interpretation of the immunohistochemical staining pattern and careful scrutiny of the cytological features and biopsy specimens in three of four cases led them to reclassify three cases into poorly differentiated squamous cell carcinomas. All adenocarcinomas, large-cell carcinomas, and metastatic adenocarcinomas were p63 negative. Positive staining was seen in 9/16 tumors designated as non-squamous cell carcinomas; these tumors were not classified further into distinct histological categories[25].

Esther conde et al (2010), in their study confirmed a previous microarray study, in which they found that one of the top differentially expressed genes between adenocarcinomas and squamous cell carcinomas is p63. They analysed the value of P63 immunohistochemistry in reducing the number of large cell carcinoma diagnoses in surgical specimens. They investigated the potential of P63 IHC to minimize the proportion of carcinoma NOS (not otherwise specified) in a prospective series of small tumor samples. They studied p63 staining of 33 adenocarcinomas, 99 squamous cell carcinomas, 20 large cell carcinomas and 32 carcinoma NOS. They proved that P63 IHC was differentially expressed in Squamous Cell Carcinomas when compared to Adenocarcinomas[26].

Thus from the above studies it is understood that p63, a recently discovered member of the p53 family has been shown to be important in the

development of epithelial tissues. It does not correlate with the expression of proliferation markers and thus it is not a cell proliferation marker. p63 plays a role in squamous cell carcinomas of the lung, head and neck, and cervix, and its expression is increased in these tumors. p63 plays a major role in lung tumorigenesis. It is used to identify lung tumors with squamous differentiation. It can also be used in pulmonary cytologic samples of nonsmall cell carcinomas to identify squamous differentiation.

In this study, the expression of p63 in varying grades and histologic types of lung malignancies in fibreoptic lung biopsy specimens at Department of Pathology, Stanley Medical College has been studied. p63 expression in various lung carcinomas in our population is then correlated with the above studies.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry involves two disciplines – immunology and histology. Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. These differences can specifically identify the lineage of cell population and define biologically distinct populations of cells within the same lineage.

Immunohistochemistry started in 1940 when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and colleagues in 1974 showed it was possible to demonstrate antigens in routinely processed tissues. Antigen retrieval technique was introduced by Shi and associates in 1991. Antigen retrieval technique is a simple method that involves heating paraffin processed sections at high temperature before IHC staining.

The use of antibody in IHC depends on the sensitivity and specificity of the antigen – antibody reaction and the Hybridoma technique provides limitless source of highly specific antibodies.

Blocking non – specific background staining

Background staining is due to either non specific binding or presence of endogenous enzymes. Non specific binding with polyclonal primary antibody is minimized by pre incubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature (almost complete abolition of endogenous peroxidase activity). Endogenous alkaline phosphatase is blocked by addition of 0.1 M concentration of levamisole to the enzyme substrate solution.

Detection systems

Antibodies are labeled or flagged by some method to permit visualization – these include fluorescent substances, enzymes forming colored reaction with suitable substrate (light microscopy) or heavy metals (electron microscopy).

Methods of IHC

Direct labeling method

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of multiple antigens which require separate incubation with respective antibodies.

Indirect labeling method

Enzymes are labeled with the secondary antibody, which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versality, higher working dilution of primary antibody, secondary antibodies against primary antibodies of different species and easy to prepare.

Avidin biotin techniques

High affinity binding between biotin and avidin is used in this procedure. Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme. The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantages of this technique is that the endogenous biotin produces non specific background staining.

Avidin biotin conjugate procedure

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse raddish peroxidase conjugate. This is a more sensitive method.

Biotin streptavidin system

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

Immunogold silver staining technique

This is used in ultrastuctural immunolocalisation. Gold particles are enhanced by the addition of several layers of metallic silver. The fine silver deposits in the background create confusion when small amounts of antigen are identified.

Polymeric method

This technique permits binding of large number of enzyme molecules to a secondary antibody via the dextran backbone. Advantages of this technique are increased sensitivity, minimized non specific background staining and a reduction in the total number of assay steps.

Tissue fixation, processing and antigen retrieval techniques

Tissues for IHC undergo fixation, dehydration and paraffin embedding.

Fixation

This is a critical step as the preservation of morphology is essential for interpretation of IHC. 10% buffered neutral formalin is commonly used because of the following advantages.

1. Good morphological preservation
2. Cheap
3. Sterilizes tissues
4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrieval techniques.

Antigen retrieval

This procedure involves unmasking of the antigens. Following techniques can be used.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Microwave and trypsin antigen retrieval technique
4. Pressure cooker antigen retrieval

MATERIALS AND METHODS

Source of the data

A total of 105 fibreoptic lung biopsy specimens were received in the Department of Pathology, Stanley medical college from Government Hospital of Thoracic Medicine, Tambaram Sanatorium during the year July 2008 to September 2010.

Study design & Plan

Longitudinal prospective and retrospective study.

The patients who were included in this study were screened for predetermined inclusion and exclusion criteria. Selected patients underwent through consent protocols. Brief clinical history and examination was done with predetermined proforma.

Inclusion Criteria

All patients undergoing fibreoptic endoscopic biopsies, irrespective of age and sex were included for the study

Exclusion Criteria

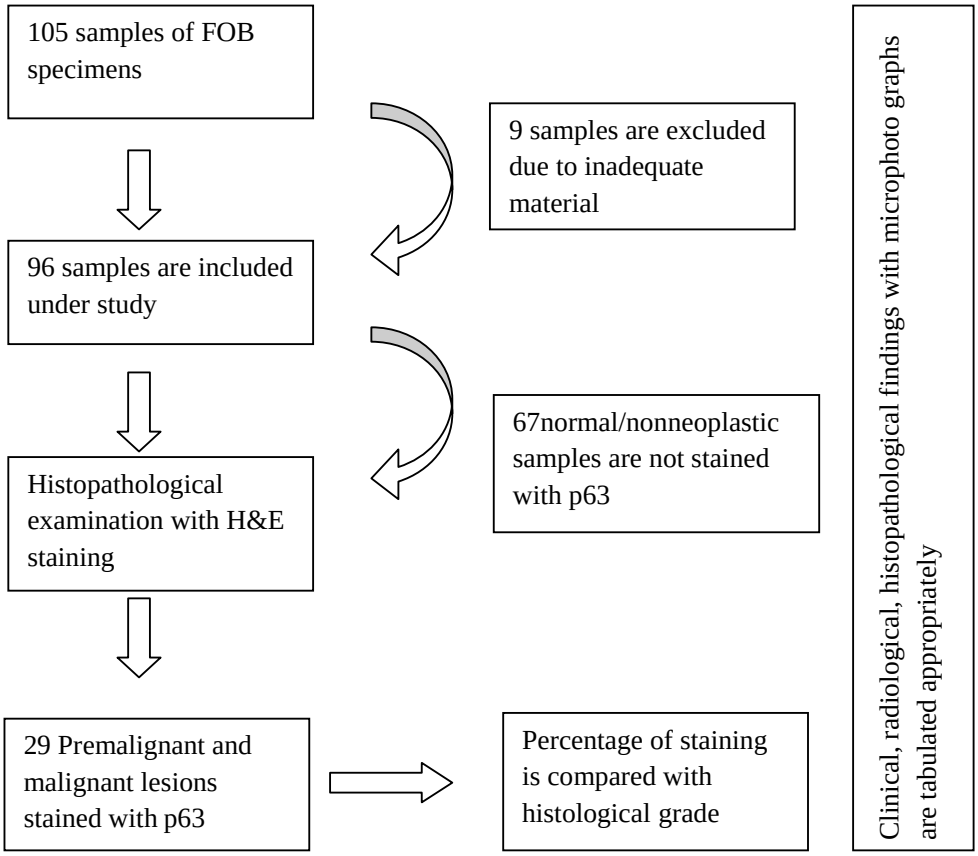
Patients with inadequate biopsy material were excluded from the study.

Methods of data collection

Out of 105 cases adequate material was obtained in about 96 cases. Clinical history, radiological and necessary hematological findings were tabulated.

The tissues obtained by FOB were processed and sections were cut at 5 microns. Haematoxylin and eosin staining of the sections was done and histopathological diagnosis was made. Necessary microphotographs were taken.

The various premalignant and malignant lesions were graded histopathologically. Immunostaining of the p63 was studied in all neoplastic lesions. Percentage of p63 staining correlated with histological grade. Results were tabulated and conclusion was drawn.


105 samples of FOB specimens
9 samples are excluded due to inadequate material
96 samples are included under study
67normal/nonneoplastic samples are not stained with p63
Histopathological examination with H&E staining
29 Premalignant and malignant lesions stained with p63
Percentage of staining is compared with histological grade
Clinical, radiological, histopathological findings with microphoto graphs are tabulated appropriately

Flow chart

Method of tissue preparation for IHC

10% buffered formalin was used for fixing the specimens, the tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and sections of 5 microns thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using microwave technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HPR (horse raddish peroxidase) polymer technique.

HPR polymer technique

The coated slides were taken through the following stages

1. Treatment with peroxidase block – for inhibiting endogenous peroxidases in the tissue for 20 minutes
2. Wash in TRIS buffer for 5 minutes
3. Application of power block – blocks non specific antigen antibody reaction – 20 minutes
4. Blot dry the excess power block
5. Application of primary antibody for 60 minutes

6. Wash in TRIS buffer for 5 minutes thrice
7. Application of super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction
8. Application of SS label – secondary antibody from goat with the tagged horse radish peroxidase enzyme for 30 minutes
9. Wash thrice in TRIS buffer
10. Application of DAB (diamino benzidine) chromogen for 5 minutes – this is cleaved by the enzyme to give the coloured product at the antigen sites
11. Wash in distilled water for 5 minutes
12. The slides are counterstained with hematoxylin
13. Air dried and mounted with DPX (distrene dibutyle pthalide in xylol)

p63 nuclear staining pattern and staining intensity ranged from strong to moderate to weak. Staining was focal rather than extensive and confluent. Extent of staining varied from multiple positive foci to scattered or rare foci.

METHODS OF SCORING

Included measurements of the intensity of staining or percentage of positive cells or a combination of the two.

SCORE FOR INTENSITY OF STAINING OF p63

0 – no staining

1 – weak staining

2 – moderate staining

3 – strong staining

This would give a maximum score of 300 if 100 percent of tumor cells show strong positivity.

SCORE FOR PROPORTION OF POSITIVE CELLS

0 – less than 5% positive nuclei

1 – 5 to 25% positive nuclei

2 – 26 to 75% positive nuclei

3 – over 75% positive nuclei

PERCENTAGE METHOD

Positivity of cells was defined regardless of staining intensity. More than 10% of positive cells represented the cut off between negativity and positivity.

OBSERVATION AND RESULTS

A total of 96 cases were studied. Morphology showed the following results.

NORMAL	**45**
NON NEOPLASTIC LESIONS	**22**
Non specific inflammation	8
Chronic inflammation	9
Granulomatous lesion	3
Other infections (candidiasis, aspergillosis)	2
PRE NEOPLASTIC LESIONS	**6**
Mild dysplasia (fig. 1,2)	2
Moderate dysplasia(fig. 3,4)	3
Severe dysplasia(fig. 5,6)	1
NEOPLASTIC LESIONS	**23**
Squamous cell carcinoma(fig. 7,8,9,10)	12
Adenocarcinoma(fig. 11,12)	8
Bronchioloalveolar carcinoma (mucinous type)	1
Bronchioloalveolar carcinoma (non mucinous type)	1
Large cell carcinoma	1

AGE AND SEX DISTRIBUTION OF PATIENTS UNDERGOING FIBREOPTIC LUNG BIOPSY

TABLE NO 1

Age	Non Malignant			Pre Malignant			Malignant			Total		
	M	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**
21 TO 30	4	4	8	0	0	0	0	0	0	4	4	8
31 TO 40	7	3	10	0	0	0	2	0	2	9	3	12
41 TO 50	19	1	20	2	0	2	6	1	7	27	2	29
51 TO 60	20	1	21	1	0	1	5	0	5	26	1	27
61 TO 70	8	5	13	2	0	2	6	1	7	16	6	22
71 TO 80	2	2	4	1	0	1	0	1	1	3	3	6
81 TO 90	0	0	0	0	0	0	1	0	1	1	0	1
TOTAL	60	16	76	6	0	6	20	3	23	86	19	105

AGE AND SEX DISTRIBUTION OF LUNG MALIGNANCIES

TABLE NO 2

Age	Squamous Cell Carcinoma			Adeno Carcinoma			Others			Total		
	M	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**
21 TO 30	0	0	0	0	0	0	0	0	0	0	0	0
31 TO 40	2	0	2	0	0	0	0	0	0	2	0	2
41 TO 50	3	0	3	2	0	2	1	1	2	6	1	7
51 TO 60	2	0	2	2	0	2	1	0	1	5	0	5
61 TO 70	4	0	4	2	1	3	0	0	0	6	1	7
71 TO 80	0	0	0	0	1	1	0	0	0	0	1	1
81 TO 90	1	0	1	0	0	0	0	0	0	1	0	1
TOTAL	12	0	12	6	2	8	2	1	3	20	3	23

Age range of patients undergoing fibreoptic lung biopsies was from 21 to 85. The age range of squamous cell carcinoma was from 35 to 85 and adenocarcinoma was from 45 to 75.

Both squamous and adenocarcinomas occurred in males predominantly.

CLINICAL PRESENTATION OF LUNG MALIGNANCIES
SQUAMOUS CELL CARCINOMA
TABLE NO 3

CLINICAL PRESENTATION	NO OF CASES	%
COUGH	7	58.33%
HEMOPTYSIS	4	33.33%
BREATHLESSNESS	10	83.33%
LOSS OF WEIGHT	11	91.67%
CHEST PAIN	6	50%

ADENOCARCINOMA
TABLE NO 4

CLINICAL PRESENTATION	NO OF CASES	%
COUGH	6	75%
HEMOPTYSIS	0	0%
BREATHLESSNESS	5	62.50%
LOSS OF WEIGHT	8	100%
CHEST PAIN	3	37.50%

Cough, breathlessness and weight loss were the most common clinical presentation of both squamous and adenocarcinoma.

ASSOCIATION OF MALIGNANCIES WITH SMOKING

SMOKING ASSOCIATION IN NON MALIGNANT AND MALIGNANT LESIONS

TABLE NO 5

	H/O SMOKING	NO H/O SMOKING	TOTAL
MALIGNANT LESIONS	17	12	29
NON MALIGNANT LESIONS	42	34	76
TOTAL	57	48	

Yates corrected chi square – 0.0081; p value – 0.9282(not significant)

SMOKING ASSOCIATION IN SQUAMOUS AND NON SQUAMOUS MALIGNANCIES

TABLE NO 6

	H/O SMOKING	NO H/O SMOKING	TOTAL
SQUAMOUS CELL CARCINOMAS	9	3	12
NONSQUAMOUS CELL CARCINOMAS	3	8	11
TOTAL	12	11	

Yates corrected chi square – 3.5010; p value – 0.0613(not significant).

Squamous cell carcinomas were most commonly associated with history of smoking which is statistically insignificant probably due to reduced sample size.

IMAGING FINDINGS

SQUAMOUS CELL CARCINOMA

TABLE NO 7

XRAY FINDINGS	NO OF CASES	%
HILAR MASS	5	41.65%
CONSOLIDATION	3	25%
OPACITY	1	8.33%
CAVITY	1	8.33%
RIB METASTASIS	1	8.33%
NORMAL	1	8.33%

ADENOCARCINOMA

TABLE NO 8

XRAY FINDINGS	NO OF CASES	%
OPACITY	5	62.50%
CONSOLIDATION	1	12.50%
HILAR PROMINANCE	1	12.50%
PLEURAL EFFUSION	1	12.50%

Most common x ray finding of squamous cell carcinomas was found to be hilar mass. Other less common findings are consolidation, opacity and cavity formation. Most common x ray finding of adenocarcinomas was found to be opacity. Other less common findings are consolidation, hilar prominence, pleural effusion.

FIBREOPTIC BRONCHOSCOPIC FINDINGS

SQUAMOUS CELL CARCINOMA

TABLE NO 9

FOB FINDINGS	NO OF CASES	%
GROWTH	10	83.33%
UNHEALTHY MUCOSA	1	8.33%
NECROTIC MATERIAL	1	8.33%

ADENOCARCINOMA

TABLE NO 10

FOB FINDINGS	NO OF CASES	%
GROWTH	6	75%
NARROWING OF BRONCHUS	2	25%

Most of the carcinomas (squamous and adeno) presented with endobronchial growth at fibreoptic bronchoscopy.

p63 EXPRESSION IN MALIGNANCIES

TABLE NO 11

S.NO	BIOPSY NO	HISTOLOGICAL TYPE	p63 SCORE
1	1157/09	SEVERE DYSPLASIA	3
2	1367/09	MODERATE DYSPLASIA	2
3	1368/09	POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	0
4	1465/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	3
5	5676/09	ADENOCARCINOMA	0
6	5677/09	ADENOCARCINOMA	0
7	2429/09	ADENOCARCINOMA	0
8	2702/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	2
9	2860/09	LARGE CELL CARCINOMA	0
10	2861/09	ADENOCARCINOMA	0
11	3430/09	BRONCHIOLOALVEOLAR NONMUCINOUS CARCINOMA	0
12	4025/09	ADENOCARCINOMA	0
13	4321/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	3
14	4454/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1
15	4455/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	3
16	4603/09	MILD DYSPLASIA	1
17	4936/09	POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1

S.NO	BIOPSY NO	HISTOLOGICAL TYPE	p63 SCORE
18	5033/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1
19	5138/09	MILD DYSPLASIA	1
20	5515/09	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1
21	405/10	BRONCHIOLOALVEOLAR MUCINOUS CARCINOMA	0
22	624/10	MODERATE DYSPLASIA	2
23	625/10	MODERATE DYSPLASIA	2
24	791/10	ADENOCARCINOMA	0
25	1039/10	ADENOCARCINOMA	0
26	2030/10	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	2
27	2068/10	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	3
28	1041/10	MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1
29	2027/10	ADENOCARCINOMA	0

p63 EXPRESSION IN SQUAMOUS AND NON SQUAMOUS MALIGNANCIES

TABLE NO 12

p63 EXPRESSION	POSITIVE	NEGATIVE	TOTAL
SQUAMOUS CELL CARCINOMAS	11	1	12
NON SQUAMOUS CELL CARCINOMAS	0	11	11
TOTAL	11	12	23

Yates corrected chi-Square 12.5484; p value – 0.0004(significant)

Most of the preinvasive and invasive lesions with squamous differentiation showed nuclear positivity for p63(fig.13 to 18). Other carcinomas were negative for p63(fig.19)

p63 SCORE IN PRE INVASIVE LESIONS

TABLE NO 13

p63 SCORE	0	1	2	3	TOTAL
MILD DYSPLASIA	0	2	0	0	2
MODERATE DYSPLASIA	0	0	3	0	3
SEVERE DYSPLASIA	0	0	0	1	1
TOTAL	0	2	3	1	6

p63 score showed progressive increase through mild to severe dysplasia. p value cannot be determined due to the smaller sample size.

p63 SCORE IN SQUAMOUS CELL CARCINOMAS

TABLE NO 14

p63SCORE	0	1	2	3	TOTAL
MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	0	3	2	5	10
POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA	1	1	0	0	2
TOTAL	1	4	2	5	12

p63 score ranged from 0 to 3 in different histologic grades of squamous cell carcinomas. p value cannot be determined due to the smaller sample size.

DISCUSSION

Lung cancer is the most common fatal malignancy in men and women. Lung cancer has a peak incidence in the group 50 to 70 years of age[27] whereas in this study, the age range of lung malignancies was found to be 35 to 85 and peak incidence was around 40 to 70 years of age. Lung cancer is more common in males than in females. In this study also it is more common in males. This may be due to less use of medical services by females in our population[28].

Local and systemic symptoms of most lung cancers are related to mass effect of the tumor. Local symptoms include cough, hemoptysis, dyspnoea and chest pain. Systemic symptoms include weight loss, cachexia and pain attributable to metastases[29]. In concordance with literature, the most common clinical presentations in this study were found to be cough, breathlessness and weight loss.

All cell types of lung cancer are associated with smoking. The strongest associations are with small cell and squamous cell carcinomas[31]. Similarly, in this study, squamous cell carcinomas were found to be most commonly associated with history of smoking.

Performing a chest radiograph is the first step if a patient reports with symptoms that may suggest lung cancer. This may reveal an obvious mass, widening of the mediastinum (suggestive of spread to lymph nodes there),

atelectasis (collapse), consolidation (pneumonia), opacity, cavity, pleural effusion[30]. Accordingly, in this study, the most common x ray findings were found to be hilar mass, opacity and consolidation.

Tissue needs to be obtained to confirm the diagnosis of lung cancer. Flexible bronchoscopy is an invasive, nonsurgical approach used to obtain tissue. Flexible bronchoscopy has a high diagnostic yield for endoscopically visible lesions. Most common findings at fibreoptic bronchoscopy were growth, purulent secretions, inflamed mucosa and necrotic material[32]. In this study also, the most common findings at fibreoptic bronchoscopy were growth, unhealthy mucosa, necrotic material and narrowing of bronchus.

The incidence of lung cancer seems to be on increase in Asia and many other parts of the world and currently death caused by lung cancer is the leading cause of neoplasia related mortality world wide[33]. This may be due to lack of early detection strategies in diagnosis of lung tumors. Genomic abnormalities may play a major role in malignant transformation and tumor progression[34].

p63 is a transcription factor that transactivates p53 target genes [35] and induces apoptosis when expressed in cells [36]. Although p63 was recently discovered, it is the most ancient member of the p53 family [37].

p63 genomic sequence is found in chromosome 3q27. Chromosome 3q26-ter amplification, which includes the *p63* gene locus, is one of the most

prevalent genomic abnormalities in solid tumors and is likely to play a critical role in tumorigenesis. Amplification of chromosome 3q has been described in squamous epithelial transformation from the lung [39], head and neck [40,41], esophagus [42], bladder [43], cervix [44,45], and stomach [46]. It is demonstrated that the presence of 3q amplification alone allows the distinction between squamous and adenocarcinoma in more than 75% of cases [47].

p63 functions as a potent transcriptional repressor and dissociates from promoter binding sites of key growth inhibitory genes during normal human keratinocyte differentiation[38]. Thus, *p63* gene overexpression may have important implications in lung tumorigenesis.

In this study, fibre optic lung biopsies received at the Department of Pathology, Stanley Medical College were analysed and expression of p63 was studied in varying histologic types and grades of lung carcinomas.

A total of 105 specimens were received during the period of July 2008 to September 2010. 96 samples had adequate material and were included in the study.

29 cases were diagnosed to be preinvasive and invasive lesions.

There were 6 pre invasive lesions (mild, moderate and severe dyplasias), 12 squamous cell carcinomas, 8 adenocarcinomas, 1 in each of

bronchioloalveolar carcinoma mucinous and non mucinous type and 1 large cell carcinoma. P63 immunoprotein status was studied in these cases.

All invasive and preinvasive lesions of squamous differentiation showed nuclear positivity for p63 immunoprotein. Scoring was done according to the percentage of cells which showed p63 positivity in each lesion.

p63 score showed progressive increase through mild to severe dysplasia. This is in accordance with the data previously reported by Pelosi *et al.* [48] and Sniezek *et al.* [49] in a small series of head and neck and lung tumors. Also, Pierre P. Massion et al.[50], in his study showed that, in preinvasive tumors, p63 staining involved not only the basal but also the supra-basal layers and followed a pattern consistent with severity of histological grade. In invasive tumors, p63 was seen throughout the whole sheet of invasive tumor. It is apparent that p63 expression increases progressively from preinvasive to invasive lesions during the transformation of squamous epithelia.

91.6% (11/12) of squamous cell carcinomas showed p63 expression. p63 score ranged from 0 to 3 in different histologic grades of squamous cell carcinoma. It was found that p63 overexpression were extremely prevalent in squamous cell carcinomas. This is in accordance with a recent study, in which Pelosi *et al.* [48] examined the percentage of p63-positive cells by IHC, yet did not find an association between p63 expression and survival. But the majority

of cancer cells (80%) stained for p63 in squamous cell carcinomas. He found p63 immunoreactivity in 92.4% (109/118) of squamous cell carcinomas.

Poorly differentiated tumors showed decreased expression of p63 immunostaining. This may be due to the fact that as the normal cell transforms to dysplastic cells, they develop p63 genomic amplification. As the neoplastic cells become less differentiated, p63 expression is decreased. Thus, p63 may be an important marker of cell differentiation and confer survival advantage[50].

All adenocarcinomas showed no expression of p63. This is in accordance with Pierre P. Massion et al[50], who showed that p63 gene amplification occurs in the majority of squamous cell carcinomas, rarely in adenocarcinomas. Also, Charles J. Di Como et al[18], in his study showed that p63 expressed itself predominantly in basal cell and squamous cell carcinomas, as well as transitional cell carcinomas, but not in adenocarcinomas. Thus p63 immunostaining can be used to distinguish between squamous and non squamous carcinomas. However, due to smaller sample size, the results observed in this study are inconclusive. Further studies involving larger sample size are necessary to confirm the association between the degree of squamous differentiation and p63 grading.

As p63 gene is located in chromosome 3, genes in the chromosome 3q amplicon may open a window for identification of potential targets for molecular intervention in preinvasive and invasive lung cancer. P63 plays a

critical role in early development of squamous cell carcinoma and thus p63 amplification may prove to be an excellent biological marker of squamous cell tumor progression.

SUMMARY AND CONCLUSION

96 adequate samples of fibreoptic lung biopsy specimens received during the period between July 2008 to September 2010 were taken for the study. Demographic and risk factor analysis of the cases were done. Histopathological examination of all the cases was done. Immnohistochemical analysis of p63 antibody was done.

Lung carcinomas had a peak incidence in the group 40 to 70 years of age and was more common in males than in females. Most common clinical presentations were found to be cough, breathlessness and weight loss. Squamous cell carcinomas were found to be most commonly associated with history of smoking. The most common X ray findings were found to be mass lesion, opacity and consolidation. The most common findings at fibreoptic bronchoscopy were growth, unhealthy mucosa, necrotic material and narrowing of bronchus.

Histopathological examination showed 29 cases of pre invasive and invasive lesions - 6 pre invasive lesions (mild, moderate and severe dyplasias), 12 squamous cell carcinomas, 8 adenocarcinomas, 1 in each of bronchioloalveolar carcinoma mucinous and non mucinous type and 1 large cell carcinoma. p63 immunoprotein status was studied in these cases and scoring was done according to the percentage of cells which showed p63 positivity in each lesion.

Most of the squamous cell carcinomas showed nuclear positivity for p63. p63 score showed progressive increase through mild to severe dysplasia. p63 score ranged from 0 to 3 in different histologic grades of squamous cell carcinoma. Poorly differentiated tumors showed decreased expression of p63 immunostaining.

This confers that p63 immunostaining can be used to differentiate squamous and adenocarcinomas. It is also an important biological marker for squamous cell tumor progression and it can confer survival advantage.

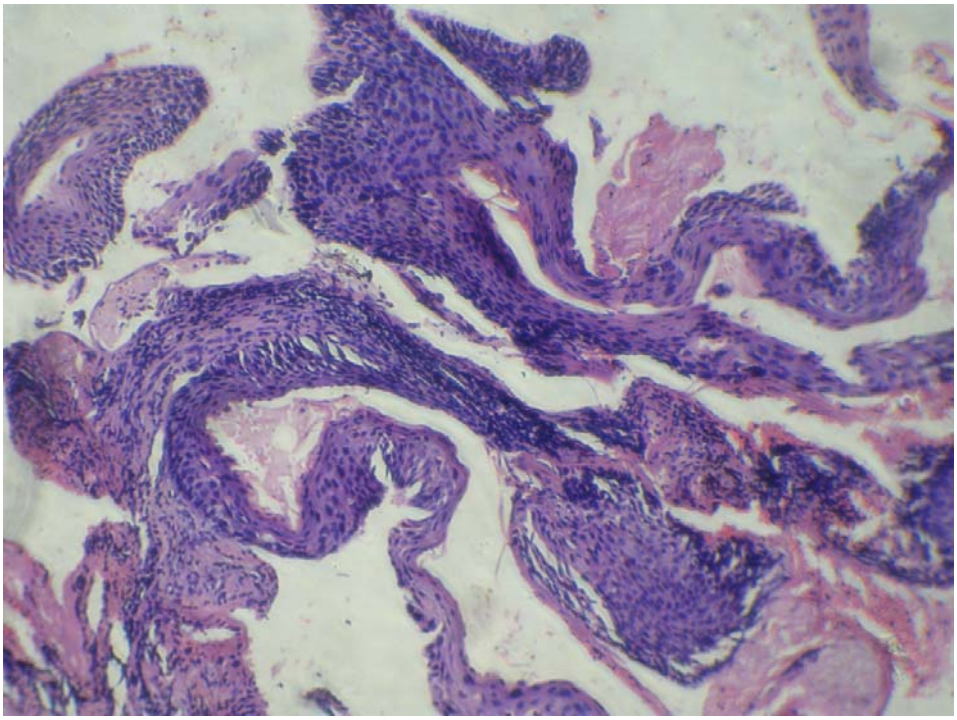


Fig : 1 Mild Dysplasia - 100x

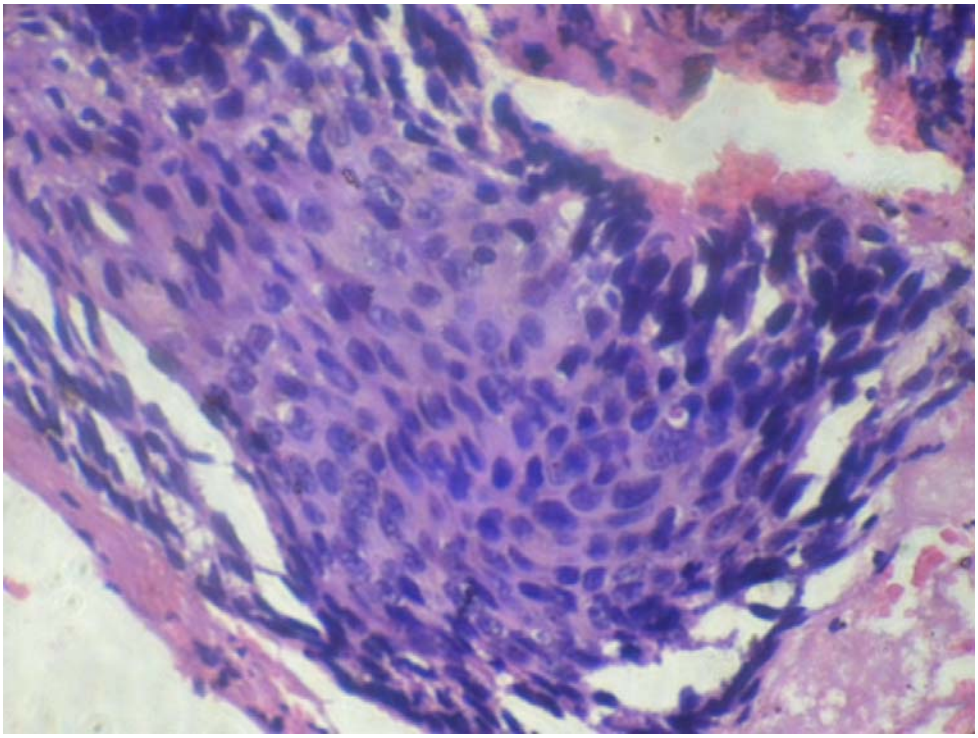


Fig : 2 Mild Dysplasia - 400x

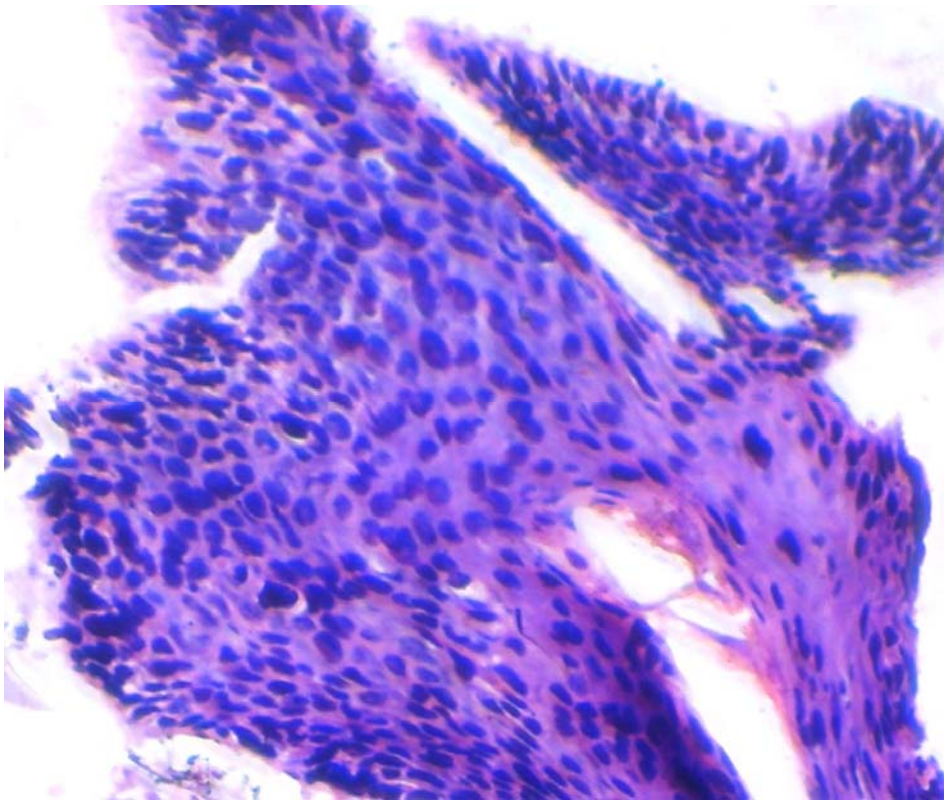


Fig : 3 Moderate Dysplasia - 100x

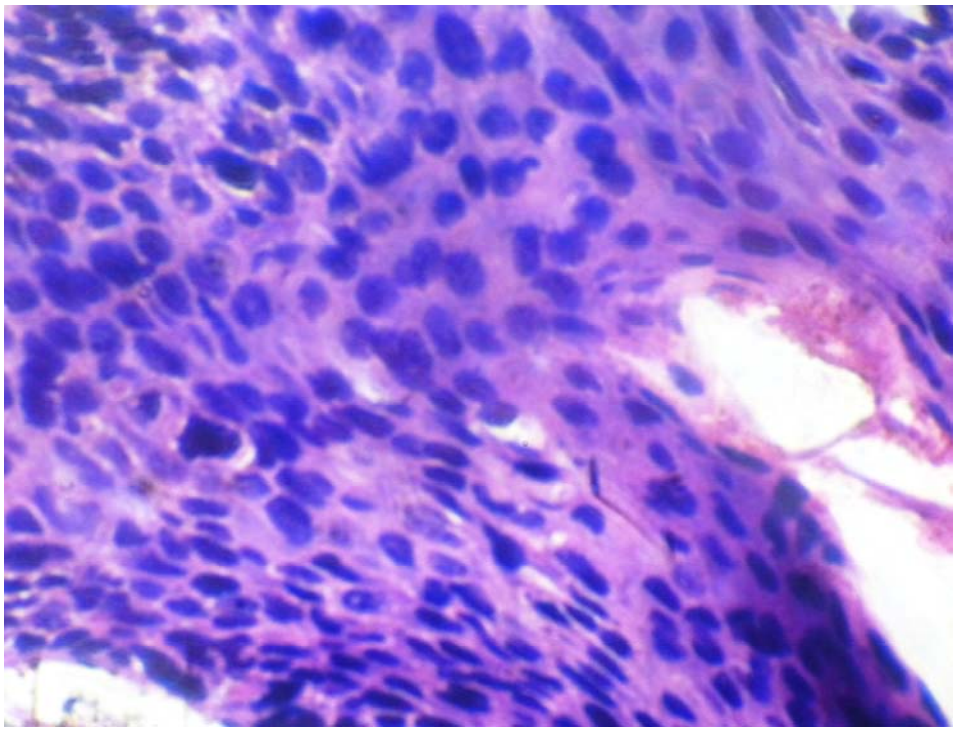


Fig : 4 Moderate Dysplasia - 400x

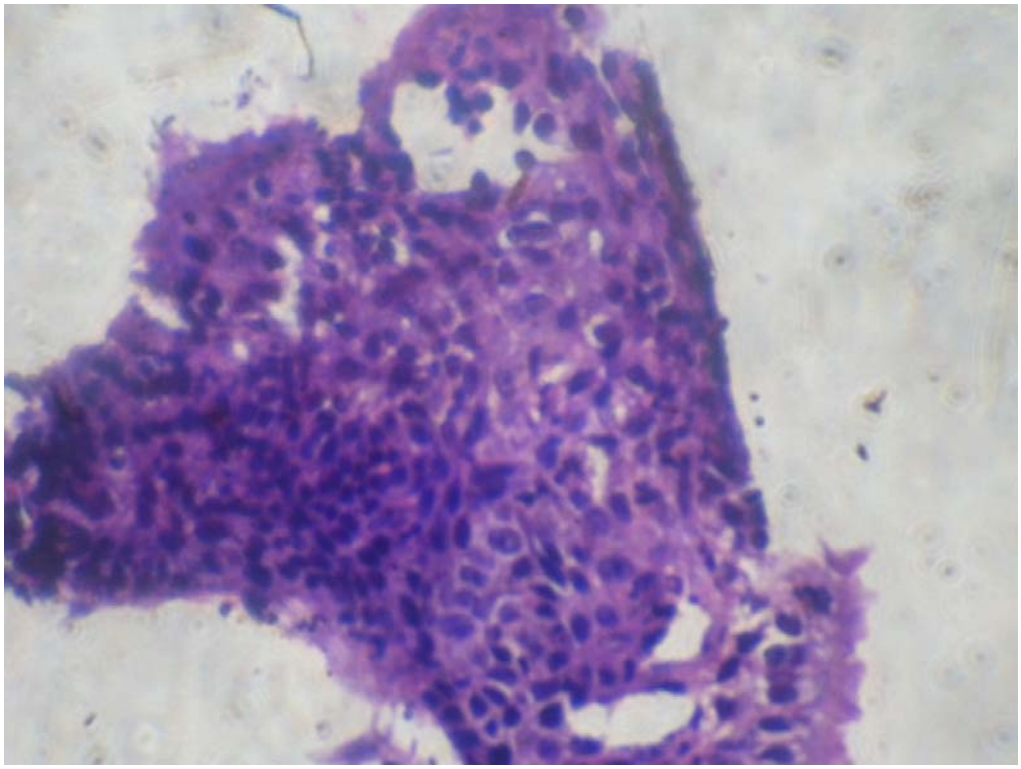


Fig : 5 Severe Dysplasia - 100x

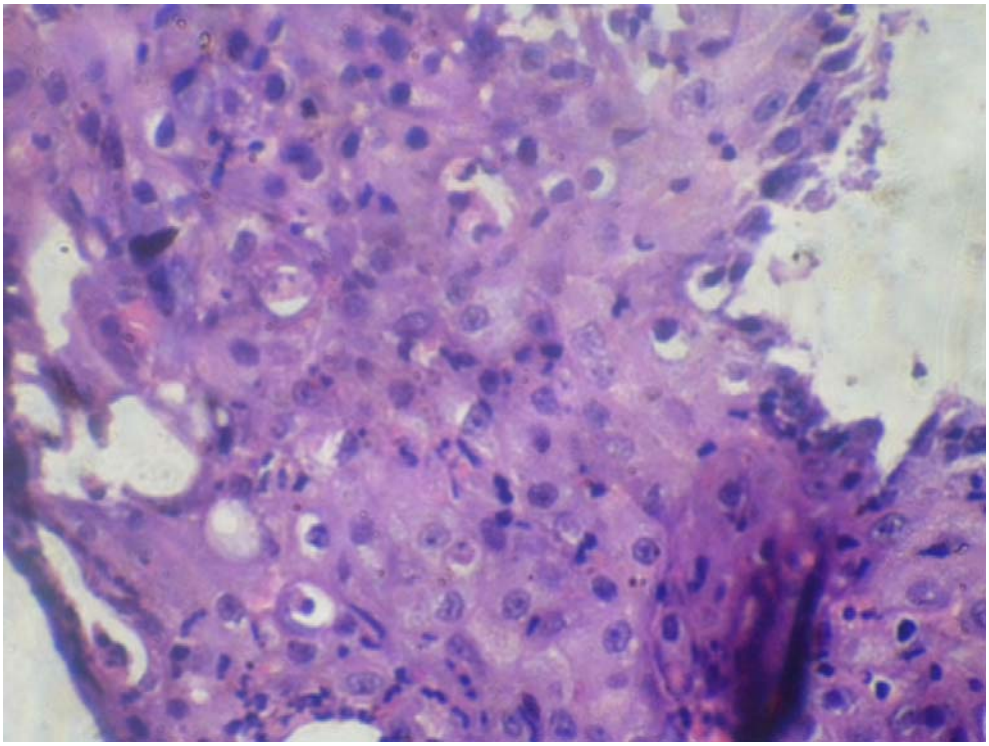


Fig : 6 Severe Dysplasia - 400x

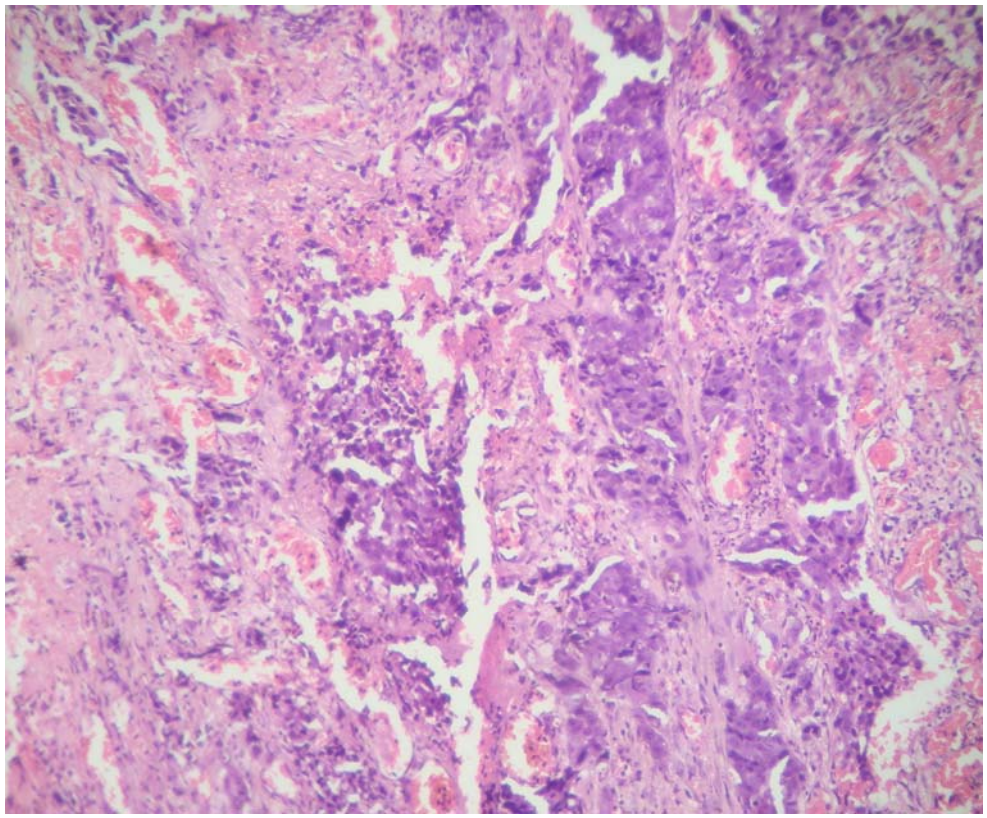


Fig : 7 Squamous Cell Carcinoma, Moderately Differentiated – 100x

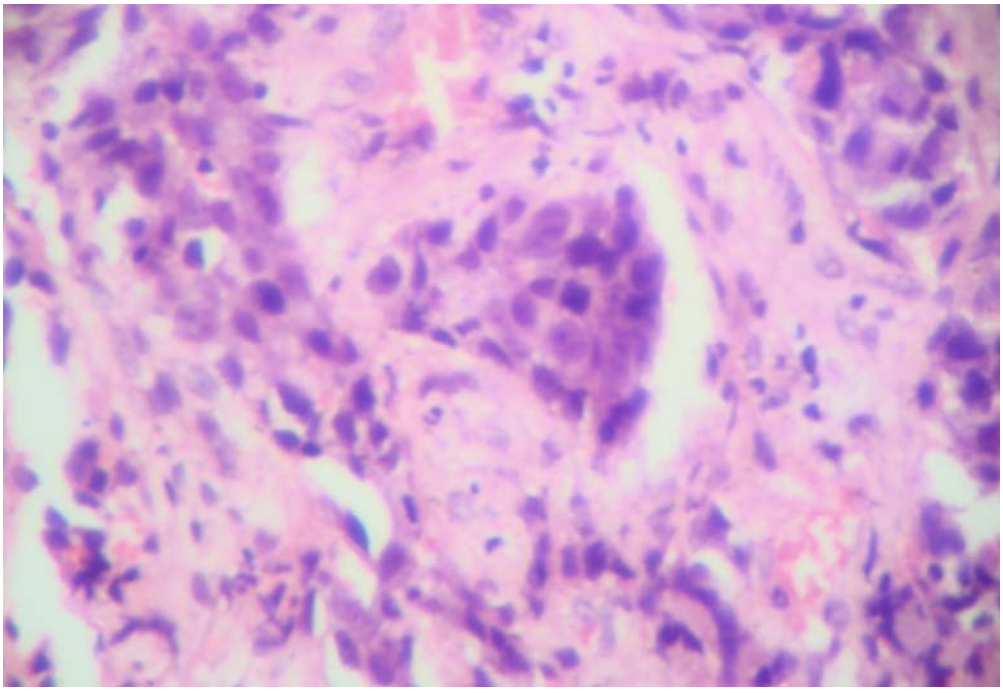


Fig : 8 Squamous Cell Carcinoma, Moderately Differentiated – 400x

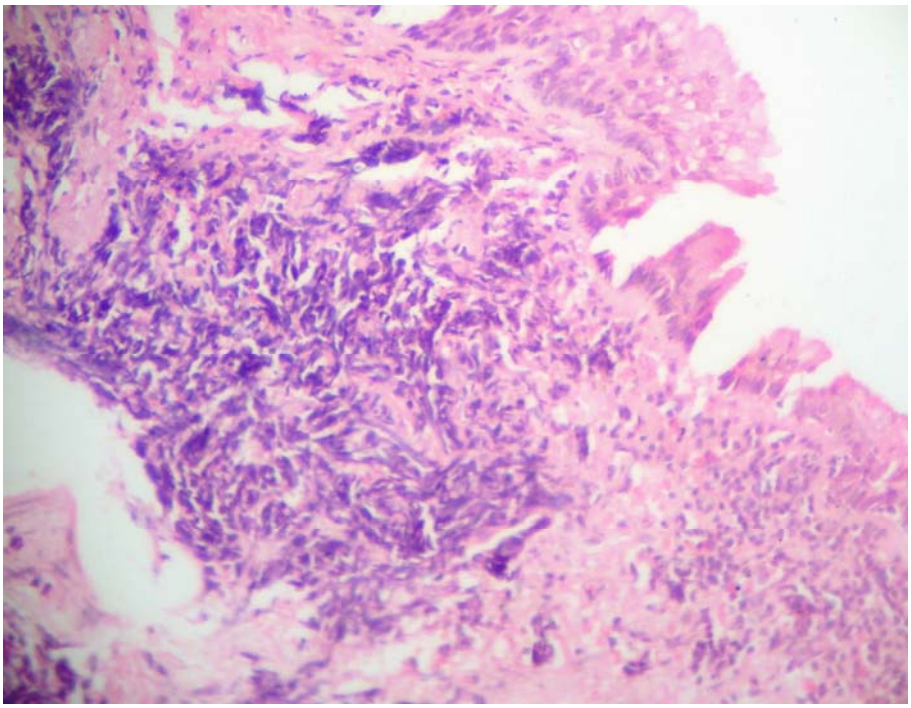


Fig : 9 Squamous Cell Carcinoma, Poorly Differentiated – 100x

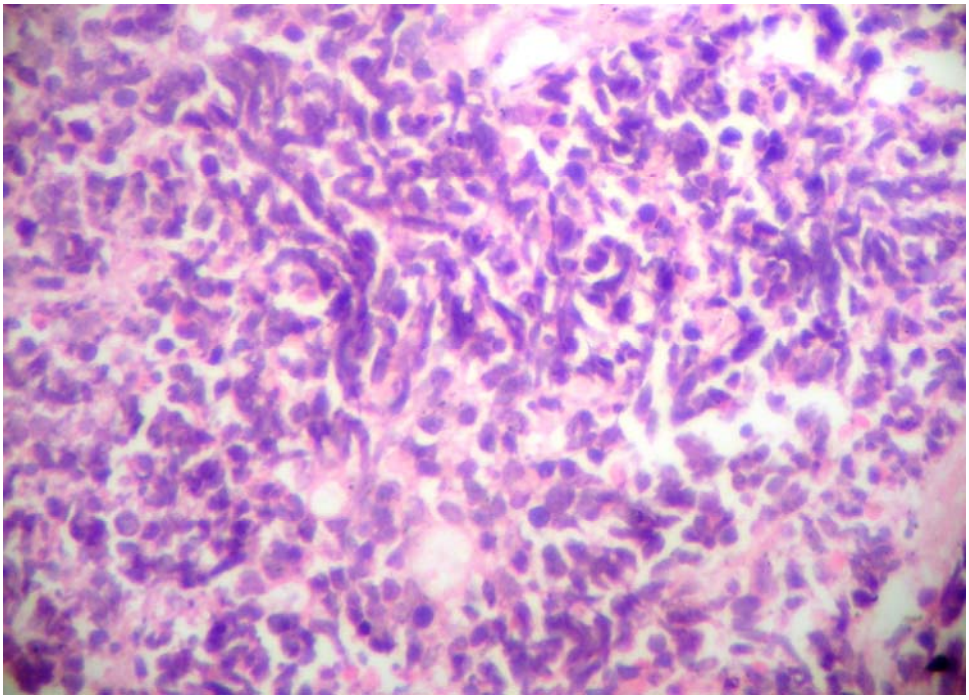


Fig : 10 Squamous Cell Carcinoma, Poorly Differentiated – 400x

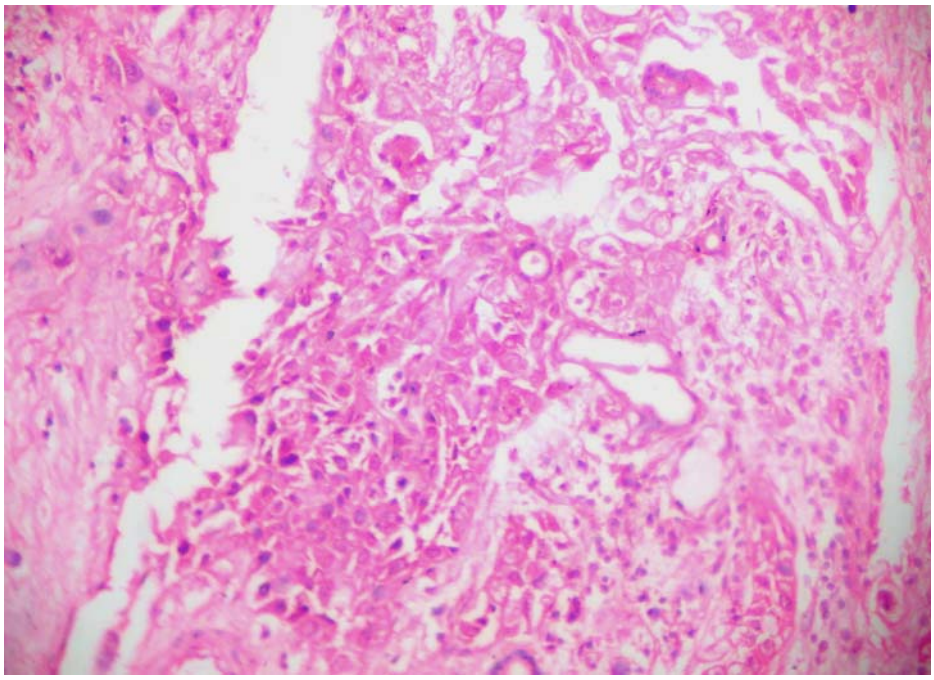


Fig : 11 Adenocarcinoma – 100x

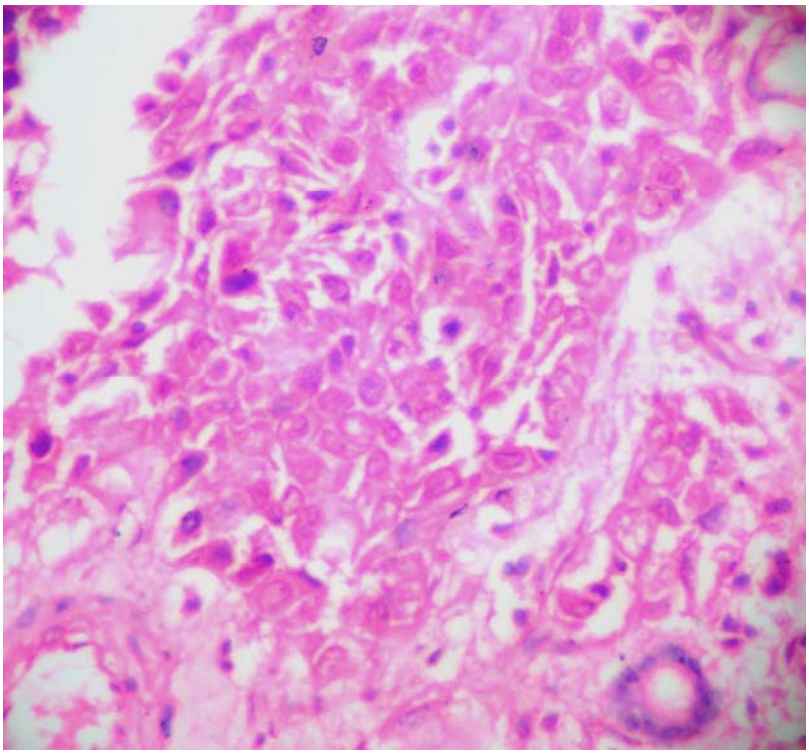


Fig : 12 Adenocarcinoma – 400x

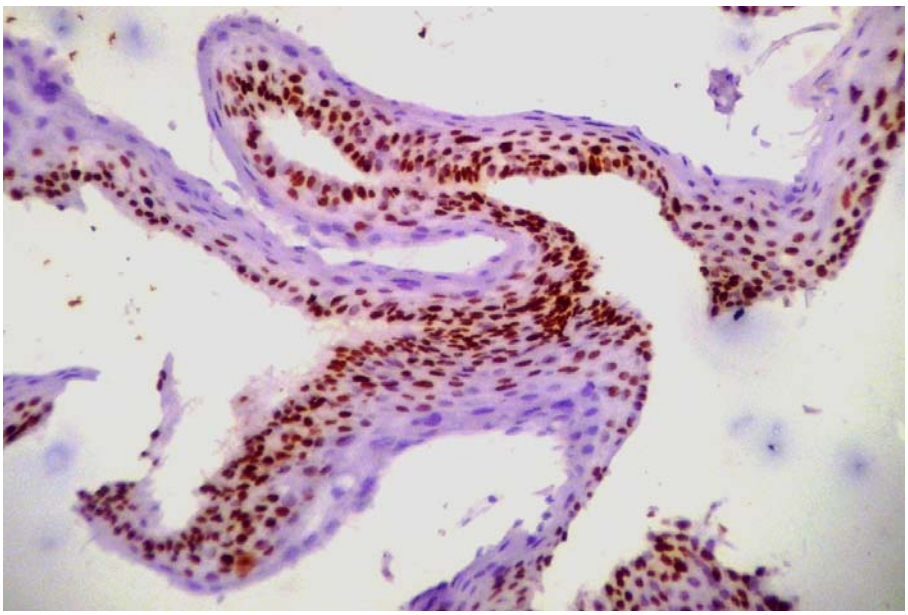


Fig : 13 Mild Dysplasia – p63 Score 1

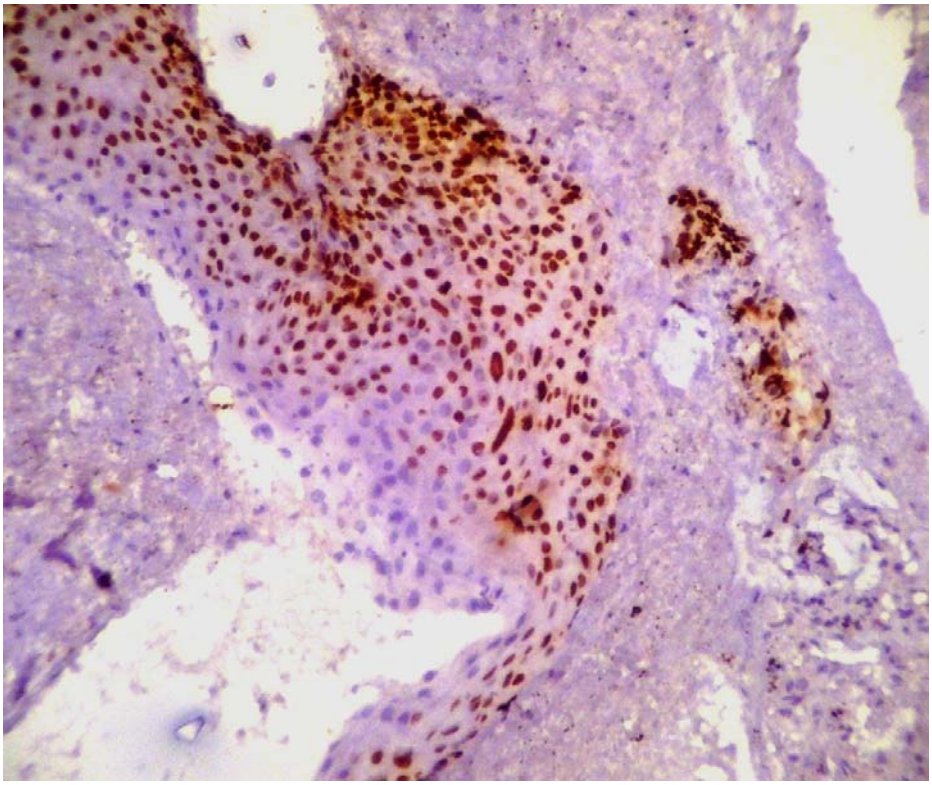


Fig : 14 Moderate Dysplasia – p63 Score 2

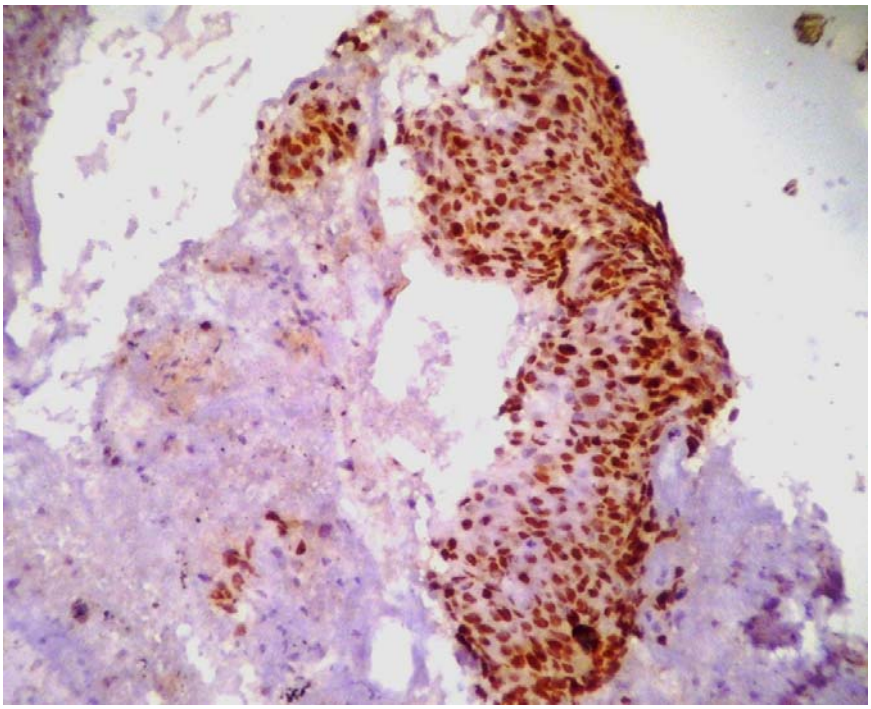


Fig : 15 Severe Dysplasia – p63 Score 3

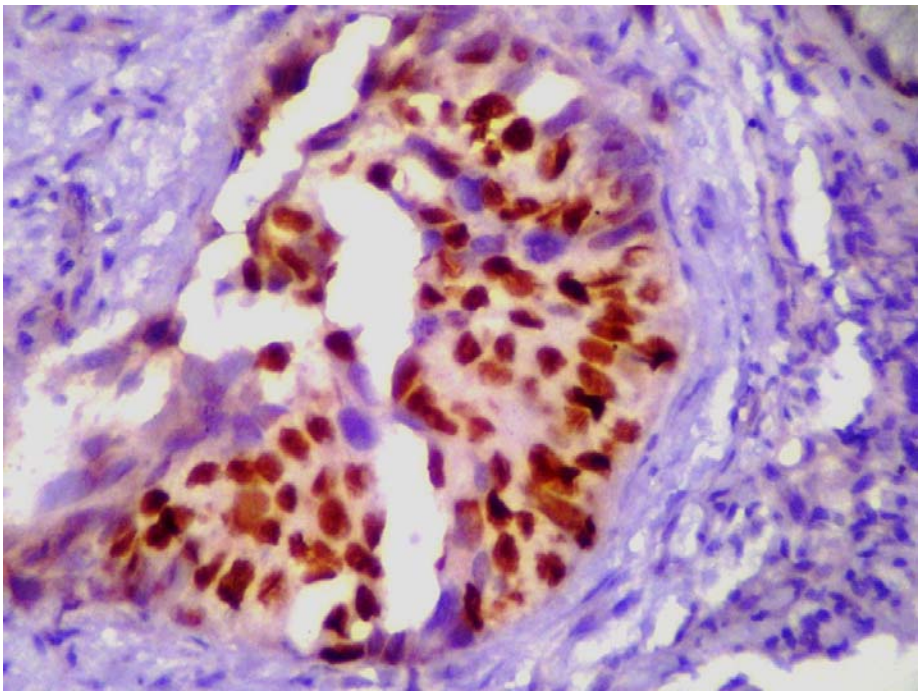


Fig : 16 Squamous Cell Carcinoma, Moderately Differentiated – p63 Score 3

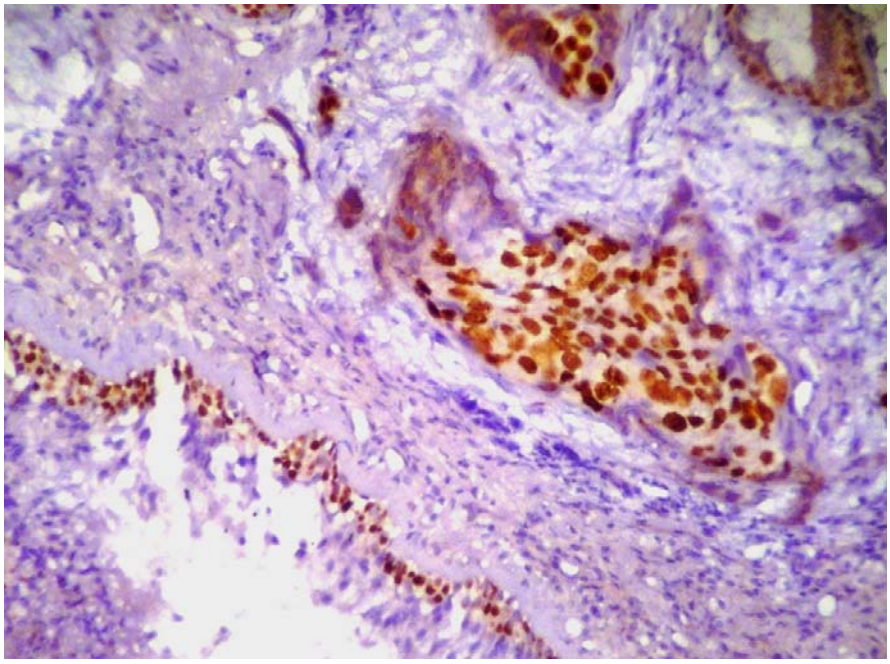


Fig : 17 Squamous Cell Carcinoma, Moderately Differentiated – p63 Score 3

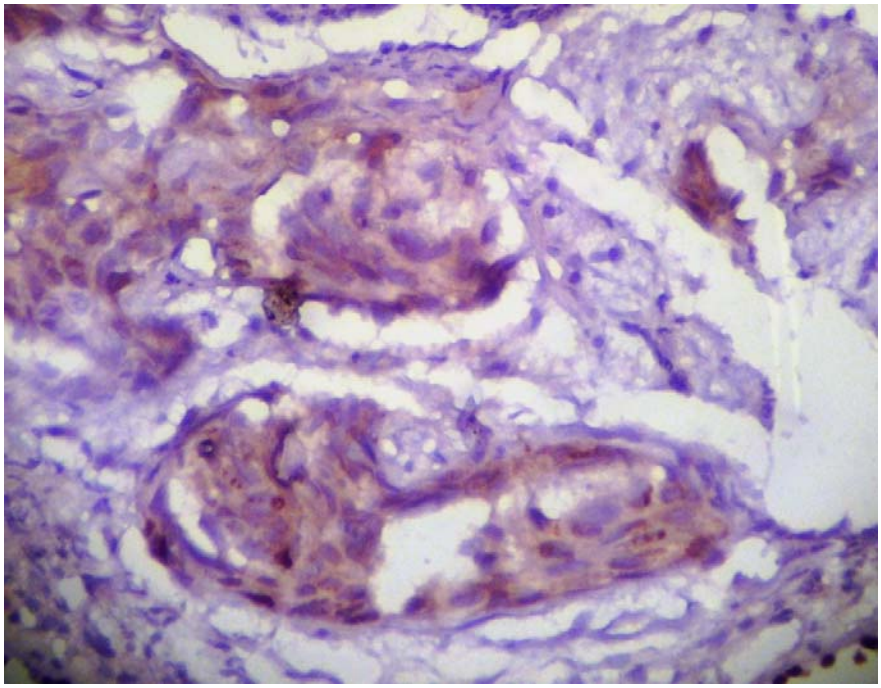


Fig : 18 Squamous Cell Carcinoma, Poorly Differentiated – p63 Score 0

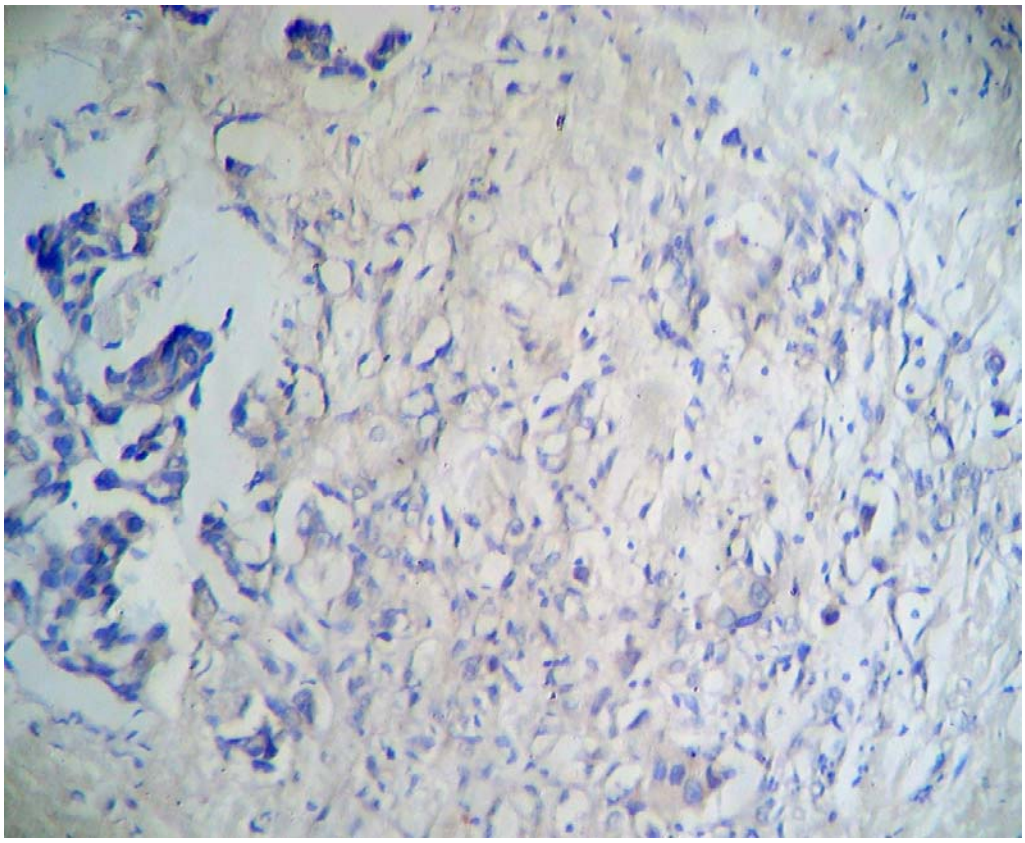


Fig : 19 Adenocarcinoma – p63 Score 0

S. No	AGE	SEX	HP NO	COUGH	SPUTUM	HEMOPTYSIS	CHEST PAIN	BREATHLESSNESS	LOSS OF WEIGHT	H/O SMOKING	XRAY FINDINGS	FOB FINDINGS	HP FINDINGS	HP GRADE	p63 SCORE
1	52	M	68/09	Y	Y	N	N	N	N	Y	HAZINESS	UNHEALTHY MUCOSA	normal		
2	77	F	210/09	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	non specific inflammation		
3	36	M	211/09	Y	Y	Y	Y	Y	N	N	CYSTIC CHANGES	N	non specific inflammation		
4	60	M	212/09	Y	Y	Y	N	N	Y	Y	OPACITY	HYPERTROPHIED MUCOSA	granulomatous lesion		
5	65	M	506/09	Y	Y	N	N	N	N	Y	OPACITY	PURULANT SECRETIONS	candidiasis		
6	56	M	561/09	Y	Y	N	N	N	N	Y	HAZINESS	INFLAMMED MUCOSA	squamous metaplasia		
7	72	M	819/09	Y	Y	N	Y	Y	N	Y	FIBROSIS	INFLAMMED MUCOSA	chronic Inflammation		
8	50	M	890/09	Y	Y	Y	N	N	N	N	N	HYPERTROPHIED MUCOSA	normal		
9	38	M	943/09	Y	Y	N	N	N	N	Y	HAZINESS	UNHEALTHY MUCOSA	normal		
10	72	M	1157/09	Y	Y	N	Y	Y	Y	Y	OPACITY	NECROTIC MATERIAL	severe dysplasia		3+
11	65	M	1367/09	Y	Y	N	Y	Y	Y	N	CONSOLIDATION	NARROWING OF BRONCHUS	moderate dysplasia		2+
12	70	M	1368/09	Y	Y	N	Y	Y	Y	Y	N	GROWTH	squamous cell carcinoma	poorly differentiated	0
13	85	M	1465/09	Y	Y	N	Y	Y	Y	N	OPACITY	NECROTIC MATERIAL	squamous cell carcinoma	moderately differentiated	3+
14	54	M	5145/08	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	non specific inflammation		
15	68	M	5146/08	Y	Y	N	Y	Y	N	Y	HAZINESS	NECROTIC MATERIAL	inadequate material		
16	47	M	5147/08	Y	Y	N	N	N	N	N	HAZINESS	HYPERTROPHIED MUCOSA	chronic inflammation		
17	54	M	5422/08	Y	Y	Y	N	N	Y	Y	N	INFLAMMED MUCOSA	non specific inflammation		
18	45	M	5676/08	Y	Y	N	N	N	Y	Y	OPACITY	GROWTH	adenocarcinoma	poorly differentiated	0
19	51	M	5677/08	Y	Y	N	N	Y	Y	N	CONSOLIDATION	GROWTH	adenocarcinoma	poorly differentiated	0
20	74	F	5981/08	Y	Y	N	Y	Y	N	Y	FIBROSIS	INFLAMMED MUCOSA	chronic Inflammation		
21	58	M	5982/08	Y	Y	Y	N	Y	N	N	CAVITY	N	normal		
22	55	M	5983/08	Y	Y	N	N	N	N	N	CONSOLIDATION	NARROWING OF BRONCHUS	inadequate material		
23	57	M	1601/09	Y	Y	Y	N	Y	N	N	CAVITY	N	normal		
24	45	M	1682/09	Y	Y	N	N	N	N	N	HAZINESS	HYPERTROPHIED MUCOSA	chronic inflammation		
25	57	M	1683/09	Y	Y	N	N	N	Y	Y	OPACITY	NARROWING OF BRONCHUS	inadequate material		
26	65	M	1892/09	Y	Y	N	Y	Y	N	Y	HAZINESS	NECROTIC MATERIAL	inadequate material		
27	60	M	1893/09	Y	Y	N	Y	Y	Y	N	CAVITY	PURULANT SECRETIONS	inadequate material		
28	40	M	2076/09	Y	Y	Y	N	N	Y	Y	N	INFLAMMED MUCOSA	non specific inflammation		
29	47	M	2308/09	Y	Y	Y	N	N	Y	Y	N	HYPERTROPHIED MUCOSA	non specific inflammation		
30	70	M	2429/09	Y	Y	N	N	N	Y	N	OPACITY	GROWTH	adenocarcinoma	moderately differentiated	0
31	23	M	2566/09	Y	Y	Y	N	Y	N	N	HAZINESS	N	normal		
32	60	M	2702/09	N	N	N	Y	Y	Y	Y	CONSOLIDATION	GROWTH	squamous cell carcinoma	moderately differentiated	2+
33	50	M	2860/09	N	N	N	Y	Y	Y	N	OPACITY	NARROWING OF BRONCHUS	large cell ca		0
34	61	M	2861/09	N	N	N	N	Y	Y	Y	HILAR PROMINENCE	NARROWING OF BRONCHUS	adenocarcinoma	poorly differentiated	0
35	50	M	3428/09	Y	Y	Y	N	N	N	Y	CYSTIC CHANGES	N	normal		
36	60	M	3430/09	N	N	N	N	N	Y	N	N	UNHEALTHY MUCOSA	BAC, mucinous type		0
37	57	M	3431/09	Y	Y	N	N	Y	Y	Y	N	HYPERTROPHIED MUCOSA	normal		
38	65	M	3775/09	Y	Y	Y	N	N	N	Y	CYSTIC CHANGES	N	normal		
39	48	M	3973/09	Y	Y	N	Y	Y	Y	Y	OPACITY	GROWTH	inadequate material		
40	55	M	3973/09	Y	N	N	Y	Y	N	N	FIBROSIS	HYPERTROPHIED MUCOSA	normal		
41	59	M	3972/09	Y	Y	Y	N	N	N	Y	CAVITY	N	normal		
42	48	M	4025/09	N	N	N	Y	Y	Y	N	PLEURAL EFFUSION	GROWTH	adenocarcinoma		0
43	55	M	4026/09	Y	Y	N	N	N	N	Y	CONSOLIDATION	UNHEALTHY MUCOSA	normal		
44	50	M	4320/09	Y	Y	Y	N	N	N	Y	CYSTIC CHANGES	N	normal		
45	45	M	4321/09	Y	N	Y	N	Y	Y	Y	HILAR MASS	GROWTH	squamous cell carcinoma	moderately differentiated	3+
46	65	M	4454/09	Y	N	Y	Y	N	N	Y	RIB METZ	GROWTH	squamous cell carcinoma	moderately differentiated	1+
47	65	M	4455/09	N	Y	Y	N	Y	Y	Y	HILAR MASS	GROWTH	squamous cell carcinoma	moderately differentiated	3+
48	61	M	4603/09	N	N	N	N	N	N	Y	HILAR PROMINENCE	HYPERTROPHIED MUCOSA	mild dysplasia		1+
49	40	M	4656/09	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	non specific inflammation		
50	70	M	4796/09	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
51	49	M	4935/09	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
52	60	M	4936/09	Y	N	N	N	N	Y	N	CAVITY	UNHEALTHY MUCOSA	squamous cell carcinoma	poorly differentiated	1+
53	72	M	4937/09	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	normal		
54	28	M	4938/09	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
55	35	M	5033/09	N	Y	Y	N	Y	Y	Y	HILAR MASS	GROWTH	squamous cell carcinoma	moderately differentiated	1+

56	58	M	5034/09	Y	Y	N	N	N	N	Y	CONSOLIDATION	N	normal		
57	46	M	5138/09	N	N	N	N	N	N	Y	HILAR PROMINENCE	HYPERTROPHIED MUCOSA	mild dysplasia		1+
58	50	M	5515/09	N	N	N	Y	Y	Y	Y	CONSOLIDATION	GROWTH	squamous cell carcinoma	moderately differentiated	3+
59	24	F	5746/09	Y	Y	N	Y	Y	Y	N	CAVITY	PURULANT SECRETIONS	inadequate material		
60	50	M	5978/09	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
61	25	F	5979/09	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
62	49	M	87/10	N	N	Y	N	N	Y	Y	N	N	normal		
63	45	M	245/10	N	N	Y	N	N	Y	Y	N	N	normal		
64	48	F	405/10	Y	Y	N	N	N	Y	N	OPACITY	NARROWING OF BRONCHUS	BAC,non-mucinous type		0
65	32	F	543/10	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
66	49	M	623/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
67	60	M	624/10	Y	Y	N	N	N	Y	Y	OPACITY	NARROWING OF BRONCHUS	moderate dysplasia		2+
68	45	M	625/10	Y	Y	N	Y	Y	N	Y	HAZINESS	NECROTIC MATERIAL	moderate dysplasia		2+
69	52	M	626/10	N	N	Y	N	N	Y	Y	N	N	normal		
70	40	M	627/10	N	N	Y	N	N	Y	Y	N	N	normal		
71	33	F	790/10	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	nonspecific inflammation		
72	75	F	791/10	Y	Y	N	Y	Y	Y	N	OPACITY	GROWTH	adenocarcinoma		0
73	48	F	792/10	Y	Y	N	Y	Y	N	N	HAZINESS	NECROTIC MATERIAL	inadequate material		
74	30	M	1037/10	Y	Y	N	Y	Y	Y	N	CAVITY	PURULANT SECRETIONS	inadequate material		
75	21	M	1038/10	N	N	Y	N	N	Y	Y	N	N	normal		
76	52	M	1039/10	Y	Y	N	Y	Y	Y	Y	OPACITY	GROWTH	adenocarcinoma		0
77	70	F	1040/10	N	N	Y	N	N	Y	Y	N	N	normal		
78	40	M	1041/10	Y	N	N	N	Y	Y	Y	HILAR MASS	GROWTH	squamous cell carcinoma	moderately differentiated	1+
79	35	M	1549/10	Y	Y	N	Y	Y	Y	N	CAVITY	PURULANT SECRETIONS	aspergillosis		
80	58	M	1579/10	Y	Y	Y	N	Y	Y	N	HILAR MASS	NARROWING OF BRONCHUS	granulomatous lesion		
81	60	F	1653/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
82	70	F	2027/10	Y	N	N	N	N	Y	N	OPACITY	NARROWING OF BRONCHUS	adenocarcinoma		0
83	63	F	2028/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
84	47	M	2029/10	N	N	Y	N	N	Y	Y	N	N	normal		
85	42	M	2030/10	Y	N	N	N	Y	Y	Y	HILAR MASS	GROWTH	squamous cell carcinoma	moderately differentiated	2+
86	70	M	2068/10	N	N	N	Y	Y	Y	N	CONSOLIDATION	GROWTH	squamous cell carcinoma	moderately differentiated	3+
87	29	F	2187/10	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	chronic inflammation		
88	39	F	2188/10	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
89	60	M	2189/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
90	65	F	2190/10	N	N	Y	N	N	Y	Y	N	N	normal		
91	63	F	2191/10	N	N	Y	N	N	Y	Y	N	N	normal		
92	65	M	2269/10	Y	N	N	N	N	Y	N	CAVITY	UNHEALTHY MUCOSA	granulomatous lesion		
93	40	M	2391/10	Y	Y	N	Y	Y	N	Y	FIBROSIS	INFLAMMED MUCOSA	chronic Inflammation		
94	47	M	2392/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
95	42	M	2393/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
96	63	F	2028/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
97	70	M	4796/09	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
98	29	F	2187/10	N	N	Y	Y	N	Y	N	BRONCHIECTASIS	N	chronic inflammation		
99	45	M	2391/10	Y	Y	N	Y	Y	N	Y	FIBROSIS	INFLAMMED MUCOSA	chronic Inflammation		
100	50	M	4320/09	Y	Y	Y	N	N	N	Y	CYSTIC CHANGES	N	normal		
101	56	M	4026/09	Y	Y	N	N	N	N	Y	CONSOLIDATION	UNHEALTHY MUCOSA	normal		
102	65	M	2190/10	N	N	Y	N	N	Y	Y	N	N	normal		
103	50	M	623/10	Y	Y	N	N	N	N	N	HAZINESS	N	normal		
104	55	M	5978/09	Y	Y	N	Y	Y	N	Y	CAVITY	UNHEALTHY MUCOSA	normal		
105	45	M	245/10	N	N	Y	N	N	Y	Y	N	N	normal		