A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN LUNG CANCERS

Dissertation submitted in

partial fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

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MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

CERTIFICATE

This is to certify that this Dissertation entitled "**A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN LUNG CANCERS"** is the bonafide original work of **Dr.VAMITHA.P.S**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the TamilnaduDr.M.G.R Medical University to be held in April 2016.

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DECLARATION

I, **Dr.Vamitha.P.S,** solemnly declare that the dissertation titled "**A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN LUNG CANCERS**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr.S.Pappathi, M.D., DCH.,** Professor of Pathology, Institute of Child Health, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place : Chennai

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INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To
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Dear Dr.P.S.Vamitha,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study of expression of EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) in lung cancers". No.08102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

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

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

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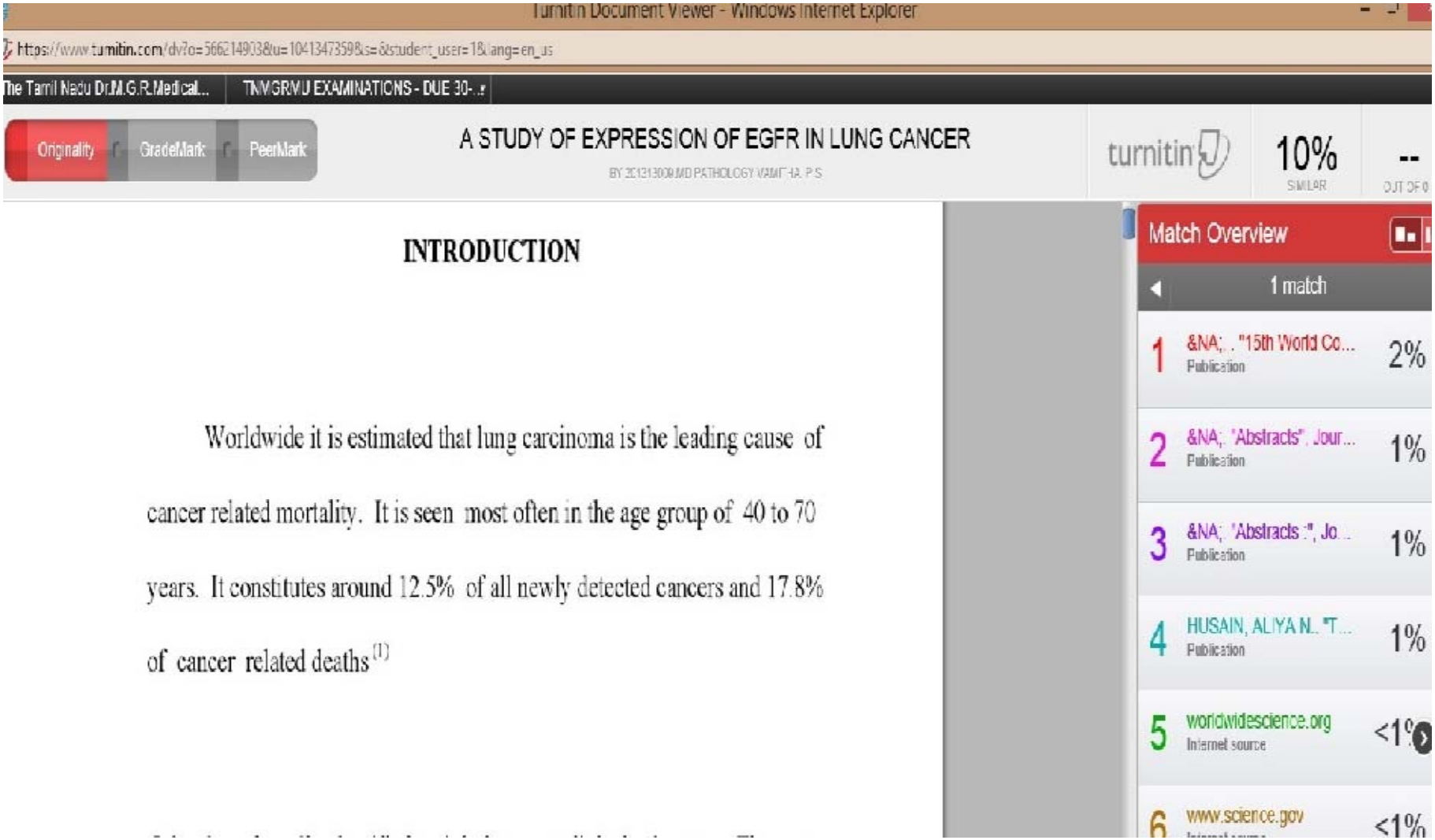
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INTRODUCTION
Worldwide it is estimated that lung carcinoma is the leading cause of cancer related mortality. It is seen most often in the age group of 40 to 70 years. It constitutes around 12.5% of all newly detected cancers and 17.8% of cancer related deaths [1]
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ABBREVIATIONS

SCC	:	Squamous cell carcinoma
ADC	:	Adenocarcinoma
EGFR	:	Epidermal growth factor receptor
WHO	:	World Health Organisation
IASLC/ATS/ERS	:	International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society
NSCLC	:	Non Small Cell Lung Carcinoma
NSCLC-NOS	:	Non Small Cell Lung Carcinoma-not otherwise specified
TTF-1	:	Thyroid Transcription Factor-1
IHC	:	Immunohistochemistry
H & E	:	Hematoxylin& Eosin
CIS	:	Carcinoma in situ
BAC	:	Bronchoalveolar Carcinoma
AIS	:	Adenocarcinoma Insitu
MIA	:	Minimally Invasive Adenocarcinoma

CONTENTS

ABSTRACT

INTRODUCTION:

Lung cancer is a highly aggressive malignancy causing high morbidity and mortality. An increasing incidence of lung cancer has been observed in India. For those with non-small cell lung cancer and patients with more advanced disease, targeted therapy has been a cornerstone of treatment. Several molecular markers in lung cancer has been introduced in the recent past. They are recent topics of interest which has emerged not only as a prognostic marker but also as markers to predict therapy response especially the EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR). The expression of this marker forms important criteria for prognosis and therapy of lung carcinomas. The aim of this study is to assess the patterns of expression of EGFR and to assess the clinical and morphological characteristics of lung carcinomas.

AIMS AND OBJECTIVES:

The aim of this study is to assess the expression of Epidermal Growth Factors Receptor (EGFR) in lung cancers. To compare the expression of EGFR with clinicopathological parameters in lung carcinoma. To assess the prognostic and predictive values of EGFR

MATERIALS AND METHODS:

Out of 412 lung specimens received during the two year study period, 178 cases were non-small cell lung carcinoma. 60 cases are randomly selected for assessing EGFR expression which includes 20 cases each of adenocarcinoma, squamous cell carcinoma and non-small cell lung carcinoma-not otherwise specified(NSCLC-NOS).

RESULTS:

68.33% of the cases are positive for EGFR expression. 90.48% of the females are positive for EGFR expression96.67% of the EGFR positive cases are smokers and 40% are non smokers. Among the EGFR positive histological subtype, adenocarcinoma is the most common type with 43.90% followed by squamous cell carcinoma with 36.59% & NSCLC-NOS with 19.51%.

CONCLUSION:

The identification of EGFR expression gives a fascinating opportunity for the development of tyrosine kinase inhibitors against non-small cell lung cancers. It is very clear from the comparison of various studies from our studies that EGFR expression is more common in females, never smokers and adenocarcinoma histological type. EGFR, being a poor prognostic factor, its expression is very important to identify the tyrosine kinase inhibitors sensitivity. Hence it is very important to find the association between EGFR expression and its clinocopathological parameters in order to select the patients for targeted therapy like erlotinib, gefitinib for advanced lung cancers.

In conclusion, it is recommended that EGFR expression should be a routine test after lung resection for all non-small lung carcinoma especially adenocarcinoma and squamous cell carcinoma for better treatment for the patients.

Key words: EGFR, Adenocarcinoma, Tyrosine Kinase inhibitors, Non-small cell lung cancers

INTRODUCTION

Worldwide it is estimated that lung carcinoma is the leading cause of cancer related mortality. It is seen most often in the age group of 40 to 70 years. It constitutes around 12.5% of all newly detected cancers and 17.8% of cancer related deaths [(1)].

It has been broadly classified mainly into two clinical subgroups. They are

1. Non- small cell carcinoma of lung and
2. Small cell carcinoma of lung, with the incidence of about 80- 85% and 15- 20% respectively[(2)].
3. Non- small cell lung carcinomas are further sub typed as
4. Adenocarcinoma (40% of lung cancers),
5. Squamous cell or epidermoid carcinoma (25-30%),
6. Large cell or undifferentiated carcinoma (10-15%),
7. Adenosquamous type
8. Sarcomatoid type (less common types) [(2)]

Thus it is clear that Non small cell lung cancer constitutes for more than 85% of lung malignant cases out of which adenocarcinoma is the most common subtype.Only 15% of lung cancer patients present with localised disease. Most patients present with advanced disease at the time of announcement of the disease.

For those with non small cell lung cancer and patients with more advanced disease, targeted therapy has been a cornerstone of treatment The prognostic factors are age,sex,smoking status,stage,histology, histological grade.Several molecular markers in lung cancer has been introduced in the recent past. They are recent topics of interest which has emerged not only as a prognostic marker but also as markers to predict therapy response especially the EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR). The expression of this marker forms important criteria for prognosis and therapy of lung carcinomas.The aim of this study is to assess the patterns of expression of EGFR and to assess the clinical and morphological characteristics of lung carcinomas.

AIMS

AND

OBJECTIVES

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- To compare the expression of EGFR with clinicopathological parameters in lung carcinoma.
- To assess the prognostic and predictive values of EGFR

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

Epidemiology

Lung carcinoma has become very frequent cause of mortality and morbidity in and around the world and it has become the most important cause of death in industrialized countries. Though lung cancer has been strongly associated with the usage of tobacco, it is increasing in females in the recent years. It is said that more than 90% of patients are over the age of 40 years at the time of diagnosis[3]

1.8 million new cases have been diagnosed in 2012 with highest age standardized rates in central Europe & eastern Asia. Incidence rate is low in western & middle Africa.It is calculated that nearly one in every five cases of death is due to lung cancer which accounts for 19.4% of all deaths[4]

In India, lung carcinoma is more common and severe among males. The incidence rate is relatively low among Indian Women[4]

Etiology & Pathogenesis

Tobacco Smoking

Among all the risk factors estimated, smoking is the most important etiological factor associated with this malignancy. There are many evidences to prove that there is a strong statistical association between the increased incidence rates of lung cancers. Most of the carcinomas of lung are associated with smoking.

It depends upon the smoke inhalation depth, number of packets of cigarettes smoked eachday and the duration of smoking years. We all know that procarcinogens are converted to carcinogens through activation of P450 monooxygenase enzymes. Cigarette smoke has P450 monooxygenase enzyme system which has increased capacity to activate procarcinogens.

Pipes and cigars smoking also increases the risk.

There is a strong linear correlation between the appearance of epithelial living changes and tobacco smoking intensity. The changes seen in order are squamous metaplasia, Carcinoma in Situ and invasive carcinoma[5]. Second hand smoke or passive smoking also increases the risk[6,7,8].

The etiology of lung carcinoma in multifactorial as explained by the lung cancers in non-smokers. Environmental and genetic factors also play important role.

Air pollution

Air pollution increases the risk of lung cancer. Air particulates in smog, when exposed chronically causes lung irritation, inflammation and repair. Repeated inflammation can lead to lung cancer. Radan, which is a radioactive gas causes increased risk of lung cancer and it has been estimated to be the second most important cause for lung cancer in Unites States[9,10,11].

Industrial hazards

Industrial exposures like chromium, arsenic, uranium, nickel, asbestos increases the risk of developing lung malignancies. Uranium, which is a weak radioactive substance, causes four times increased risk.

The latent period before the exposure of asbestos and lung cancer is 10-30 years. Asbestos workers are at 50-90 times increases risk than the non-smokers[12,13].

Radiation therapy

Patients who are given radiation therapy as treatment for malignancies like Hodgkin disease and breast carcinoma are at increased risk for lung carcinoma[14].

Geographical location

Areas like South-America and South-Asia where people are exposed to increased levels of arsenic in drinking water are at increased risk[14]. Certain histological types are seen in particular geographical areas than the others, as for example adenocarcinoma is more common in the Unites States and squamous cell carcinoma is more common in white women when compared to black women.

Molecular genetics

Oncogenic ‘driver’ mutations which when accumulate can cause neoplastic transformation of the epithelial cells of lung. There are various histological subgroups and each of them have distinct molecular features.

Adenocarcinoma

They are characterized by gain of function mutation in EGFR, ALK, ROS, MET and RET.

Small Cell Carcinoma

Often shows loss of function in TP53 and chromosome 3p deletion.

Squamous cell carcinoma

These are characterized by losses in 3p, 9q, 17p loss of expression of retinoblastoma (RB) gene.

ANATOMY AND HISTOLOGY OF LUNGS:

There are two main components of the lung parenchyma, bronchi and bronchioles and the alveoli. They are paired intrathoracic organs. There are three lobes on the right lung and 2 on the left lung. There is a rudimentary appendage from the upper lobe of left lung which is called lingulae.

Trachea divides into 2 bronchi which in-turn divides into bronchioles, terminal & respiratory bronchioles. The respiratory tract is lined by pseudostratified ciliated columnar epithelium.

Type 1 & type 2 pneumocytes line the alveoli. Neuroendocrine cells, Clara cells, alveoli cells, ciliated cells and goblet cells are other cell types in bronchial-bronchiolar epithelium.

ORIGIN

The site of origin for lung cancer refers to the type of tissue from which the cancer cells develops[15,16]. Usually lung cancer is categorized by its site of origin into hilar and peripheral types, as these structures from where the disease originates are different . The majority of the early lung cancers arising in hilar regions are squamous cell carcinoma, whereas those early stage cancers arising in the peripheral areas of lung are adenocarcinomas[17]. Adenocarcinomas usually originates in glandular tissue whereas squamous cell carcinoma originates in the tissue which lines the organs and tubes of the lungs called epithelial tissues[18]. NSCLCs such as adenocarcinomas and large cell lung carcinoma are located typically in the peripheral areas of lungs and can present as either solitary nodule or masses[17]. Squamous cell carcinoma and small cell carcinoma are normally found to arise in the central portions of the lung and may be misdiagnosed as collapsed lung (Atelectasis) or pneumonia[17]. Small cell carcinoma are usually located in the main bronchi. this type of malignancy appears to originates from the Kulchitsky cells, which in turn is a component of the bronchial epithelium[17]

Histological types

There are several histopathological subgroups of lung cancer.

The major categories are

- Squamous cell carcinoma
- Small cell carcinoma
- Adenocarcinoma
- Adenosquamous carcinoma
- Large cell carcinoma
- Sarcomatoid carcinoma
- Carcinoid tumor
- Carcinoma of salivary gland type
- Unclassified carcinoma

Squamous cell carcinoma

This particular histological type has strong association with exposure to tobacco smoke and genetic association like loss of 3p, 9q & 17p. Among all the histological types of lung carcinoma, SCC has high association with TP53 mutation. Most of the patients present as hilar/perihilarmass. But they can also be found in peripheral & subpleurallocation[19,20]. If present in such location, tumor cells may fill the alveolar Lumina in a lepidic like fashion[21]. Malignant cells are seen exfoliated in the sputum or brushing cytology. They can undergo necrosis along with cavitation. Sometimes they present as intrabronchial polypoid masswith few minor extra bronchial spread[22,23].

The tumor is also seen to penetrate into the wall of bronchus and infiltrate into the peribronchial tissue.

In H&E examination, diagnosis is based on cell atypia and invasiveness. It is characterized by keratinization with or without intercellular bridges. These features are more prominent in well-differentiated tumors whereas mitotic activity and atypia increase in poorly differentiated tumors.

Thus they are divided into well, moderate & poorly differentiated carcinoma on the basis of degree of keratinization. There are various subsets like

- Small cell variant
- Clear cell variant
- Well-differentiated papillary variant
- Basaloid variant
- Spindle cell/carcinosarcoma

Variants of scc:

Papillary variant:

This variant of squamous cell carcinoma shows exophytic and endo-bronchial growth invasion in most of the cases. But sometimes limited intra-epitheial spread without invasion is seen[(24)]

Clear cell variant:

This variant of scc contains most malignant cells featuring classical clear cytoplasm[(25)]

Small cell variant:

These are poorly differentiated SCC with small tumour cells which retains the morphological characteristics of NSCLCs but with focal squamous differentiation[26,27]

Basaloid variant:

This squamous cell carcinoma variant shows peripheral palisading of nuclei which is the prominent finding and it usually presents with very aggressive clinical course. Squamous cell carcinoma have better survival rate than adenocarcinoma[28]

Electron microscopy shows abundant tonofilaments and basal lamina formation[29,30].

On IHC, there is strong reactivity for low & high molecular weight keratins & involucrin[31,32,33]. Also positive for vimentin EMA, S100, desmocollin-3[34]& glypican-3.

Adenocarcinoma

They are seen most commonly in over half of all lung carcinomas in females and a few percentage in males[35,36]. Its incidence is increasing in the last 2 decades, most of them present as poorly circumscribed grey-yellow lesions.

Sometimes, a small peripherally presenting adenocarcinoma spreads extensively into the pleural space and coats both the pleural layers simulating the appearance of diffuse mesothelioma(Pseudomesotheliomatous carcinoma)[37-40].

Rarely they can be present as a large endobronchial polypoid mass[41]. Sometimes a large number of cases of adenocarcinoma arising in association with a peripheral scar or Honey combing may show areas of atypical bronchiolar and alveolar proliferation in neighboring air spaces[42-47].

On microscopy they show a spectrum of differentiation which on one extreme blends with bronchio alveolar carcinoma and other end with undifferented large cell carcinoma. The formation of tubular or papilla and secretion of mucin are the two signs of glandular differentiation. Depending on the predominance of these features, they can be subdivided into acinar, solid, papillary, micro papillary, signed ring type. Rare variants are adenocarcinoma with goblet cell type[48,49,50], hepatoid type[51,52]adenocarcinomawith choriocarcinoma foci[53], adenocarcinoma with rhabdoid features[54], microcystic adenocarcinoma[55], and adenocarcinoma with massive lymphocytic infiltration[56]. On IHC, they are positive for low molecular weight keratin, CEA, EMA, & members of MUC family[57-60]. They are associated with genetic features like TP53 alteration, p16/CDKN2A inactivation, disruption of RB pathway[61], loss of 3p, KRAS , EGFR and C-MET mutation.

Histological subtypes of adenocarcinoma:

1. **Mixed Type** :

Most common type of adenocarcinoma representing 80% of resected specimens.

2. **Acinar Pattern**:

It contains tubules and acini composed of columnar or cuboidal cells which may secrete mucin[28]

3. **Papillary Pattern** :

In this type secondary and tertiary papillary structures are seen which replaces the underlying lung architecture. Tissue invasion and necrosis may be present. The lining cells may be mucinous and non-mucinous secreting cuboidal to columnar cells. Micropapillary pattern of adenocarcinoma, are usually prognostically unfavourable varieties[62]

4. **Bronchoalveolar pattern** :

In this pattern malignant cells will grow along the alveolar structures (this is known as lepidic growth). but without vascular, stromal, or pleural invasion[28]

5. **Solid Pattern**:

This variety composed usually of polygonal cell sheets which lacks tubules acini and papillae but mucin is seen in atleast five tumour cells.

Large cell carcinoma

Undifferentiated or large cell carcinomas are pleomorphic malignant epithelial tumors without any squamous or glandular differentiation[63]. Its location along with electron microscopy and IHC features suggests that they mostly resemble adenocarcinoma[64-68]. Some cases are associated with marked peripheral

eosinophilia or leukocytosis[69,70] due to granulocyte colony-stimulating factor produced by the tumor.

The variants of this tumor are

- Giant cell carcinoma
- Lymphoepithelioma like carcinoma
- Large cell neuroendocrine carcinoma
- Non-small cell lung carcinoma with neuroendocrine features.
- Combined large cell neuroendocrine carcinoma.
- Basaloid carcinoma
- Clear cell carcinoma
- Large cell with rhabdoid phenotype.

Variants :

1.**Large cell neuroendocrine carcinoma :**

This type constitutes 3% of lung cancers[71]. The malignant cells are arranged in various patterns such as organoid, nesting, trabecular rossetes or peritubular palisading patterns[28,72]. The cells are usually large with abundant cytoplasm and nucleus shows prominent nucleoli.

2. **Combined large cell neuro endocrine carcinoma :**

This tumor shows combination of features of squamous cell carcinoma, adenocarcinoma, giant cell carcinoma and may be spindle cell carcinoma too.

3.**Basaloid carcinoma:**

Here, the tumor cells are arranged in many patterns as nodular, solid, trabecular, and invasive growth pattern . Peripheral palisading of cells may be noted. The cells are monomorphic, small cuboidal to fusiform with nuclei showing moderate hyperchromatism[73]

4.**Lymphoepithelioma like carcinoma:**

They show growth pattern, with tumor cells having large vesicular nuclei, and prominent nucleoli. This type of carcinoma show heavy lymphatic infiltration[71,72]

5. **Clear cell carcinoma:**

Large polygonal tumour cells with clear, foamy cytoplasm[74,75]

6. **Large cell with rhabdoid phenotype:**

Rhabdoid cells containing tumour in which this rhobdoid cells should constitute atleast 10% of tumour cells.

Small Cell carcinoma

They constitute 10-20% of all lung cancers and most of them are males with the median age of 60 and most of them are smokers. Typically, they present in central position of the lung, but rarely in peripheral location[76,77].

The epithelial cells are very small, with scant cytoplasm, granular nuclear chromatin and inconspicuous nucleoli. Azzopardi effect which is characterized by

staining of vascular walls by intense basophilic staining due to encrustation caused by DNA from necrotic tumor cells which are frequently present.

Electron microscopy shows densecore neurosecretory granules.

Immunohistochemically, they are positive for Bcl_2 and mutation of p63 and RB tumor suppressor genes are commonly present.

Combined small cell carcinoma :

A tumour with characteristics of small cell variety with additional small components of either squamous cell carcinoma or adenocarcinoma. This type usually appears as hilar or periphral mass lesions or often presents with mediastinal lymphadenopathy and or lobar lung collapse.

AdenoSquamous carcinoma

This type of lung cancers shows evidence of malignant features in both squamous and glandular differentiation in an almost equivalent amount[78]. They account for less than 10% of all lung tumors. Most of them are seen located peripherally and associated C scar.

Sarcomatoid carcinoma & carcinosarcoma

It is a family of carcinoma having sarcoma like features when it contain predominantly of spindle shaped cells and proved on EM and IHC, they are called spindle cell or sarcomatoid carcinoma[79-83].

Grossly, they present as intraparenchymal or intrabronchial polypoidal mass.

On H&E, epithelial elements are present along with sarcoma like component which can resemble fibro sarcoma, MFH like appearance chondrosarcoma, osteosarcoma, RMS or angiosarcoma.

On IHC, they are positive for pankeratin, EMA and p63.

Clear Cell Carcinoma

It is a type of lung carcinoma composed of cells predominantly with clear cytoplasm. These clear cells contain abundant glycogen.

Adenocarcinoma and related tumors

They can present as single or multiple modules and a diffuse pneumonic like infiltrate[84-86].

On microscopy, they can be divided into mucinous and non- mucinous type.

The mucinous type has a typical glistening appearance on gross.

Microscopically, the tumor is composed of well differentiated mucin containing columnar cells in a ‘lepidic’ fashion without stromal invasion.

The non-mucinous type has a gray-white foci of parenchymal consolidation. Microscopically, they also show lepidic pattern without stromal infiltrate and the tumor cells are cuboidal rather than columnar and have brighter eosinophilic cytoplasm nuclear atypia and nucleolar prominence is greater than that seen in mucinous type. PAS positive eosinophilic intranuclear inclusions are seen.

Other rare types of lung cancers:

There are many rare types of lung cancers like sarcomas, lymphomas, adenoidcystic carcinoma, mucoepidermoid carcinoma and epithelial-myoepithelial carcinoma.

Precursor lesions

It has been said for a long time that squamous cell lung malignancies have a long preclinical stage where the lesion progresses from mild, moderate & severe dysplasia to carcinoma in situ, mircoinvasive carcinoma and frank invasive carcinoma.

Grossly they show granularity, papillation and loss of rugae. On H&E, they show full thickness involvement with intact basement membrane. Loss of heterozygosity, at 3p and 9q are seen in squamous metaplasia and dysplasia followed by TP53 mutation in carcinoma in situ. Squamous dysplasia / carcinoma in-situ:

This represents the precursors of SCC of lung.

- **Atypical Adenomatous hyperplasia (AAH) :**

This adenomatous hyperplasia represents the precursor lesion for adenocarcinoma . it usually seen in peripheral lesions and

- **Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia:**

This may progresses to carcinoids. Other possible pre-neoplastic lesions are Squamous metaplasia (which progresses to squamous dysplasia,carcinoma in situ),

Adenomatous hyperplasia (precursor to AAH), Basal cell hyperplasia, pulmonary fibrosis, angiogenic squamous dysplasia, etc[87]

No precancerous lesion is identified for small cell carcinoma so far. But sometimes precursors of NSCLSs such as squamous dysplasia or carcinoma in-situ be seen in the nearby airway mucosa[87]

Clinical features:

Lung malignancies usually present with symptoms like cough, hemoptysis, chest pain, hoarseness of voice, loss of weight, loss of appetite, new onset of wheeze (commonly seen in squamous cell carcinoma).

Small cell carcinoma present with symptoms according to the site of distant metastasis.

Adenocarcinoma are usually asymptomatic and it is more often a incidental radiological finding.

Obstructive pneumonia, pleural effusion, chest pain, back pain are the local effects of the tumor and they are due to mediastinal invasion by the tumor.

Entrapment of nerves like recurrent laryngeal nerve can cause hoarseness, horner syndrome is due to sympathetic nervous system and diaphragmatic paralysis is due of entrapment of phrenic nerve.

Superior vena cava obstruction can cause superior vena cava syndrome.

Cardiac tamponade and pericarditis are caused due to pericardial involvement.Dysphagia occurs due to oesophagus involvement.

The symptoms due to metastasis depends on the organ of involvement by the tumour. Infiltration of liver, pancreas and adrenals cause symptoms like loss of weight and abdomen pain.

Bone pain is present if it involves the bone.Neurological symptoms like headache, dizziness and vomiting are due to the central nervous system involvement by the tumour.

There are many paraneoplastic syndromes associated with lung carcinomas like hypercalcemia, hypocalcemia, carcinoid syndrome, cushing syndrome, gynacomastia etc

Systemic manifestations:

Antibodies directed against the neuronal calcium channels[88] cause Lambert-Eaten syndrome. It causes symptoms like neuropathy, acanthosis nigricans, hypertrophic pulmonary osteoarthropathy. The hematological symptoms caused are anemia, thrombocytopenia, eosinophilia, leukemoid reaction, leucoerythroblastosis etc.

Disease course:

Dysplasia usually preceeds the disease course for years which is followed by carcinoma in situ which lasts for several years and presents as a mass which is aymptomatic in the beginning and presents with symptoms in the latter. The lesion presents with hard grey white lesions with some areas of hemorrhage and necrosis[18].

Lung carcinomas can present as lesion within the lumen. It can penetrate the wall of the bronchus and involves the peribronchial tissues, carina and mediastinum.

Local spread to the pleura, pericardium and the regional lymphnodes like tracheal, bronchial and mediastinal lymph nodes can occur.

Lymphatic and hematogenous routes are the ways of spread to cause distant metastasis. Most of the tumors metastasise early except squamous cell carcinoma which metastasise late.

Distant metastasis is common to the adrenals which constitutes more than 50% and followed by liver, , bone and brain respectively[89].

Role of Imaging studies in lung cancer:

Imaging should be done both pre operatively and post operatively for the following reasons

- To find a suspicious lesion in the lung which turn out to be malignant
- Used for the staging of the disease
- To assess the effectiveness of the treatment
- To look for recurrence of the tumor

There are various imaging modalities that are used for diagnosing of lung malignancies. They are

X-ray:

It is the most preliminary and basic investigation used for identifying lung malignancies. It helps to assess the involvement of main bronchi and trachea. It also helps in identifying lymphadenopathy, mediastinal invasion and pleural effusion.

Computed tomogram (CT):

It helps to localize the site of involvement of tumour. The size and shape of the tumor can be assessed. Also the regional sites of involvement of the tumor and metastatic sites of involvement like liver, brain, bone adrenal can be assessed.

Magnetic resonance imaging(MRI):

It can identify lesions of metastatic sites like brain, spinal cord etc.

Ultrasonogram (USG):

It helps to detect pleural effusion. It also guides for investigations like thoracocentesis and for biopsy of peripheral lung lesions for tissue biopsy.

Positron emission tomography(PET SCAN):

It is a method of nuclear imaging which can detect the biochemical changes in the body tissues.

More commonly it is used as a whole body scan which detects the tumor recurrence and early breast lesions.

Staging of lung cancer has been improved with the advancement of this technique[(90-92)].

Bone scan:

It is used to identify bone involvement.

Investigations for lung cancer:

The gold standard test for diagnosis of lung malignancies is by histopathological examination of lung tissue specimens. The diagnostic modalities used are

- ✓ Cytology of sputum specimens
- ✓ Thoracocentesis
- ✓ Flexible bronchoscopy
- ✓ Transthoracic needle aspiration
- ✓ Thoracotomy[(93)]
- ✓ Video assisted thoracoscopy
- ✓ Excision biopsy of nodes

The treating physician should determine the suspicious type of lung cancer in order to select the appropriate test procedure.

Patients suspected to have early lung cancer can be evaluated with thoracotomy. Histopathological examination and staging can also be estimated with this method.

Sputum examination:

For sputum cytology examination, atleast three sputum samples are examined[94,95]. Adequacy of sputum sample is identified by the presence of alveolar macrophage, squamous cells

Minimum of three sputum samples are examined. It is a noninvasive test. Hence other procedures can be proceeded if it is negative. Sputum cytology examination is very important for patients presenting with hemoptysis. It is recommended for centrally located tumours. Specificity of sputum cytology in the diagnosis of lung cancer is about 98%. Sensitivity for centrally located tumors is 71% and peripherally located tumors is less than 50%[96,97]. It is also helpful to diagnose squamous cell carcinoma.

Thoracocentesis:

In patients presenting with pleural effusion, thoracocentesis can be done. Pleural fluid can be evaluated for malignancies by careful sampling. The sensitivity and specificity of diagnosing lung cancer using thoracocentesis is 80% and 90% respectively.

Excision biopsy of lymph node:

If the lymph node is in a accessible site, excision biopsy of the entire node is done to for histopathological examination.

Flexible bronchoscopy:

The sensitivity of this test is 88%. Flexible bronchoscopy is done by passing the bronchoscope through the bronchial lumen and advancing the scope to visualize the lesion, if any. Bronchial wash and tissue for examination is collected.

According to De Wever W et al[(90)], catheter placement into the patients lung should not be attempted without the assistance of computed tomography. The sensitivity and specificity of the investigation depends on on the site of location of tumor and its size. The sensitivity of diagnosing centrally located tumor is higher than the lesions presenting peripherally which are 90% and 70% respectively.

Transthoracic needle aspiration:

CT or fluoroscopy guided transthoracic aspiration can be done with the appropriate needle size. This is the recommended procedure of choice for peripherally located lesions. The sensitivity and specificity of this method is 90% and 97% respectively. This procedure is done in cases where the transneedle aspiration is not conclusive for a patient with peripheral tumor. Also done for patients who are not suitable for surgery. Pneumothorax which is the most common complication of this procedure is seen in 25% to 30% of patients undergoing this procedure.

Video assisted thoracoscopy:

Tumors of size less than 2 cm that are peripherally located pleural tumors, pleural effusion can be done with video assisted thoracoscopy. Endoscopies help to assess and visualize the space between the lung and peripheral pleura. It helps in identifying small lesions in the interpleural space and to take tissue biopsy. It is also useful to resect the tumours at the early stage. The major advantage of this procedure is that we can prevent the attempt of thoracotomy.

Thoracotomy:

If the tumor is resectable, thoracotomy is the recommended method for diagnosis of early stage disease[(90)].

Biopsy

With the advent of fiber optic bronchoscopy, it has dramatically expanded the potential of bronchoscopic biopsy. The instrument is easily inserted and can be well accepted by the patients. If the clinical suspicion is high and bronchoscopic biopsy fails to establish the diagnosis of carcinoma, an exploratory thoracotomy can be done.

Cytology

Sputum and/or bronchial brushings can make a diagnosis in 80-90% of patients with lung carcinoma. False positive diagnosis are seen in infarct, bronchiectasis, mycotic infection, viral pneumonia, irradiation changes and lipoid

pneumonia. Macrophages, altered alveolar lining cells or mesothelial cellscan be misinterpreted as malignant.

The reports can be as

- Unsatisfactory (no macrophages)
- 'Negative' (no abnormal cells)
- 'Benign atypia' (epithelial bronchial cells with hyperplastic & metaplastic changes secondary to inflammation)

  Suspicious but not diagnosed (Indication to repeat)
- Positive for malignant cells.

Frozen section

It is mostly recommended for peripheral lesions where the tumor can be excised with a margin of normal lung. Also when it is proved to be a benign lesion like hamartoma or organizing pneumonia.

The two important contribution of frozen section for lung carcinoma are mapping of mediastinal & hilar lymph modes and estimating the bronchial margins for tumor cells.

Screening:

As there are many histological patterns of lung cancer, a single biomarker for diagnosis is a great challenge. Many biomarkers are evaluated. An effective screening should be such that it should identify cases at an early stage so that survival can be improved. Moffitt cancer research[98,99] centre has a project which

emphasizes on screening procedure. Monoclonal antibodies is one of the screening method which can be followed. Our recent research topic of interest has been the identification of monoclonal antibodies and their staining intensity. The expression of these genetic and protein markers help in understanding the cell of origin and their tumour biology. Mutation of a particular gene can cause specific epithelial malignancies. These mutated genes induces abnormal cell growth and proliferation. An early marker used for the early detection of lesions of lung cancer in sputum cytology is Heterogenous nuclear ribonucleoprotein(hnRNP). There are many datas which explains the that hnRNP is expressed in most lung cancers before any morphological abnormality could be identified.

There are many other lung markers which are useful in for cancer screening. They are tumor suppressor genes like p53, p16, p21, Rb, proto-oncogenes like c-myc, K-ras, HER2 NEU, HGF, growth factors are TGF-b, FDGF, apoptotic factors like Bcl-2, factors of angiogenesis like VEGF and gene amplification factor like HER-2[(98)]. These markers are very useful for identifying lung carcinomas. Also these markers are used for the identification of prognostic and theraupeutic markers. There are many biological markers which are found to be strongly associated with various lung malignancies. 30% of non small cell lung cancer is strongly associated with retinoblastoma gene and nearly 100% of cases of small cell lung carcinoma is associated with retinoblastoma gene.

There are various clinical trials conducted on a large scale to find out the efficacy of screening methods in lung carcinoma. One such trial is the one conducted by National Cancer institute. The main focus of this study was to identify the

effective methods for screening and to identify the mortality and morbidity rates of various specific types of lung malignancies. The main disadvantage encountered in this trial was that the conventional x-rays fail to diagnose lung neoplasms at an early stage[(100)]. National Lung Screening Trial is another such kind of trial for evaluating the screening methods. This trial compares the efficacy of conventional x-rays and spiral CT scans for the screening of lung cancers. It has been evaluated that spiral CT scans can detect lung nodules which cannot be picked up by conventional x-rays. Thus spiral CT scans are proved to diagnose lung cancers at early stage and thus helpful to detect lung malignancies.

Spread and metastasis

Lung cancer spreads by direct extension proximally & distally along the bronchus of origin and invades the trachea. Pleural effusion is very common when they invade the chest wall and diaphragm. Invasion of blood vessels can sometimes cause extensive tumor emboli.

Lymph node metastasis first occur in the hilar region followed by mediastinum, supraclavicular groups, axillary & subdiaphragmatic sites.

Distant metastasis are seen in liver, other parts of lung, adrenal, bone, kidney & Bone marrow.

CLASSIFICATION OF LUNG CANCER:

In the past lung tumors has been classified into small cell carcinoma of lung and non small cell lung malignancies based on the available modality of treatment options. But in the recent past, due to the availability of personalized medicine and targeted therapies, it is important to identify the specific subtypes of non small cell subtypes so that advanced treatment options can be used. The discovery of specific gene alterations, particularly those responding to tyrosine kinase inhibitors has necessitated the need for revising this older classification. The most frequent molecular alterations encountered in lung carcinomas are epidermal growth receptor(EGFR), K-RAS genes, rearrangements of anaplastic lymphoma kinase (ALK) genes. The expression of EGFR genes correlates with better response to treatment modalities particularly in adenocarcinoma. K-RAS mutations is also expressed in 15-30% of lung adenocarcinomas which is a marker to denote the resistance of lung carcinoma to tyrosine kinase inhibitors. This approach has led to the necessity of inclusion of molecular genetic classification[101,102].

WHO CLASSIFICATION OF LUNG CANCER:

(ANNEXURE II)

The most widely used method of histological classification is proposed by World Health Organisation(WHO).

Though it is widely accepted method of classification, it has some interobserver variability for which certain subtypes are not associated with molecular characteristics.

It is broadly classified as malignant epithelial neoplasms, biphasic epithelial mesenchymal neoplasms, mesenchymal neoplasms, lymphoproliferative neoplasms and metastatic tumors. It is explained in ANNEXURE.

IASLC/ATS/ERS Classification in small biopsy and cytology:

(ANNEXURE III)

Treatment:

There are four basic modalities of treatment for the trearment of lung cancer. They are

- Surgery
- Chemotherapy
- Radiotherapy and
- Targeted therapy

The treatment for different types of lung cancers depends on the histological type of lung cancer, stage at presentation, patients preference, side effects of particular treatment and health of the patient.

The standard therapy done for operating non-small cell lung carcinoma is complete surgical excision through thoracotomy[(103-105)]. Combined modality treatment is done for tumors of high grade. Radiation therapy can be done with effective results to control local growth of lung cancer.

The various types of surgeries done are lobectomy, segmentectomy, pneumonectomy and wedge resection.

Chemotherapy and concurrent radiotherapy can be done for patients with early stage small cell lung carcinoma.

Surgery can no longer be done once the tumour has started spreading beyond the hemithorax and metastasized to the lymph nodes. Chemotherapy and radiotherapy are the only modality of treatment in such cases.

The two types of radiotherapies given in lung cancer are radioisotope therapy and external beam radiotherapy.

The chemotherapy drugs used are platinum based cisplatin, carboplatin and non platinum based drugs like Doceraxel, Paclitaxel, Gemcitabine etc.

Chemotherapy is the only modality of treatment in distant metastasis.

Targeted therapy is of much benefit when given to appropriate patients. In targeted therapies, treatment is given against the specific genes or specific proteins.

Currently it is recommended that EGFR mutation to be documented in lung cancer cases. It can be done by mutant specific EGFR antibodies or PCR. EGFR mutations are seen in 10-15% of cases of non small cell lung cancer. It is most commonly seen in adenocarcinoma type of lung malignancies.

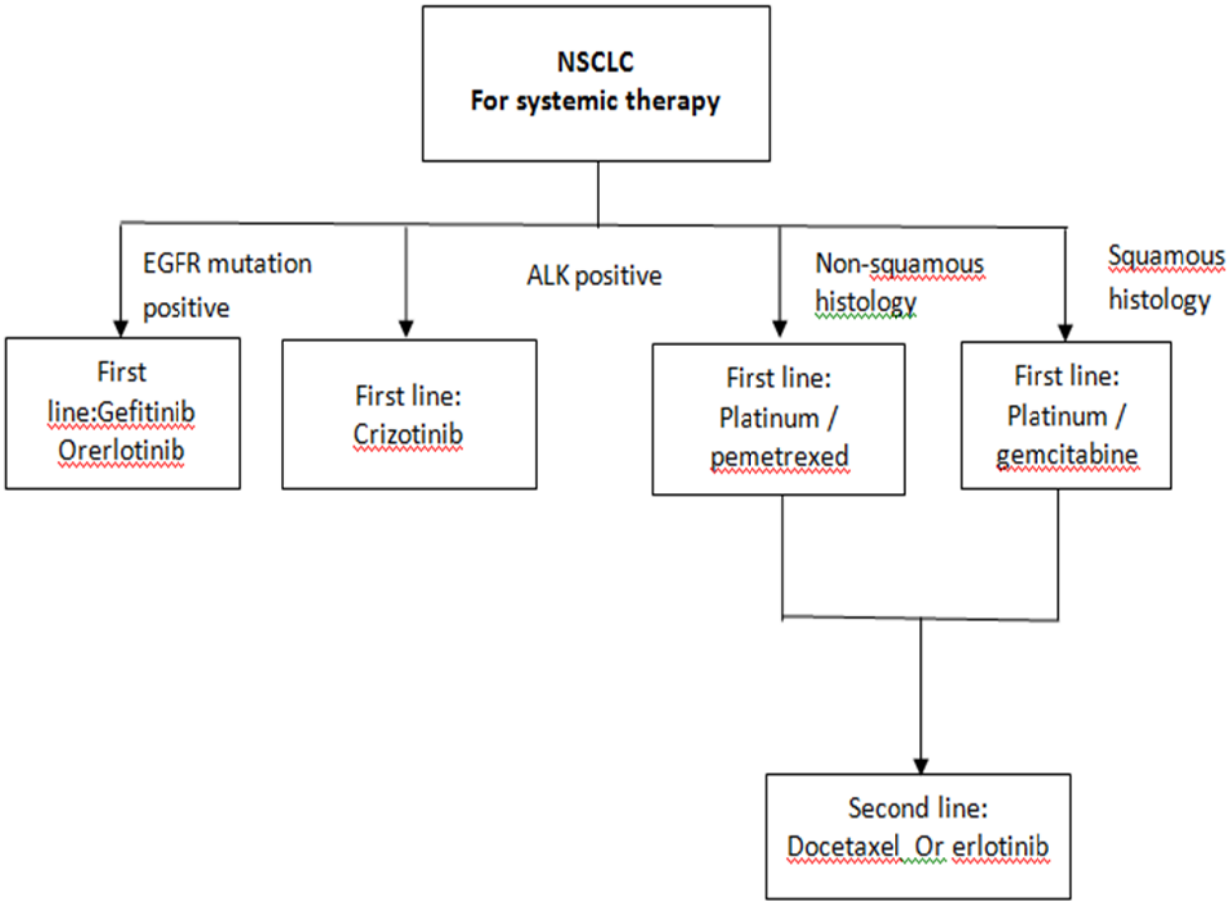
There is a significant advancement in the treatment of lung cancer due to tyrosine kinase inhibitors like gefitinb and erlotinib which acts as targeted therapy in patients with EGFR mutation.

Another targeted therapy is vascular endothelial growth factor inhibitor such as bevacizumab. This antiangiogenesis therapy blocks the development of new blood vessels and helps in treatment management.

Around 5% of non small cell lung carcinoma is is associated with rearrangements of ALK gene. It is seen commonly in non smokers and in patients with adenocarcinoma.

There are many other markers for targeted therapy like like BRAF, FGFR, HER2, K-RAS, ROS1.

Non small cell lung carcinoma- treatment in advanced stage:



Prognostic factors:

As most of the screening programs are unsuccessful in identifying malignancies at early stages, early detection of cancer is not possible in all cases. Early detection of cancer is helpful in improving the survival. As early detection of cancer is quite difficult for lung malignancies, it has become one of the most lethal of al malignancies. Mortality rate is increased when compared to other malignancies

like colon, prostate and breast malignancies. The major drawback of this malignancy is that most cancers are asymptomatic till the advanced stage. American cancer society has estimated that only 15% of the lung malignancies are identified at early stage. The five year survival of lung carcinoma is 15%. It is because of lack of effective screening programmes.

There are many factors which affect the prognosis of lung cancer

Age :

Patients less than 40 years have poor prognosis. This is because they present with advanced disease and aggressive behaviour[106].

Sex:

Women have higher incidence of advanced lesions & adenocarcinoma is the most common type of histological type and have worse prognosis

Location:

Tumors of superior pulmonary sulcus have a better prognosis than others. Squamous cell malignancies that are located peripherally have better prognosis than the carcinomas that are located centrally[107-110].

Stage:

TNM staging is the single most important parameter for lung cancer[111,112].

Tumor size:

Large size tumors have worse prognosis than the small size tumors of same histological type[113]. In adenocarcinoma type of lung malignancies showing both in-situ and invasive component, the site of invasive component is the single most important predictor of survival rate.

Histological type & Differentiation:

The most curable type of lung carcinoma is squamous cell carcinoma[114-116].The five years survival rate for well differentiated carcinoma is 40%, for moderately differentiated tumors it is 20%, and for the poorly differentiated malignancies, it is 7%.

In adenocarcinoma type, micropapillary pattern has worst prognosis[117-119].

Prognosis of BAC is found to be better than ordinary adenocarcinoma.

Large cell carcinoma having tumor giant cells have worse prognosis.

Small cell carcinomas have worse prognosis compared to other type of lung malignancies. Their five year survival rate is less than 2%.

Chest wall invasion:

The risk of operative mortality is higher than in cases with chest wall invasion[120].

Blood vessel invasion:

They have ominous prognostic significance.

Pleural effusion:

The pleural fluid positive for tumor cells is a poor prognostic indicator[121].

Presence of scar:

Tumors with scar has worst prognosis than tumors without scar.

Lymph mode involvement:

It is one of the most important prognostic factors.

Inflammatory reaction:

The presence of lymphoplasmacytic infiltrate is a favorable prognostic sign[122].

Rhabdoid features:

It has a very aggressive behavior[123].

TTF-1:

Strong expression of this factor has better survival[124-126].

CD 117:

Its expression indicates high risk of tumor proliferation and aggressivity.

Oncogene expression:

Increased expression of RAS, p21 and NMYC predicts poor prognosis.

P53 and HER2neu:

P53 and HER2neu overexpression is associated with poor prognosis.

RECOMMENDATIONS BY IASLC/ATS/ERS NEW MULTIDISCIPLINARY INTERNATIONAL CLASSIFICATION, FOR SMALL BIOPSY AND CYTOLOGY SPECIMENS WERE:

- For cytology as well as small biopsy specimens, if a clear differentiation can be done, which satisfies the standard morphologic criteria, further specific typing of NSCLC into squamous cell carcinomas and adenocarcinomas can be done with morphology alone.
- The term NSCLC - NOS must be used as infrequently as possible and it should only be used if the diagnosis cannot be made out by morphology and /or by special staining / IHC.
- When small biopsy / cytology specimen is used in addition with special stains for diagnosis, it should be clearly noted whether the diagnosis is achieved with only light microscopy or in combination with special stains.
- The term non-squamous cell carcinoma which is used by clinicians, should not be used by pathologists while reporting. Pathologists should report NSCLC only as ADC , SQCC and NSCLC - NOS.
- The tissue specimens received by pathologists should be used judiciously and preserved to the maximum, as more tissues will be needed for further molecular studies[127-129]
- In small biopsies / cytology specimens, if any invasive pattern is found in adenocarcinoma ,it is to be reported as a lepidic growth pattern . The term minimally invasive ADC and ADC- in situ should not be used.

- The term large cell carcinoma, should be used only in resected specimens as thorough sampling of tumour is not possible in small biopsy/cytology specimens.
- • If the tumor shows sarcomatoid features characterised by malignant giant cells or spindle cells with nucleus showing pleomorphism, it should be classified according to guidelines above as NSCLC favouring ADC or NSCLC favouring SCC based on features of glandular pattern or squamous features respectively. when these features are absent it is to be reported as NSCLC - NOS with a word about sarcomatoid features..
- Only if the tumor shows neuro endocrine morphology, neuro endocrine IHC markers are performed.
- Further classification of NSCLC- NOS is possible with the use of IHC, into NSCLC favouring ADC and NSCLC favouring SCC.
- It is advised to use minimal stains for further subclassification of NSCLC-NOS.
- It is recommended to use only one marker for adenocarcinoma or one marker for squamous cell carcinoma.
- Currently, the single best marker for diagnosing adenocarcinoma is TTF-1. Staining with diastase - periodic acid schiff, alcian blue/ PAS stains or mucicarmine also play a role in diagnosing adenocarcinoma.
- The specific marker for diagnosing SCC is Polyclonal p40 rather than the monoclonal p63 . p40 is likely to surpasses p63 as a best IHC marker in diagnosing squamous cell carcinoma.

- In NSCLC -NOS , the cases which shows TTF-1 positive and /or mucin positive, but p40 and p63 negative are termed as NSCLC favouring adenocarcinoma. similarly those cases with p40 and/or p63 positive but TTF-1 and mucin stain negative are termed as NSCLC favouring SCC with comment on whether special stains are used to arrive at diagnosis.
- In case, one population of tumour cells show TTF-1 reactivity and another population of tumor cells show positive for squamous cell markers, possibility of adenosquamous carcinoma should be considered.
- But if TTF-1 as well as p40 are negative and fails to show any squamous or glandular morphology, the diagnosis still remains as NSCLC-NOS.

ALTERATIONS SUGGESTED BY IASLC/ERS/ATS INTERNATIONAL CLASSIFICATION OF LUNG MALIGNANCY IN RESECTED SPECIMENS:

(ANNEXURE IV)

1. The term bronchoalveolar carcinoma is discarded.

In the new multidisciplinary classification, BAC is discarded. Originally, broncho alveolar carcinoma is defined as a non-invasive lesion, but since then, it is used to denote broad group of tumours which includes

- Nonmucinous BAC. This is defined as solitary non invasive small peripheral adenocarcinoma. This type will have 100% 5 year survival rate[(130)]

- Minimally invasive small peripheral adenocarcinoma with 5 year survival upto 100%[131,132]
- Invasive adenocarcinoma with mixed subtype.
- Nonmucinous and mucinous adenocarcinoma, which is known as BAC earlier.
- Advanced mucinous adenocarcinoma(stage 4) with low survival rate.
- In the new multidisciplinary classification, ‘BAC’ is referred to as "former BAC"

2. New concepts were introduced for

- Small solitary peripheral adenocarcinoma with size less than or equal to 3cm,with pure lepidic growth without invasion with 100% disease specific survival as adenocarcinoma in situ(AIS).
- Small, solitary peripheral adenocarcinoma with size less than or equal to 3cm, with predominantly lepidic growth with invasion, with 100% disease specific interval as minimally invasive adenocarcinoma(MIA).

3. Former invasive adenocarcinoma with mixed subtype is replaced by predominant pattern..

- According to 2004 WHO classification more than 90% of lung adenocarcinoma are of mixed subtypes. In this new international classification this mixed subtype is replaced with predominant pattern. It is recommended to choose one predominant pattern based on recording of patterns in 5% increments.

4. In multiple lung adenocarcinoma patients it is recommended comprehensive histological subtyping of heterogenous, complex lung adenocarcinoma to determine if the tumours are synchronous, metachronous or metastasis.

5. It recommends the term lepidic predominant adenocarcinoma in place of previously classified as mixed subtype ,predominantly non mucinous BAC.

6. New histological type of Micropapillary predominant adenocarcinoma is introduced . This is associated with poor prognosis.

7. In new international classification, invasive mucinous adenocarcinoma, fetal, enteric and colloid adenocarcinomas are introduced as new variants.

Acccording to Edwards et al , only 10-15% of lung cancer patients undergo resection and the preoperative diagnosis confirmed. So treatment for most of the patients is based on diagnosis with small biopsy/cytology specimens alone.

According to Suprun et al,

• The criteria for diagnosis of SCC are

The presence of Keratin formation and/or Intercellular bridges. In case if the tumor lacks such features, the intraepithelial in- situ like extensions along the bronchus are present in SCC. Both adeno and small cell carcinoma do not replace the bronchial epithelium to a considerable extent. And most of these cases are either well differentiated or moderately differentiated. This feature aids in histological typing of lung cancers in small biopsy specimens.

The grading of squamous cell carcinoma cannot be done in small biopsy specimens.

• The diagnosis of adenocarcinoma seems to be more challengeable as the presence of mucin and gland formation are frequently not present in small biopsies, which calls for mucin stains to demonstrate the presence of glandular elements.

• According to the study of Edwards et al, the diagnosis of large cell carcinoma is possible only with resected specimens and not on small biopsies. This is because, they are the poorly differentiated forms of adenocarcinoma, squamous cell carcinoma, or neuroendocrine carcinoma and also most major types of lung cancers contain foci of features of large cell carcinoma .

• Recent data showed that the high percentage (30-50%) of NSCLC-NOS has been diagnosed in small biopsies. Data from the registry of epidemiology surveillence shows the increasing frequency of this diagnosis.

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR is a trans-membrane glycoprotein belonging to erbB family of closely related receptor tyrosine kinases. It has a transmembrane protein binding extracellularly and intracellular tyrosine kinases and other regulatory domains. On binding of specific ligands, normally functioning EGFR undergoes conformational change and phosphorylation causing signal transduction and cell proliferation and inhibition of apoptosis[(133)].

The advent of EGFR receptor tyrosine kinase inhibitors gefinitb and erlotinib has revolutionized the treatment of lung cancer patients in advanced cases. A large phase III trial ofnearly 1700 advanced lung cancer patients treated with gefitinib or

placebo was conducted. It did not have survival benefit for all patients treated with lung cancer[134]. However, there was a significant survival advantage in non-smoking Asian women with adenocarcinoma historical type.It has been estimated that EGFR is most likely expressed in solid growth pattern which suggest that there is a strong association between EGFR amplification and aggressiveness.

It is suggested that EGFR expression can be arrested by IHC, PCR and F1SH. Positive predictive values for each of them are 6.5-8.2%, 7-100% and 11-89%[133] respectively.

IHC as tool for diagnosis:

Paraffin blocks with 4 mm serial sections[135] from surgical biopsy specimens or cytology cell blocks can be used for EGFR protein expression. Chromogen is used to identify positive result in diaminobenzidine.

Negative internal control can be provided by normal epithelial and stroma IHC scoring for EGFR is as follows.

Tumour cell staining membrane is considered to be specific for the interpretation of the tumour.

Score 0 is given for tumours that has no staining of tumour cells, score 1 weak membrane staining in more than 10% of tumor cells, score 2 for moderate staining in more than 10% of tumor cells and score 3 for tumor cells with strong intensity in more than 10% of tumour cells.

IHC done for EGFR has approximate sensitivity of 88% and specificity of 88%. Squamous cell carcinoma is said to have strong staining with more number of positive tumor cells than adenocarcinoma.

It is estimated that IHC positive tumors show strong EGFR expression whereas IHC negative tumors have low or no expression.

The introduction of targeted therapies has greatly revolutionized the treatment of advanced lung carcinomas. Two oral drugs which are used to inhibit EGFR are Gefitinib & Erlotinib and they have been recently approved for use in advanced non-small cell lung cancer. Hence clinical, morphological and molecular factors can predict the response rate to these drugs. However standardization of techniques and more studies regarding this concept are expected in future.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study is a retrospective study of lung cancers in large resected specimens and small biopsies conducted in the Institute of pathology,Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between July 2013 to June 2015.

A total of 33042 cases were submitted to our department the Institute of pathology, Madras medical college during the period of July 2013 to June 2015 for histopathological examination. Out of them, 412 were lung cases. Among them 39 cases were pneumonectomy specimens, 67 were lobectomy specimens and 306 were small biopsies (Transbronchial, Endobronchial, Open, Ultrasound guided biopsies and Computed tomography guided core biopsies).

INCLUSION CRITERIA:

- All lobectomy specimens of lung cancer
- All small biopsies of lung cancer

EXCLUSION CRITERIA:

- Benign lesions
- other non neoplastic lesions

METHOD OF DATA COLLECTION

Detailed history of the patients regarding age, sex, site, tumor location, radiological findings, FOB findings, cytological findings were obtained for all the cases. Out of these

Material Inadequate for opinion	-	42
Non neoplastic lesions	-	137
Suspicious of malignant cells	-	40
Malignancy	-	193

Among the malignant cases,

NSCLCs	-	178 cases
Small cell lung carcinoma	-	9
Others	-	6

These 178 cases of Non small cell lung carcinoma cases were reviewed and sub classified on the basis of H&E morphology according to WHO criteria for classification . Tumours were sub typed as adenocarcinoma if it has features of gland formation and/ or mucin production, squamous cell carcinoma if it has features of keratinazation or intercellular bridges and large cell carcinoma (undifferentiated non small cell carcinoma) if it lacked both glandular or squamous patterns. Out of these 178 cases, 77 cases are Squamous cell carcinoma, 63 cases are

adenocarcinoma, 38 cases are NSCLC-NOS which includes poorly differentiated carcinoma 9 cases are small cell carcinoma and 4 cases are neuroendocrine carcinoma.

Among all these cases 20 cases of squamous cell carcinoma, 20 cases of Adenocarcinoma, 20 cases of NSCLC - NOS were randomly selected for study purpose.

All 60 cases of non small cell lung carcinomas (NSCLC) which were selected randomly were included for further evaluating and comparing the clinical and morphological parameters and to study the efficency of marker

(EGFR). Various clinical parameters like age, sex, site, location of tumor, radiological findings, cytology and histopathological diagnosed were assessed.

Five micron thick paraffin sections were cut and procedures were done for IHC study of EGFR.

IMMUNOHISTOCHEMICAL EVALUATION:

Antigen	Vendor	Species	Dilution	Positive control
EGFR	Pathnsitu	Rabbit	Ready to use	Lung squamous cell carcinoma

Immunohistochemistry was done as per protocol given in ANNEXURE V.

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization(membrane), percentageof cells stained and intensity of reaction.

In this study, for evaluation of EGFR protein, expression greater than 10% of the tumour cells by the tumour marker is considered as positive. Cases with no focal areas of positive staining and with less than 10% staining are considered negative. (ANNEXURE VI).

STATISTICAL ANALYSIS:

The clinicopathological relationship with EGFR expression was analysed by Chi-square test.

The statistical analysis for this study was carried out via SPSS software, version 21 .

The p values of less than 0.05 is considered to be stastically significant.

OBSERVATION

AND

RESULTS

OBSERVATION AND RESULTS

In my study period of two years from july 2013 to june 2015, a total of 33042 specimens were obtained in the Institute of Pathology, Madras Medical College for histopathological examination.

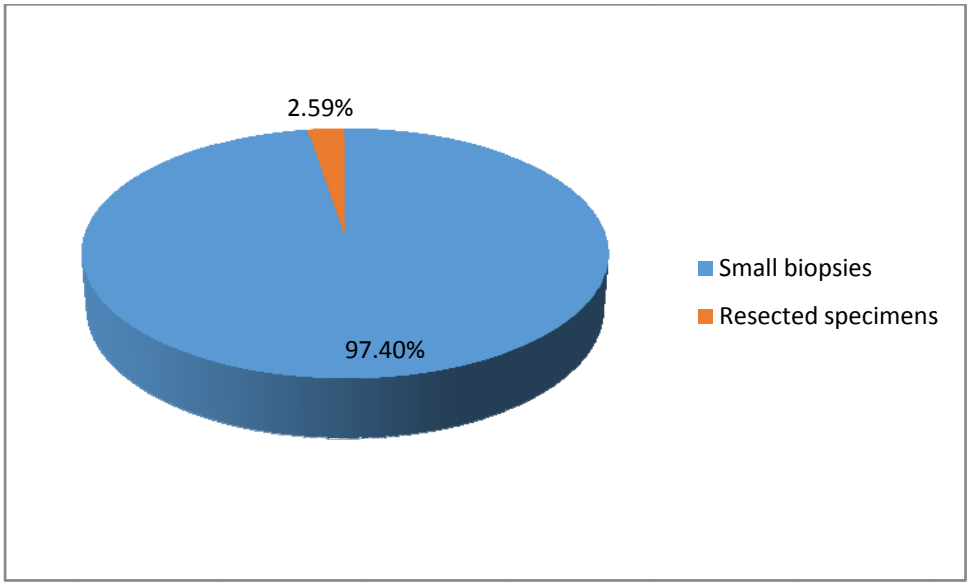
Totally 412 lung specimens were reveived. Out of these 188 are small biopsies and 5 are resected specimens.

It is shown in (TABLE :1 CHART :1)**.**

TABLE 1: TYPES OF LUNG SPECIMEN

	Total	**Percentage**
Small biopsies	188	97.40%
Resected specimens	5	2.59%
Total	193	100%

CHART: 1 TYPES OF LUNG SPECIMEN



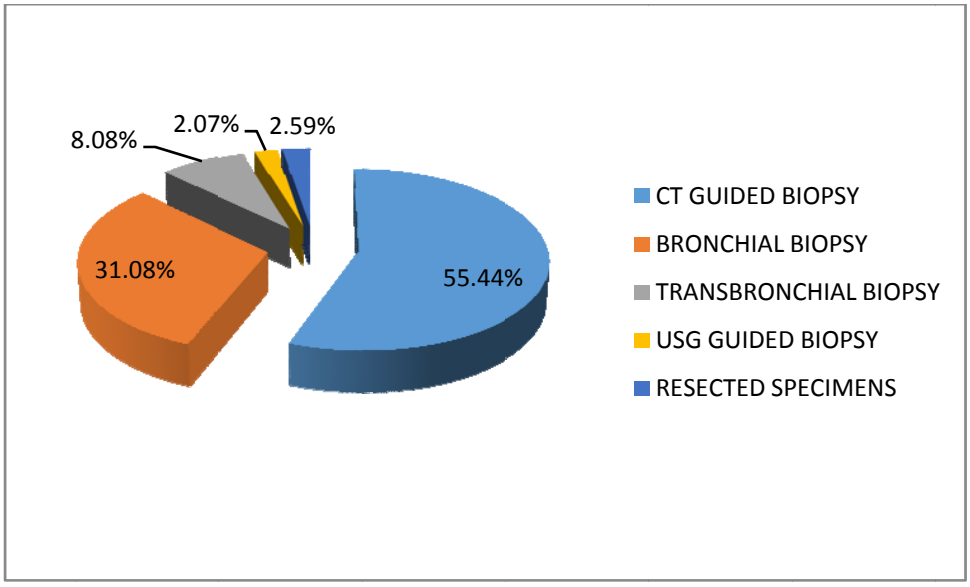
Among these cases, total number of CT guided biopsy are 107 , bronchial biopsy specimens are 60 , USG guided biopsy specimens are 4 , transbronchial specimens are 17, resected specimens are 5

(TABLE :2, TABLE :2)

TABLE:2 PROCEDURE DONE

SPECIMEN	Total	PERCENTAGE
CT GUIDED BIOPSY	107	55.44%
BRONCHIAL BIOPSY	60	31.08%
TRANSBRONCHIAL BIOPSY	17	8.08%
USG GUIDED BIOPSY	4	2.07%
RESECTED SPECIMENS	5	2.59%

CHART 2: PROCEDURE DONE



Thus CT guided biopsy is the most common type of specimen received in our institute and constitutes 55% of total Procedures done. Lung resected specimens accounts for the least number of specimens received constituting 2% .

Of the 193 malignant cases reported, Squamous cell carcinoma constituting the number of 77, 64 were adenocarcinoma, nine cases are small cell carcinoma, four cases are neuroendocrine tumors, 37 cases of NSCLC-NOS carcinoma and two cases are large cell carcinoma.

The most common type of lung malignancy received is Non small cell lung carcinomas (NSCLC) which includes squamous cell carcinoma, adenocarcinoma, poorly differentiated carcinoma which constitutes a total of 178 cases with a percentage of 92.22%. small cell carcinomas constituted 9 cases that accounts for 4.66%.

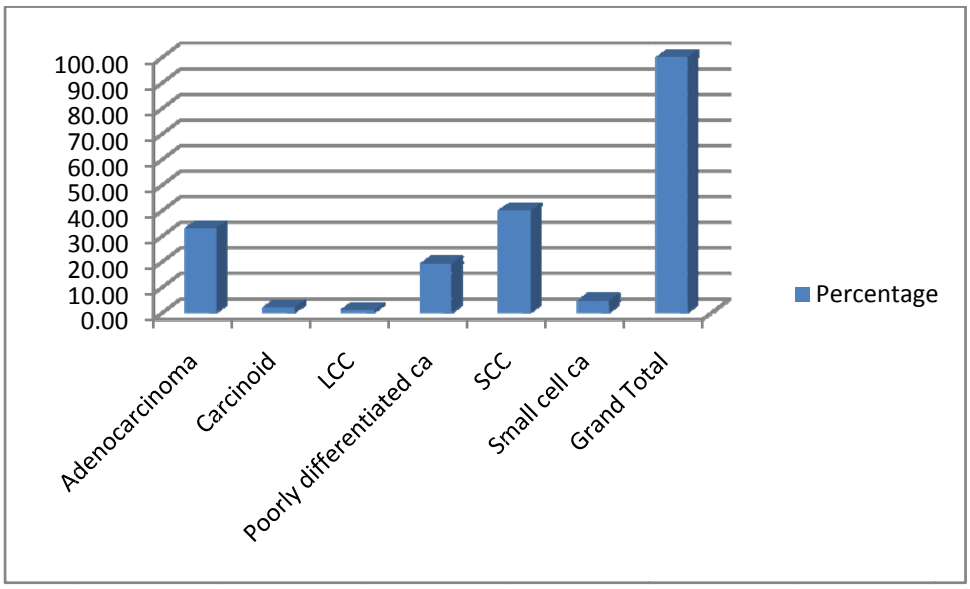
Apart from this metastatic carcinoma constitutes 12 cases and carcinoid constitutes 4 cases with 2%.

TABLE:3, CHART:3

TABLE 3: HISTOLOGICAL TYPES OF LUNG CARCINOMA

HPE DIAGNOSIS	Total	Percentage
Adenocarcinoma	64	33.01
Carcinoid	4	2.07
LCC	2	1.04
NSCLC-NOS	37	19.17
SCC	77	39.90
Small cell ca	9	4.66
Grand Total	**193**	**100.00**

CHART 3: HISTOLOGICAL TYPES OF LUNG CANCER



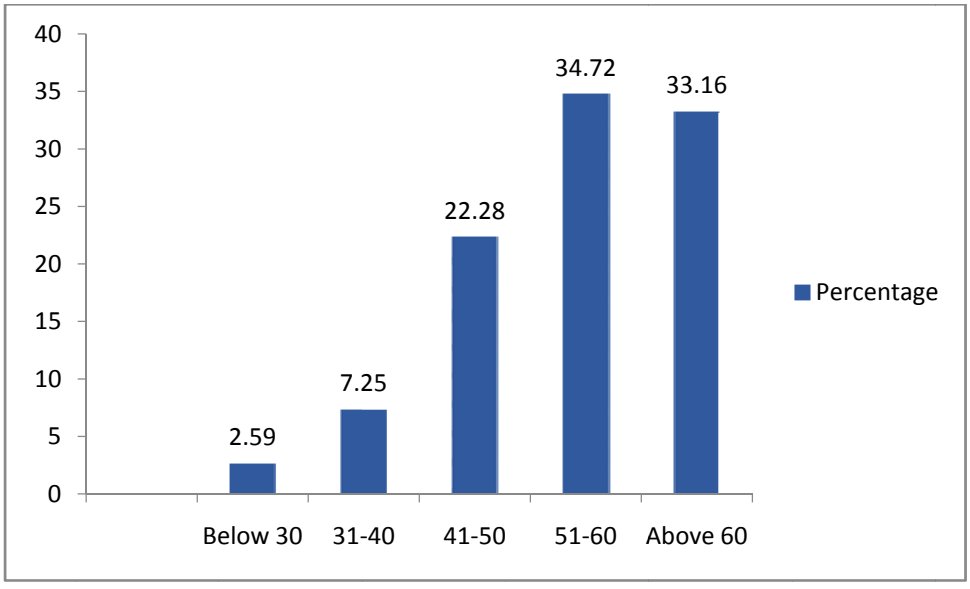
Peak incidence for all type of lung cancers is 51-60 years. In This study the youngest age at presentation is 29 years. The mean age is 55.15years . 64 cases are observed in the age group above 60 years. More than 90% of the cases are seen between 40 and 70 years.

(TABLE:4 CHART:4)

TABLE 4: AGE WISE DISTRIBUTION OF LUNG CARCINOMAS

Age	Total	Percentage
Below 30	5	2.59
31-40	14	7.25
41-50	43	22.28
51-60	67	34.72
Above 60	64	33.16
Grand Total	**193**	**100**

CHART 4: AGE WISE DISTRIBUTION OF LUNG CANCERS



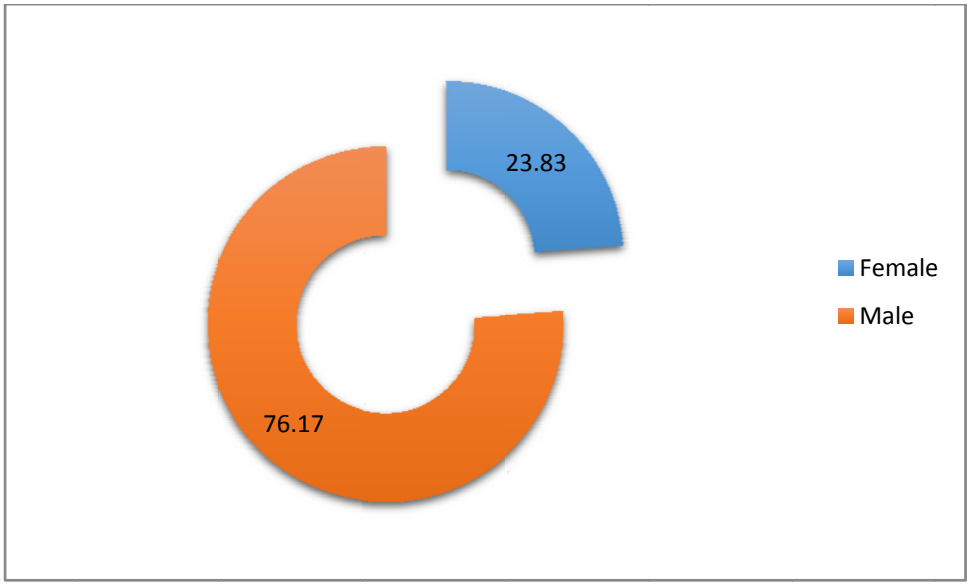
Among the total 193 cases, 147 cases are males and 46 cases are females constituting 76.17% and 46% respectively.

(TABLE :5 CHART:5)

TABLE 5: SEX WISE DISTRIBUTION OF LUNG CANCERS

SEX	Total	Percentage
Female	46	23.83
Male	147	76.17
Grand Total	193	100

CHART 5: SEX WISE DISTRIBUTION OF LUNG CANCER



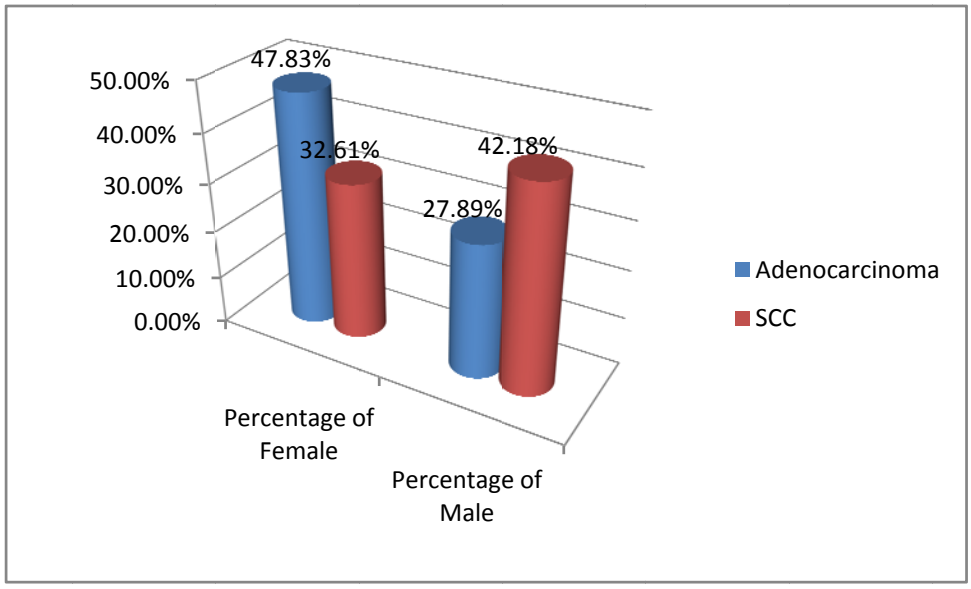
In this study it is observed that both squamous cell carcinoma and adenocarcinoma are more Common in males than in the females. But when compared to squamous cell carcinoma, the Females are more commonly affected by adenocarcinoma with near equal incidence in males.

TABLE: 6, CHART: 6

TABLE: 6 SEX WISE DISTRIBUTION OF ADENOCARCINOMA AND SCC

HPE Diagnosis	Female	Percentage of Female	Male	Percentage of Male	Grand Total
Adenocarcinoma	22	34.92%	41	65.07%	63
SCC	15	19.48%	62	80.51%	77
Grand Total	37		103		

CHART:6 SEX WISE DISTRIBUTION OF ADENOCARCINOMA AND SCC



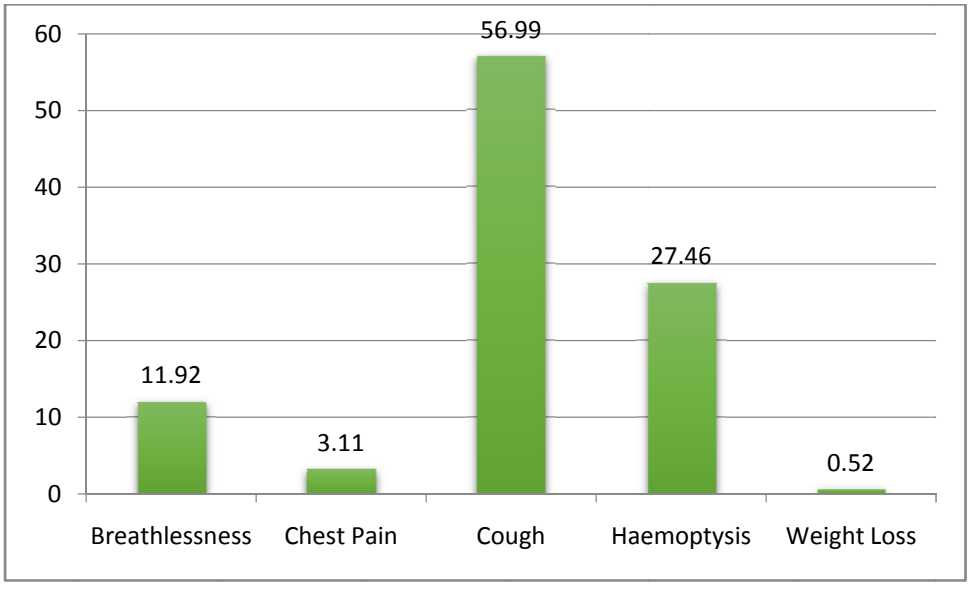
Cough, hemoptysis and breathlessness are the most common symptom of presentation for all types of lung cancers.

TABLE:7, CHART:7

TABLE 7: CLINICAL FEATURES OF LUNG CANCER

CLINICAL FEATURES	Total	Percentage
Breathlessness	23	11.92
Chest Pain	6	3.11
Cough	110	56.99
Haemoptysis	53	27.46
Weight Loss	1	0.52
Grand Total	193	100.00

CHART 7: CLINICAL PRESENTATION OF LUNG CANCERS



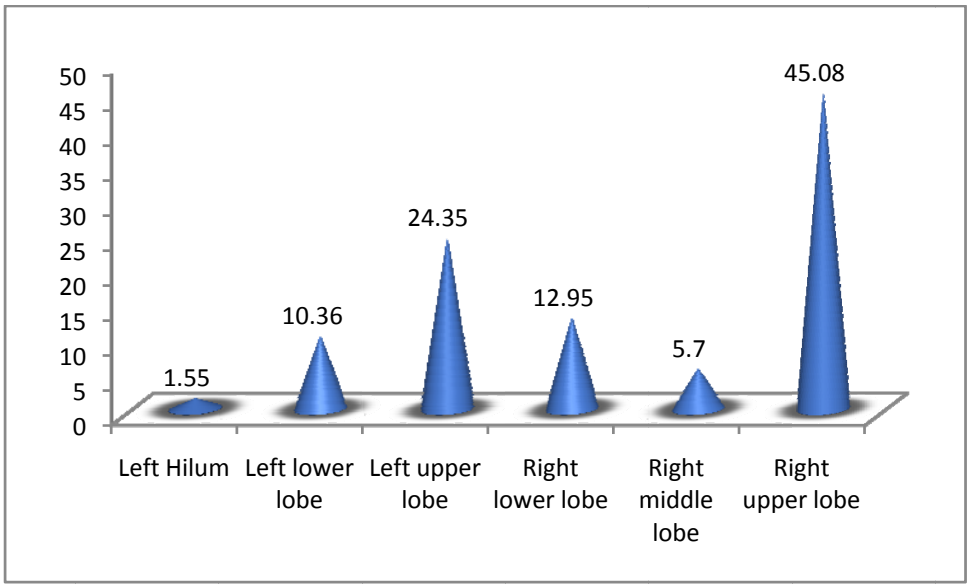
Of all the cases of lung carcinoma, the most common location of tumour is right upper lobe (45.08%) followed by left upper lobe (24.35%). Least common site of location is left hilum (1.55%). In this study, right lung was more commonly affected than left lung.

TABLE:8, CHART:8

TABLE 8: SITE DISTRIBUTION OF LUNG CARCINOMA

SITE	Total	Percentage
Left Hilum	3	1.55
Left lower lobe	20	10.36
Left upper lobe	47	24.35
Right lower lobe	25	12.95
Right middle lobe	11	5.70
Right upper lobe	87	45.08
Grand Total	**193**	**100.00**

CHART 8: SITE DISTRIBUTION OF LUNG CARCINOMA



In this study, with the available radiological details, mass lesions and opacity are proved to be malignant. Some cases which are diagnosed as non-neoplastic lesions radiologically such as cavitary lesions, consolidation changes etc also turned out to be malignant pathologically.

(TABLE:9 CHART:9)

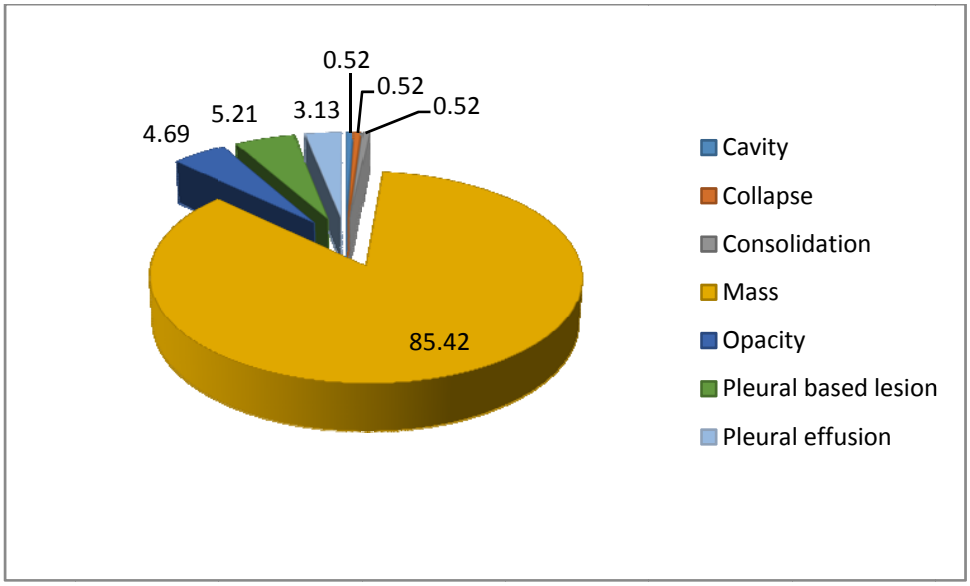
CHART 9: VARIOUS RADIOLOGICAL FINDINGS IN LUNG CANCER

XRAY,CT,MRI	Total	Percentage
Cavity	1	0.52
Collapse	1	0.52
Consolidation	1	0.52
Mass	164	85.42
Opacity	9	4.69
Pleural based lesion	10	5.21
Pleural effusion	6	3.13
Grand Total	**192**	**100.00**

Most common radiological finding was found to be mass lesion which constitutes 164 cases making a total percentage of 85.42%. other less common findings are cavity, collapse and consolidation which constitutes only0.5% of cases each.

(TABLE :10 CHART:10)

CHART: 10 VARIOUS RADIOLOGICAL FINDINGS IN LUNG CANCER



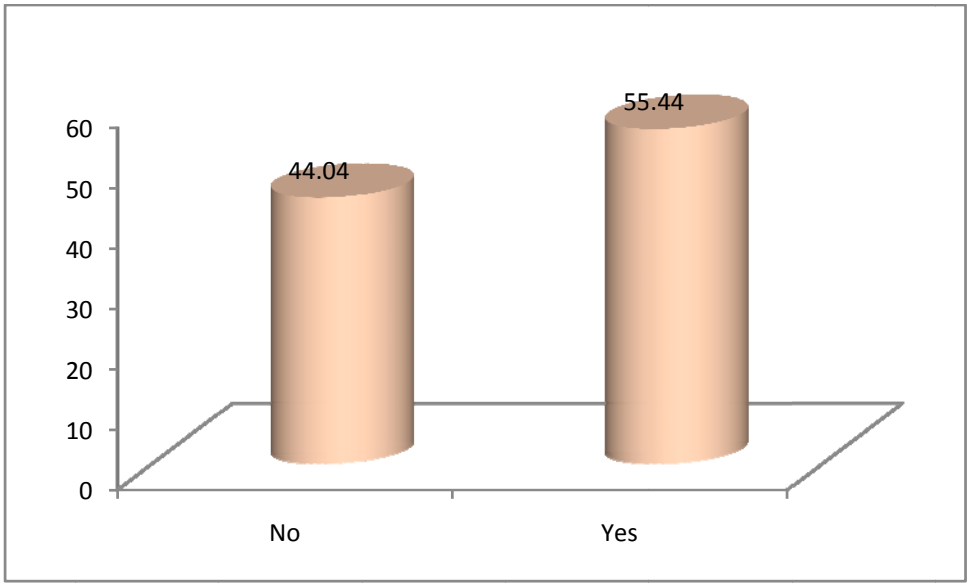
In this study 55% of the cases are smokers and 44.04 % of cases are Nonsmokers

(CHART :11 TABLE :11)

TABLE: 11 DISTRIBUTION OF SMOKERS AND NONSMOKERS IN LUNG CANCER CASES

SMOKING	Total	Percentage
No	85	44.04
Yes	107	55.44
Grand Total	**193**	**100**

CHART:11 DISTRIBUTION OF SMOKERS AND NONSMOKERS IN LUNG CANCER CASES



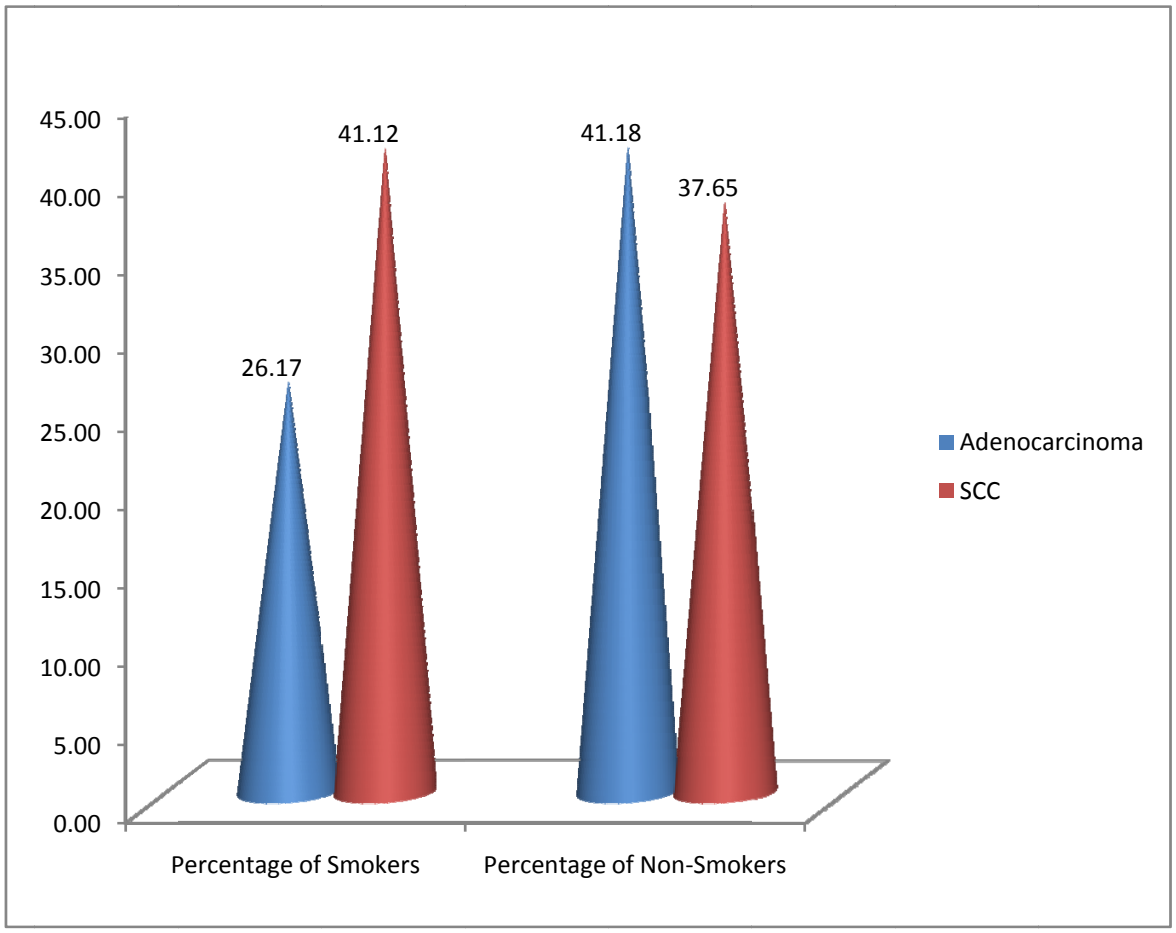
In this study, adenocarcinoma are more common in nonsmokers than in the smokers and squamous cell carcinomas are most commonly seen in the smokers than in the nonsmokers.

(CHART:12 TABLE:12)

TABLE :12 SMOKING ASSOCIATION IN SQUAMOUS CELL AND ADENOCARCINOMA

HPE Diagnosis	Smoker	Percentage of Smokers	nonsmokers	Percentage of Non-Smokers
Adenocarcinoma	28	26.17	35	41.18
SCC	44	41.12	32	37.65
Grand Total	**72**		**67**	

CHART:12 SMOKING ASSOCIATION IN SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA



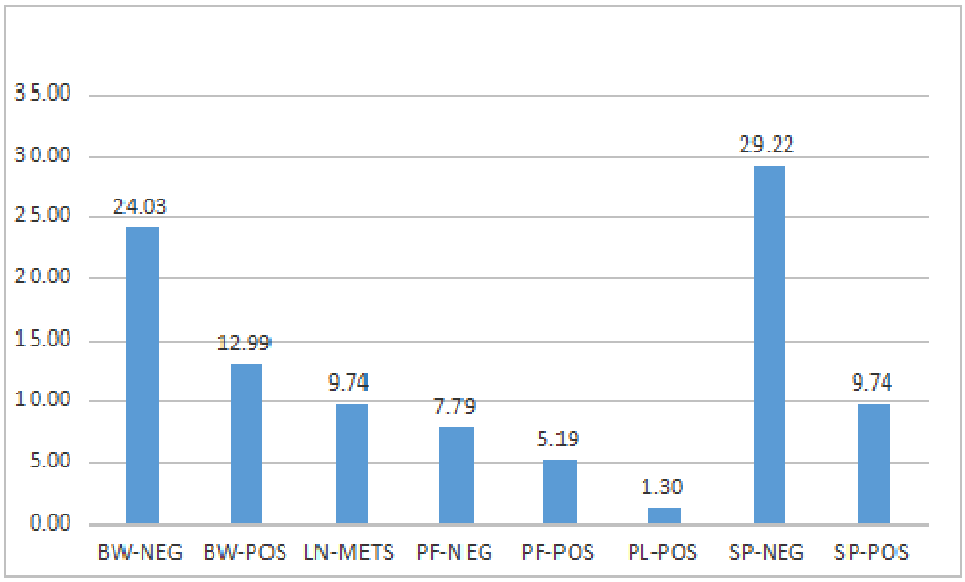
The cytological presentation of lung cancers was analysed in our study. It is found that 29.22% of the cases are sputum negative. 24.03% of the cases are negative for bronchial wash and 12.99% of the cases are positive for tumour cells in bronchial wash.

(TABLE:13 CHART:13)

TABLE: 13 CYTOLOGICAL FINDINGS IN LUNG CARCINOMA

CYTOLOGY	Total	Percentage
BW-NEG	37	24.03
BW-POS	20	12.99
LN-METS	15	9.74
PF-NEG	12	7.79
PF-POS	8	5.19
PL-POS	2	1.30
SP-NEG	45	29.22
SP-POS	15	9.74
Grand Total		**100**

CHART:13 CYTOLOGICAL FINDINGS IN LUNG CARCINOMA



According to WHO classification, Non-small cell lung carcinoma are divided into Squamous cell carcinoma, Adenocarcinoma, NSCLC-NOS. In our study,Squamous cell carcinoma is the most common type which accounts for 39.90% with the total of 193 in our 2 years study. Adenocarcnima constitutes 63(32.64%), NSCLC-NOS constitutes 37 cases(19.17%).

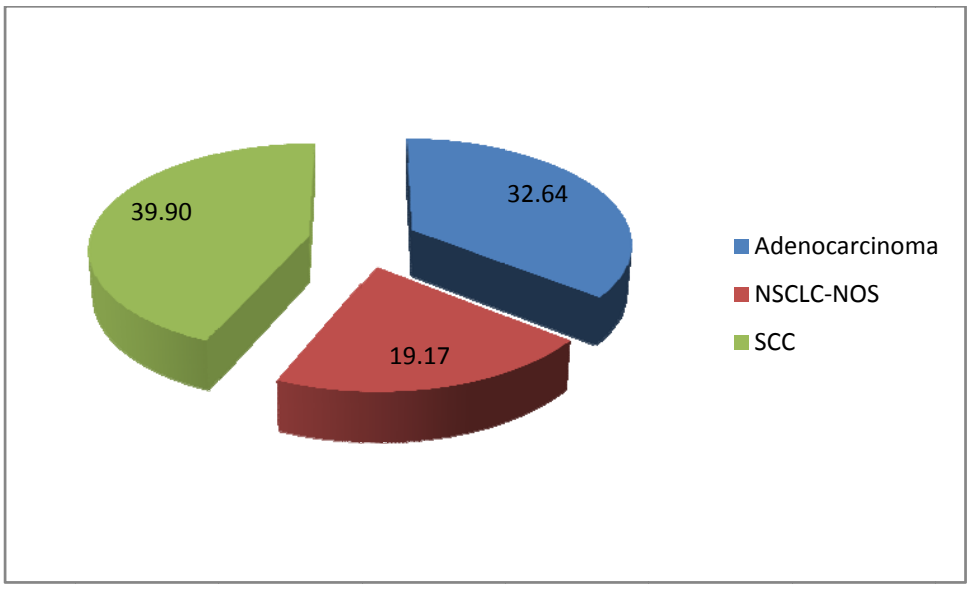
(TABLE:14 CHART:14)

TABLE 14:NON SMALL CELL LUNG CARCINOMA SUB TYPE ACCORDING TO WHO CLASSIFICATION

HPE DIAGNOSIS	Total	Percentage
Adenocarcinoma	63	32.64
NSCLC-NOS	37	19.17
SCC	77	39.90
Grand Total	**193**	**100.00**

CHART 14: NON SMALL CELL LUNG CARCINOMA SUB TYPES

ACCORDING TO WHO CLASSIFICATION



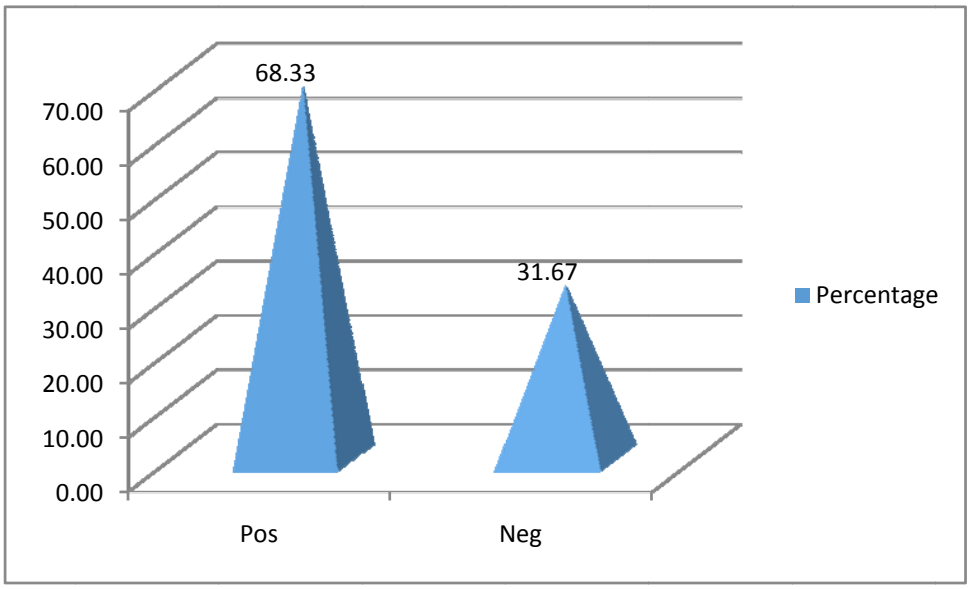
In our study, IHC for EGFR is done for randomly selected 20 cases each of Adenocarcinoma, NSCLC-NOS and squamous cell carcinoma was done. It was estimated that EGFR is positive in 41 cases which constitutes 68.33%. EGFR is negative in 19 cases with a percentage of 31.67

(TABLE:15 CHART:15)

TABLE 15:DISTRIBUTION OF EGFR

EGFR	Total	Percentage
Pos	41	68.33
Neg	19	31.67
Grand Total	**60**	**100.00**

CHART 15: DISTRIBUTION OF EGFR

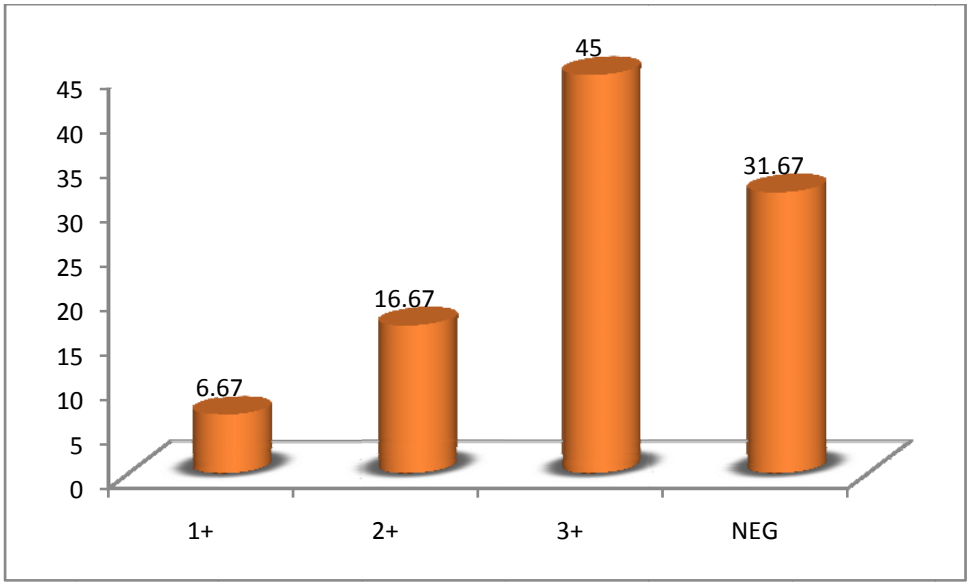


EGFR is graded according to the percentage of positive tumor cells and the intensity of staining.Acordingly it is graded as 1+, 2+, 3+ & Negative. 27 cases showed 3+ grade with a percentage of 45. 10 cases showed 2+ grade with 16.67%. 6.67% of cases showed grade 1+ with the total number of 4 cases.(TABLE:16 CHART:16)

TABLE 16:DISTRIBUTION OFGRADING OF EGFR

EGFR	Total	Percentage
1+	4	6.67
2+	10	16.67
3+	27	45.00
NEG	19	31.67
Grand Total	**60**	**100.00**

CHART 16: DISTRIBUTION OF GRADING OF EGFR



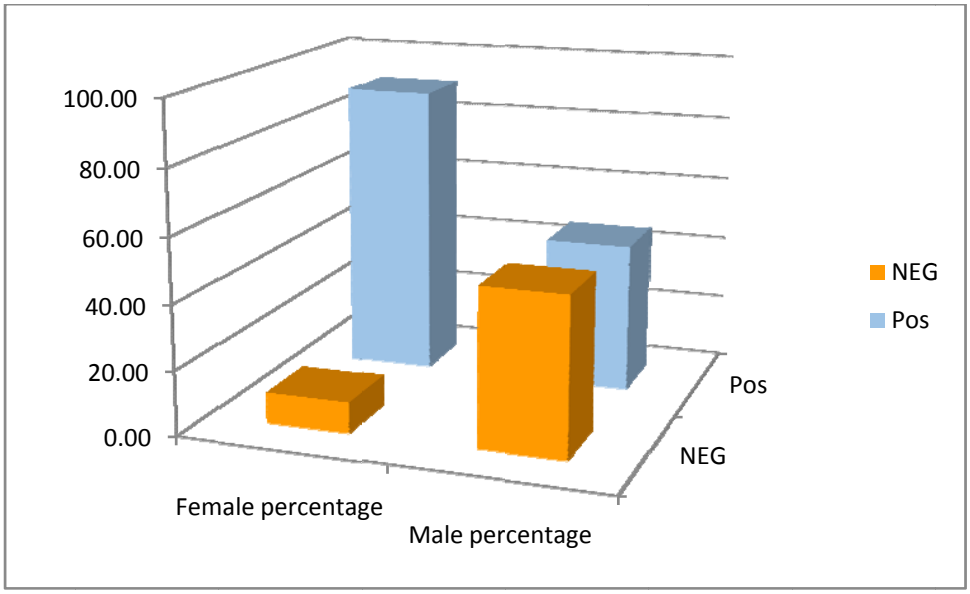
In our study, we compared the distribution of EGFR in males and females. It is estimated that 90.48% of females were positive for EGFR and9.52% were found negative.46.15% of males were positive for EGFR and 48.72% were found to be negative.(TABLE:17 CHART:17)

TABLE 17:DISTRIBUTION OF EGFR IN MEN AND WOMEN

EGFR	Females	Female percentage	Males	Male percentage	Grand Total
NEGATIVE	2	9.52	19	48.72	19
POSITIVE	21	90.48	18	46.15	41
Grand Total		**100**		**100.00**	**60**

p value - 0.000613

CHART:17 DISTRIBUTION OF EGFR IN MEN AND WOMEN



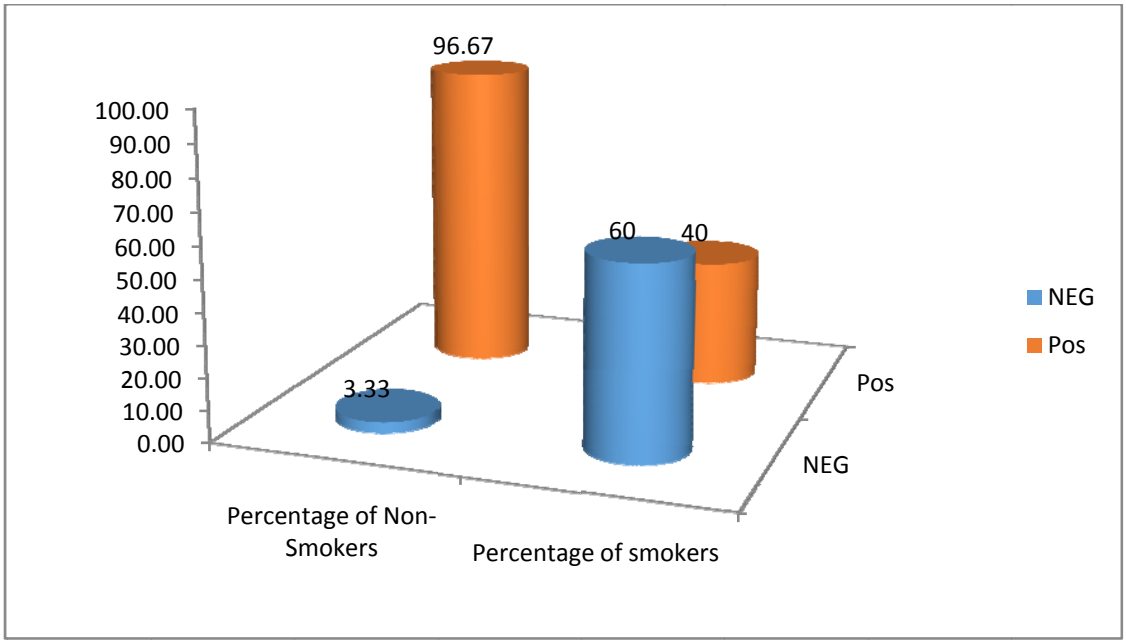
The distribution of Smokers and non-smokers were compared with EGFR expression.It is estimated that12 smokers were positive for EGFR and 18 found negative.29 non-smokers were found to be positive for EGFR expression and 1 was found to be negative.(TABLE:18CHART:18)

TABLE 18:DISTIBUTION OF EGFR IN SMOKERS AND NON-SMOKERS

EGFR	NO	Percentage of Non-Smokers	YES	Percentage of smokers	Grand Total
NEG	1	3.33	18	60	19
POS	29	96.67	12	40	41
Total	**30**	**100**	**30**	**100**	**60**

p value - 0.0000023

CHART 18: DISTIBUTION OF EGFR IN SMOKERS AND NON-SMOKERS



The distribution of EGFR in various age groups were compared. One case found to be positive for EGFR in age group less than 30. 6 were found to be positive in age group between 31-40. 11 were found to be positive in the age group between 41-50. 12 were found to be positive in the age group between 51-60. 11 were found to be positive in age group between 61-70. No case was positive above 70 years of age.

No negativity was found for age group below 40 for EGFR expression. 7 cases were found negative in age group between 41 and 50. 6 were found negative in age group between 51 and 60. 4 were found negative for age group between 61 and 70. 2 were found to be negative for age group above 70.

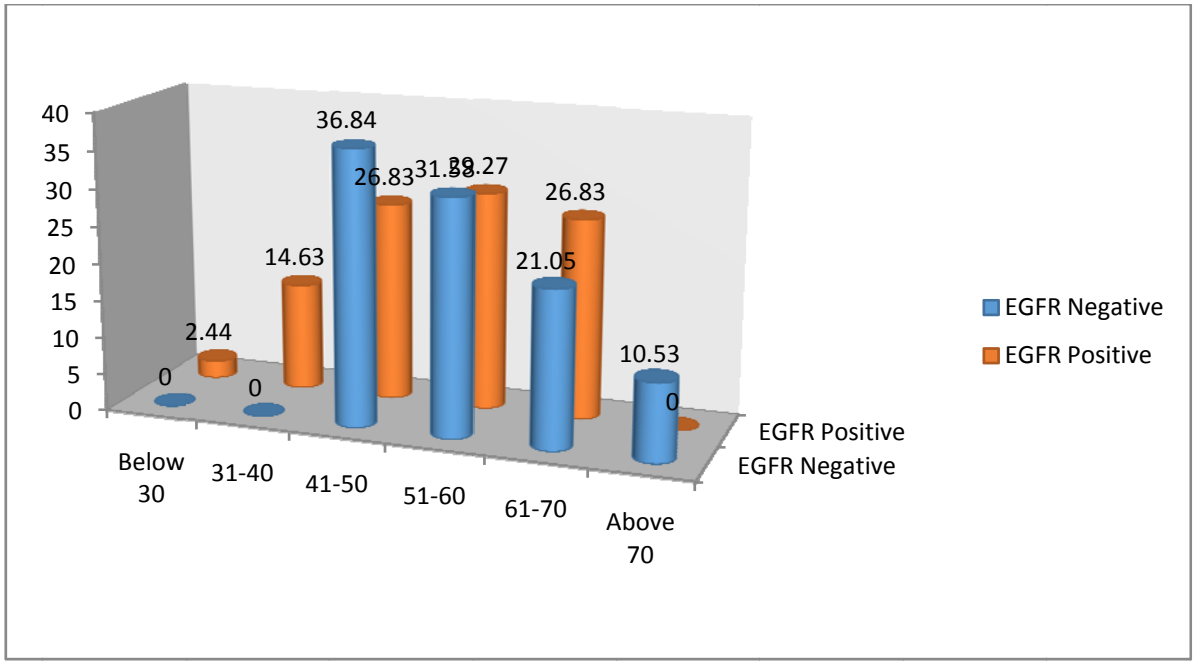
(TABLE:19 CHART:19)

TABLE 19: AGE WISE DISTRIBUTION OF EGFR

Age	NEGATIVE	EGFR Negative	POSITIVE	EGFR Positive	Grand Total
Below 30	0	0	1	2.44	1
31-40	0	0	6	14.63	6
41-50	7	36.84	11	26.83	18
51-60	6	31.58	12	29.27	18
61-70	4	21.05	11	26.83	15
Above 70	2	10.53	0	0	2
Total	**19**	**100**	**41**	**100**	**60**

p value - 0.146071

CHART 19: AGE WISE DISTRIBUTION OF EGFR



The distribution of EGFR in various histological types of non-small cell lung cancer was compared and it was estimated that 18 cases of adenocarcinoma, 8 cases of poorly NSCLC-NOS and 5 cases of SCC were found to be positive for EGFR expression.2 cases of Adenocarcinoma, 12 cases of NSCLC-NOS and 5 cases of SCC were found to be negative for EGFR expression.

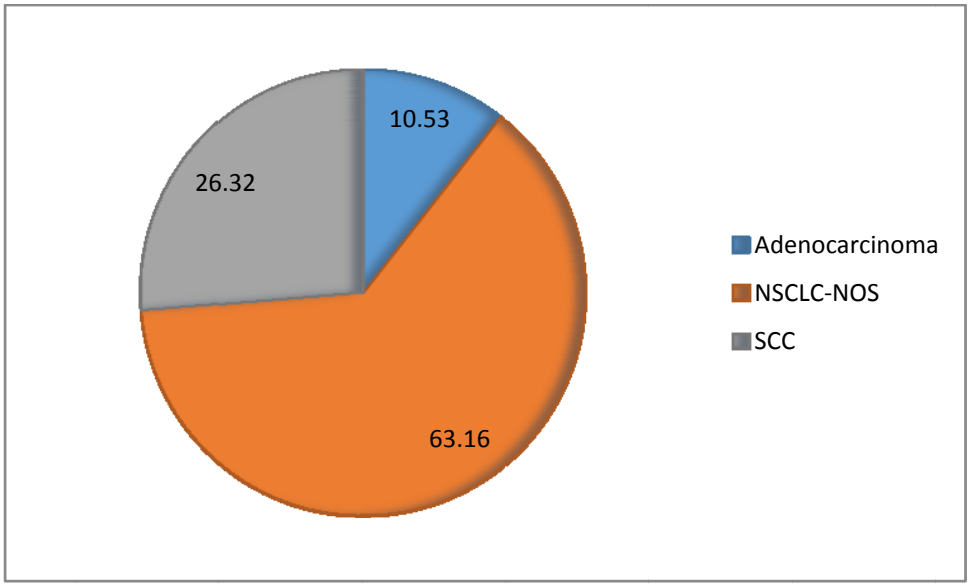
(TABLE:20 CHART:20)

TABLE 20: DISTRIBUTION OF EGFR IN NON SMALL CELL LUNG CANCER

HPE Diagnosis	NEG	EGFR Neg	Pos	EGFR pos	Grand Total
Adenocarcinoma	2	10.53	18	43.90	20
NSCLC-NOS	12	63.16	8	19.51	20
SCC	5	26.32	15	36.59	20
Grand Total	**19**	**100**	**41**	**100**	**60**

p value - 0.002277393

CHART 20: DISTRIBUTION OF EGFR IN NON SMALL CELL LUNG CANCER



GROSS

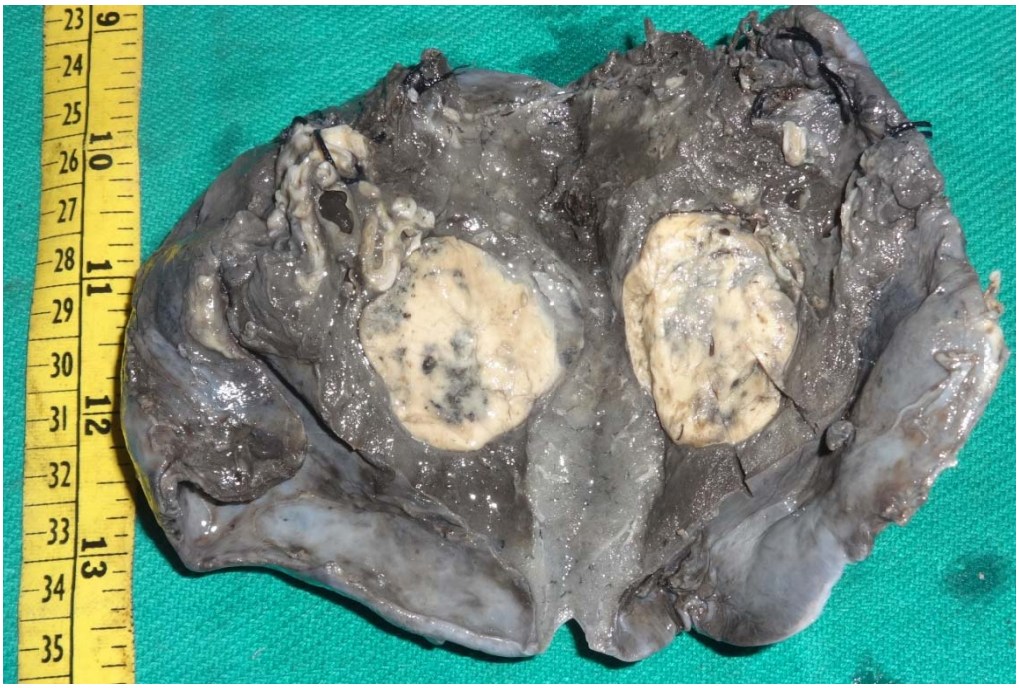


FIGURE 1: ADENOCARCINOMA OF LUNG. BIOPSY NO. 878/14

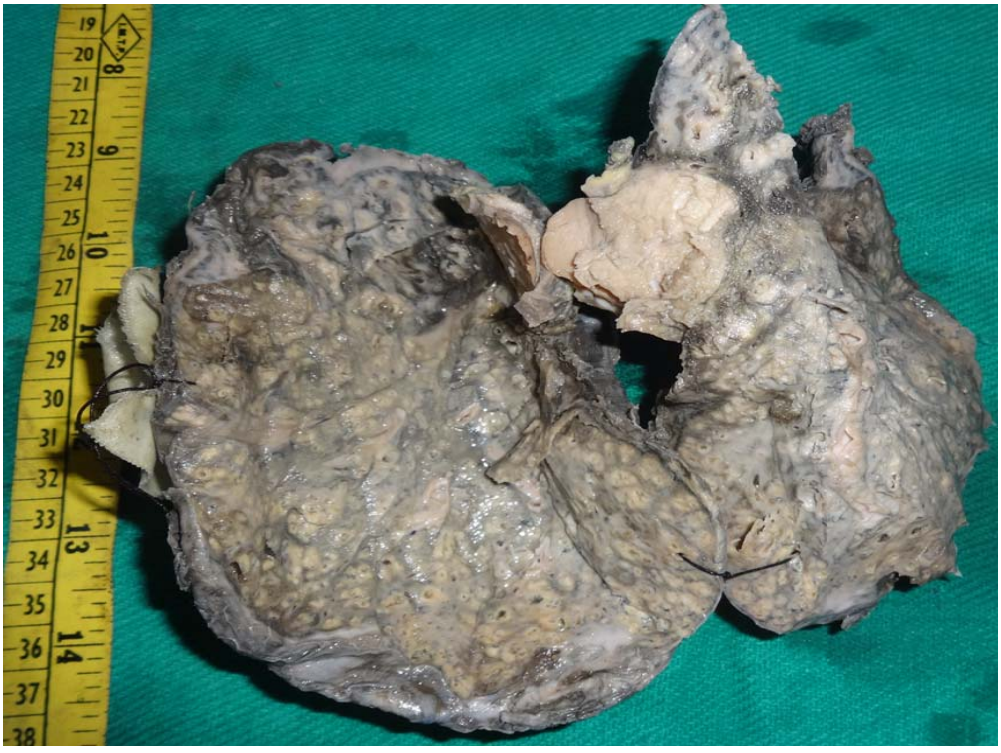


FIGURE 2: LUNG CARCINOID. BIOPSY NO. 9676/15

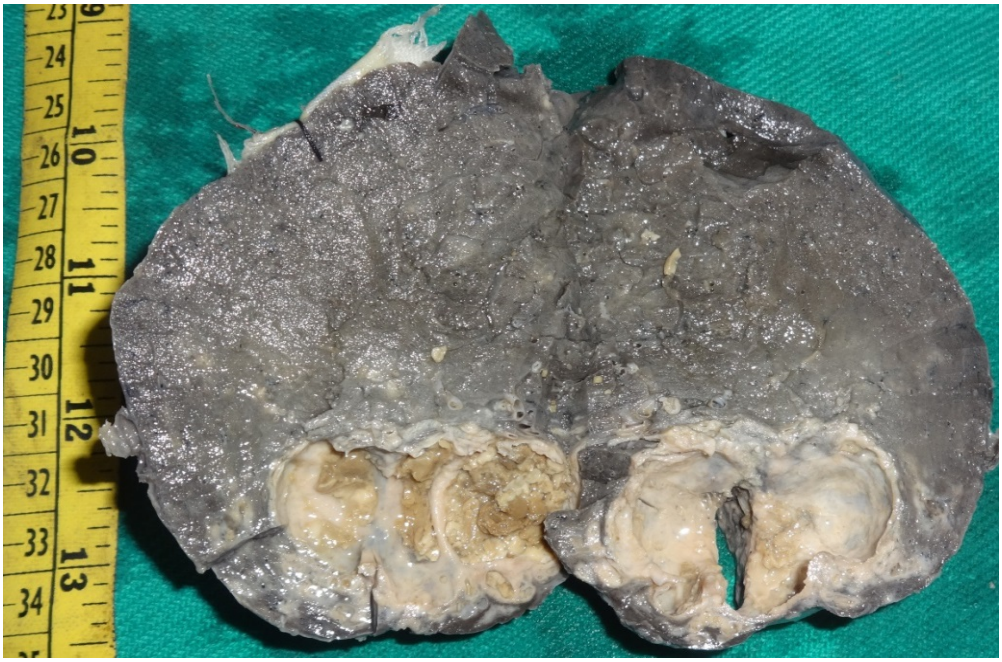


FIGURE 3: SQUAMOUS CELL CARCINOMA PRESENTING AS CAVITARY LESION

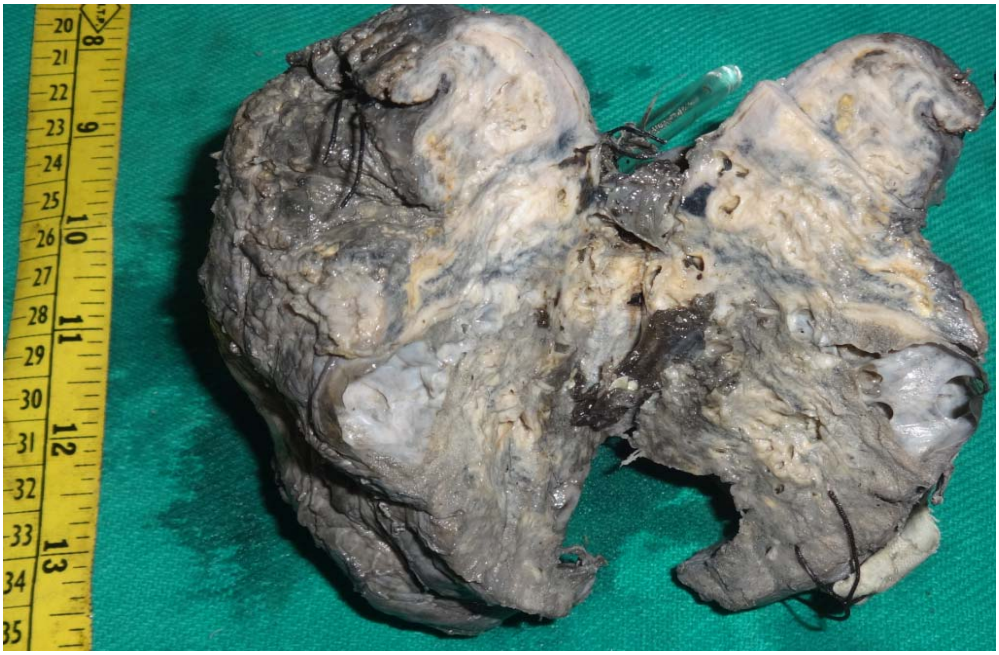


FIGURE 4: SQUAMOUS CELL CARCINOMA-LUNG

SQUAMOUS CELL CARCINOMA

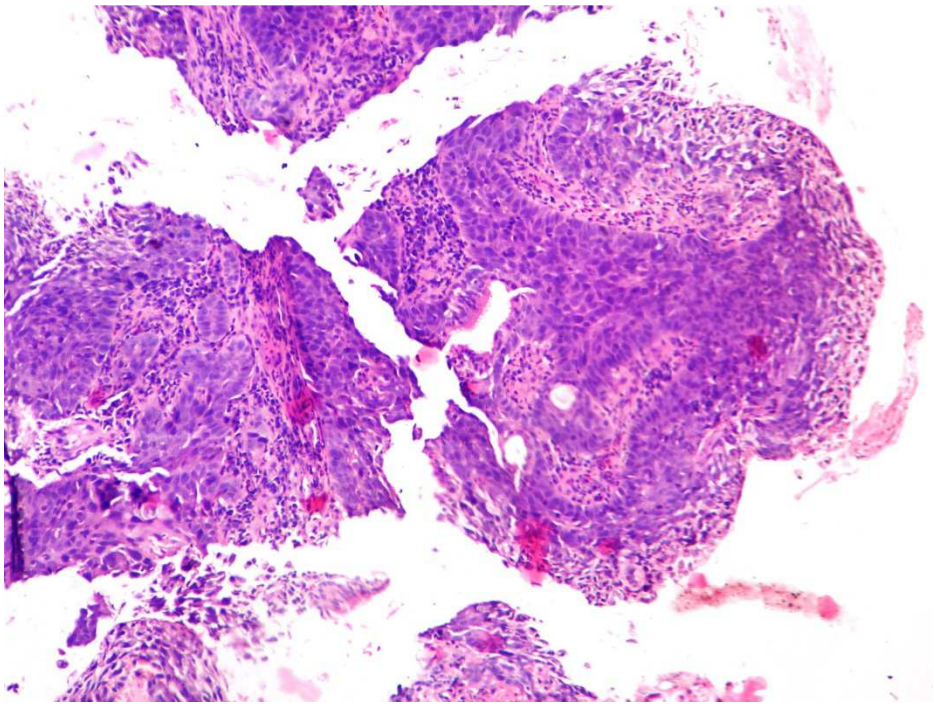


FIGURE 5 : MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA 100X HPE NO: 3071/15

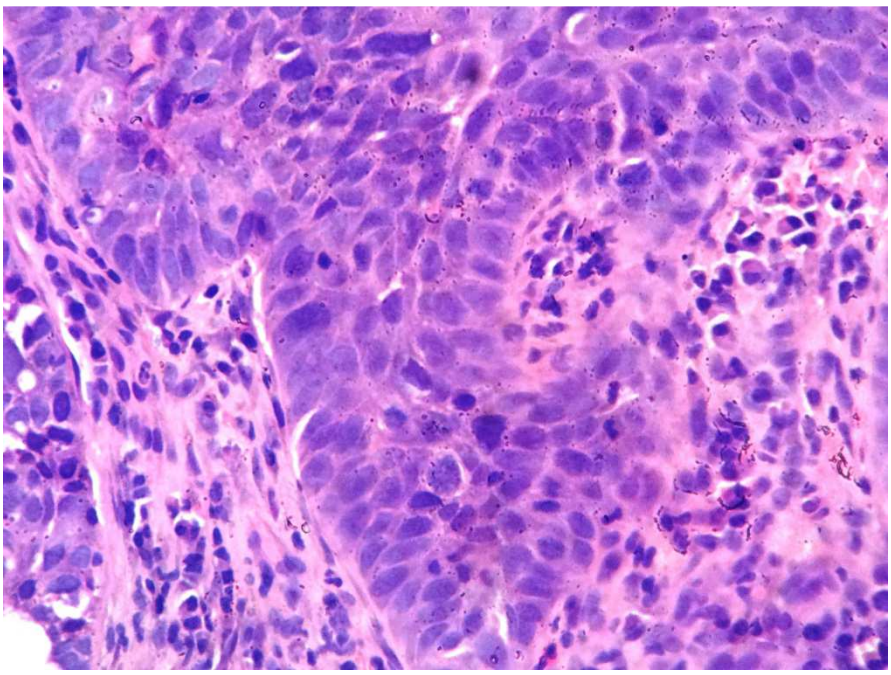


FIGURE 6 : MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA 400X HPE NO: 3071/14

ADENOCARCINOMA

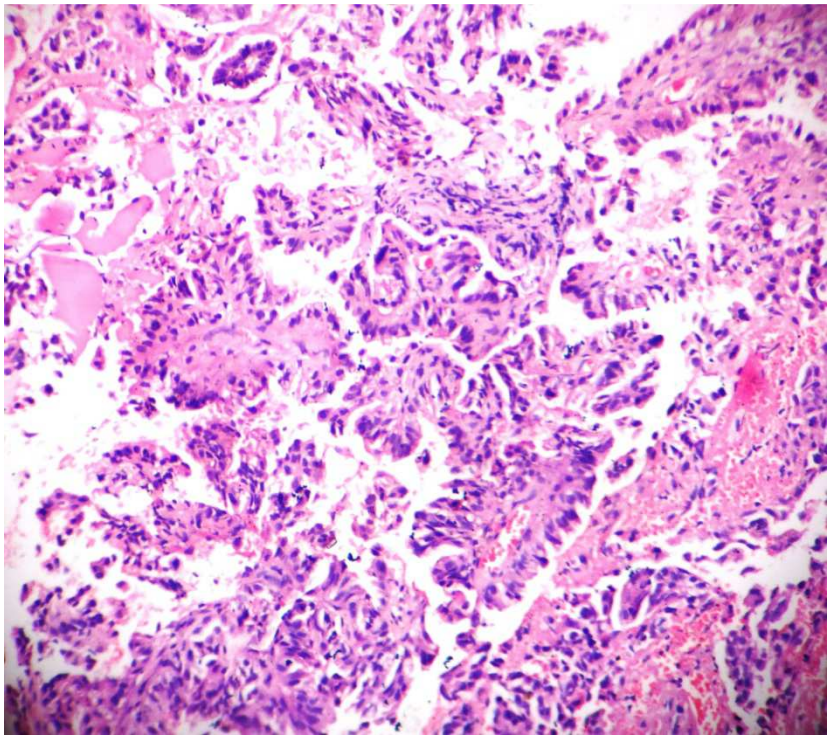


FIGURE 7 : MODERATELY DIFFERENTIATED ADENOCARCINOMA 100X HPE NO. 99/15

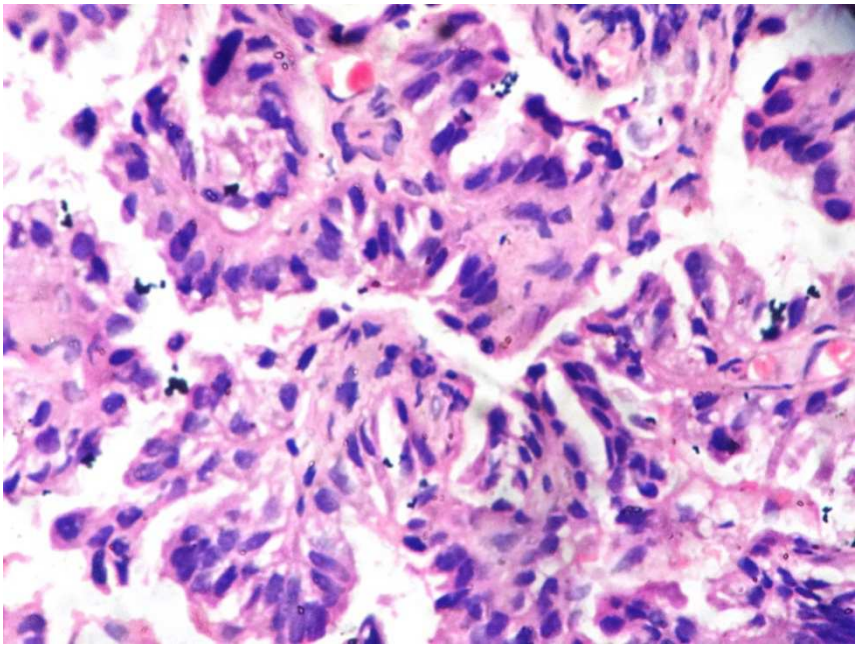


FIGURE 8 : MODERATELY DIFFERENTIATED ADENOCARCINOMA 400X HPE NO. 99/15

NON-SMALL CELL LUNG CARCINOMA-NOS

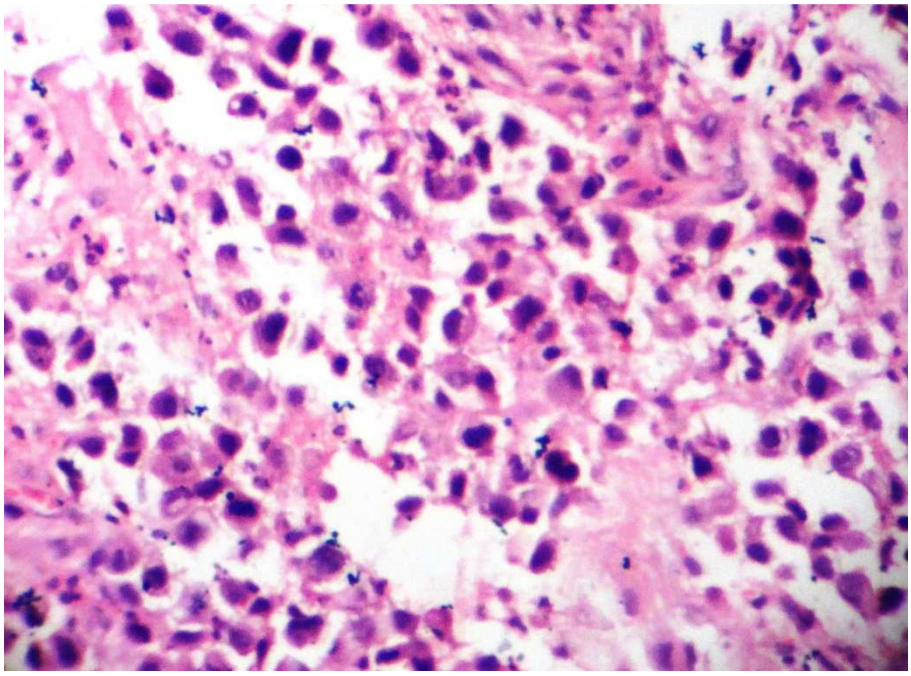


FIGURE 9 : NON SMALL CELL LUNG CARCINOMA-NOS

100X HPE NO. 3225/15

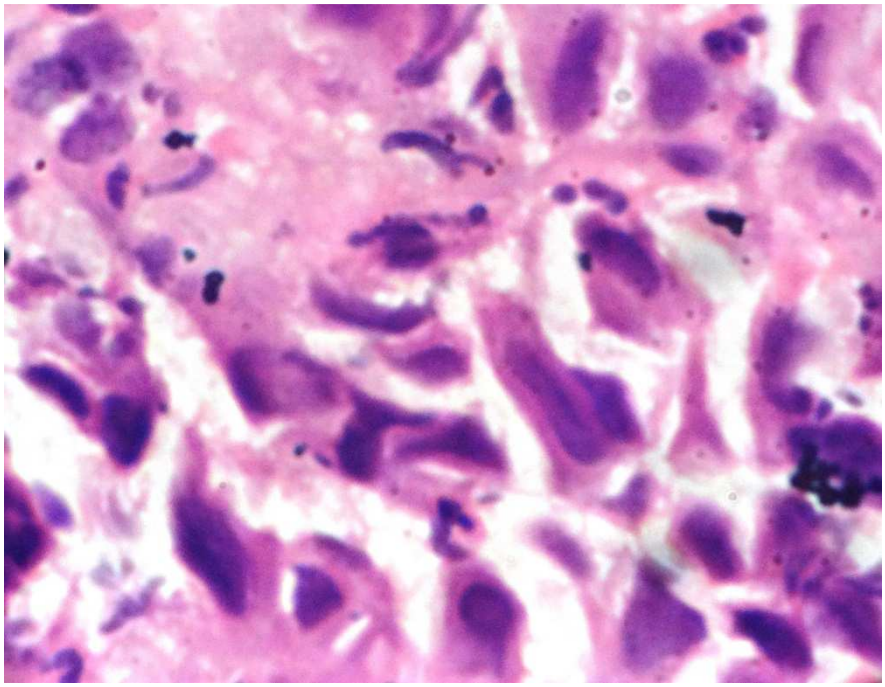


FIGURE 10 : NONSMALL CELL LUNG CARCINOM-NOS

400X HPE NO. 3225/15

EGFR EXPRESSION

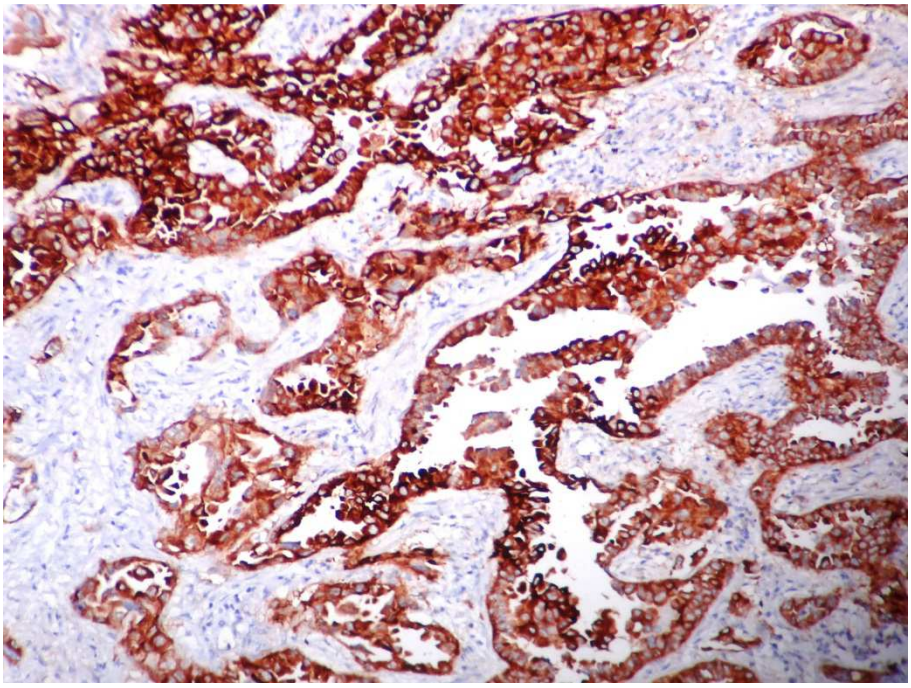


FIGURE 11 : STRONG MEMBRANE POSITIVITY OF EGFR IN ADENOCARCINOMA 100 X HPE NO.98/15

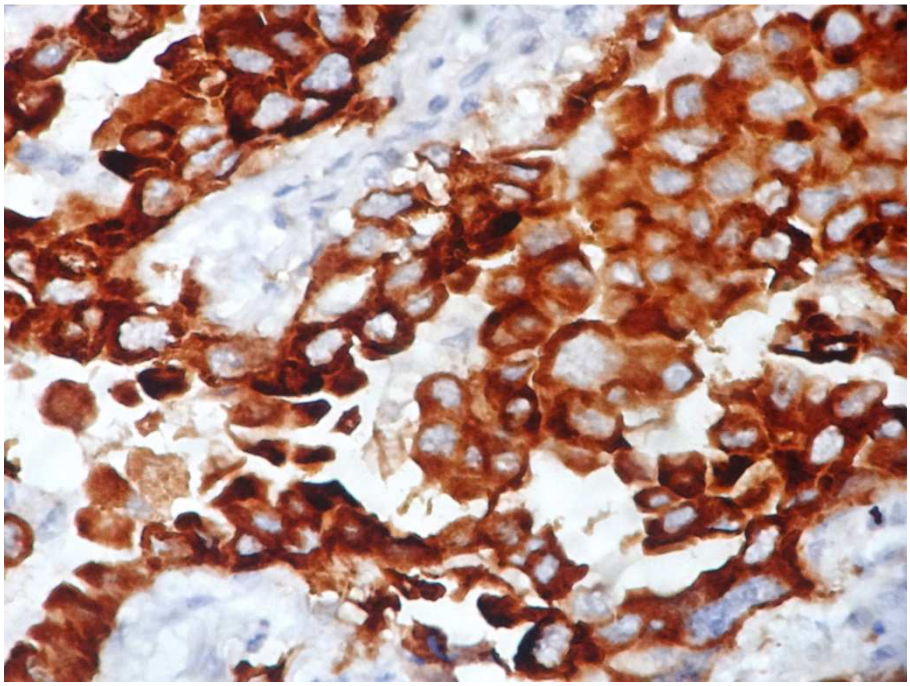


FIGURE 12 : STRONG MEMBRANE POSITIVITY OF EGFR IN ADENOCARCINOMA 400 X HPE NO. 98/15

EGFR EXPRESSION

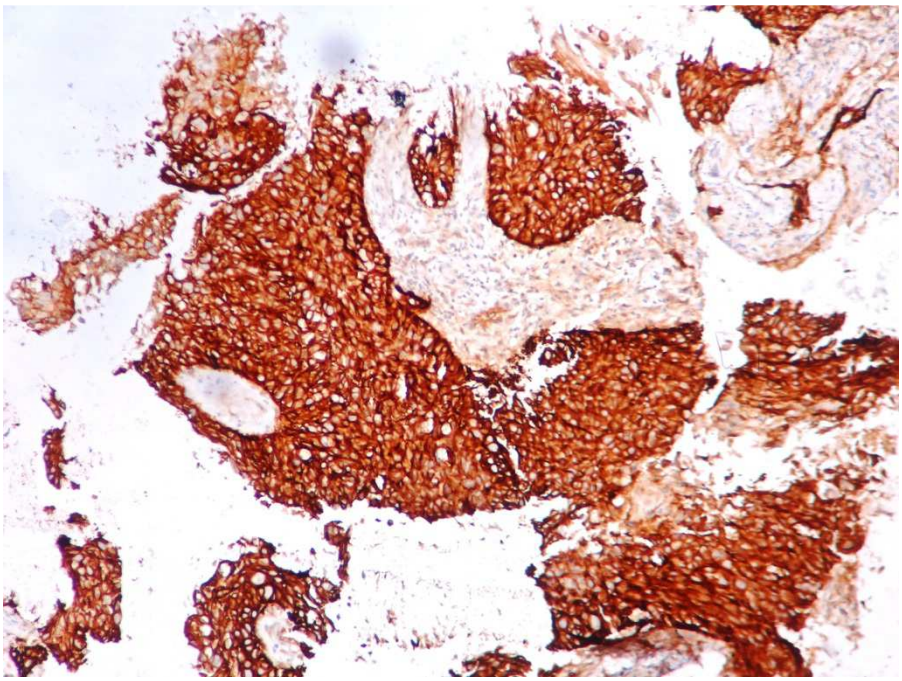


FIGURE 13 : STRONG MEMBRANE STAINING OF EGFR IN SQUAMOUS CELL CARCINOMA 100 X HPE NO. 312/15

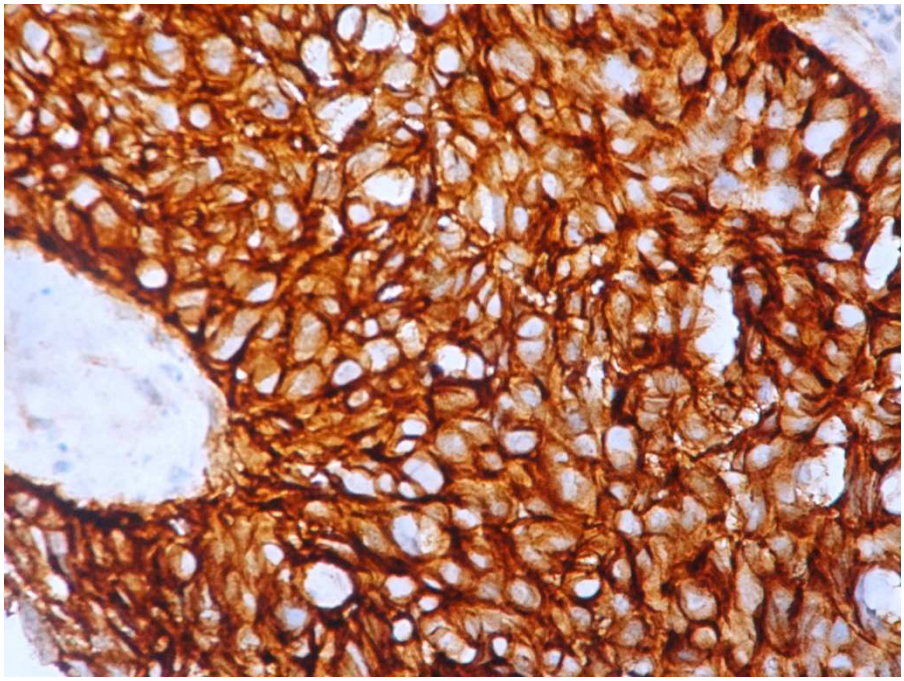


FIGURE 14 : STRONG MEMBRANE STAINING OF EGFR IN SQUAMOUS CELL CARCINOMA 100 X HPE NO. 312/15

CYTOLOGY

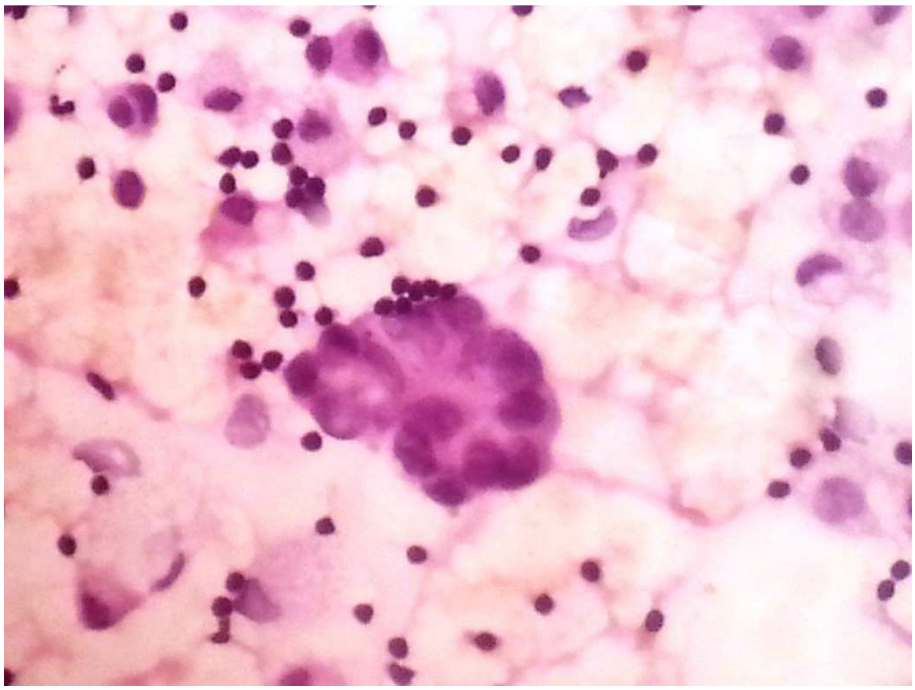


FIGURE 15 : BRONCHIAL WASH; PLEOMORPHIC MALIGNANT CELLS IN GROUPS AND IN SINGLES. CYTOLOGY NO. 1264/15

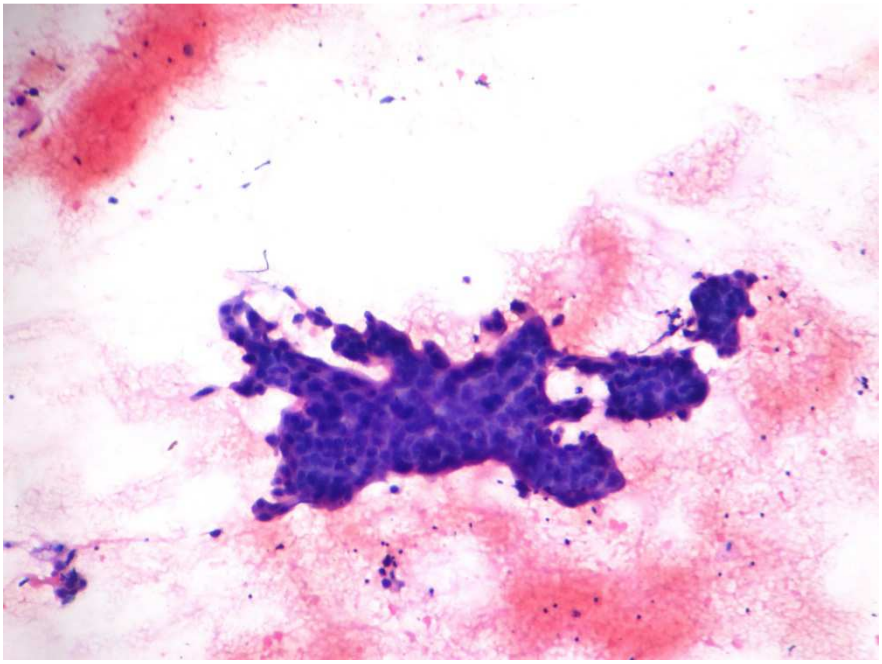


FIGURE 16 : LYMPH NODE FNAC; METASTATIC SCC DEPOSITS IN LYMPH NODE 400X FNAC NO. 1544/15

DISCUSSION

DISCUSSION

Worldwide, lung cancer is the most common cause of cancer and cancer related Mortality and the common chemotheraupeutic drugs which are used today do not have adequate Efficacy and specificity.

Lung cancer is more predominant in males which constitutes 10.9% of all cases of Cancer and it constitutes around 13% of all cancer related mortality.

Lung cancer is more predominant in 4^{th} to 6^{th} dacade of life. Also the prognosis of lung cancer is very poor.

Among all the histological types of lung carcinoma, more than 85% are non small cell lung cancer. Adenocarcinoma is the most common type of lung carcinoma. Until the recent past, the histological subclassification has no clinical or theraupeutic significance. But recently with the advent of newer drugs and targeted therapies, it is very essential to subtype non small cell lung cancer because it has direct effect on treatment and prognosis.

Non small cell lung cancer has to be subclassified accurately into squamous cell Carcinoma, adenocarcinoma, non small cell lung cancer-NOS. It is necessary to classify them as EGFR expression should be tested in adenocarcinoma and other types of lung cancer as EGFR tyrosine kinase inhibitors form the primary treatment for adenocarcinoma lung.

Most of the lung cancers are in advanced stage at presentation. So the resection rate is very low most which in probably 15-20% of cases and targeted therapies are the mainstay of treatment for such cases.

Madras medical college is a tertiary referral centre and institute for pathology.This is a retrospective study of lung cancers conducted for two years between july 2013 to june 2015. A total of 193 lung cancer specimens were found in this study period among the total 412 lung specimens received . Among the entire lung cancer specimens, 33.25% of cases are non neoplastic and 46.84% of cases are malignant. More than 85% of lung cancers in my study is nonsmall cell lung cancer. Small cell lung carcinoma accounts for 4.66% of cases. This is in accordance with the study done by Navada S et al which states that around 80-85% of lung cancer cases are non small cell lung carcinoma and thus it is the most common type.

Most of the lung cancers present in the age group between 50-80 years of age with a percentage of 77.88%. less then 3% of cases are seen in the age group less than 30 years. This is in correlation with the study done by Delik Erman et al[136] and others. The median age group in our study is 55 years.

Median age of lung cancer compared in various studies compared with the current study

Study	Median age
Xu J et al[137]	58
Jing C et al[138]	62
Zhang J et al[139]	61
Yin X et al[140]	61
Current study	**55**

In our study lung cancer is more common in males with a percentage of 76.17. ItObserved that the incidence of lung cancer in squamous cell carcinoma and adenocarcinoma areMore common among males. But females are more commonly affected by adenocarcinoma than by squamous cell carcinoma. This is accordance with the study conducted by Churg AM et al[141].A study by Kiyohara et al[142] has estimated that the ability of DNA repair is relatively low in Females when compared to males and they are said to have increased susceptibity for lung cancer.

Pecerntage of gender distribution in other studies compared with the current study

	Cases(n)	**MALES(%)**	**FEMALES(%)**
An S et al[143]	524	361(68.89%)	163(31.10%)
Liu Y et al[144]	251	138(54.98%)	113(45.01%)
Feng Q et al[145]	309	184(59.54%)	125(40.45%)
Sun L et al[146]	301	174(57.80%)	127(42.19%)
current study	**193**	**147(76.17%)**	**46(23.83%)**

Cough is the most common presenting symptom in our study with a percentage of 56.99. The other common symptoms are hemoptysis and breathlessness with 27.46% and 11.92% respectively. According to the study conducted by Bach PB et al[147], the common symptoms are cough, hemoptysis, breathlessness, hoarseness of voice, significant loss of weight and recurrent respiratory infections like bronchitis, pneumonia.

Most of the time, the symptoms of lung cancer are not specific. In many patients, the Lung cancer has already spread beyond the origin site by the time they seek medical attention.

In our study 55.44% of cases are smokers and 44.04% of the cases are non smokers. Squamous cell carcinoma is strongly associated with smoking with 44%. And most of the adenocarcinoma patients are non smokers with 35%. This is in accordance with the study conduceted by Satcher D et al[148] who estimated that smoking is the most common cause for both males and females and it is more strongly associated with squamous cell carcinoma.

Comparison of smoking incidence in various studies with the current study

Studies	Cases(n)	Smokers(%)	Nonsmokers(%)
Li Y et al[149]	208	130(62.5%)	78(37.5%)
Lai Y et al[150]	697	366(52.91%)	331(47.48%)
Zhang J et al[139]	454	279(61.45%)	175(38.54%)
Sun L et al[146]	301	173(57.47%)	128(42.52%)
current study	**193**	**187(55.44%)**	**85(44.04%)**

In our study, squamous cell carcinoma is the most common histological subtype of lung Cancer with 39.90% followed by adenocarcinoma which is 33.01%. the recent study by Lortet et al[151]in 2014 has stated that adenocarcinoma incidence is on the increasing side and it has surpassed those of SCC though historically SCC is the most common subtype.

Comparison of histological subtypes with various studies:

HISTOLOGICAL SUBTYPES	Voporciyan AA et al[152]	Delik Erman et al[136]	current study
Adenocarcinoma	40%	20-30%	**33.01%**
Squamous cell carcinoma	30%	40-60%	**39.90%**
Large cell carcinoma	9%	5-10%	**1.04%**
Small cell lung cancer	10-15%	5-15%	**4.66%**
Carcinoid	<5%	5%	**2.07%**

Squamous cell carcinoma:

In our study, squamous cell carcinoma is the most common type of nonsmall cell lung cancer. It is diagnosed histologically based on the keratinformation and the appearance ofintercellular bridges. These specific features arenot seen in other carcinomas such as adenocarcinoma or small cell carcinoma.

Among the 193 malignant cases in our study, 77 cases come under this category with a percentage of 39.90% and showed a male predominance with 62cases with a percentage of 80.51%.

Out of the total 77 cases of squamous cell carcinoma, 44 cases are smokers with a percentage of 41.12%. 37.67% of cases are non smokers.

Adenocarcinoma :

In our study, next to squamous cell carcinoma, adenocarcinoma is the most Common subtype. The presence of glandular features and mucin production are the characteristic features of this subtype.

Out of the total 193 malignant cases, 33.01% of cases come under this category(n=64). Out of the 64 cases 41 cases are males and 22 cases are females with a 65.07% and 34.92% respectively.

The most common radiological finding is the presence of mass lesion which has a total of 164 cases with 85.42%. the other radiological findings are opacity, pleural effusion and pleural based lesion. This is in accordance with the study conducted by Minna, JD et al.

Right upper lobe is the most common site of involvement in our study with 45.08%(n=87). It is followed by left upper lobe with 24.35%. The right lung is more commonly involved than the left lung with 63.73% and 36. 26% respectively. This is in accordance with the study done by Vivekanand N et al in which right lung cancers are more common than the cancers of left lung.

Expression of EGFR:

Lung cancer is the leading cause of cancer related mortality in industrialised Countries. Most of the cases present with advanced disease. It is therefore essential to identifyThe prognostic factors and treatment modalities for effective management.

EGFR plays an important role in motility, invasion and angiogenesis of tumourCells. It is thus considered to be a poor prognostic factor for survival in non small lung cancer.

EGFR positivity among non small cell lung cancers:

In our randomly selected 60 cases of non small cell lung cancer, 68.33% of cancers arepositive for EGFR expression. The various studies results are as follows,

	EGFR positivity
Gao J et al[153]	36.7%
Sun LN et al[146]	32.9%
She Juan An et al[154]	28.4%
Feng Q et al[145]	22.2%
Yuanyang Lai et al[155]	33.7%
Current study	**68.33%**

Comparison of EGFR expression in males and females in other studies compared with the current study:

In our study, EGFR seems to be increasingly expressed among females with a percentage of 90.48. 46.15% of the males are positive for EGFR expression. This is in accordance with the study done by ShuaiWang et al[156], a meta analysis study which statesthat EGFR expression is significantly low in females.

This variable is statistically significant in our study as indicated by the p value 0.000613($p<0.05$) obtained by t test.

EGFR expression in males and females in other studies compared with our study:

	FEMALES	**MALES**
Yan Liu et al[157]	63.7%	49.3%
Yuan Yang Lai et al[155]	57.5%	22.7%
Ying Li et al[158]	47.5%	15%
Feng Q et al[145]	39.2%	30.4%
current study	**90.48%**	**46.15%**

Comparison of EGFR expression among smokers and non smokers:

It is estimated in our study that 96.67% of the EGFR positive cases are nonsmokers. This is in accordance with the study conducted by Yaxiong Zhang et al[159]. This study states that EGFR positive patients are never smokers which is defined as the persons who smoked less than 100 cigarettes in his lifetime.

	Smokers	**Nonsmokers**
Yingli et al[158]	13.9%	42.3%
She Juan An et al[154]	12.4%	40.9%
Feng Q et al[145]		40.2%
Yuanyang et al[155]	30.3%	37.5%
current study	**40%**	**96.67%**

This is statistically significant in our study p value of 0.0000023($p<0.05$)

Agewise distribution of EGFR:

In our study, 29.27% of the EGFR positive patients are in the age group between 51 and 60 years. 73.17% of the cases are less than 60 years of age and 26.83% of the patients are above 60 years. The study by Yuanyang et al[155] says that 37.3% of the patients are above 60 years of age and 30.7% of the patients are less than 60 years of age. Studies by Feng Q et al has suggested that most EGFR positive patients are less tha 60 years. This variable is not statistically significant in our study with a p value of 0.146071($p>0.05$).

EGFR expression in various histological subtypes:

In our study, EGFR expression is more commonly seen in adenocarcinoma with a percentage of 43.90%. 36.59% of squamous cell carcinomas are positive for EGFR expressionand 19.51% of NSCLC-NOS are positive foe EGFR expression. The comparison with various studies are as follows

	Adenocarcinoma	**Squamous cell carcinoma**	**NSCLC-NOS**
Yan Liu et al[160]	61.1%	29.7%	40%
Yuanyang Lai et al[155]	52.9%	14.5%	
Ying Li et al[158]	44.2%	8%	
She Juan An et al[154]	40.3%	4.4%	
Feng Q et al[145]	38.8%		
Our study	**43.90%**	**36.59%**	**19.51%**

The comparison of EGFR distribution with various histological subtypes is statisticallySignificant in our study with p value of 0.00227393($p<0.05$).

Limitations of the study:

- These patients are selected from the tertiary care centre and it is not a population basedStudy. Hence the study population might not represent the general population
- Gene expression profiling gives more accurate values than the immunohistochemistry.Since it is expensive, it cannot be applied to all the patients.
- Since this is a retrospective study, targeted therapy was not given. Hence the prognosticInfluence cannot be ascertained.

SUMMARY

SUMMARY

- This study on lung cancer is a retrospective study conducted in theInstitute of Pathology, MADRAS MEDICAL COLLEGE, Chennai during the period of july2013 to june 2015.
- Among the total 412 lung specimens received, 137 cases are non neoplastic, 40 cases are suspicious of malignancy and193 cases are malignant.
- Among the 193 malignant cases, 97.405 of the cases are small biopsiesand 2.59% of the cases are resected specimens. The types of small biopsies reveived are CTguided biopsy, bronchial biopsy, transbronchial biopsy and USG guided biopsy.
- Among the malignant cases, 178 cases are non small cell lung cancer Which vastly outnumber small cell lung carcinoma cases(9).
- The distribution of squamous cell carcinoma and adenocarcinoma are39.90% and 33.01% respectively. Squamous cell carcinoma is more commonly seen in thanAdenocarcinoma, NSCLC-NOS accounts for 19.17% of cases.
- The mean age of presentation of lung cancer is 55.15 years. The youngest age at presentation is 29 years.
- Among the 193 malignant cases, 76.17% of the cases are men and23.83% of the cases are women with increased incidence of squamous cell carcinoma in men and adenocarcinoma seen more commonly in women.

- Cough is the most common presenting complaint with 56.99% followedby breathlessness and hemoptysis.
- The most common location of tumour is the right upper lobe. Right lungis more commonly involved than the left lung.
- 85.42% of the patients present with mass radiologically. The non neoplastic radiological findings like cavity and collape have turned to be malignant in few cases.
- 55.44% of the cases are smokers and 44.04% of the cases arenonsmokers with strong smoking association in squamous cell carcinoma with 41.1%
- Sixty cases of non small cell lung cancer are randomly selected fromeach cateogory for EGFR expression by IHC. 68.33% of the cases are positive for EGFR expression.90.48% of the females are positive for EGFR expression and 46.15%of the males showed positivity with p value- 0.000613
- 96.67% of the EGFR positive cases are smokers and 40% areare non smokers. p value- 0.0000023
- 29.27% of the EGFR positive patients are in the age group between51 and 60 years.
- Among the EGFR positive histological subtype, adenocarcinoma is the most common type with 43.90% followed by squamous cell carcinoma with 36.59% & NSCLC-NOS with 19.51%. p value- 0.0022.

PATIENT CHARACTERISTICS

CHARACTERISTICS	NUMBER	%
AGE		
<60	129	66.84%
>60	64	33.16%
GENDER		
Male	147	76.17%
Female	46	23.83%
SMOKING STATUS		
Never	107	55.44%
Ever	85	44.04%
HISTOLOGY		
Adenocarcinoma	64	33.01%
SCC	77	39.90%
NSCLC-NOS	37	19.17%
LCC	2	1.04%
Carcinoid	4	2.07%

n=193

CHARACTERISTICS IN RELATION TO EGFR:

CHARACTERISTICS	NUMBER	%	p-value
AGE			
<60	30	73.17%	**0.146071**
>60	11	26.83%	
GENDER			
Male	18	46.15%	**0.000613**
Female	21	90.48%	
SMOKING STATUS			
Never	29	96.67%	**0.0000023**
Ever	12	40%	
HISTOLOGY			
Adenocarcinoma	18	43.90%	**0.0027**
SCC	15	36.59%	
NSCLC-NOS	8	19.51%	

n = 60

CONCLUSION

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As most of the lung cancer cases present at advanced age, the introduction of targeted therapies particularly EGFR has revolutionise the treatment of lung cancer patients.

There is a heightened interest in targeted therapy against lung cancers particularlyNon small cell lung cancer. The identification of EGFR expression gives a fascinating opportunity for the development of tyrosine kinase inhibitors against non small cell lung cancers.

It is very clear from the comparison of various studies from our studies that EGFR expression is more common in females, never smokers and adenocarcinoma histological type.

EGFR, being a poor prognostic factor, its expression is very important to identify the tyrosine kinase inhibitors sensitivity.

Hence it is very important to find the association between EGFR expression and its clinocopathological parameters in order to select the patients for targeted therapy like erlotinib, gefitinib for advanced lung cancers.

In conclusion, it is recommended that EGFR expression should be a routine test after lung resection for all non small lung carcinoma especially adenocarcinoma and squamous cell carcinoma for better treatment for the patients.

ANNEXURES

ANNEXURE-I

PROFORMA

Case number	:	Name	:
HPE number	:	Age	:
IP number	:	Sex	:
Clinical diagnosis	:		
Complaint	:		
Radioimaging	:		
FOB findings	:		
Site of lesion	:	Right upper lobe/middle lobe/lower lobe Left upper lobe/hilum/lower lobe	
Specimen	:	CT guided biopsy/Bronchial biopsy/USG guided biopsy/Open biopsy	
MICROSCOPY	:		
Special stain	:		
IHC			
EGFR	:	Positive / Negative	

DIAGNOSIS :

ANNEXURE : II

WHO CLASSIFICATION FOR LUNG CANCER

Malignant epithelial tumours
Squamous cell carcinoma
Papillary
Clear cell
Small cell
Basaloid

Small cell carcinoma
Combined small cell carcinoma

Adenocarcinoma
Adenocarcinoma, mixed subtype
Acinar adenocarcinoma
Papillary adenocarcinoma
Bronchioloalveolar carcinoma
Nonmucinous
Mucinous
Mixed nonmucinous and mucinous or indeterminate Solid adenocarcinoma with mucin production
Fetal adenocarcinoma
Mucinous ("colloid") carcinoma
Mucinous cystadenocarcinoma
Signet ring adenocarcinoma
Clear cell adenocarcinoma

Large cell carcinoma
Large cell neuroendocrine carcinoma
Combined large cell neuroendocrine carcinoma
Basaloid carcinoma
Lymphoepithelioma-like carcinoma
Clear cell carcinoma
Large cell carcinoma with rhabdoid phenotype

Adenosquamous carcinoma

Sarcomatoid carcinoma
Pleomorphic carcinoma
Spindle cell carcinoma
Giant cell carcinoma
Carcinosarcoma
Pulmonary blastoma

Carcinoid tumour
Typical carcinoid
Atypical carcinoid

Salivary gland tumours
Mucoepidermoid carcinoma
Adenoid cystic carcinoma
Epithelial-myoepithelial carcinoma

Preinvasive lesions
Squamous carcinoma in situ
Atypical adenomatous hyperplasia
Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Mesenchymal tumours
Epithelioidhaemangioendothelioma
Angiosarcoma
Pleuropulmonaryblastoma
Chondroma
Congenial peribronchialmyofibroblastic tumour
Diffuse pulmonary lymphangiomatosis
Inflammatory myofibroblastic tumour
Lymphangioleiomyomatosis
Synovial sarcoma
Monophasic
Biphasic
Pulmonary artery sarcoma
Pulmonary vein sarcoma

Benign epithelial tumours
Papillomas
Squamous cell papilloma
Exophytic
Inverted
Glandular papilloma
Mixed squamous cell and glandular papilloma
Adenomas
Alveolar adenoma
Papillary adenoma
Adenomas of the salivary gland type
Mucous gland adenoma
Pleomorphic adenoma
Others
Mucinous cystadenoma

Lymphoproliferative tumours
Marginal zone B-cell lymphoma of the MALT type
Diffuse large B-cell lymphoma
Lymphomatoidgranulomatosis
Langerhans cell histiocytosis

Miscellaneous tumours
Harmatoma
Sclerosinghemangioma
Clear cell tumour
Germ cell tumours
Teratoma, mature
Immature
Other germ cell tumours
Intrapulmonary thymoma
Melanoma
Metastatic tumours

ANNEXURE : III

PROPOSED IASLC/ATS/ERS CLASSIFICATION FOR SMALL BIOPSIES/CYTOLOGY

2004 WHO Classification	**SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS**
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	Morphologic Adenocarcinoma pattern clearly presents:Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification)
No 2004 WHO counterpart – most will be solid adenocarcinomas	Morphologic adenocarcinoma patterns not present (supported by special stains): Non-small cell carcinoma, favor adenocarcinoma
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with Fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloidal pattern
Signet Ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	Morphologic squamous cell pattern clearly presents: Squamous cell carcinoma
No 2004 WHO counterpart	Morphologic squamous cell patterns not present (supported by stains): Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma

LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)
Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNE
Large cell carcinoma with N morphology(LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
ADENOSQUAMOUS CARCINOMA	Morphologic squamous cell and adenocarcinoma patterns present: Non-small cell carcinoma, NOS, (comment that glandular and squamous components are present
No counterpart in 2004 WHO classification	Morphologic squamous cell or adenocarcinoma patterns present and stains are conflicting (TTF1 and p63 positive) or suggest the other pattern is also present Non-small cell carcinoma, NOS, comment that glandular and squamous differentiation seen by IHC) Comment (for either setting): this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	Poorly differentiated NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

ANNEXURE:IV

IASLC/ATS/ERS CLASSIFICATION OF LUNG ADENOCARCINOMA IN RESECTION SPECIMENS

PREINVASIVE LESIONS

A typical adenomatous hyperplasia

Adenocarcinoma in situ (≤3 cm formerly BAC)

-Nonmucinous

-Mucinous

-Mixed Mucinous/Non-Mucinous

MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm lepidic predominant tumor with ≤5 mm invasion)

-Non mucinous

-Mucinous

-Mixed Mucinous/Non-Mucinous

INVASIVE ADENOCARCINOMA

Lepidic predominant (formerly non-mucinous BAC pattern, with >5 mm invasion)

Acinar predominant

Papillary predominant

Micropapillary predominant

Solid predominant with mucin production

VARIANTS OF INVASIVE ADENOCARCINOMA

Invasive mucinous adenocarcinoma (formerly mucinous BAC)

Colloid Fetal (low and high grade)

Enteric

ANNEXURE-V

Immunohistochemistry procedure:

Slide Preparation:

1. Sections with a thickness of 4 μ were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated for overnight at 58ºC.
3. The sections were deparaffinised in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes for 2 changes.
5. Then the sections were washed with tap water for 10 minutes.
6. The slides are then immersed in distilled water upto 5 minutes.

Antigen Retrieval:

1. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 minutes. This step unmasks the antigenic determinants of fixed tissue sections.
2. The slides were then cooled to room temperature for 20 minutes and washed with tap water for 5 minutes.
3. The slides were then rinsed with distilled water for 5 minutes.
4. then the slides were washed with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
5. Peroxidase block was then applied for 10 minutes.
6. The slides then were washed in phosphate buffer for 5 minutes x 2 changes.
7. Sections were covered with protein block for 5 minutes.

Antibody application:

1. The sections were drained (without washing) and appropriate primary antibody is applied and incubated for 30 minutes.
2. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.
3. The slides were covered with Primary antibody amplifier for 10 minutes.
4. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.
5. The slides were covered with HRP micropolymer Quanto for 10 minutes.
6. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.

Chromogen application:

1. DAB substrate was prepared by diluting 1 drop of DAB Quantochromogen to 1 ml of DAB Quanto buffer.
2. DAB substrate solution was applied on the sections for 5 minutes.
3. wash the slides then in distilled water for 2 minutes.
4. counterstain the section with Hematoxylin for 2 seconds.
5. wash the slides in running tap water for 5 minutes.
6. air dry the slides, cleared with xylene and mounted with DPX.

Alternate methods of antigen retrieval

- Pressure cooker antigen retrieval
- Microwave and trypsin antigen retrieval

ANNEXURE VI

GRADING OF EGFR:

0 – negative, no detectable staining.

1+ faint membrane staining in more than 10% of tumour cells.

2+ moderate and continuous membrane staining in more than 10% of tumour cells.

3+ strong and continuousmembrane staining in more than 10% of tumour cells.

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MASTER CHART

S. NO	HPE NO	AGE	SEX	SITE	SMOKING	CLINICAL FEATURES	XRAY, CT,MRI	CYTOLOGY	SPECIMEN	HPE DIAGNOSIS	EGFR
1	7455/14	70	F	1	N	1,2	O	PF-POS	3	A	3+
2	7542/14	55	F	1	N	1,3.5	M	PF-POS	1	A	2+
3	9164/14	42	M	1	Y	1,3.5	M	SP-NEG	5	A	1+
4	1485/14	47	M	1	Y	1,6	M	BW-POS	1	A	2+
5	6766/14	57	F	5	N	2,5	M	BW-NEG	2	A	3+
6	9357/14	50	M	6	Y	1,4	M	LN-METS	3	A	NEG
7	11553/14	67	M	1	Y	2,4	M		1	A	3+
8	7859/14	58	F	4	N	2,5	PE	PF-NEG	1	A	2+
9	8096/14	50	F	4	N	2,5	M		5	A	3+
10	11215/14	60	M	4	Y	2,4,5	M	BW-POS	3	A	3+
11	98/15	55	M	1	N	1,3,4	M	PF-NEG	1	A	3+
12	99/15	60	M	1	Y	1,2,5	PF	SP-POS	3	A	2+
13	2973/15	62	M	4	N	1,4	M		1	A	3+
14	224/15	34	F	1	N	1,2,5	M		1	A	3+
15	608/15	62	M	4	Y	1,6	PB	BW-NEG	3	A	NEG
16	1829/15	63	F	2	N	3,4	M		1	A	3+
17	2856/15	36	M	4	Y	1,2	M		1	A	3+
18	1112/15	60	F	1	N	1,5	M		1	A	3+
19	2335/15	60	M	4	Y	1,3	PB	SP-POS	1	A	3+
20	2973/15	62	F	4	N	1,3,5	M	SP-POS	1	A	3+
21	3044/15	45	F	1	N	2,4,5	M, RIB	PF-POS	1	S	2+
22	10611/14	75	M	6	Y	3,4,5	M,PE	PF-NEG	1	S	NEG
23	10911/14	55	M	4	Y	1,6	M,RIB		1	S	2+
24	10968/14	65	F	1	N	1,5	M,F,C	BW-POS	1	S	3+

25	11529/14	48	F	7	N	1,3,4	M, RIB	BW-POS	2	S	3+
26	08/15	70	M	5	Y	2,3,4	M,COL	SP-NEG	1	S	NEG
27	72/15	45	F	4	N	2,3,5	M,MNI		1	S	3+
28	34/15	62	F	4	N	1,2	M		1	S	3+
29	312/15	70	M	4	N	1,3,5	M	BW-NEG	1	S	3+
30	317/15	56	M	1	Y	1,4,5	M	BW-NEG	1	S	NEG
31	10292/14	50	M	4	Y	1,5	M		1	S	2+
32	1328/14	55	M	2	N	1,3,4	M,MNC	SP-POS	2	S	1+
33	2482/15	64	M	4	Y	1,4,5	M	SP-NEG	3	S	NEG
34	3029/15	48	M	4	N	1,2,5	M		2	S	3+
35	2426/15	31	M	4	N	1,3,4,5	M	BW-NEG	2	S	3+
36	2379/15	40	M	1	N	3,4,5	M,MNI, COL		1	S	2+
37	3187/15	45	M	1	Y	2,3,4	M,CAV	BW-POS	2	S	1+
38	3666/15	55	M	1	Y	1,2,5	M		1	S	NEG
39	4308/15	65	F	1	N	1,4,5	M	SP-NEG	1	S	3+
40	4271/15	55	F	4	N	1,3	M	LN-METS	3	S	3+
41	10582/14	46	M	1	Y	1,2,4	M,RIB	SP-NEG	1	NSCLC-NOS	NEG
42	9166/14	70	M	1	Y	1,3.5	M		1	NSCLC-NOS	1+
43	9912/14	62	M	1	Y	1,3	M	BW-NEG	5	NSCLC-NOS	NEG
44	9821/14	59	M	1	Y	2,4	M,MNI	SP-POS	3	NSCLC-NOS	NEG
45	11506/14	45	M	1	Y	2,5	M	SP-NEG	2	NSCLC-NOS	NEG
46	10480/14	60	M	2	N	2,3,4	M	SP-POS	1	NSCLC-NOS	3+
47	3033/15	55	M	4	Y	1,2	M,CN	LN-METS	1	NSCLC-NOS	NEG
48	2427/15	47	M	1	Y	1,5	M	BW-NEG	1	NSCLC-NOS	3+
49	3071/15	68	F	1	N	3,4,5	M	SP-NEG	2	NSCLC-NOS	3+
50	3225/15	49	M	4	Y	1,2,4	M,CN	LN-METS	1	NSCLC-NOS	NEG

51	3676/15	42	M	1	Y	1,2,4	P	SP-NEG	5	NSCLC-NOS	NEG
52	9880/14	50	M	1	Y	2,3,5	M	SP-NEG	1	NSCLC-NOS	NEG
53	6474/13	74	M	1	Y	1,2	M	LN-METS	2	NSCLC-NOS	NEG
54	7205/13	72	M	3	Y	1,2	M.PF	PF-POS	3	NSCLC-NOS	NEG
55	8317/13	40	F	5	N	2,3	M	SP-POS	1	NSCLC-NOS	3+
56	9789/13	53	M	5	Y	1,2,3	M	BW-POS	2	NSCLC-NOS	NEG
57	10452/13	30	F	4	N	2,3	M	SP-NEG	4	NSCLC-NOS	2+
58	10570/13	48	F	1	N	1,5	O	LN-METS	1	NSCLC-NOS	3+
59	10724/13	34	F	3	N	2,3,4	M	SP-NEG	2	NSCLC-NOS	2+
60	10847/13	56	M	1	Y	1,2	M	BW-NEG	1	NSCLC-NOS	NEG
61	878/14	44	M	4	Y	1,4	P	SP-NEG	4	C	
62	1014/14	56	M	4	Y	3	M,F	BW-POS	2	A	
63	1054/14	62	M	5	N	1,3	M	BW-NEG	2	A	
64	1130/14	42	M	5	Y	1,6	M,F		1	C	
65	1181/14	50	M	1	N	4	M,COL	BW-NEG	1	S	
66	1565/14	65	M	1	Y	1	O,M	BW-NEG	1	S	
67	1597/14	60	M	1	N	1,2,3	M	SP-NEG	1	S	
68	1630/14	56	M	4	Y	1,2	O,M	SP-NEG	1	A	
69	1744/14	52	F	1	N	1,2,3	M		1	A	
70	1830/14	55	M	1	Y	1,2	PE	PF-NEG	2	NSCLC-NOS	
71	1917/14	58	M	5	Y	1	O	BW-NEG	2	NSCLC-NOS	
72	1858/14	23	M	4	Y	1,2,3	M	BW-NEG	5	S	
73	2031/14	45	F	4	N	2,3	PE	PF-POS	1	A	
74	2043/14	64	M	4	Y	5	PE	PF-NEG	1	S	
75	2120/14	48	M	4	Y	2,3,5	M	SP-NEG	1	S	
76	2204/14	54	M	4	N	1	M,F	SP-NEG	1	A	

77	2256/14	67	M	1	N	1,2	M	BW-NEG	1	S	
78	2262/14	51	M	4	Y	1,2,3	M	SP-NEG	1	NSCLC-NOS	
79	2312/14	56	F	2	N	1,2	CAV	BW-NEG	1	S	
80	2340/14	65	M	2	Y	2,3,4	M,PE	PF-NEG	1	S	
81	2344/14	63	M	5	Y	4	M	BW-NEG	3	S	
82	2400/14	52	F	1	Y	1,2,3	M, RIB	LN-METS	1	S	
83	2521/14	75	M	1	Y	1,2	M	BW-NEG	1	S	
84	2527/14	62	M	1	N	1,3	M		1	S	
85	2531/14	56	F	1	Y	1,3,4,5	M	SP-POS	1	A	
86	2532/14	50	F	4	N	1,2,3	COL	SP-NEG	1	S	
87	2618/14	61	M	1	Y	1,2	M		1	A	
88	2644/14	70	M	4	Y	1,4,5	PB	BW-NEG	1	S	
89	2732/14	55	M	1	Y	1	M	BW-POS	1	NSCLC-NOS	
90	2881/14	60	M	1	N	1,2	M,CN	LN-METS	1	A	
91	3101/14	60	F	4	Y	1,4,5	M		1	A	
92	3104/14	60	M	4	Y	4	M	SP-NEG	2	S	
93	3140/14	70	M	1	Y	2,3	M,PE	PF-NEG	2	NSCLC-NOS	
94	3314/14	60	M	1	Y	1,3,4,5	PB	SP-NEG	2	S	
95	3411/14	65	F	3	Y	1,2,4	M	SP-NEG	3	S	
96	3740/14	60	M	4	Y	1,3	CON,M	BW-NEG	2	S	
97	3836/14	58	M	1	Y	1,3,4	M		2	NSCLC-NOS	
98	3990/14	55	M	1	Y	2,3,4	M	SP-NEG	4	S	
99	9208/14	55	M	1	Y	1	M,CAV		3	NSCLC-NOS	
100	9657/14	50	M	3	Y	1,2,3	M,CN	LN-METS	1	A	
101	9808/14	42	F	1	N	2,3,4	M	SP-NEG	1	A	
102	9880/14	33	F	5	N	2,3,5	M,COL		1	NSCLC-NOS	

103	5048/14	54	M	3	Y	1,3,4,5	M, RIB	SP-POS	2	NSCLC-NOS	
104	5080/14	40	F	4	N	1,2,3	M	BW-NEG	2	A	
105	5130/14	40	F	1	N	2,3	M	BW-POS	2	NSCLC-NOS	
106	5166/14	57	M	1	Y	2,4,5	M	BW-POS	3	A	
107	5341/14	37	M	1	N	3,4	M	BW-NEG	2	A	
108	5451/14	58	M	5	N	3,4	M,MNC	SP-NEG	1	Small cell ca	
109	5486/14	65	M	1	Y	2,4,5	M,MNI	LN-METS	1	S	
110	5642/14	70	M	2	Y	1,2,4	M,F,C	SP-NEG	1	NSCLC-NOS	
111	5276/14	50	M	2	Y	3,4,5	M	BW-NEG	2	S	
112	5441/14	63	M	1	N	2,5	M		1	A	
113	5527/14	67	M	3	N	2,3,4	M	SP-NEG	1	A	
114	5618/14	77	M	1	Y	4	M, RIB	BW-POS	2	S	
115	5658/14	67	M	1	N	1,2	M,COL	BW-NEG	2	A	
116	5718/14	63	M	3	Y	1,2,4	M,CAV	BW-NEG	2	A	
117	5788/14	63	M	3	N	1,2	M	SP-NEG	1	S	
118	5913/14	58	M	1	Y	2,4,5	M	BW-NEG	2	NSCLC-NOS	
119	5995/14	55	M	1	N	3	M		1	S	
120	5997/14	50	M	1	N	3	M	SP-NEG	1	S	
121	6008/14	65	M	3	Y	4	M,F	BW-POS	2	S	
122	6041/14	70	M	1	N	2,4,5	M,PE	PF-NEG	1	NSCLC-NOS	
123	6167/14	60	M	5	Y	1,3,4,5	M	BW-POS	3	S	
124	6359/14	55	M	1	Y	2,3,4	M	SP-NEG	2	S	
125	6606/14	55	M	3	N	2,3,4	PB	SP-NEG	1	Small cell ca	
126	6678/14	75	M	1	Y	1,2,3	M	SP-NEG	1	A	
127	6719/14	55	M	4	Y	2,4,5	M,COL	BW-POS	2	Small cell ca	
128	6799/14	50	M	1	N	1,2	M	SP-NEG	1	NSCLC-NOS	

129	6970/14	64	M	3	N	1,2	M	BW-NEG	3	S	
130	6984/14	65	M	1	N	1,2,3	M	BW-POS	2	Small cell ca	
131	7021/14	71	M	1	Y	1	M	BW-NEG	2	S	
132	7202/14	46	M	1	N	1,2,3	M		1	S	
133	7771/14	52	M	3	N	1,2	M, RIB		1	A	
134	8028/14	67	M	1	Y	1,2,3	M,PE	PF-NEG	1	S	
135	8274/14	47	F	5	N	2,3,5	M	BW-POS	2	S	
136	8338/14	65	M	3	Y	3,4,5	M		1	S	
137	8352/14	64	M	1	Y	1,2	M	BW-POS	2	A	
138	8454/14	47	M	5	Y	2,3,5	M	SP-POS	1	S	
139	8483/14	29	F	3	N	1,3	M	BW-NEG	1	A	
140	8605/14	73	M	2	Y	2,4,5	M,F		2	S	
141	8860/14	56	F	3	N	2,4	M	SP-NEG	2	A	
142	8943/14	43	M	5	Y	3	M,CAV	SP-NEG	1	S	
143	9038/14	67	M	3	Y	1,2,4	O		2	Small cell ca	
144	9121/14	55	M	1	Y	2,3	M,MNC	BW-POS	2	A	
145	11105/14	30	F	1	N	1,3,4,5	M,PE	PF-NEG	1	S	
146	12042/14	38	F	1	Y	3	M,CN	LN-METS	2	A	
147	12066/14	55	M	3	N	2,3,4	O	SP-NEG	2	S	
148	12068/14	42	M	3	Y	2,4	M,PE	PF-POS	1	S	
149	12093/14	68	M	1	Y	3,4,5	M	SP-POS	1	S	
150	6223/13	55	M	3	Y	2,3	O		1	NSCLC-NOS	
151	6431/13	53	F	1	N	1,2	M	SP-POS	2	Small cell ca	
152	6478/13	55	M	1	Y	2,3,4	M	BW-NEG	2	S	
153	6592/13	65	M	3	Y	3,4,5	O,M	SP-NEG	1	A	
154	6618/13	63	M	1	Y	2,3	M	BW-NEG	3	NSCLC-NOS	

155	6690/13	52	F	4	N	1,2,5	M,PE	PF-POS	1	NSCLC-NOS	
156	8324/13	67	M	2	Y	1,2,4	M, RIB	SP-NEG	1	S	
157	8467/13	62	M	1	N	2,3	M	SP-NEG	1	LCC	
158	8525/13	58	M	1	Y	3,4,5	M,CAV		2	S	
159	8574/13	60	M	1	N	1,3,4	M,F	SP-NEG	1	A	
160	8603/13	50	F	4	N	1,3,4	M	LN-METS	1	A	
161	8663/13	60	F	4	N	1,2,3	M,CAV		2	A	
162	8907/13	70	F	1	N	1	M	SP-NEG	1	A	
163	9030/13	54	M	1	Y	1,3,4	M,CN	LN-METS	2	Small cell ca	
164	9031/13	65	M	3	Y	3,4,5	M	BW-NEG	2	S	
165	9090/13	65	M	4	Y	2,3,4	M		1	S	
166	9416/13	65	M	4	N	1,2	M	SP-POS	1	A	
167	9417/13	55	M	4	Y	2,3	M,F	SP-NEG	1	A	
168	9467/13	55	M	1	Y	4	M,MNC	SP-NEG	1	A	
169	9593/13	54	M	3	N	3,4,5	M,MNI, COL		1	A	
170	9597/13	55	M	4	Y	1,3,4	M,PE	PL-POS	2	LCC	
171	9780/13	55	M	5	N	1,3,4,5	M	SP-NEG	2	A	
172	9782/13	42	M	5	N	1,2,4	M,PE	PF-POS	1	A	
173	10058/13	65	M	1	N	1,2	M	BW-NEG	2	NSCLC-NOS	
174	10181/13	45	F	1	N	1,2,3	M,PE	PF-NEG	4	A	
175	10524/13	56	M	4	Y	2,3,4	M		1	S	
176	10525/13	42	M	3	Y	2,3	M,MNC	BW-POS	2	A	
177	10569/13	67	M	1	Y	1,3	M	SP-POS	2	A	
178	10650/13	50	M	1	Y	1,2,4	M	LN-METS	1	A	
179	10763/13	47	M	3	Y	2,3,4	M,MNI	LN-METS	1	S	
180	10995/13	49	M	5	N	1,3,4,5	M	BW-NEG	2	S	

181	409/15	51	M	4	N	1,2,3	M	BW-NEG	3	Small cell ca	
182	1580/15	70	M	4	Y	3,4,5	PB		2	Small cell ca	
183	2104/15	67	M	3	Y	1,2	M, RIB	SP-NEG	1	S	
184	2779/15	40	M	1	Y	3,4	M,COL	SP-POS	1	A	
185	2913/15	32	M	5	Y	1,3,4,5	M		1	S	
186	2914/15	42	M	1	Y	1,2	P	BW-NEG	2	C	
187	2946/15	58	M	2	N	2,4,5			1	S	
188	2974/15	41	M	5	N	3	M	BW-POS	2	S	
189	3879/15	35	F	2	N	2,3	M,PE	PL-POS	2	S	
190	4166/15	20	M	1	Y	3,4	P	BW-NEG	2	C	
191	4320/15	43	F	4	N	2,3,5	M	BW-NEG	1	S	
192	4356/15	56	M	5	N	3,4	M	SP-NEG	2	S	
193	4494/15	57	M	1	N	2,3	M,PE	PF-NEG	1	A	

KEY TO MASTER CHART

HPE NO : Histopathological examination number

M	:	Male
F	:	Female

Site

1	:	Right upper lobe
2	:	Right middle lobe
3	:	Right lower lobe
4	:	Left upper lobe
5	:	Left lower lobe
6	:	Left hilum

Smoking

Y	:	Yes
N	:	No

C/F : Clinical Features

1	:	Cough
2	:	Hemoptysis
3	:	Breathlessness
4	:	Chest pain
5	:	Weight loss

Radiological features:

M	:	Mass
ME	:	Mediastinal invasion
PE	:	Pleural effusion
CN	:	Cervical node metastasis
MNI	:	Ipsilateralmediastinallymphnode involvement
MNC	:	Contralateral mediastinallymphnode involvement
O	:	Opacity

F	:	Fibrosis
CON	:	Consolidation
CAV	:	Cavity
SCN	:	Supraclavicular lymphnode metastasis
RIB	:	Rib erosion
P	:	Polypoidal lesion
SP	:	Sputum
BW	:	Bronchial wash
PF	:	Pleural fluid
NEG	:	Negative
POS	:	Positive

Specimen:

1	:	Computed tomogram guided biopsy
2	:	Bronchial biopsy
3	:	Transbronchialbipsy
4	:	Ultrasonogram guided biopsy
5	:	Resected specimens
HPE diagnosis	:	Histipathological examination diagnosis
A	:	Adenocarcinoma
S	:	Squamous cell carcinoma
NSCLC-NOS	:	Non small cell lung cancer-not otherwise Specified.
C	:	Carcinoid
LCC	:	Large Cell Carcinoma
P	:	Poorly differentiated carcinoma.

INFORMATION SHEET

Title : A study of expression of EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) in Lung Cancers

Your specimen has been accepted.

- We are conducting a study on lung cancersamong patients attending Institute of Pathology, Madras Medical College,Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to diagnose the expression of a special marker (EGFR) in lung cancers.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

INFORMED CONSENT FORM

Title of the study**: A study of expression of EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) in Lung Cancers**

Name of the Participant :
Name of the Principal (Co-Investigator) :
Name of the Institution : Institute of Pathology, Madras Medical College.
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"A study of expression of EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) in Lung Cancers"

I have read and understood this consent form and the information provided to me.

1. I have had the consent document explained to me.
2. I have been explained about the nature of the study in which the resected endometrial tumors will be subjected to immunohistochemistry and histopathological examination.
3. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
4. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
5. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
6. I have understand that my identity will be kept confidential if my data are publicly presented
7. I have had my questions answered to my satisfaction.
8. I have decided to be in the research study.

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

For adult participants:
Name and signature / thumb impression of the participant (or legal representative if participant incompetent)
Name _____ Signature_____ Date_____

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

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

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

Name _____ Signature_____ Date_____