COMPARISON OF NAPSIN A VERSUS THYROID TRANSCRIPTION FACTOR - 1 IN THE TYPING OF LUNG CARCINOMA

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BRANCH - III

INSTITUTE OF PATHOLOGY
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CHENNAI

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CERTIFICATE

This is to certify that this dissertation entitled **"Comparision Of Napsin A Versus Thyroid Transcription Factor -1 In The Typing Of Lung Carcinoma"** is the original work of **Dr. R. MANIBARATHI,** in partial fulfilment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2018.

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DECLARATION

I, **Dr. R. MANIBARATHI,** solemnly declare that the dissertation titled **"COMPARISION OF NAPSIN A VERSUS THYROID TRANSCRIPTION FACTOR-1 IN THE TYPING OF LUNG CARCINOMA"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **PROF. DR. RAJAVELU INDIRA,** M.D., Director & Professor of Pathology, Department of pathology, Institute of Social Obstetrics & Government Kasturba Gandhi Hospital for Women and Children, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. R. MANIBARATHI**

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CERTIFICATE OF APPROVAL

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Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"COMPARISION OF NAPSIN A VERSUS TTF-1 IN THE TYPING OF LUNG CARCINOMA " NO.20092016 .**

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC ,Ch-3	Deputy Chairperson
3. Prof. Sudha Seshayyan, MD., Vice Principal, MMC.Ch- 3.	Member Secretary
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8. Thiru.S.Govindasamy., B.A.B.L., High Court, Chennai-1	Lawyer
9. Tmt.ArnoldSaulina, MA., MSW.,	Social Scientist

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.

Member Secretary - Ethics Committee

MEMBER SECRETARY
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ABBREVIATIONS

ADC	**:**	**AdenoCarcinoma**
ALK	**:**	**Anaplastic lymphoma Kinase**
ATS	**:**	**American Thoracic Society**
ERS	**:**	**European Respiratory Society**
EGFR	**:**	**Epidermal growth Factor Receptor**
SqCC	**:**	**Squamous Cell Carcinoma**
SCC	**:**	**Small Cell carcinoma**
NSCLC	**:**	**Non small Cell Lung Cancer**
H & E	**:**	**Hematoxylin & Eosin**
IASLC	**:**	**International Association For The Study of Lung Cancers**
IHC	**:**	**Immuno Histo Chemistry**
LCC	**:**	**Large Cell carcinoma**
PAS	**:**	**Periodic Acid Schiff**
TTF- 1	**:**	**Thyroid Transcription Factor-1**

CONTENTS

INTRODUCTION

INTRODUCTION

Lung cancer is the most frequently diagnosed major cancer in the world and most common cause of cancer related death. [1] Lung cancer is the most frequently diagnosed cancer and leading cause of cancer mortality in the world [1].

Previously, it was sufficient to diagnose primary lung carcinoma as either NSCLC or SCC for treatment purpose. With the development of new, successful treatments for adenocarcinoma, it is essential to diagnose the type of NSCLC whenever possible [1].

Routine sections stained with H&E remain the most common method by which lung cancers are classified; however typing of NSCLC and the more poorly differentiated tumours is often hard to achieve by H&E alone. Immunohistochemistry has emerged as a powerful, adjunctive tool for the differential diagnosis of lung carcinomas [1].

TTF-1 is a favoured marker for lung adenocarcinoma but has limited sensitivity and specificity. Napsin A is a functional aspartic proteinase that may be an alternative marker for primary lung ADC [1, 2].

AIMS & OBJECTIVES

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1. To study the prevalence of napsin A in lung cancer tissues, compared with another marker, thyroid transcription factor-1 (TTF-1), which has already recognized as a useful marker for lung adenocarcinoma
2. To compare the usefulness of napsin –A with TTF-1 for the identification of primary lung adenocarcinoma.
3. To evaluate their utilization in the identification of primary and metastatic lung cancer.
4. To evaluate the association of their expression with other clinicopathological parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY[3]:

The lungs are the primary organs of respiratory function. Within the thoracic cavity, The lungs lie either side of the mediastinum. Each lung is covered by a pleural cavity, which is formed by the visceral and parietal pleura. The lungs are roughly cone in shape. They have an apex, base, and three borders, three surfaces. The Left lung is slightly smaller than right due to the presence of the heart. Hilum comprises a bronchus, two pulmonary veins, pulmonary artery, bronchial vessels, pulmonary plexus of nerves and lymphatic vessels.

Bronchial tree comprises Trachea - Right and Left Bronchus - Lobar bronchi - Segmental bronchi – Terminal bronchiole - respiratory bronchiole – Alveoli.

The right lung comprises 3 lobes and 10 segments. 3 in the right upper lobe (apical, anterior, medial),2 in right middle lobe (medial and lateral), and 5 in the right lower lobe (superior, medial, anterior, lateral, posterior).

The left lung comprises 2 lobes and 8 segments. 4 in the left upper lobe (apicoposterior, anterior, superior lingual, and inferior lingual) and 4 in the left lower lobe (superior, anteromedial, lateral, and posterior).

HISTOLOGY [4]:

Lung parenchyma consists of airway (bronchi/bronchioles) and alveoli. The pulmonary lobule, (terminal respiratory unit) contains 3-5 terminal breonchioles, alveolar ducts and alveoli; It is the smallest anatomic unit.

The entire respiratory tree is lined by pseudostratified, tall, columnar, ciliated epithelial cells with neuroendocrine cells, mucous secreating goblet cells in the wall of trachea and bronchi, basal cells, clara cells and inflammatory cells except vocal cord and alveoli.

Alveoli is almost exclusively lined by type I and type II pneumocytes. Type I pneumocytes is 95% which is flattened, Type II is 5% which produces surfactant, and during repair, type II pneumocytes give rise to type I pneumocytes.

Number of Clara cells increases towards terminal bronchiole, have secretory function. It is the main progenitor cell after bronchiolar injury and have apical PAS + diastase resistant secretory granules. Neuro endocrine cells are numerous in neonatal bronchial and bronchiolar epithelium; in adults it is rare except as small clusters within epithelium of bronchi and bronchioles. Submucous glands comprises serous and mucus cells with myoepithelial lining and with age may have oncocytic change.

LUNG CANCER:

The lung cancer is the uncontrolled abnormal growth of cells which line the air passages[5].

Lung cancer was first discovered in 1838 by A German pathologist named Johannes Muller. The association between lung cancer and smoking was demonstrated by a German doctor named Fritz Lickint in 1929[5,6].

Sir Richard Doll and Austin Hill published an article which confirmed the link between smoking and lung cancer [5, 6].

EPIDEMIOLOGY:

In 2012 Lung cancer death constitutes around 1,590,000 persons and currently it is the leading cause of cancer death worldwide. The mortality rates vary across the world, and follow the smoking trends [7].

By region, there is the highest lung cancer mortality rates (per 100,000) in 2012 among males, were in Central and Eastern Asia (47.6) and Eastern Asia (44.8) and among females, in Northern America (23.5) and Northern Europe (19.1); the lowest rate were found in sub-saharan africa among both males (4.4) and females (2.2)[7,8].

Depending on smoking prevalence, lung cancer mortality rate may be a mixture of decreasing, stable or increasing trends[6,7].

IN INDIA:

Lung cancer constitutes 6.9% of all new cancer cases and 9.3% of all cancer related death [9, 10]. the highest incidences was reported from Mizoram [8, 9, 10, 11]. Delhi, Chennai and Bengaluru show a increasing trends of lung cancer. [8, 9].

RECENT TREND IN INCIDENCE:

There has been a shift in the distribution of NSCLC over the past 4 decades. Prior to the 1970s, squamous cell carcinoma was the most common histological type. However, after 1975, adenocarcinoma has been significantly increased and it remains the predominant subtype. [12, 13, 14]

Previously adeno carcinoma was thought to be confined to smokers. Recent studies showed that adenocarcinoma is not only confined to smokers but as occurs in non smokers as well, suggested that non-smoking related factors also plays a role in pathogenesis of adenocarcinoma.[13,14].

AGE AND SEX:

The median age for a diagnosis of lung cancer is 72. More mommon in males with M:F- 2:1. Women tend to develop lung cancer 2 years earlier than men.

ETIOLOGY AND PATHOGENESIS:

- **Smoking:** 90% of all lung cancer results from tobacco exposure. The tobacco related products smoked in India are Bidi, Cigarettes, Hooka

and mixed. [15]. Of which Bidi is found to be more carcinogenic followed by Hooka.[16,17,18]

- **Passive smoking:** Exposure to Environmental tobacco smoke during childhood is found to be strongly associated with increase risk.
- **Occupational risk:**[20, 21] Exposure to Asbestos, Arsenic, Nickel, Uranium, Chromium and rarely Acrylonitrite, berrylium, and dimethyl sulphate associated with lung cancer.
- **Genetics of lung cancer:** [22, 23] The ras and myc family proto oncogene activation and tumor suppressor genes inactivation was found to be associate with lung cancer.
- **Dietary factors:** β-carotene, Flavonoids, isothiocyanates were found to have a protective role[24] Smoking and Vitamin A deficiency, animal food products, dairy products have a predisposing effect. [25-28].
- **Air pollution:**[29- 36] Coal smoke, incense smoke and kerosene consists many carcinogens like SO2, CO, TSP, B(a)P, radon, thoron also found to be associated with lung cancer development.

CLINICAL PRRESENTATION:

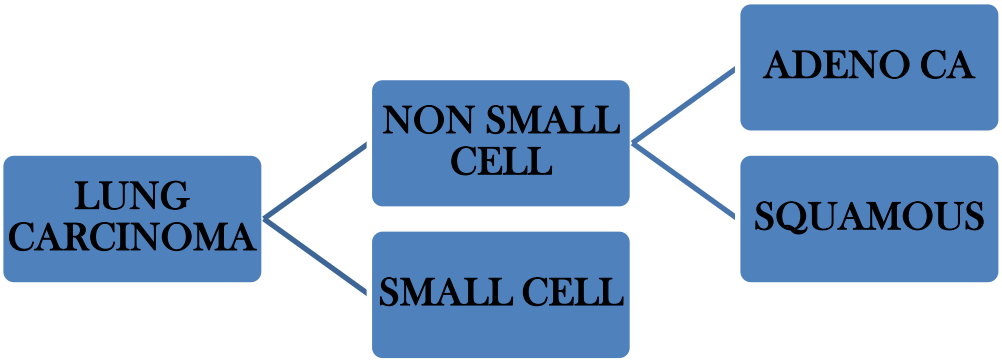
The most common symptoms are

- Cough
- weight loss
- Chest pain
- increased sputum production

- hemoptysis
- malaise
- fever
- Symptoms results from paraneoplastic manifestations.

CLASSIFICATION OF LUNG CANCER:

WHO classification of Lung tumours 2015 is given in Annexure-



Lung cancer is divided into small cell cancer and non- small cell cancer (NSCLC). NSCLC accounts for 80% of all lung cancers and is comprised of Adenocarcinoma(ADC), Squamous cell carcinoma (SqCC), and large cell carcinoma. [(2)] Traditionally, it was enough to differentiate small cell carcinoma from NSCLC, as NSCLC subtyping had not been shown to predict any differences in patient outcomes. Recent advances in molecular biology have led to an increase in target-specific chemotherapeutic therapies that require the subcategorization of NSCLCs.

The International Association for the study of Lung Cancer, the American Thoracic Society, and the European respiratory Society (IASLC/ATS/ERS) has outlined a new classification of lung ADCs based on a multidisciplinary approach. They have outlined the importance of further classifying NSCLCs as either ADCs or SqCCs, since ADCs should be tested for Epidermal growth Factor (EGFR) and Anaplastic Lymphoma Kinase (ALK) fusion gene mutations, as targeted chemotherapeutic agents can be used with greater efficacy[37].

Lung adenocarcinomas are often associated with EGFR mutations, and can be effectively treated with tyrosine kinase inhibitors such as geftinib.[38,39]. ADCs have been shown to have improved outcomes when compared to SqCCs, when treated with pemetrexed therapy, which inhibits specific enzymes in purine and pyramidine synthesis. Finally the distinction between ADCs and SqCCs can avoid potentially hazardous outcomes, as life threatening hemorrhages have been rarely reported when patients with SqCCs are treated with bevacizumab, a vascular endothelial growth factor inhibitor [40].

NEW PATHOLOGIC CLASSIFICATION OF LUNG CANCER:

From 2004 WHO Classification, there is numerous important changes have been made in the 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus and Heart[41]. The most significant changes are

- Use of Immunohistochemistry

- Integration of molecular testing
- New classification for small biopsies and cytology

New Terminology and Criteria for Classification of Major Lung Cancer Types in Small Biopsies and Cytology:

In the previous 1967, 1981, and 1999 WHO classifications, lung cancer s are classified mainly based on resection specimens.[41,42,] Cytology was included for the first time in the 2004 WHO classification. The percentage of NSCLC cases diagnosed as NSCLC-NOS has been as high as 30% to 50%[43,44,45]. So far there have been no established standardized criteria or terminology for the diagnosis of lung cancer in small biopsies or cytology. However, because of the need for molecular testing and eligibility for specific therapies, now the situation has changed.

In prior WHO classifications the diagnosis of lung cancer was mainly based on light microscopy.[46, 47] Mucin is the only special stain recommended in the 1967 and 1981 WHO classification.First time Immunohistochemistry was introduced in the 1999 WHO classification for 3 main tumors:

(1) large cell neuroendocrine carcinoma,

(2) sarcomatoid carcinomas,

(3) separation of malignant mesothelioma from carcinoma.

In the 2004 WHO classification in addition to these three tumors its usefulness was expanded in the diagnosis of many other tumors as well.

Adenocarcinoma:

Adenocarcinomas are characterized by glandular differentiation by manifesting 1 or more architectural features. These are

- lepidic
- acinar
- papillary,
- Micropapillary
- solid patterns.

The pattern has to mentioned in the report if it is present in the tumour.. Tumor cells may have homogenous basophilic cytoplasm, granular or foamy cytoplasm, often with cytoplasmic vacuoles The nuclei are eccentrically placed with fine granular chromatin to hyperchromatic nuclei. They may have macronucleoli. So finally,

NSCLC with gland formations or mucin productions were classified as adenocarcinomas[41,46,47].

Squamous cell carcinoma:

Squamous differentiation is characterized by 3 morphologic features:

- keratinization,
- pearls,
- intercellular bridges.

The cells have round to ovoid to elongated contours with sharply defined cell borders with dense cytoplasm. Cells with long cytoplasmic tails and ‘‘tadpole’’ configurations

may be seen. Nuclei are centrally situated and hyperchromatic with dense homogenous chromatin with pyknotic nuclei with inconspicuous nucleoli. So finally,

The NSCLC with keratinization and intercellular bridges were classified as Squamous cell carcinoma[46,47].

The NSCLC that lacked these specific histological features were classified under waste basket category of Large cell carcinoma. Distinguishing small cell carcinoma from Non small cell carcinoma was important for planning treatment protocol. Until mid 2000s NSCLC subtyping was of less importance for determine treatment protocol. TTF-1 was introduced in routine practice in the early 2000s. In the absence of routine histological criteria, IHC was used to subtype NSCLCs in resected specimens[44,45].

The 2015 WHO classification revised this past approach. In this classification it is mandatory to differentiate Squamous cell Carcinoma from Adenocarcinoma both in resected as well as in small biopsies. Thus IHC is recommended for both resected and small biopsies in 2015 WHO classification.[41]

Because of the recent advances in cancer therapy, WHO 2015 propose to use IHC to further classify the cancers previously diagnosed as large cell carcinoma. When possible, it is essential to minimize the diagnosis of NSCLCs.[48,49]

The large cell carcinoma category is reduced to those NSCLCs that cannot be classified as adenocarcinoma or squamous cell carcinoma by histology, mucin stain and IHC. It includes

- Those large cell carcinoma with null IHC features.
- Those large cell carcinoma with ambiguous IHC features.
- Those large cell carcinoma with no IHC available.[49]

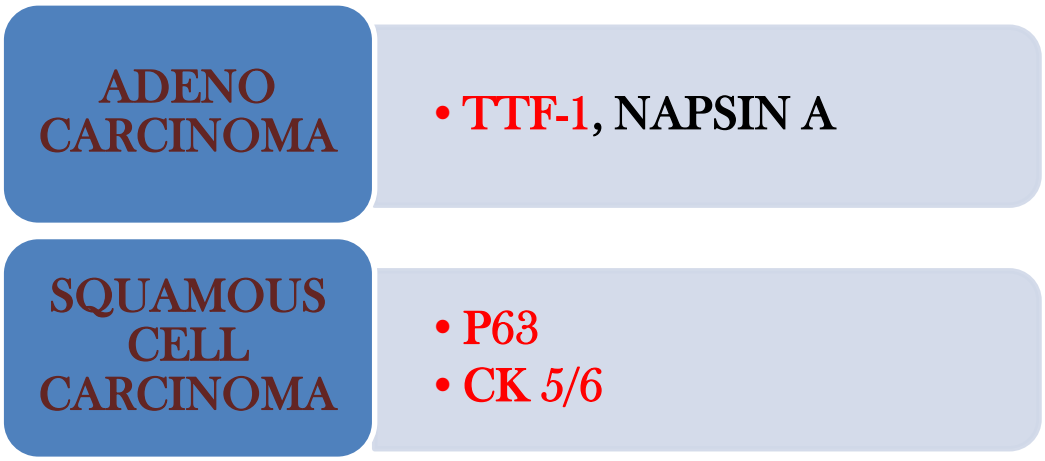
In 2015 WHO classification new criteria for the diagnosis of lung cancer based on small biopsy and cytology are introduced.[49]

	Pathologic Features	**New Terminology and Diagnosis**
1.	Morphologic adenocarcinoma patterns clearly present	Adenocarcinoma (Describe identifiable patterns present)
2.	Morphologic adenocarcinoma patterns not present, but supported by special stains (TTF 1 positive)	NSSLC favours adenocarcinoma
3.	Morphologic squamous cell patterns clearly present	Squamous cell carcinoma
4.	Morphologic squamous cell pattern not present, but supported by special stains (p 63 or CK5/6)	NSSLC favours Squamous Cell Carcinoma.
5.	No clear adenocarcinoma, squamous or neuroendocrine morphology or staining patterns	NSCC NOS

If the malignancy is well differentiated one, it is easy to subtype the carcinoma either as ADCs or SqCCs. Well differentiated ADCs exhibits glandular formations where as well differentiated SqCCs exhibits Keratin pearl formation and intercellular bridging. If the tumor is poorly differentiated, making a definite diagnosis is not easy, even for experienced pathologists. If the classification of NSCLCs cannot be achieved with cytological/histological criteria alone, Immunohistochemistry (IHC) should be employed[49].

With the use of relatively specific marker, Thyroid Transcription Factor-1 (TTF-1), lung primary can be separated from a metastasis in certain extent. Another lung specific marker is napsin a (Nap-A), that complements TTF-1 in identifying a primary lung carcinoma, also helpful in subtyping NSCLC, and helps to distinguish NSCLC, particularly poorly differentiated adenocarcinoma, from small cell carcinoma(SCC)[1,42].

Without the use of immunohistochemistry markers, it is difficult to subtype a lung cancer on small biopsies that may not show differentiation because of poor sampling, small amount of tumor tissue, crush artifact or cell dispersals[43,44]. In that situation we can use panel of IHC markers to subtype. The basic panel should include atleast one marker specific for adenocarcinoma and one specific marker for squamous cell carcinoma. The commonly used basic panel of markers for subtyping includes TTF 1, P63 and CK 5/6. In most of the cases these basic panel of markers are enough for subcategorization.



TTF-1:

TTF-1 also called as thyroid specific enhancer binding protein. It regulates transcription activity of thyroid, lung (surfactant proteins A, B and C, Clara cell secretory protein) and diencephalon specific genes[51,52]. It is positive in normal lung type II pneumocytes and clara cells, thyroid follicular and parafollicular C cells. It is a nuclear marker. TTF-1 is positive in lung carcinoma (small cell - 90 %, adenocarcinoma – 75%, large cell – 40 %, Squamous cell – 5 %. Also expressed in hyperplastic and neoplastic thyroid tissue, but less common in undifferentiated thyroid carcinomas[53,54]. Other tumours positive for TTF-1 includes primary thyroid cancers and small cell carcinoma of various organs. TTF1 regulates the cell proliferation and new vessels formation, thereby promotes the cancerisation[51,52,53,54].

NAPSIN A:

Napsin is an aspartic proteinase of the pepsin family involved in the maturation of surfactant protein B. It is found primarily in lung and kidney[51].

It is expressed in type 2 pneumocytes, alveolar macrophages, renal tubules and exocrine glands and ducts in the pancreas[42, 55,56]. It is cytoplasmic marker. It is useful as an individual marker or as a part of panel to distinguish lung adenocarcinoma from squamous cell carcinoma. Also useful in identifying metastatic disease with unknown primary as originating in lung[42,50]. It is superior to TTF 1 in distinguishing metastatic pulmonary from non pulmonary adenocarcinoma in cell blocks of pleual fluid and in distinguishing primary lung adenocarcinoma from other carcinomas particularly primary lung small cell carcinoma and primary thyroid carcinoma[50, 55,56,57,58].

P63:

P63 is a recently discovered marker of p53 family involved in development of epithelial tissues[49]. Normally P63 is expressed in bronchial reserve cells and metaplastic squamous epithelial cells. It is a nuclear marker[49]. P63 is consistently expressed in SqCC in the lung. But it is also expressed in a subset of adenocarcinoma and large cell carcinomas but with weak low level of positivity. A cut-off value of >10% tumour cell positivity is taken for categorization of squamous cell carcinoma[49].

Cytokeratin 5/6:

Cytokeratin 5/6 is a sensitive marker for squamous differentiation. Ck5/6 is normally expressed in basel cell of bronchial epithelium. CK5/6 positivity is cytoplasmic. It is mainly used to distinguishing mesothelioma from pulmonary adenocarcinoma[49].

P40:

P40 is a more specific marker than P63 for squamous cell carcinoma. Because it shows virtually no overlap in adenocarcinoma. Hence this p40 may replace p63 as the best immunohistochemical squamous marker.[49,59,60]

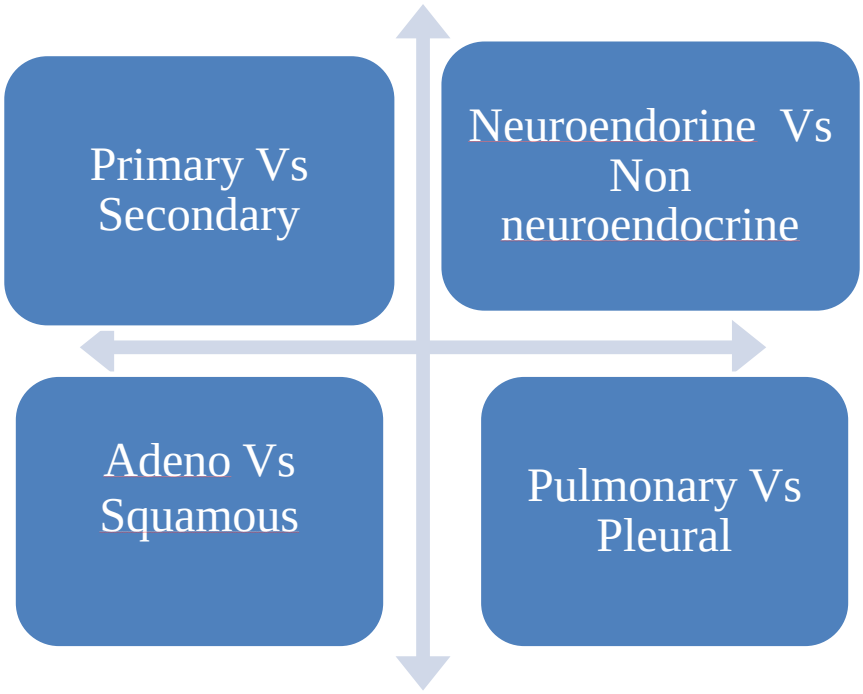
Although p63 frequently show nuclear positivity in most of squamous cell carcinomas, it may show patchy or weak staining in 20% to 30% of adenocarcinomas. This immunophenotype actually indiacates the good prognosis adenocarcinoma. But usually it has been misinterpreted as squamous differentiation.[61,62]

To preserve the tissue for molecular studies, only limited initial panel of one adeno and one squamous marker shold be used. Or else we can use cocktail of one nuclear and one cytoplasmic marker (TTF1/Cytokeratin 5/6). [49]

Application of Immunohistochemistry in lung pathology:

Whenever we deals with lung small biopsies we have to go in a systematic stepwise manner.

1. We have differentiate lung primary from secondary tumours
2. Within the lung primary whether it is neuroendocrine or non neuroendocrine
3. Distinguishing Adenocarcinoma from Squamous cell carcinoma
4. Distinguishing pulmonary malignancy from pleural malignancies.



NEUROENDORINE VS NON NEUROENDOCRINE NEOPLASM

IHC is helpful for confirmation of neuroendocrine differentiation. But its utility is limited for separating individual NeuroEndocrine Tumour from each other. Upto 20% of NSCLCs show positivity for Neuro Endocrine markers.

LARGE CELL NEUROENDOCRINE CARCINOMA:

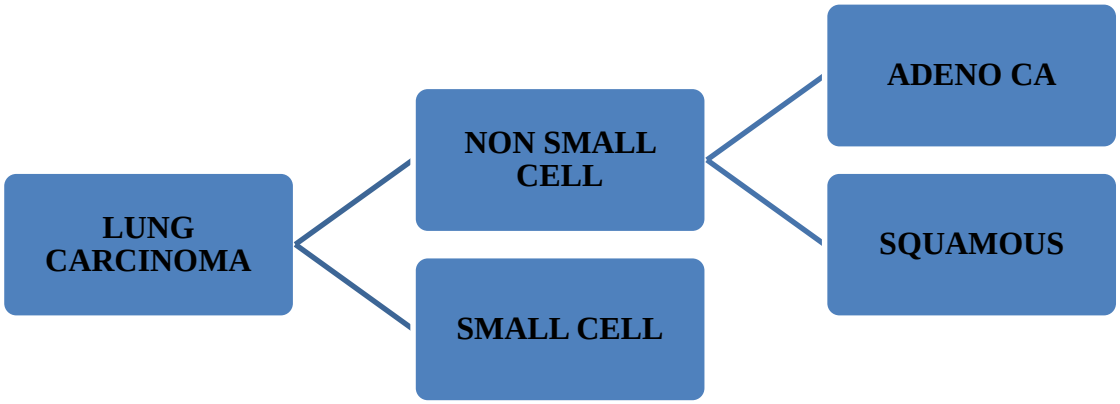
The diagnosis of large cell NEC shouldnot be made without the IHC profile.

- **Large cell carcinoma with NE architecture without NE staining is categorized as LARGE CELL CARCINOMA WITH NEUROENDOCRINE ARCHITECTURE**

- **Large cell carcinoma with NE staining without NE architecture is categorised as LARGE CELL CARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION**

ADENOCARCINOMA VS SQUAMOUS CELL CARCINOMA:

Based on the typical morphological features they should be differentiate from each other.



Squamous Cell carcinoma histological features:

Keratinization

Keratin pearls

Intercellular bridges

Adenocarcinoma histological features:

Glandular differentiation

JUDICIOUS USE OF IMMUNOHISTOCHEMICAL STAINS:

Usually as a basic panel TTF1 for adenocarcinoma and either P63 or CK5/6 for SqCCA is used in most of the laboratories. With the use of these markers we can get four different immunohistochemical profile[49].

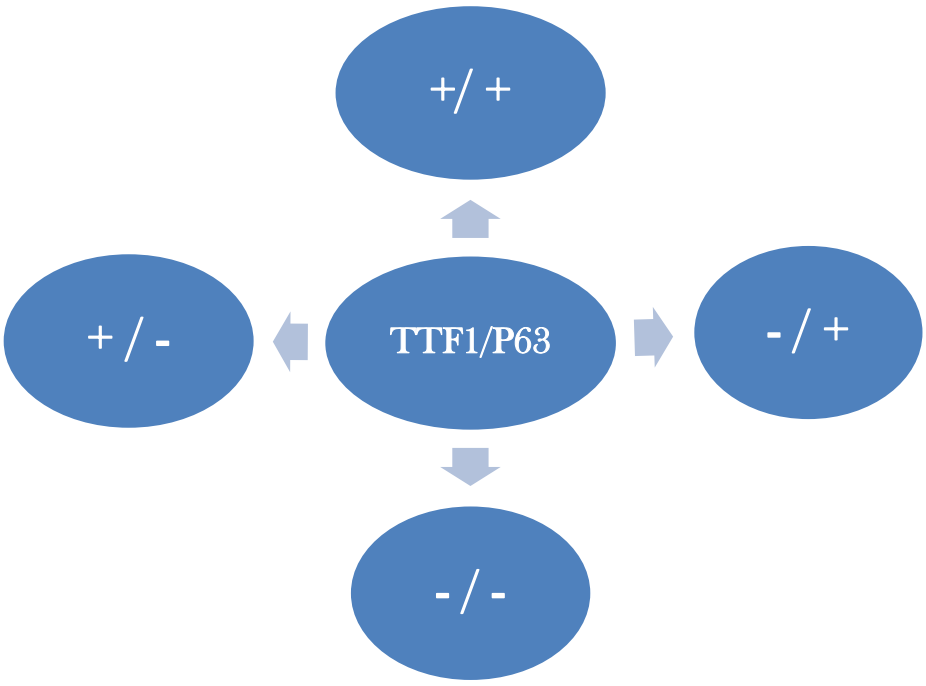
- TTF1 Positive & P63 Negative

  (Positive adeno marker with Negative Squamous marker)
- TTF1 Negative & P63 Positive

  (Negative adenomarker with positive squamous marker)
- TTF1 Positive & P63 Positive

  (Adeno and squamous marker - both positive)
- TTF1 Negative & P63 Negative

  (Adeno and squamous marker – both negative)



INTERPRETATION OF TTF1 & P63 PROFILE:I

- **Cases positive for an adeno marker with a negative squamous marker:**

  should be classified as NSCLC, favor adenocarcinoma [49]

- **Cases positive for a squamous marker, with a negative adeno marker**

  should be classified as NSCLC, favor squamous cell carcinoma, And with the above impression, a comment should be mentioned to specify whether the differentiation was detected by light microscopy and/or by special stains.

These 2 markers are generally mutually exclusive.[44,49]

- **Cases positive for both adeno and squamous markers**

  In these cases first we have to assess whether these two markers were expressed by same population of tumor cells or different population of tumor cells.

  If positive in same tumor population, despite any expression of squamous marker, if the tumor is positive for TTF1 it should be ckassified as NSCLC favours adenocarcinoma.[44,49,59,63,64]

  If TTF-1 reactivity is present in one population of tumor cells and another population is positive for squamous markers, this may raise the possibility of adenosquamous carcinoma[49,64,65,66,67,68]. To classify the tumour under adenosquamous category, the tumour should contain

atleast 10 % of each component. This quantification can be done only in resected specimens. So the diagnosis of adenosquamous carcinoma should not be made in the small biopsy specimens[49.69].

- **Cases negative for both adeno and squamous markers:**

For these case, it should proceeded with cytokeratin stain to confirm the histiogenis of the tumor whether it is a carcinoma or not. If a keratin stain is negative, it excludes carcinoma. So other tumors exhibiting epithelioid morphology, such as melanoma,lymphoma, malignant mesothelioma, or epithelioid hemangioendothelioma has to be considered. To exclude this S100, CD45, or CD31 may be used.[49,70]

TTF 1 negativity rules out the primary lung adenocarcinomas. In these cases metastasis from colon and breast has to be considered. To exclude these primary origin, CDX-2, cytokeratin 20, estrogen receptor, or progesterone receptor expression may be needed. Thoroug clinical evaluation to exclude a metastasis from other sites should be done[49,71].

TTF-1 positivity and CDX2 negativity may be seen in Invasive mucinous adenocarcinomas or colloid adenocarcinomas. So clinical correlation is needed in such tumors to exclude a metastasis from other sites such as the pancreas or colon.[49,71]

Algorithm for Subclassification of Poorly Differentiated Non-small Cell Lung Carcinomas Using Immunohistochemical Staining in Lung Biopsies [69]

TTF1	P63	CK5/6	DIAGNOSIS
+	+	-	Adenocarcinoma
-	+ / -	+	Squamous cell carcinoma
-	Diffusely positive	-	Squamous cell carcinoma
-	Focally positive	-	Poorly differentiated NSCLC NOS
-	-	-	Poorly differentiated NSCLC NOS

METASTASES TO THE LUNGS

Metastatic malignant neoplasms are the most common form of secondary lung tumours.[72,73,77] Lung metastasesae identified in 30- 55 % of all cancer patients Almost any cancer has propensity to spread to the lungs, but most common tumours are

Bladder cancer

Colon cancer

Breast cancer

Prostate Cancer

Renal cancer

Primary lung cancers mostly metastasize to the adrenal glands, liver, brain and bone.

Benign Cancers Metastasizing to Lung: [74,75,76,77]

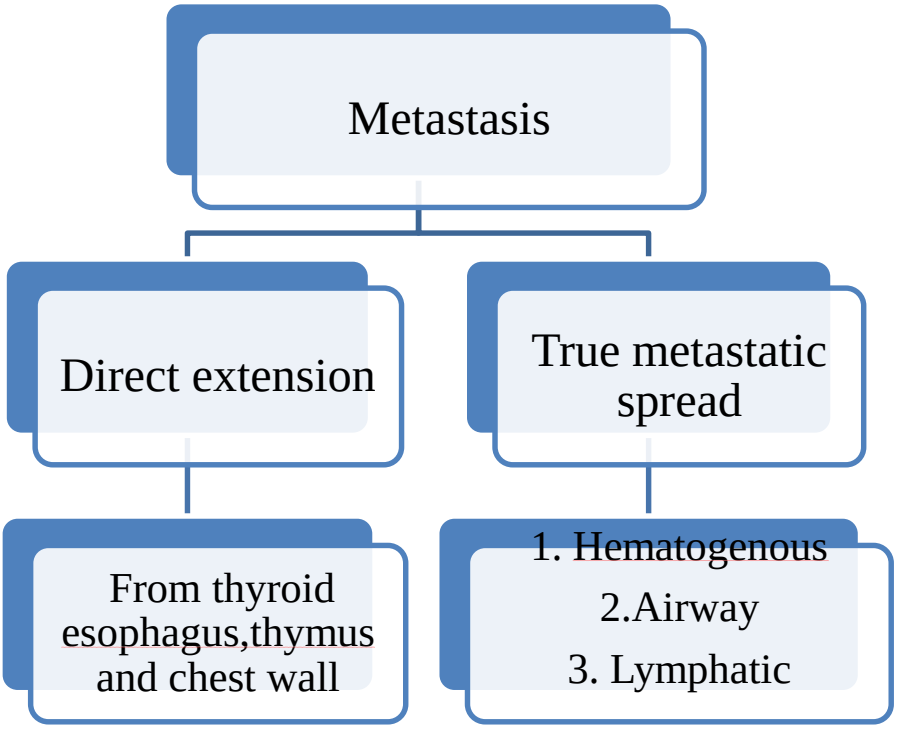
Leiomyoma

Meningioma

Thymoma

Giant Cell tumour of bone

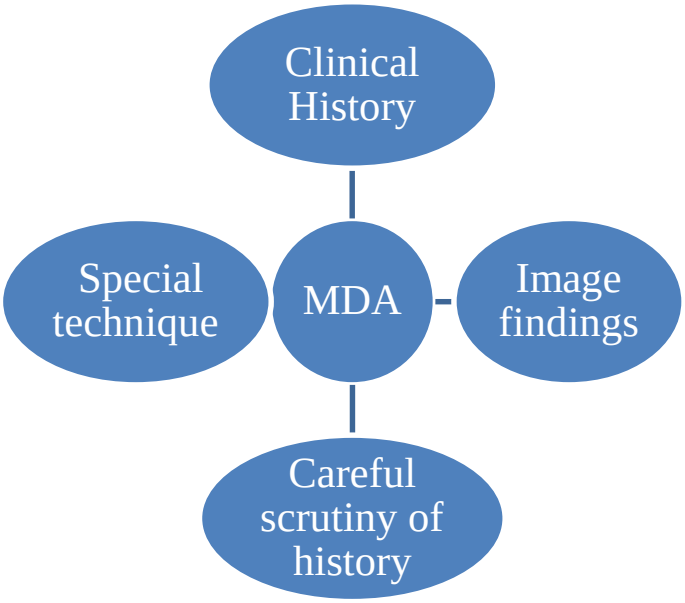
MECHANISM OF CANCERS SPREAD TO LUNG: [72,73,74]



Metastatics can be either due to direct or contiguous extension or true metastatic spread via blood vessels, lymphatic route or along the airway.

DISTINGUISHING A PRIMARY LESION FROM A METASTASIS TO THE LUNG:

It may be difficult on the basis of morphology alone, and need a multidisciplinary approach [72, 73, and 77].



Clinical Presentation & Image findings:

- Multiple bilateral peripheral nodules.(Classical)
- As a solitary coin lesion (Rare - 9% of cases) [73, 77].
- Endobronchial metastasis simulating primary bronchogenic carcinoma (unusual)
- Milliary spreads of microscopic tumor nodules simulate an infectious process.
- Growth along the alveolar walls,simulates a primary bronchioloalveolar carcinoma –lung.

To rule out the other primaries also certain organ specific IHC markers can be used. CK7/ CK20 panel is primarily used for distinguishing metastasis from primary in every organ. Lung cancers mainly have the profile of CK7 positive CK20 Negative. But in the lung by using this CK7 / CK 20 panel, we cannot rule out malignancies metastasizing from certain primaries. [78]

By using CK7/CK20 panel we can exclude Metastasis from prostate, kidney and ovary.

In addition to lung primary, colon, breast and ovary have similar CK7 Positive / CK 20 Negative profile.

PRIMARY	CK7	CK20
Lung	Positive	Negative
Ovary	Positive	Negative
Colon	Positive	Negative
Breast	Positive	Negative

But organ specific antibodies can be used to exclude theses primaries.

- For breast cancers ER and GCDFP can be used.
- For Ovary ER, inhibin can be used.
- For adrenal tumors Inhibin is a specific marker.
- For prostate PSA is used.

And in all of these primaries TTF1 is negative except in primary from thyroid malignancies. But napsin will be negative in primary from thyroid [77, 78].

SIMPLIFIED PANEL FOR DISTINGUISHING PRIMARY FROM SECONDARY:

Organ	TTF1	CK7	CK20	ER	PSA	GCDFP	INHB	CD10	RCC
Lung	+	+	-	-	-	-	-	-	-
Colon	-	+	-	-	-	-	-	-	-
Breast	-	+	-	+	-	+	-	-	-
Prostate	-	-	-	-	+	-	-	-	-
Kidney	-	-	-	-	-	-	-	+	++
Adrenal	-	-	-	-	-	-	+	-	-
Ovary	-	+	-	+	-	-	+	-	-

PLEURAL TUMORS VERSUS PRIMARY LUNG TUMOURS:

Lung cancers especially those located peripherally can simulate mesothelioma radilogically as well as in some occasions histopathologically. So IHC can be used to distinguishing these mesothelioma from primary adenocarcinoma lung [79, 80].

IHC markers to differentiate Pleural versus primary lung AdCC[81,82]:

MESOTHELIOMA	ADENOCARCINOMA LUNG
CK 5/6 Calretinin Mesothelin WT1 Podoplanin	TTF 1 CEA CD15 MOC 31

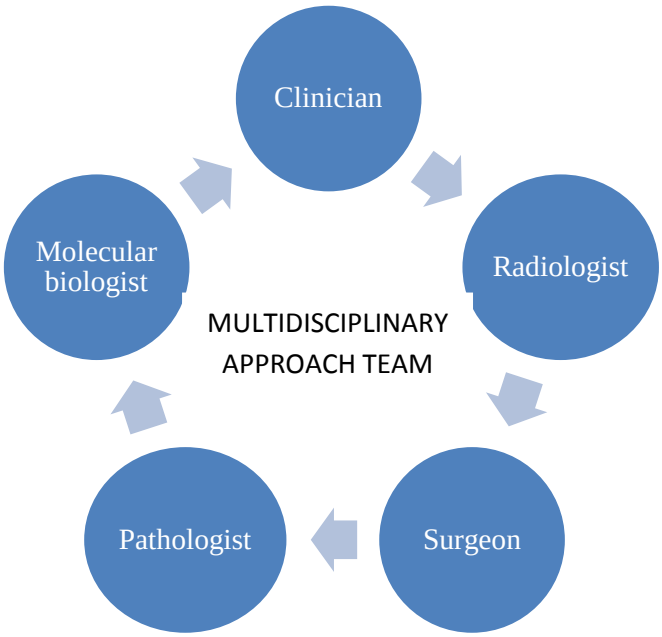
MULTIDISCIPLINARY APPROACH FOR LUNG CANCER DIAGNOSIS:

Multidisciplinary approach is needed for diagnosing lung cancers. The new concepts in IASLC / ATS / ERS classification are the direct result of the multidisciplinary approach,which includes clinicians, molecular biologists, radiologists, surgeons and pathologist. To achieve a corresct diagnosis and management all specialists need to work together.

Each institution must have a multidisciplinary strategy that addresses

1. how to best obtain these small specimens,
2. how to process them in the pathology laboratory,
3. how to preserve material for molecular testing,
4. how to send specimens to the molecular laboratory for expedited testing
5. how to report the results in a pathology report.

It is useful to have a multidisciplinary committee to develop this strategy and to keep lines of communication open in order to monitor issues as they arise in an ongoing fashion. Pathologists should take a leadership role in this process.



PERSONALIZED MEDICINE IN LUNG CANCER :

The pathologist's role in diagnosing lung cancers is becoming more important, because lung cancer therapy is becoming personalized for individual patients nowadays [(49)].

Therapy is based on

1. the histologic cell type and subtypes of lung cancer (adenocarcinoma versus squamous
2. molecular status
    - epidermal growth factor receptor [EGFR] mutation

- anaplastic lymphoma kinase [ALK] rearrangement in adenocarcinoma

Understanding this concept is essential for the pathologists.

Since the 2004 WHO classification, there have been 4 therapeutic advances for non–small cell lung carcinoma (NSCLC)[49]. The first relates to

1. Tyrosine kinase inhibitors as first-line therapy in patients with advanced lung adenocarcinoma with EGFR mutations.[83–87]
2. Adenocarcinomas with ALK rearrangements are responsive to crizotinib.[84–90]
3. Adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS), are more responsive to pemetrexed than those squamous cell carcinoma.[91-93]
4. Squamous cell carcinoma is associated with life-threatening hemorrhage in patients treated with bevacizumab.[93,94]

MATERIALS & METHODS

MATERIALS AND METHODS

The present study is a both prospective and retrospective study of lung carcinomas conducted in the Institute of Pathology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai, during the period between September 2016 to July 2017.

A total of 22285 specimens sent to the Department of pathology during the period of September 2015 to August 2017 for histopathological examination.

Out of that 309 cases were lung specimens. Among them 50 malignant cases selected for this study.

Source of data:

The Lung carcinoma cases reported in the institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital from September 2015 to August 2017 which have been sent by the Department of surgical oncology and Department of Thoracic Medicine.

Inclusion criteria:

- Histopathological slides of biopsy proven malignant cases in which histological typing cannot be done by routine H&E sections alone.

Exclusion criteria:

- Histopathological slides of biopsy proven malignant cases in which histological typing can be done in routine H&E sections itself.
- Histopathological slides of biopsy proven non neoplastic lung lesions
- Malignancies other than epithelial tumours
- Cases with inadequate material

Method of data collection:

Detailed history of the cases regarding age, gender, clinical presentation, smoking history, image findings were obtained for all the cases of lung cancers reported during the period of study from the histopathology records.

Hematoxylin and Eosin stained 4 micron thick sections of the paraffin tissue blocks of the specimens were reviewed. Along with immunohistochemistry slides which were done for subtyping of lung carcinomas were reviewed. The markers used in our department for subtyping were TTF1 for adenocarcinoma, P63 or CK5/6 for squamous cell carcinoma and Any of the neuroendocrine markers(Synaptophysin, Chromogranin or, NSE) were used. That slides were taken from the department and reviewed.

50 cases were selected randomly from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry with a marker Napsin A.

The results were recorded with photographs.

Histological Review:

The histopathological diagnosis and its subtyping based on morphology alone was made by the senior pathologist in almost all cases.

Histopathological diagnosis was done according to the recent WHO terminology for lung carcinomas in small biopsy specimens.

Immunohistochemical evaluation:

Immunohistochemical analysis of marker for Napsin a was done in paraffin tissue blocks using super sensitive HRP polymer system based on non bioton polymeric technology.Sections with a thickness of 4 microns were cut from the paraffin tissue blocks. They were transferred to gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with rabbit monoclonal antibody (PATHNSITU) against Napsin and then the addition of secondary antibody conjugated with horse raddish peroxidase – polymer and diaminobenzidine substrate.

ANTIBODY FOR IHC:

Antigen	Vendor	Species(clone)	Dilution	Positivity	Positive control
Napsin	Pathnnsitu	Rabbit monoclonal EP205	Ready to use	Cytoplasmic positivity	Lung – type 2 pneumocytes

The step by step procedure of IHC is listed below in detail.

PREPARATION OF SLIDES:

1. 4μ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to charged slides
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 10 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 10 minutes x 2 changes.
5. The slides were then immersed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 2 minutes x 2 changes.

ANTIGEN RETRIEVAL:

7. Antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were cooled to room temperature and washed in running tap water for 5 minutes.
9. Apply peroxidase block over the sections for 10 minutes.
10. Wash the slides in wash buffer for 5 minutes x 2 changes.

ANTIBODY APPLICATION:

11. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 1 hour.
12. The slides were washed in wash buffer for 5 minutes x 2 changes.
13. The slides were covered with CRF Anti – Polyvalent HRP Polymer for 30 minutes.
14. The slides were washed in wwash buffer for 5 minutes x 2 changes.

CHROMOGEN APPLICATION:

15. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.
16. DAB substrate solution was applied on the sections for 5 minutes.
17. The slides were washed well in distilled water for 5 minutes.
18. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
19. The slides were washed in running tap water for 3 minutes.
20. The slides are air dried, cleared with xylene and mounted with DPX.

CONTROLS:

Normal type 2 pneumocytes were taken as internal control for assessing Napsin A reactivity and to avoid false negative results.

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analysed for the presence of reaction, cellular localization (nuclear / cytoplasmic/ membranous), percentage of stained slides and intensity of reaction.

Proportion Score: This study was done in small biopsy specimens. IHC staining was evaluated without exact quantification.

- Negative: No reactivity
- Focal : Labelling in the minority of cells
- Diffuse : Labelling in the majority of cells

Intensity Score:

- 0 – Negative
- 1 – weak
- 2 – intermediate
- 3 – strong

STATISTICAL ANALYSIS

The statistical analysis is peformed using IBM statistical package for social science software (SPSS) version 20. The correlation of clinicopathological parameters and comparison of napsin A expression with TTF1 expression was calculated by Pearson Chi Square test and P value less than 0.05 are considered statistically significant.

OBSERVATIONS & RESULTS

OBSERVATION AND RESULTS

The total number of lung specimens received in our institute was 309 over a period of september 2015 to august 2017 Of which non neoplastic cases were 201. Malignant cases were 108. Out of 108 malignant cases, 36 cases were diagnosed and subtyped with only light microscopy without the use of immunohistochemistry. For rest of the cases, because of the lack of typical specific features of subtypes, we proceeded with IHC markers of TTF1, CK5/6, P63, NSE, Synaptophysin, Chromagranin. With these markers subtyping was done. From that cases 50 cases were randomly chosen for this study.

TABLE 1: FREQUENCY OF LUNG CARCINOMA CATEGORIES

Diagnosis	Frequency	Percent
ADCC	23	46.0
SCC	7	14.0
SQCC	20	40.0
Total	50	100.0

TABLE - 1

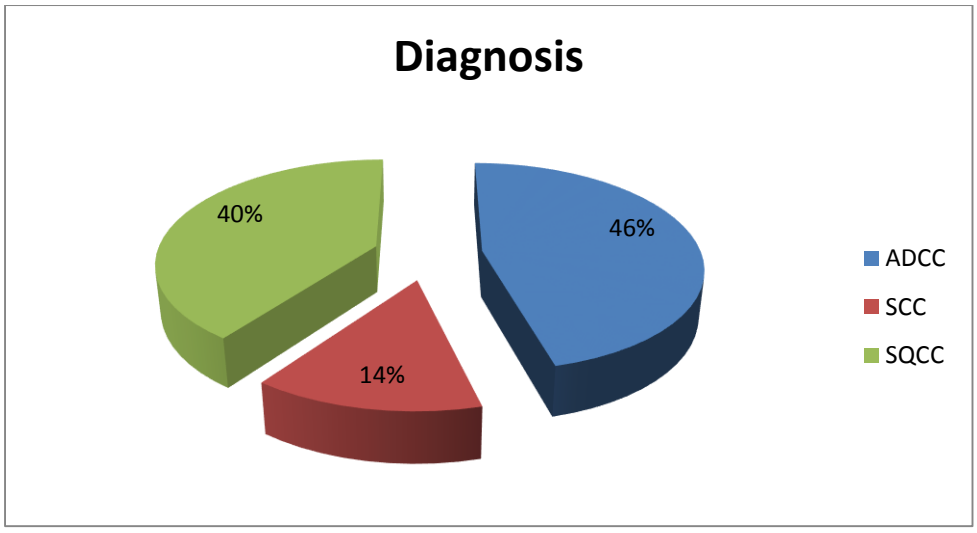


CHART - 1

It was found that adenocarcinoma had a maximum incidence of 46%. The second most common was squamous cell carcinoma accounting for 40%. Small cell carcinoma was the third most frequent subtype with relative percentage of 14% of the total cases.

TABLE 2: AGE WISE DISTRIBUTION OF TUMORS

Age range	Number of cases	Percentage (%)
41-50 years	7	14%
51-60 years	19	38%
61-70 years	17	34%
71-80 years	7	14%

TABLE - 2

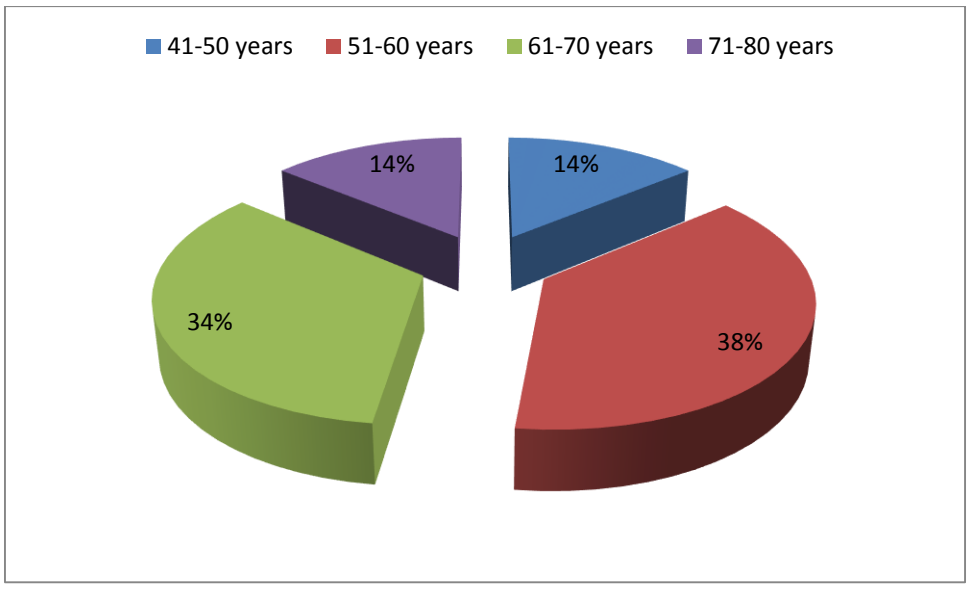


CHART - 2

It is inferred from the above table and bar diagram that there is no increase in the incidence of lung cancers with increasing age. The tumour seems to be distributed along the age group in no specific pattern.

The peak incidence was noted in a age group of 51 to 60 years, the number of patients were 19 accounting for 38% of cases. It seems to have a least incidencein the age group of 41 to 50 years and 71 to 80 years with relative percentage of 14%.

TABLE 3: THE AGE WISE DISTRIBUTION OF DIFFERENT TYPES OF LUNG CANCERS

			Diagnosis			Total
			ADCC	SCC	SQCC	
Age_range	40-50	Count	4	0	3	7
		% within Diagnosis	17.4%	0.0%	15.0%	14.0%
	51-60	Count	10	2	7	19
		% within Diagnosis	43.5%	28.6%	35.0%	38.0%
	61-70	Count	7	3	7	17
		% within Diagnosis	30.4%	42.9%	35.0%	34.0%
	71-80	Count	2	2	3	7
		% within Diagnosis	8.7%	28.6%	15.0%	14.0%
Total		Count	23	7	20	50
		% within Diagnosis	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=3.366 P=0.762

TABLE - 3

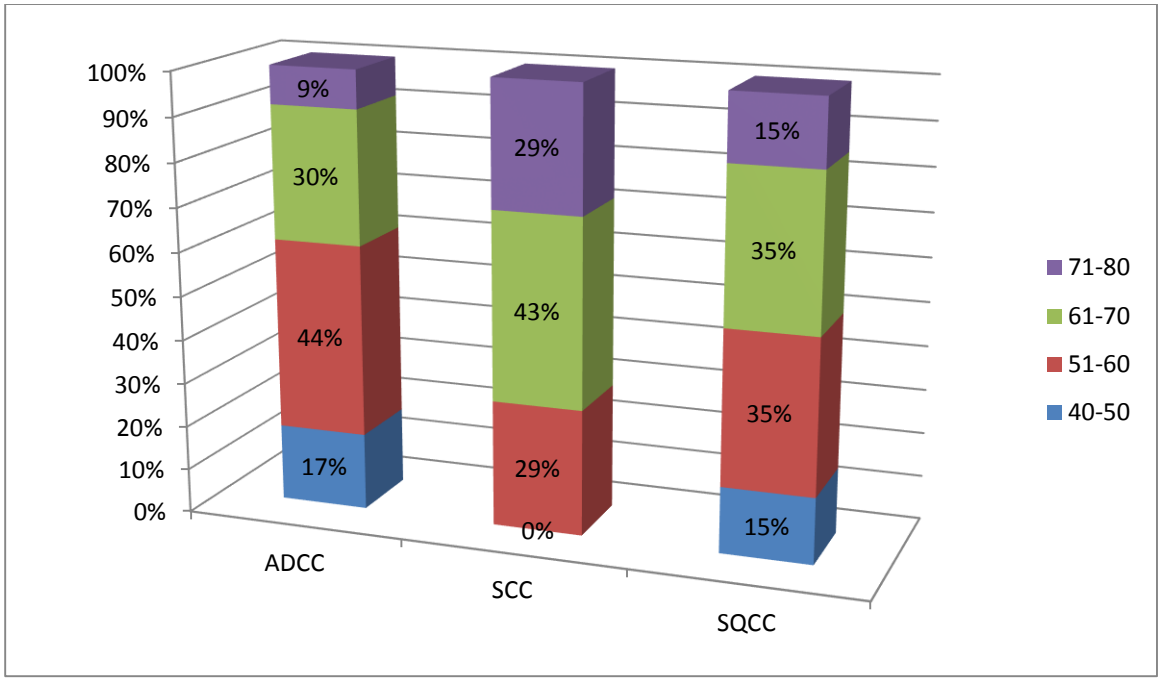


CHART - 3

Table 3 shows the percentage of individual subtypes in different age groups. The purpose of this table is to look for any specific age predeliction by different subtypes of tumors.

It was inferred from the table 3 that adenocarcinoma was most common in the age group of 51 to 60 years accounting for 43.5% (10cases). It was least common in the age group of 71 to 80 years accounting for 8.7% (2 cases).

Squamous cell carcinoma was most common in the age group of 51 to 60 years and 61 to 70 years with relative range of 35% in each group.(7 cases). It was least common in the age group of 41 to 50 years and 71 to 80 years with relative range of 15% in each group. (3 cases)

Small cell carcinoma was most common in the age group of 61 to 70 years accounting for 42.9% (3 cases), followed by second most commonly seen

in 51 to 60 years and 71 to 80 years with relative range of 28.6% in each age group. (2 cases)

Hence adenocarcinoma and squamous cell carcinoma was found to be commonly affect the age group of 51 to 60 years. Small cell carcinoma was found to be common in slightly older age group of 61 to 70 years.

TABLE 4: GENDER WISE DISTRIBUTION OF TUMOURS:

Gender	Number of cases	Percentage
Male	35	70%
Female	15	30%

Pearson Chi-Square=3.913 P=0.141

TABLE - 4

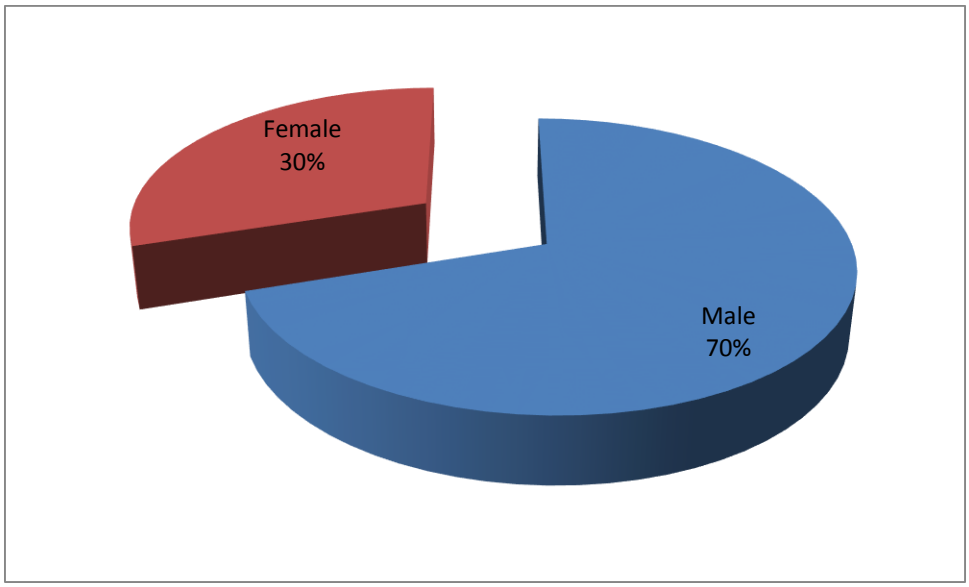


CHART - 4

The incidence of lung cancers in males was found to be 70% (35 cases). The incidence of lung cancers in female was found to be 30%(15 cases). Male to female ratio was 2.3:1. P value is 0.141. So gender has significant value in lung cancers.

TABLE 5: DISTRIBUTION OF DIFFERENT SUBTYPES OF CANCERS AMONG MALES AND FEMALES.

			Diagnosis			Total
			ADCC	SCC	SQCC	
SEX	MALE	Count	14	7	14	35
		% within Diagnosis	60.9%	100.0%	70.0%	70.0%
	FEMALE	Count	9	0	6	15
		% within Diagnosis	39.1%	0.0%	30.0%	30.0%
Total		Count	23	7	20	50
		% within Diagnosis	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=3.913 P=0.141

TABLE - 5

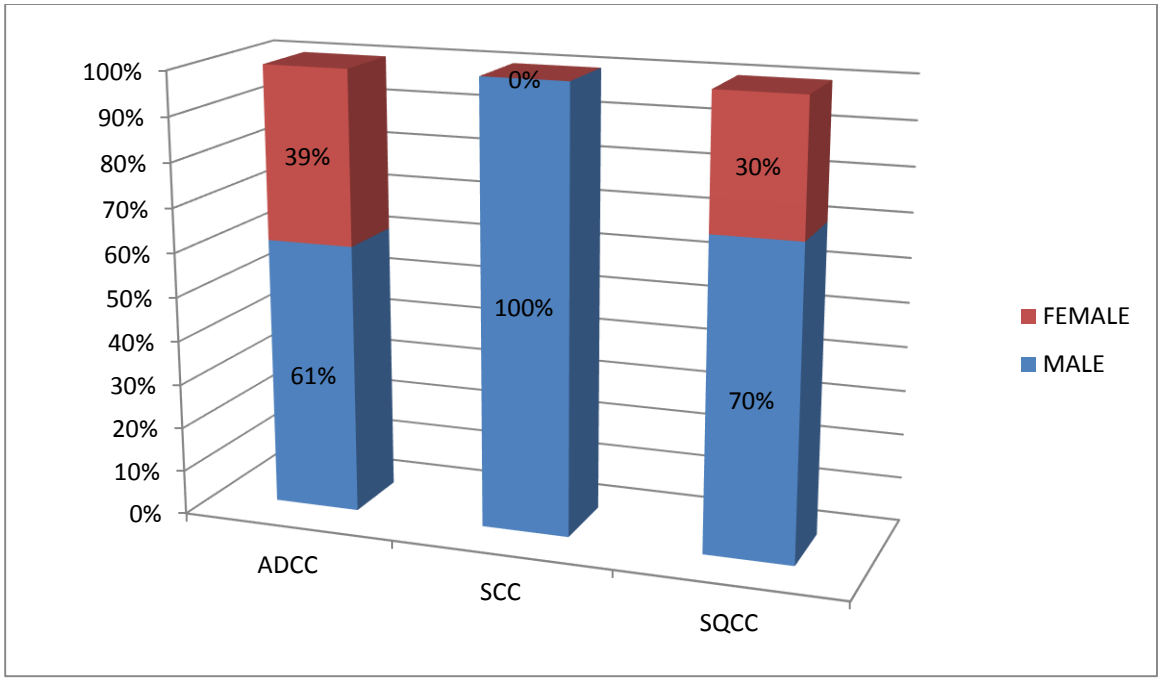


CHART - 5

It has been inferred from the table 6 that adenocarcinoma was common among males with relative percentage of 60.9% (14 cases) as compared to 39.1%(9 cases) in females.

Squamous cell carcinoma was common among males with relative percentage of 70%(14 cases) as compared to 30%(6 cases) in females.

Small cell carcinoma was found to be exclusively occurred only in males with relative percentage of 100.0%(7 / 7 cases).

TABLE 6: SIDE DISTRIBUTION IN LUNG CANCERS

Side	Number of cases	Percentage
Left	22	44%
Right	28	56%

TABLE - 6

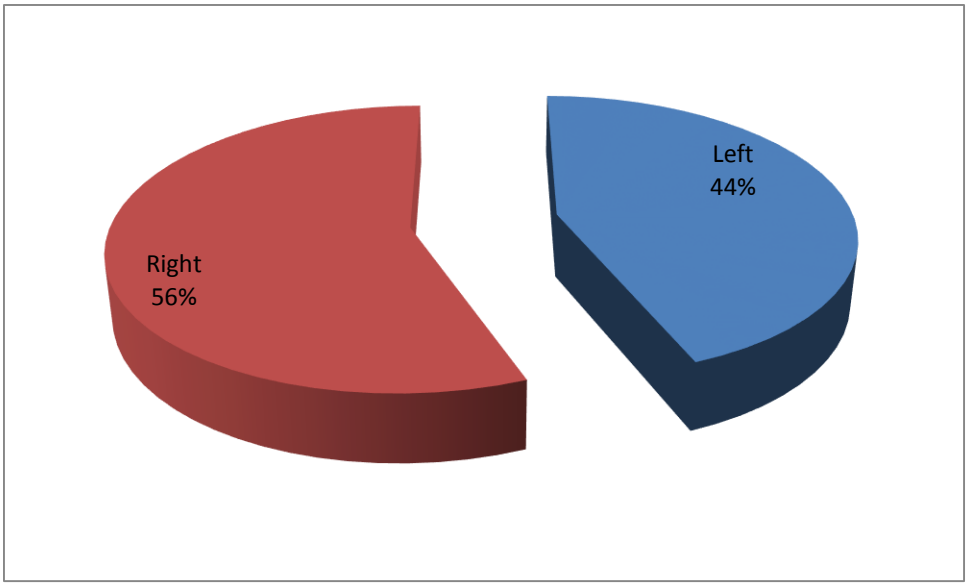


CHART - 6

From the above table it was inferrred that lung cancers found to be occur more commonly in Right lobe with relative percentage of 56% followed by left lobe with little less frequency of 44%.

TABLE 7: INVOLVEMENT OF DIFFERENT LOBES IN LUNG CANCERS

Lobe	Number of cases	Percentage
Upper	33	66%
Middle	5	10%
Lower	12	24%

TABLE - 7

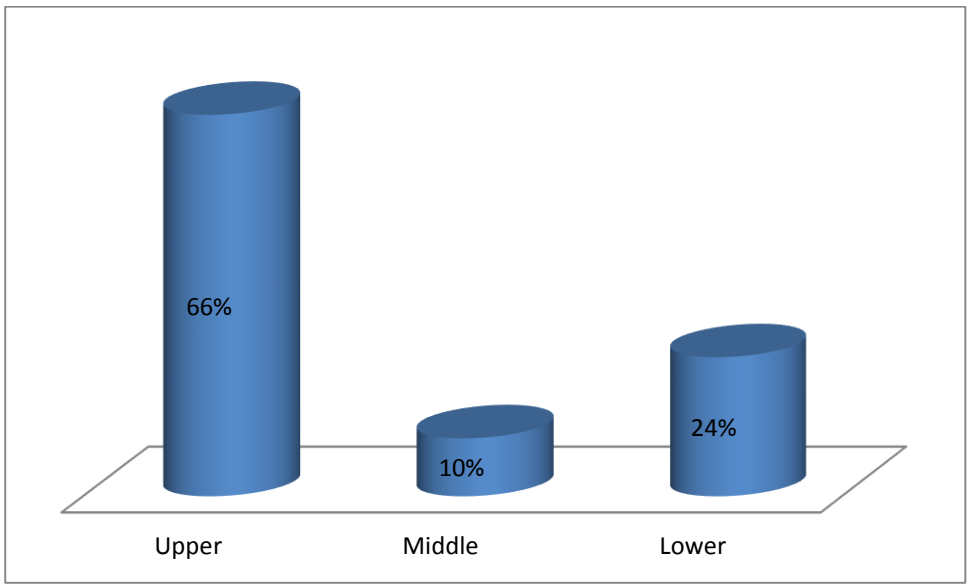


CHART - 7

The above table shows that lung cancers seems to be predominant in upper lobe with relatively higher percentage of 66% (33 / 50 cases), followed by lower lobes found to be involved in 24% of cases with least common in moddle lobe with least percentage of 10%.

TABLE 8: CLINICAL FEATURES IN LUNG CANCER

Symptoms	Number of cases	Percentage
Chest pain	10	20%
Cough	13	26%
Dyspnoea	9	18%
Hemoptysis	9	18%
No symptom	9	18%

TABLE - 8

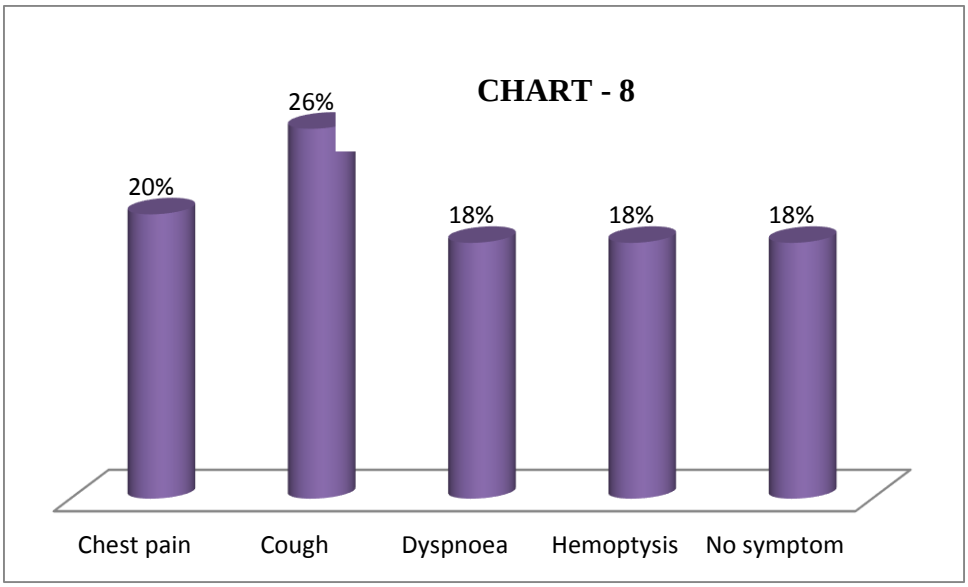


Table 8 showed the commonest symptoms in lung cancer patients. Among these five symptoms, most of the patients(26%) presented with the symptom of cough followed by chest pain in 20% of patients, followed by 18% of patients with dyspnoea, 18% with hemoptysis and 18% of patients presented with no specific symptoms.

TABLE 9: COMMON SYMPTOMS IN EACH LUNG CANCER SUBTYPE

Symptoms						
			Diagnosis			Total
			ADCC	SCC	SQCC	
Symptoms	chest pain	Count	5	1	4	10
		% within Diagnosis	21.7%	14.3%	20.0%	20.0%
	Cough	Count	6	4	3	13
		% within Diagnosis	26.1%	57.1%	15.0%	26.0%
	Dyspnoea	Count	4	0	5	9
		% within Diagnosis	17.4%	0.0%	25.0%	18.0%
	hemoptysis	Count	4	1	4	9
		% within Diagnosis	17.4%	14.3%	20.0%	18.0%
	No symp	Count	4	1	4	9
		% within Diagnosis	17.4%	14.3%	20.0%	18.0%
Total		Count	23	7	20	50
		% within Diagnosis	100.0%	100.0 %	100.0%	100.0%

Pearson Chi-Square=5.706 p=0.680

TABLE - 9

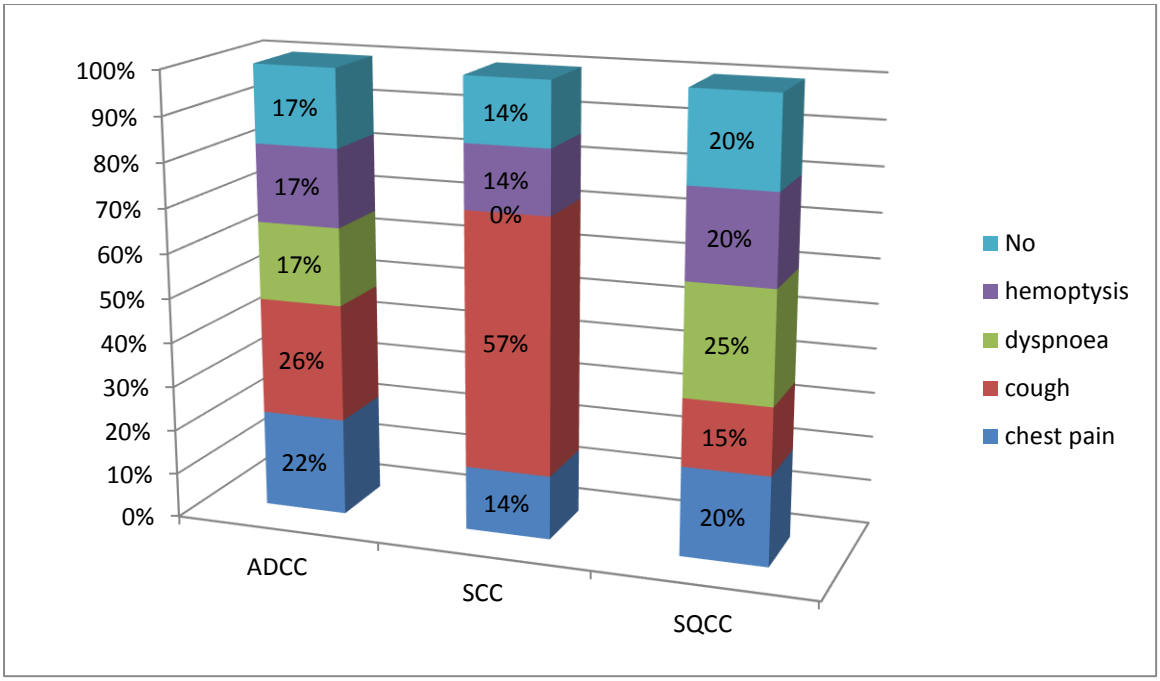


CHART - 9

Table 9 showed commonest presentation in each lung cancer subtype. In lung adenocarcinoma cough followed by chest pain was found to be commonest presentation with relative percentage of 26.1% and 21.7% respectively.

Among the squamous cell carcinoma patients, Dyspnoea is the commonest presenting complaints with 25% relative percentage.

Among the small cell carcinoma patients cough is the predominant symptoms with relative percentage of 57.1%.

TABLE 10: SMOKING HISTORY IN LUNG CANCER PATIENTS.

Smoking history	Number of cases	Percentage
Present	31	62%
Absent	19	38%

TABLE- 10

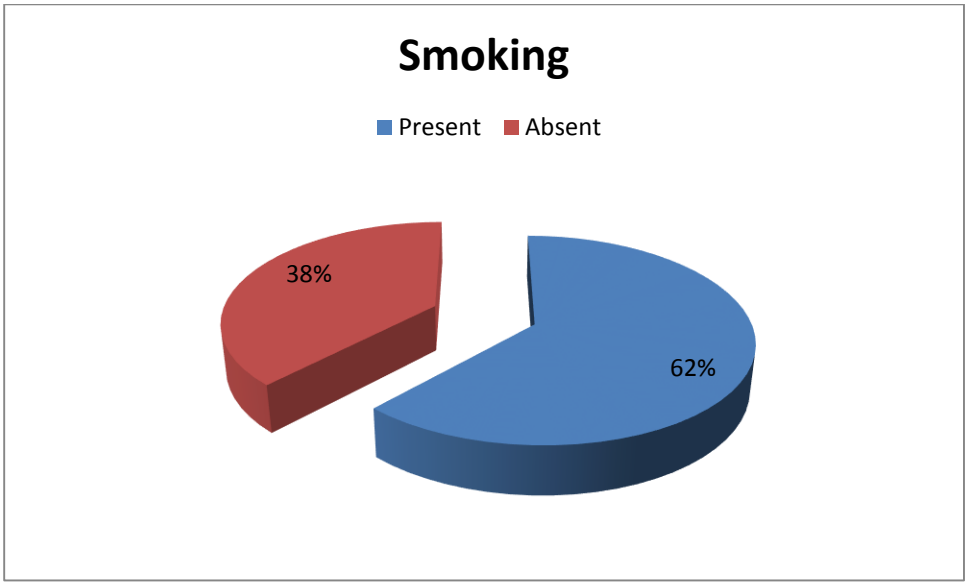


CHART - 10

It was inferred from table 10 that lung cancers were common in smokers with 62% accounting for 31 cases out of 50 cases. 38% of non smokers developed lung cancers accounting for 19 out of 50 cases.

TABLE 11: SMOKING HISTORY IN LUNG CANCER SUBTYPES

			Diagnosis			Total
			ADCC	SCC	SQCC	
Smoking	NO	Count	12	0	7	19
		% within Diagnosis	52.2%	0.0%	35.0%	38.0%
	YES	Count	11	7	13	31
		% within Diagnosis	47.8%	100.0%	65.0%	62.0%
Total		Count	23	7	20	50
		% within Diagnosis	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=6.328* p=0.042

TABLE -11

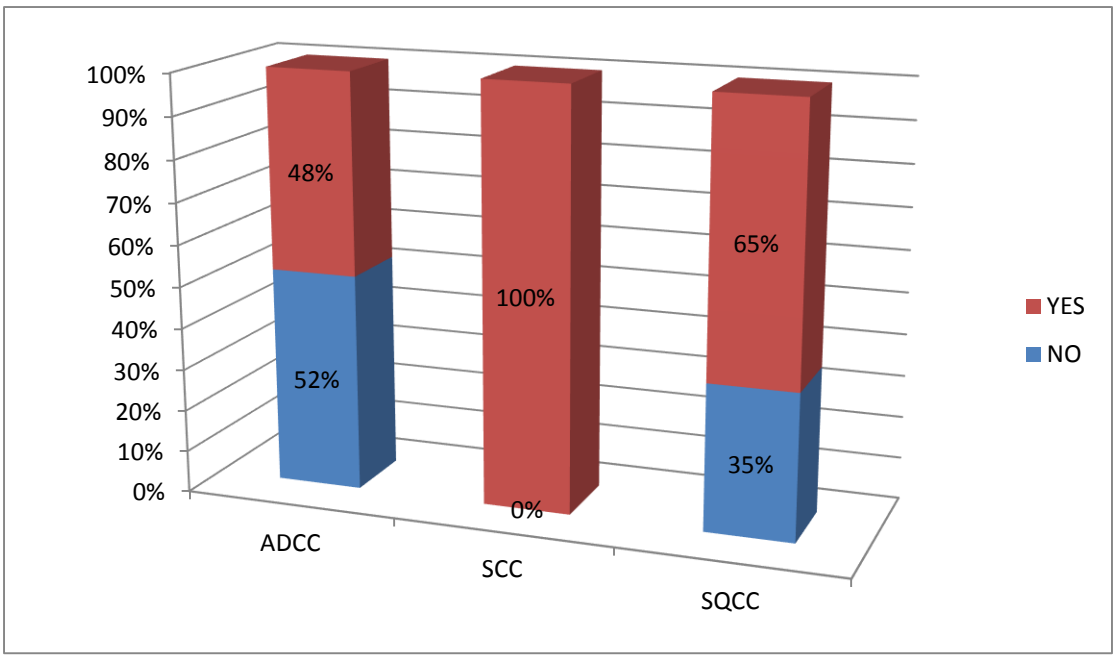


CHART – 11

Table 11 showed that adenocarcinoma seems to be commonly occurs in non smokers with relative percentage of 52.2% (12 cases) in contrast to 47.8% in smokers (11 cases).

Squamous cell carcinoma and small cell carcinoma seems to be common in smokers with relative percentage of 65% (13 cases) and 100.0% (7 cases) respectively.Small cell carcinoma was found to be occur exclusively only in smokers.

TABLE 12: IMAGE FINDINGS IN LUNG CANCER PATIENTS

Imaging findings	Number of cases	Percentage
Consolidation	8	16%
Collapse	12	24%
Mass	27	54%
Pleural effusion	3	6%

TABLE - 12

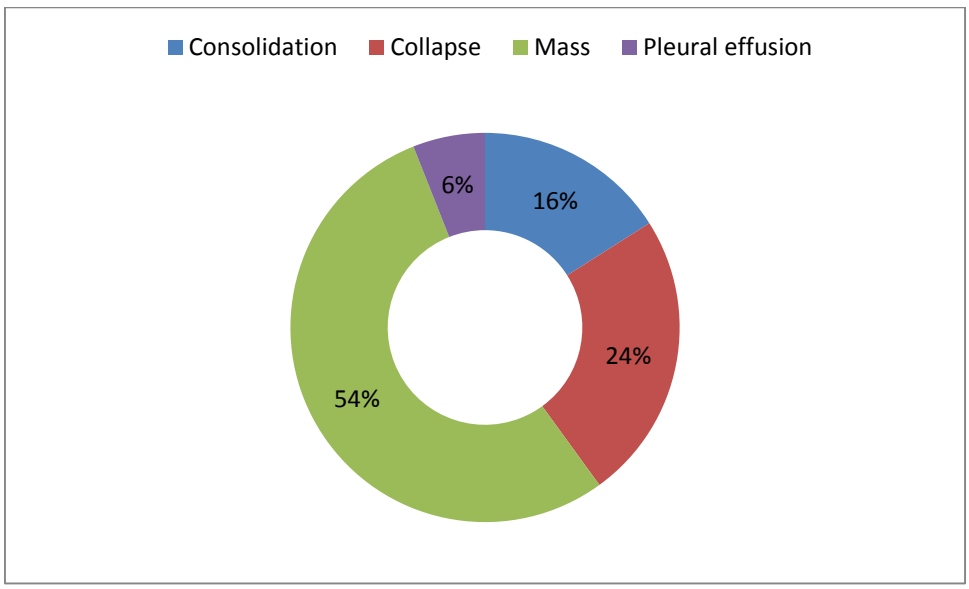


CHART - 12

From the above table, most common image findings in lung cancers was found to be lung mass with relatively highest percentage of 54% followed by collapse with 24% followed by consolidation with 16% and least common finding was pleural effusion with relative frequency of 6%.

IMMUNOHISTOCHEMICAL EXAMINATION OF LUNG CANCERS:

The expression of TTF1 and Napsin A was studied in different subtypes of lung carcinomas. A subset of 50 cases were selected randomly that constituting cases in which definitive histopathological diagnosis could not be made out by using light microscopy alone and those cases in which IHC was done and final definitive diagnosis was made out.

TABLE 13: HISTOPATHOLOGICAL DIAGNOSIS IN SELECTED CASES:

HPE diagnosis	Number of cases	Percentage
AdCC	10	20%
SqCC	6	12%
SCC	3	6%
NSCLC	16	32%
Positive for malignancy	15	30%

TABLE -13

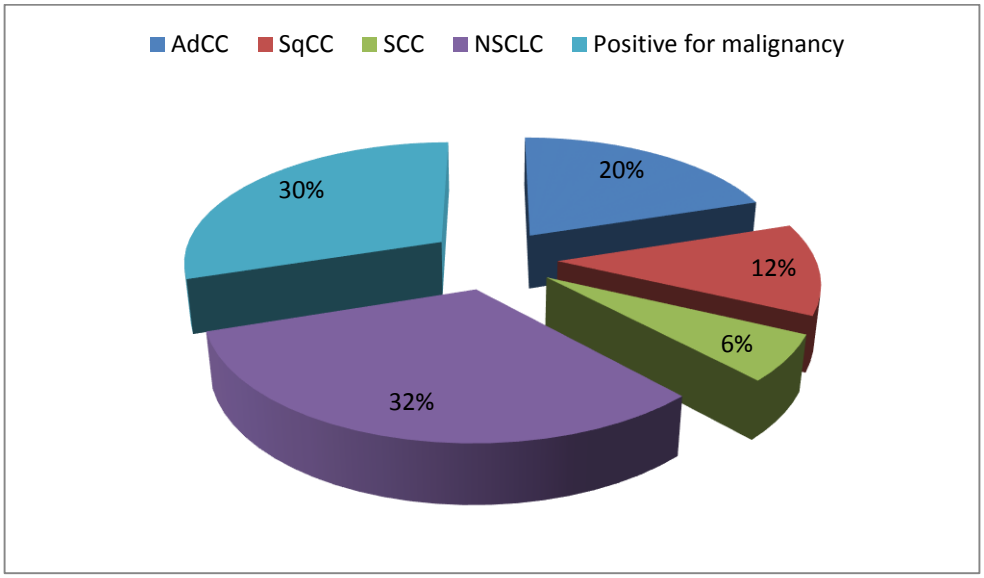


CHART - 13

From the above table it was inferred that in 32% of cases definite subtyping couldnot be done by histomorphological examination, they were diagnosed as NSCLC. In around 30 % of cases, diagnosis was given as positive for malignancy by using light microscopy alone. For rest of the cases to some extent diagnosis was made light microscopically which constituting 20%(10 cases) of cases diagnosed as adenocarcinoma, 12%(6 cases) of cases diagnosed as squamous cell carcinoma and 6%(3 cases) of cases diagnosed as small cell carcinoma.

For these cases TTF1 which was a well known already proven marker for adenocarcinoma of lung was performed to categorise adenocarcinoma. In addition to TTF1, whenever needed, squamous cell markers either CK5/6 or P63 was performed for confirmation of squamous cell carcinoma. Some cases in which even after the application of TTF1, CK5/6 or P63, neuroendocrine

markers (chromogranin, synaptophysin, NSE) were performed to diagnose small cell carcinoma.

In these cases, formalin fixed paraffin embedded sections were subjected to immunohistochemical analysis with Napsin A. The results were evaluated for both TTF1 and Napsin A in all cases and have been tabulated in the following tables and illustrated by tables also.

TABLE:14: FREQUENCY OF TTF I EXPRESSION IN LUNG CANCERS

TTF	Frequency	Percent
NEGATIVE	24	48.0
POSITIVE	26	52.0
Total	50	100.0

TABLE – XIV

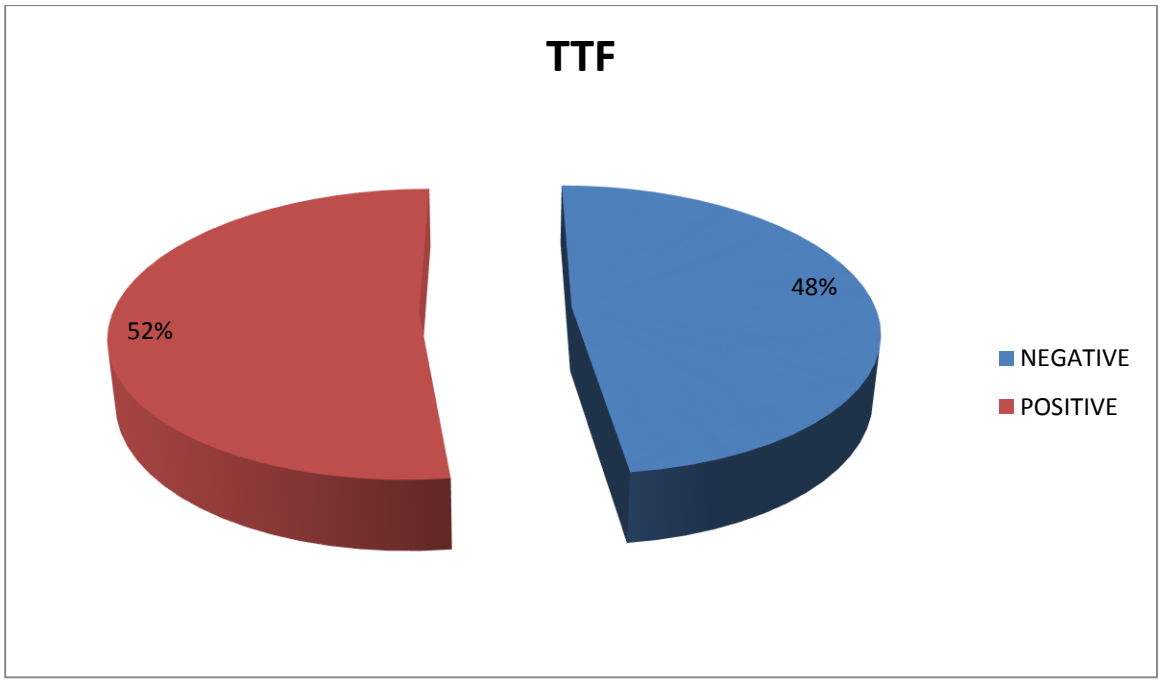


CHART - 14

From the above table, it was inferred that TTF1 was positive in totally 52% of cases (26 cases) and negative in 48% (24 cases) of cases.

TABLE:15: FREQUENCY OF TTF1 EXPRESSION IN DIFFERENT SUBTYPES OF LUNG CANCERS:

			TTF		Total
			NEGATIVE	POSITIVE	
Diagnosis	ADCC	Count	0	23	23
		% within TTF	0.0%	88.5%	46.0%
	SCC	Count	4	3	7
		% within TTF	16.7%	11.5%	14.0%
	SQCC	Count	20	0	20
		% within TTF	83.3%	0.0%	40.0%
Total		Count	24	26	50
		% within TTF	100.0%	100.0%	100.0%

Pearson Chi-Square=43.132** p<0.001

TABLE - XV

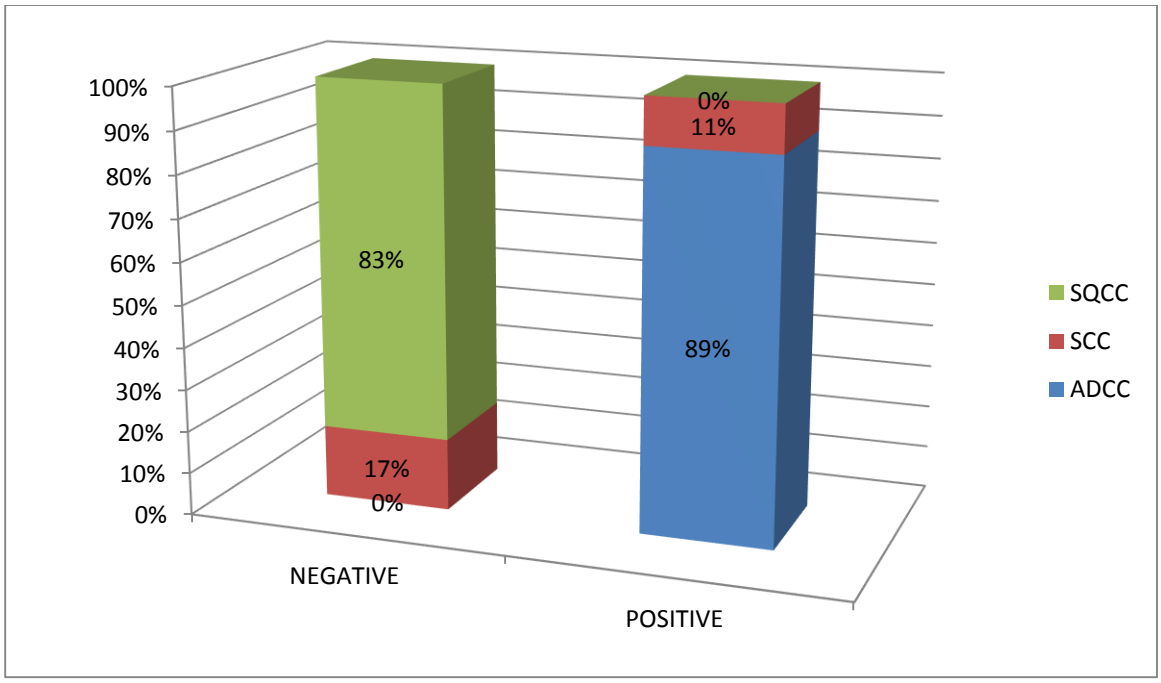


CHART - 15

The above table showed that 88.5% cases showed TTF1 positivity which were diagnosed as adenocarcinoma.. It was positive in 11.5% cases (3 cases) which were further evaluated with neuroendocrine markers and diagnosed as small cell carcinoma. Among the 50 cases accounting 4 cases for 16.7% cases showed TTF1 negativity which were further evaluated with neuroendocrine markers and diagnosed as small cell carcinoma. So it was inferred that in small cell carcinoma TTF1 can show positive expression or it may be negative. So TTF1 was not useful in separating small cell carcinoma from adenocarcinoma.

For table 15 it was found that among the 26 TTF1 positive cases 88.5% (23 cases) of cases were adenocarcinoma and 11.5 cases were small cell carcinoma.

TABLE: 16 : FREQUENCY OF NAPSIN A EXPRESSION IN LUNG CANCERS

NAPSIN	Frequency	Percent
NEGATIVE	27	54.0
POSITIVE	23	46.0
Total	50	100.0

TABLE - 16

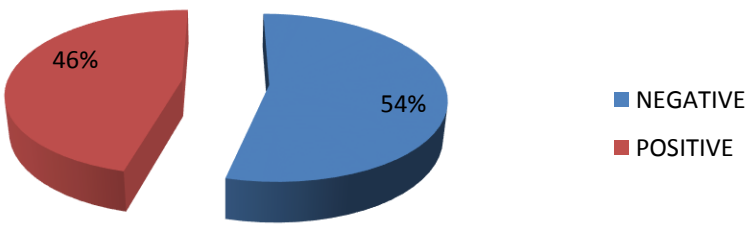


CHART - 16

From the above and chart-16 it was inferred that Napsin A was positive in 46% of cases which accounts for 23 cases and it was negative in 54% of cases which accounts for 27 cases.

TABLE: 17 : FREQUENCY OF NAPSIN A EXPRESSION IN DIFFERENT SUBTYPES OF LUNG CANCER

			NAPSIN		Total
			NEGATIVE	POSITIVE	
Diagnosis	ADCC	Count	0	23	23
		% within NAPSIN	0.0%	100.0%	46.0%
	SCC	Count	7	0	7
		% within NAPSIN	25.9%	0.0%	14.0%
	SQCC	Count	20	0	20
		% within NAPSIN	74.1%	0.0%	40.0%
Total		Count	27	23	50
		% within NAPSIN	100.0%	100.0%	100.0%

Pearson Chi-Square=50.000** p<0.001

TABLE - 17

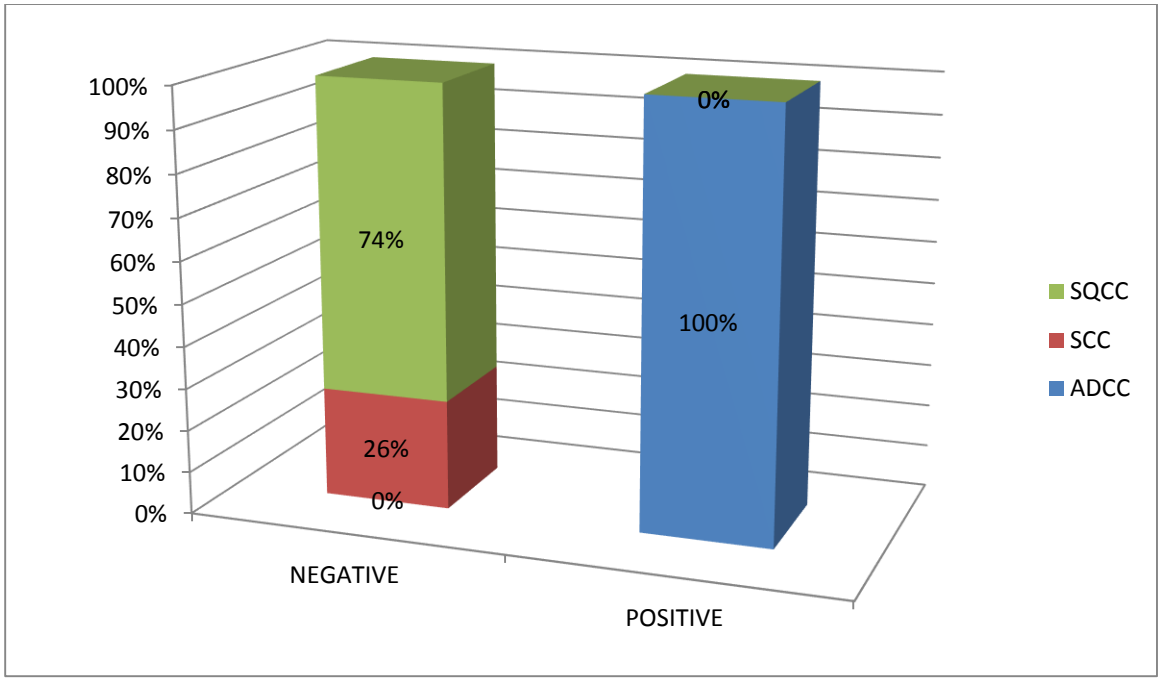


CHART - 17

From the above table it was inferred that 100% of cases that is all cases that showing napsin a positivity was adenocarcinoma. It was completely negative in squamous and small cell carcinoma. So napsin is considered as a specific marker for adenocarcinoma.

TABLE: 18: COMPARISON OF TTF1 AND NAPSIN A IN LUNG CANCERS

			TTF		NAPSIN		Total
			NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	
Diagnosis	SQCC	Count	20	0	20	0	20
		%	100.0%	0.0%	100.0%	0.0%	40.0%
	SCC	Count	4	3	7	0	7
		%	57.1%	42.9%	100.0%	0.0%	14.0%
	ADCC	Count	0	23	0	23	23
		%	0.0%	100.0%	0.0%	100.0%	46.0%
Total		Count	24	26	27	23	50
		%	48.0%	52.0%	54.0%	46.0%	100.0%

TABLE -18

From the above table it was inferred that TTF1 was positive in adenocarcinomas and in some small cell carcinomas. But it was invariably negative in squamous cell carcinomas. So with TTF1 we cannot distinguish adenocarcinoma from small cell carcinomas. Because 42.8% of small cell carcinoma cases were positive for TTF1. So almost half of the small cell carcinomas were were TTF1 positive.

Napsin A was positive in all adenocarcinomas and invariably negative in almost all cases of small cell carcinomas and squamous cell carcinomas. So napsin A can be used as a specific marker for distinguishing adenocarcinoma

from small cell carcinomas. With the Napsin A positivity we can exclude small cell carcinoma. So Napsin A can be used as exclusion marker for small cell carcinoma.

TABLE: 19: CORRELATION OF HISTOPATHOLOGICAL DIAGNOSIS WITH IHC PROVEN FINAL DIAGNOSIS

			Diagnosis			Total
			ADCC	SCC	SQCC	
HPE_DIAGNOSIS	Adenocarcinoma	Count	9	0	1	10
		% within Diagnosis	39.1%	0.0%	5.0%	20.0%
	Non Small cell Lung Carcinoma	Count	11	0	5	16
		% within Diagnosis	47.8%	0.0%	25.0%	32.0%
	Positive for malignancy	Count	3	5	7	15
		% within Diagnosis	13.0%	71.4%	35.0%	30.0%
	Small cell carcinoma	Count	0	2	1	3
		% within Diagnosis	0.0%	28.6%	5.0%	6.0%
	Squamous Cell carcinoma	Count	0	0	6	6
		% within Diagnosis	0.0%	0.0%	30.0%	12.0%
Total		Count	23	7	20	50
		% within Diagnosis	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=34.938** $p<0.001$

TABLE - 19

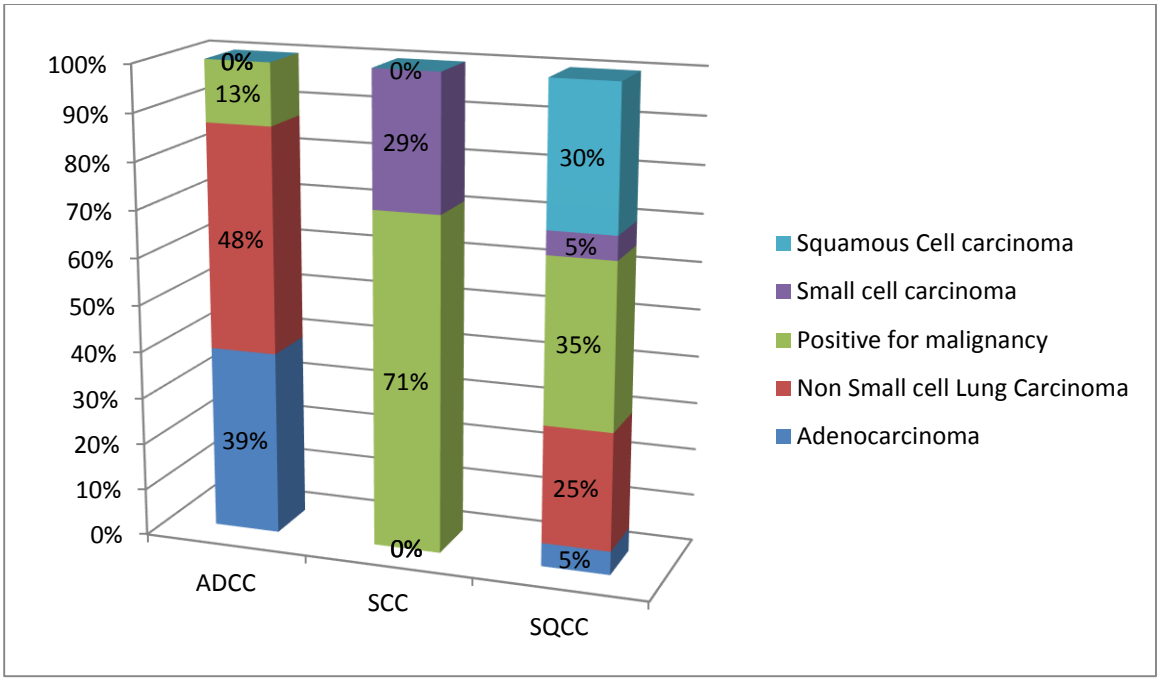


CHART - 19

From the above table it was inferred that among the 10 cases diagnosed as adenocarcinoma based on morphology alone, 9 cases were proven by IHC as adenocarcinoma. One case was turned to be squamous cell carcinoma on IHC.

It was found that 3 cases were diagnosed as small cell carcinoma based on morphology alone. Out of these three cases 2 were confirmed with IHC as small cell carcinoma. One case turned to be squamous cell carcinoma on IHC.

It was found that 6 cases were diagnosed as squamous cell carcinoma based on morphology. All cases were confirmed by IHC as squamous cell carcinoma.

Among the 50 cases further subtyping could not be done in 31 cases by morphological examination alone. They were subjected to IHC. Out of that 31 cases 14 were found to be positive for adenocarcinoma markers, 5 were found

to be positive for small cell carcinoma markers and 12 were diagnosed as squamous cell carcinoma.

TTF EXPRESSION IN ADENOCARCINOMA

			Diagnosis		Total
			ADCC	OTHERS	
TTF	POSITIVE	Count	23	3	26
		% within diagnosis	100.0%	11.1%	52.0%
	NEGATIVE	Count	0	24	24
		% within diagnosis	0.0%	88.9%	48.0%
Total		Count	23	27	50
		% within diagnosis	100.0%	100.0%	100.0%

Pearson Chi-Square=39.316** P<0.001

TABLE - 20

Statistics	**Value**	**95% CI**
Sensitivity	100.00%	85.18% to 100.00%
Specificity	88.89 %	70.84% to 97.65%
Positive Likelihood Ratio	9.00	3.10 to 26.16
Disease prevalence	46.00% (*)	31.81% to 60.68%
Positive Predictive Value	88.46% (*)	72.51% to 95.70%
Negative Predictive Value	100.00 % (*)	

CHART - 20

From the above table it was inferred that sensitivity of TTF1 to adenocarcinoma was 100% and its specificity to adenocarcinoma was 88.86%. P value was <0.001. that is TTF1 expression in adenocarcinoma was statistically significant.

NAPSIN EXPRESSION IN ADENOCARCINOMA

			Diagnosis		Total
			ADCC	OTHERS	
NAPSIN	POSITIVE	Count	23	0	23
		% within diagnosis	100.0%	0.0%	46.0%
	NEGATIVE	Count	0	27	27
		% within diagnosis	0.0%	100.0%	54.0%
TOTAL		Count	23	27	50
		% within Diagnosis	100.0%	100.0%	100.0%

Pearson Chi-Square=50.00** P<0.001

TABLE - 21

Statistic	Value	95% CI
Sensitivity	100.00%	85.18% to 100.00%
Specificity	100.00 %	87.23% to 100.00%
Disease prevalence	46.00% (*)	31.81% to 60.68%
Positive Predictive Value	100.00% (*)	
Negative Predictive Value	100.00 % (*)	

TABLE 21 (A)

From the above table it was inferred that sensitivity of napsin A in adenocarcinoma was 100% and its specificity was 100%. P value is <0.001, that is napsin A expression in adenocarcinoma was statistically significant.

TTF1 EXPRESSION IN SMALL CELL CARCINOMA

TTF1	SCC	Others
Negative	4	20
Positive	3	23

TABLE - 22

Statistic	**Value**	**95% CI**
Sensitivity	57.14%	18.41% to 90.10%
Specificity	53.49 %	37.65% to 68.82%
Positive Likelihood Ratio	1.23	0.60 to 2.52
Negative Likelihood Ratio	0.80	0.33 to 1.97
Disease prevalence	14.00% (*)	5.82% to 26.74%
Positive Predictive Value	16.67% (*)	8.89% to 29.06%
Negative Predictive Value	88.46 % (*)	75.72% to 94.96%

CHART - 22

From the above table it was inferred that TTF1 sensitivity for small cell carcinoma was 57.14% and its specificity was 53.9%.

NAPSIN A IN SMALL CELL CARCINOMA:

Napsin	SCC	Others
Negative	7	20
Positive	0	23

TABLE - 23

Statistic	**Value**	**95% CI**
Sensitivity	100.00%	59.04% to 100.00%
Specificity	53.49 %	37.65% to 68.82%
Positive Likelihood Ratio	2.15	1.56 to 2.96
Negative Likelihood Ratio	0.00	
Disease prevalence	14.00% (*)	5.82% to 26.74%
Positive Predictive Value	25.93% (*)	20.26% to 32.54%
Negative Predictive Value	100.00 % (*)	

TABLE – 23 (A)

It was inferred from the above table that Napsin A sensitivity to excluding small cell carcinoma was 100% and its specificity to exclude small cell carcinoma was 52.49%.

TTF EXPRESSION IN SQUAMOUS CELL CARCINOMA

TTF1	SQCC	Others
Negative	20	4
Positive	0	26

TABLE - 24

Statistic	Value	95% CI
Sensitivity	100.00%	83.16% to 100.00%
Specificity	86.67 %	69.28% to 96.24%
Positive Likelihood Ratio	7.50	3.01 to 18.68
Negative Likelihood Ratio	0.00	
Disease prevalence	40.00% (*)	26.41% to 54.82%
Positive Predictive Value	83.33% (*)	66.75% to 92.57%
Negative Predictive Value	100.00 % (*)	

TABLE- 24A

From the above table it was inferred that TTF 1 sensitivity to exclude squamous cell carcinoma was 100% and its specificity to exclude squamous cell carcinoma was 88.67%.

NAPSIN A EXPRESSION IN SQUAMOUS CELL CARCINOMA

NAPSIN A	SQCC	Others
Negative	20	7
Positive	0	23

TABLE – 25

Statistic	Value	95% CI
Sensitivity	100.00%	83.16% to 100.00%
Specificity	76.67 %	57.72% to 90.07%
Positive Likelihood Ratio	4.29	2.24 to 8.20
Negative Likelihood Ratio	0.00	
Disease prevalence	40.00% (*)	26.41% to 54.82%
Positive Predictive Value	74.07% (*)	59.90% to 84.53%
Negative Predictive Value	100.00 % (*)	

TABLE – 25 A

From the above table it was inferred that napsin A sensitivity to exclude squamous cell carcinoma was 100% and its specificity to exclude squamous cell carcinoma was 76.67%.

COMPARISION OF NAPSIN A WITH TTF1

		TTF		
		POSITIVE	NEGATIVE	Total
NAPSIN	POSITIVE	23	0	23
	NEGATIVE	3	24	27
	total	26	24	50

Pearson Chi-Square=39.316** p<0.001

TABLE - 27

Statistic	Value	95% CI
Sensitivity	88.46%	69.85% to 97.55%
Specificity	100.00 %	85.75% to 100.00%
Negative Likelihood Ratio	0.12	0.04 to 0.33
Disease prevalence	52.00% (*)	37.42% to 66.34%
Positive Predictive Value	100.00% (*)	
Negative Predictive Value	88.89 % (*)	73.40% to 95.87%

TABLE – 27 A

From the above table it was inferred that expression of napsin A in lung carcinoma is statistically significant.

COMPARISON OF SENSITIVITY OF NAPSIN A WITH TTF1:

SENSITIVITY	TTF1	NAPSIN A
AdCC	100%	100%
SqCC	100%	100%
SCC	57.1%	100%

TABLE - 28

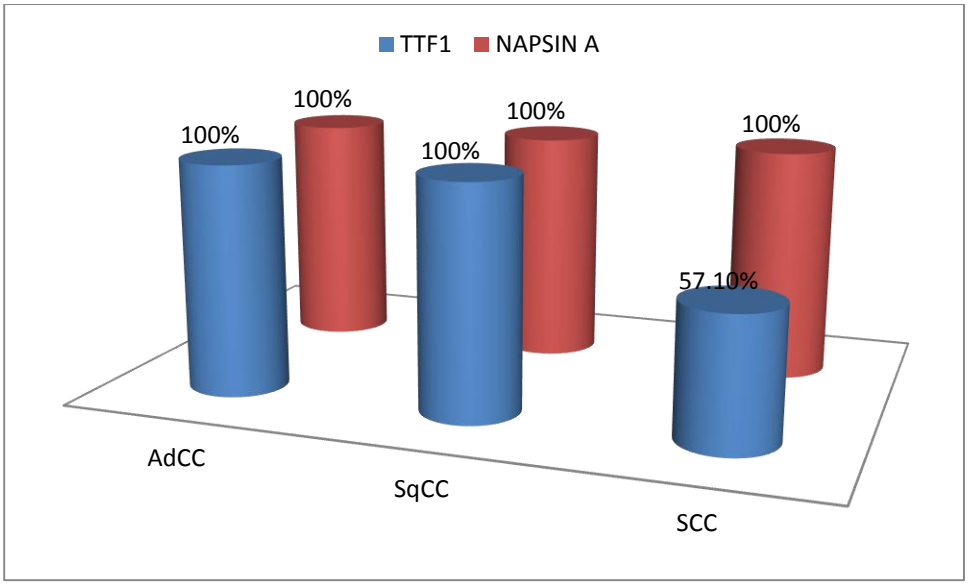


CHART – 28

From the above table it was inferred that Napsin A has comparable sensitivity with TTF1 in the subtyping adenocarcinoma.

It was inferred that sensitivity of Napsin A was comparable with TTF1 in excluding squamous cell carcinoma.

From the above table it was inferred that napsin A is more sensitive (100%) in excluding small cell carcinoma than TTF1(57.1%)

COMPARISON OF SPECIFICITY OF NAPSIN A WITH TTF1:

SPECIFICITY	TTF1	NAPSIN A
AdCC	88%	100%
SqCC	86%	76.6%
SCC	53.48%	53.00%

TABLE - 29

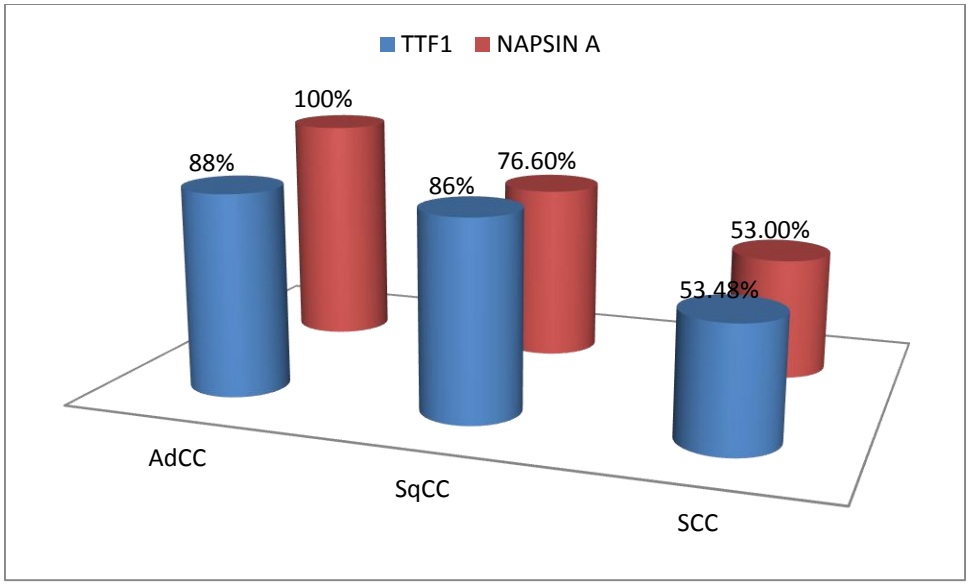


CHART - 29

From the above table it was inferred that specificity of napsin A in the subtyping of adenocarcinoma was higher(100%) than TTF1(88%).

It was inferred that specificity of napsin A (76.60%) in excluding squamous cell carcinoma was lesser than TTF1(86%).

It was inferred that specificity of napsin A (53%) in excluding small cell carcinoma was comparable with that of TTF1 (53.48%).

DISCUSSION

DISCUSSION

The incidence of lung cancer is increasing in both developed and developing countries in the present era involving both males and female population.

EPIDEMIOLOGY OF LUNG CANCERS IN GENERAL:

In the present study, histomorphological study was done for 108 cases of lung cancers while immunohistochemical evaluation was done for a subset of 50 cases. An attempt was made to check for the comparision of napsin A with TTF1 in all 50 cases including 23 cases of adenocarcinoma, 7 cases of small cell carcinoma and 20 cases of squamous cell carcinoma.

Worldwide, histological profile of lung cancer patients is seen undergoing a changing trends and adenocarinoma had replaced squamous cell carcinoma as predominant histological subtype[94,95,96].

However most of Indian studies still reports squamous cell carcinoma as a commonest type [97, 98, 99, 100], Viswanathan et al. (1962) [101], Shankar S et al. (1967) [102], Gularia et al (1971)[103], Malik et al. (1976)[104], Jindal and Behera et al.(1990)[99] from Chandigarh, Gupta RC et al. (1998)[105] and Bhattacharyya SK et al. (2010)[106] from India had reported squamous cell carcinoma as most dominant subtype.

In our study, it was found that adenocarcinoma had a maximum incidence of 46%. The second most common was squamous cell carcinoma

accounting for 40%. Small cell carcinoma was the third most frequent subtype with relative percentage of 14% of the total cases.

Our results were in concordance with recent Indian study by Mandal SK et al(2013)[107], Shankar et al (2014)[108] and Sundaram V et al(2014)[109], Mahendra Kumar et al (2016)[110] reporting adenocarcinoma as a commonest subtype.

The peak incidence was noted in a age group of 51 to 60 years, the number of patients were 19 accounting for 38% of cases. It seems to have a least incidence in the age group of 41 to 50 years and 71 to 80 years with relative percentage of 14% in each age group.

There is no increase in the incidence of lung cancers with increasing age. The tumor seems to be distributed along the age group in no specific pattern.

Adenocarcinoma was most common in the age group of 51 to 60 years accounting for 43.5%. It was least common in the age group of 71 to 80 years accounting for 8.7%

Squamous cell carcinoma was most common in the age group of 51 to 60 years and 61 to 70 years with relative range of 35% in each group. It was least common in the age group of 41 to 50 years and 71 to 80 years with relative range of 15% in each group.

Small cell carcinoma was most common in the age group of 61 to 70 years accounting for 42.9%, followed by second most commonly seen in 51 to 60 years and 71 to 80 years with relative range of 28.6% in each age group.

Adenocarcinoma and squamous cell carcinoma was found to be commonly affect the age group of 51 to 60 years. Small cell carcinoma was found to be common in slightly older age group of 61 to 70 years.

Our study results were in concordance with Pandhi N. et al (2015), Dubey N. et al (2015), Malik PS. et al (2013)[103], Koul PA.et al (2010), Sheikh S. et al(2010), Mahendra kumar. et al(2016)[110]reporting most common age group is 51-60 years.

The incidence of lung cancers in males was found to be 70%. The incidence of lung cancers in female was found to be 30%. Male to female ratio was 2.3:1. P value is 0.141. So gender has significant value in lung cancers.

It was found that adenocarcinoma was common among males with relative percentage of 60.9% as compared to 39.1% in females.

Squamous cell carcinoma was common among males with relative percentage of 70 % as compared to 30% in females.

Small cell carcinoma was found to be exclusively occurred only in males with relative percentage of 100.0%.

Our results were in concordance with Pandhi N. et al(2015), Dubey N. et al(2015), Malik PS. et al(2013)[103], Koul PA. et al(2010), Sheikh S. et al(2010), Mahendra kumar. et al(2016)[110], Baburao A. et al(2015), Sundaram V. et al(2014) [109], Mandal SK. et al(2013)[107], Bhaskarpillai B .et al(2012), Bhattacharyya et al (2010)[106], Rawat J et al(2009), Khan et al(2006), Prasad R.et al(2004) reporting that lung cancers were most commonly occurs in males.

Lung cancers found to be occurring more commonly in Right lobe with relative percentage of 56% followed by left lobe with little less frequency of 44%.

Our results were not in concordance with Mahendra kumar et al (2016) [110] reporting that lung cancers were common in right lobe.

	Present study	Mahendrakumar et al
Right lobe	56%	45%
Left lobe	44%	50%
Bilateral	-	5%

The lung cancers seems to be predominant in upper lobe with relatively higher percentage of 66%, followed by lower lobes found to be involved in 24% of cases with least common in middle lobe with least percentage of 10%.

Most of the patients (26%) presented with the symptom of cough followed by chest pain in 20% of patients, followed by 18% of patients with dyspnoea, 18% with hemoptysis and 18% of patients presented with no specific symptoms.

This is in concordance with the Mahendra kumar et al(2016)[106] reporting that most common presenting symptom is cough followed by chest pain.

In lung adenocarcinoma cough followed by chest pain was found to be commonest presentation with relative percentage of 26.1% and 21.7% respectively.

Among the squamous cell carcinoma patients, Dyspnoea is the commonest presenting complaints with 25% relative percentage.

Among the small cell carcinoma patients cough is the predominant symptoms with relative percentage of 57.1%.

Lung cancers were common in smokers with 62% and 38% of non smokers developed lung cancers.

This is in concordance with Mahendra kumar et al (2016) [101] reporting that lung cancers were common in smokers than non smokers.

	Present study	Mahendra kumar et al
Smoker	62%	81.8%
Non smoker	38%	18.2%

Adenocarcinoma seems to be commonly occurs in non smokers with relative percentage of 52.2% in contrast to 47.8% in smokers.

Squamous cell carcinoma and small cell carcinoma seems to be common in smokers with relative percentage of 65% and 100.0% respectively. Small cell carcinoma was found to be occur exclusively only in smokers.

Most common image findings in lung cancers was found to be lung mass with relatively highest percentage of 54% followed by collapse with 24% followed by consolidation with 16% and least common finding was pleural effusion with relative frequency of 6%.

Because of switch from non filtered to filtered cigarettes and altered inhalational depth, there is shift in the incidence of squamous cell carcinoma to adenocarcinoma [101]

DESCRIPTIVE STUDY OF LUNG CANCERS:

Ours is a descriptive study, including a 1 year period from 2016 to 2017. The caseswere collected in both prospective and retrospective ways. The study is hospital based; hence it does not reflect the true incidence and prevalence in the community. Follow up was not available and not analysed. Of the cases reported in our study, 50 cases were chosen randomly.

ADENOCARCINOMA:

Of the 50 cases selected for this study, adenocarcinoma constituted about 23 cases, which was 46% of total. This is in concordance with Krishnamoorthy et al [(120)], Mahendra kumar et al (2016)[(101)] reporting that most common histology was adenocarcinoma accounting for 42.6% and 40.9% respectively.

Present study	46%
Krishnamoorthy et al	42.6%
Mahendra kumar et al	40.9%

- Adenocarcinoma was most common in the age group of 51-60 years accounting for about 43.5% of all cases in this age group. The youngest being 40 years and the oldest being 77 years.This is in concordance with reporting that adenocarcinoma
- The total male patients in our study was 35, the number of females was 15. Thus the calculated male : female ratio was 2.3: 1.
- Among the adenocarcinoma, male patients were14, number of females was 9. Thus the calculated male female ratio was 1.5:1. But according to

WHO adenocarcinoma comprises 28% in men and 42% cases in women. There is slight female preponderance[121].

- The most common presentation in lung adenocarcinoma patients was cough followed by chest pain. This is in concordance with that inferred in WHO.
- Adenocarcinoma seems to be commonly occurs in non smokers with relative percentage of 52.2% (12 cases) in contrast to 47.8% in smokers (11 cases). This is in concordance with WHO estimates. The WHO estimates that 25% of lung cancer worldwide occurs in never smokers. These cancers occur more commonly in women and most are adenocarcinoma.[122]

SQUAMOUS CELL CARCINOMA:

Of the 50 cases selected for this study, squamous cell carcinoma constituted about 20 cases, which was 40% of total. This is in concordance with Mahendra kumar et al (2016)[101] reporting that most common histology after adenocarcinoma was squamous cell carcinoma accounting for 32.7%.

Present study	40%
Mahendra kumar et al	32.7%

- Squamous cell carcinoma was most common in the age group of 51-60 and 61-70 years accounting for about 35% and 35% respectively of all cases in this age group. The youngest being 42 years and the oldest being 77 years.

- Among the adenocarcinoma male patients were14, number of females was 6 accounting for 70% males and 30% of cases were females. Thus the calculated male female ratio was 2.3:1. But according to WHO squamous cell carcinoma comprises 44% in men and 25% cases in women. There is slight male preponderance[121].
- The most common presentation in lung squamous cell carcinoma patients was cough followed by chest pain. This is in concordance with that inferred in WHO.
- Squamous cell carcinoma seems to be commonly occurs in smokers with relative percentage of 65% (13 cases) in contrast to 35% in nonsmokers (7 cases). This is in concordance with WHO estimates.[122]

SMALL CELL CARCINOMA:

- Of the 50 cases selected for this study, small cell carcinoma constituted about 7 cases, which was 14% of total. This is in concordance with Mahendra kumar et al (2016)[101].
- Small cell carcinoma was most common in the age group of 61 to 70 years accounting for 42.9% (3 cases), followed by second most commonly seen in 51 to 60 years and 71 to 80 years with relative range of 28.6% in each age group. (2 cases). This is in concordance with Vanita Noronha et al(2016)[123] reporting that most common age group is 50- 59 years.
- Small cell carcinoma was found to be exclusively occurred only in males with relative percentage of 100.0%(7 / 7 cases).

- Among the small cell carcinoma patients cough is the predominant symptoms with relative percentage of 57.1%.
- Small cell carcinoma was found to be occur exclusively only in smokers.

IMMUNOHISTOCHEMISTRY IN LUNG CANCERS:

Immunohistochemistry is increasingly utilized to differentiate lung adenocarcinoma and squamous cell carcinoma. In this study we used TTF1, CK 5/6, P63 and neuroendocrine markers to subtype the lung cancers. By using this panel we lung cancers were subtyped. Napsin A marker expression was studied in 50 randomly selected cases which constituting adenocarcinoma, squamous cell carcinoma and small cell carcinoma. IHC was done in formalin fixed paraffin embedded sections.

EXPRESSION OF TTF1 IN LUNG CANCERS:

- TTF1 expression was located in the nucleus with no staining on the membrane and in the cytoplasm.
- In the present study it was inferred that TTF1 was positive in totally 52% of cases (26 cases) and negative in 48% (24 cases) of cases.
- In this present study 88.5% cases showed TTF1 positivity which were diagnosed as adenocarcinoma..
- It was positive in 11.5% cases (3 cases) which were further evaluated with neuroendocrine markers and diagnosed as small cell carcinoma.

- Among the 50 cases accounting 4 cases for 16.7% cases showed TTF1 negativity which were further evaluated with neuroendocrine markers and diagnosed as small cell carcinoma. So it was inferred that in small cell carcinoma TTF1 can show positive expression or it may be negative.
- So TTF1 was not useful in separating small cell carcinoma from adenocarcinoma.
- TTF1 was found to be positive in adenocarcinomas and in few small cell carcinomas. However TTF1 was found to be uniformly negative in all cases of squamous cell carcinoma.
- Its sensitivity and specificity to adenocarcinoma were 100% and 88.86% respectively. P value was <0.001. That is statistically significant.
- This is in concordance with other studies conducted by Bradley M. Turner et al(2012) [(50)] and Zhang et al(2010)[(2)], Sanjay Mukhopadhyay et al(2011)[(69)], Lisa M Stoll et al(2010)[(124)]

Study	Sensitivity	Specificity
Present study	100%	88.86%
Bradley M. Turner et al	64%	90%
Zhang et al	84.4%	83.9%
Sanjay Mukhopadhyay et al	80%	89%
Lisa M. Stoll et al	81%	81%

- From the present study it was inferred that TTF1 sensitivity for excluding small cell carcinoma was 57.14% and its specificity for excluding small cell carcinoma was 53.9%.

- From the present study it was inferred that TTF 1 sensitivity to excluding squamous cell carcinoma was 100% and its specificity to excluding squamous cell carcinoma was 88.67%.

EXPRESSION OF NAPSIN A IN LUNG CACERS:

- For Napsin A, only a granular cytoplasmic staining pattern was accepted as positive.
- From the present study it was inferred that Napsin A was positive in 46% of cases (23 cases) it was negative in 54% of cases (27 cases).
- From the present study it was inferred that 100% of cases that is all cases that showing napsin a positivity was adenocarcinoma. It was completely negative in squamous and small cell carcinoma. So napsin is considered as a specific marker for adenocarcinoma.
- Napsin A was positive in all adenocarcinomas and invariably negative in almost all cases of small cell carcinomas and squamous cell carcinomas. So napsin A can be used as a specific marker for distinguishing adenocarcinoma from small cell carcinomas. With the Napsin A positivity we can exclude small cell carcinoma. So Napsin A can be used as exclusion marker for small cell carcinoma.
- From the above table it was inferred that sensitivity of napsin A in adenocarcinoma was 100% and its specificity was 100%. P value is <0.001, that is statistically significant.

- Comparison with other studies such as Bradley M. Turner et al(2012) [50] and Zhang et al(2010)[2], Sanjay Mukhopadhyay et al(2011)[69], Lisa M Stoll et al(2010)[124]

Study	Sensitivity	Specificity
Present study	100%	100%
Bradley M. Turner et al	87%	97%
Zhang et al	84.9%	93.8%
Sanjay Mukhopadhyay et al	58%	100%
Lisa M. Stoll et al	65%	96%

- It was inferred that Napsin A sensitivity to exclude small cell carcinoma was 100% and its specificity to exclude small cell carcinoma was 52.49%.
- It was inferred that napsin A sensitivity to exclude squamous cell carcinoma was 100% and its specificity to exclude squamous cell carcinoma was 76.67%.

COMPARISON OF TTF1 AND NAPSIN A:

ADENOCARCINOMA:

- The expression of napsin A was significantly correlated with TTF1 in the lung adenocarcinoma.
- The senstivity of TTF1 in identifying adenocarcinoma was 100%. The sensitivity of napsin A in identifying adenocarcinoma was 100%. So both these marker have similar sensitivity for adenocarcinoma.

- The specificity of TTF1 in identifying adenocarcinoma was 88%. The sensitivity of napsin A in identifying adenocarcinoma was 100%. So napsin A was found to be more specific for adenocarcinoma than TTF1.
- Hence NAPSIN A IS AS SENSITIVE AS TTF1 & MORE SPECIFIC THAN TTF1 in the subtyping of lung adenocarcinoma.

SQUAMOUS CELL CARCINOMA:

- The expression of napsin A was significantly correlated with TTF1 in the lung squamous cell carcinoma.
- The senstivity of TTF1 in excluding squamous cell carcinoma was 100%. The sensitivity of napsin A in excluding squamous cell carcinoma was 100%. So both these marker have similar sensitivity for excluding squamous cell carcinoma.
- The specificity of TTF1 in excluding squamous cell carcinoma was 86%. The sensitivity of napsin A in excluding squamous cell carcinoma was 76.6%. So napsin A was found to be less specific for squamous carcinoma than TTF1. So with napsin A negativity alone we cannot diagnose squamous cell carcinoma.
- Hence NAPSIN A IS AS SENSITIVE AS TTF1 & LESS SPECIFIC THAN TTF1 in the subtyping of lung squamous cell carcinoma.

SMALL CELL CARCINOMA:

- The expression of napsin A was significantly correlated with TTF1 in the lung small cell carcinoma.
- The senstivity of TTF1 in excluding small cell carcinoma was 57.1%. The sensitivity of napsin A in excluding small cell carcinoma was 100%. So napsin A was found to be more sensitive than TTF1 for excluding small cell carcinoma.
- The specificity of TTF1 in excluding small cell carcinoma was 53.48%. The sensitivity of napsin A in excluding small cell carcinoma was 53%. So both these marker have similar specificity for excluding small cell carcinoma
- Hence NAPSIN A IS AS SPECIFIC AS TTF1 & MORE SENSITIVE THAN TTF1 in the subtyping of lung small cell carcinoma.

This is in concordance with other studies:

- Bradley M. Turner et al reported that Napsin A was more sensitive than TTF1 for primary lung adenocarcinoma(87% versus 64% : p valiue<0.001). Napsin Awas more specific than TTF1 for primary lung adenocarcinoma versus all metastatic tumour.(p value <0.001).
- T Ueno et al(2003) reported that napsin A is as sensitive as TTF1 and more specific than TTF1.

T Ueno et al	Sensitivity	Specificity
TTF1	84.6%	76.7%
Napsin A	84.6%	94.3%

STRENGTH AND LIMITATIONS OF THE STUDY:

STRENGTH OF THIS STUDY:

- Study was done at a tertiary care hospital in south india
- The clinicopathological aspects of lung cancers – their relative incidence, age distribution, sex predeliction, side and lobe involvement, risk factor like smoking has been enumerated and will be of value in estimating the same for a future population based study.
- The expression of napsin A in various lung cancer subtypes has been studied in this study.And it is compared with TTF1, a well known marker used in lung cancer.

LIMITATIONS OF THIS STUDY:

- This study is hospital based, hence doesnot reflect the true incidence and prevalence in the community.
- Due to the economical constraints, only limited number of cases has been studied.
- In many case, due to inadequate material from small biopsy specimens, further subtyping could not be studied.

RECOMMENDATIONS:

- New responsibility of pathologist is to preserve tissue for molecular studies.
- Along with TTF1, napsin A should be included in the panel to increase the specificity and sensitivity.
- IHC cocktail markers can be used which include one nuclear marker and one cytoplasmic marker.
- EGFR expression should be included as a routine test for all NSCLC for better treatment options for the patients.

THE DIAGNOSIS OF LUNG CANCER IN SMALL BIOPSIES SHOULD HAVE THE FOLLOWING STRUCTURE:

- Pathologic diagnosis
- Reporting of immunohistochemical and/or mucin stains
- If appropriate, a comment about the differential diagnosis

  If material has been submitted for molecular testing, this should be stated in a comment, specifying which block or slide is optimal for testing.

SUMMARY

SUMMARY

- In the present study, histomorphological study and immunohistochemical evaluation was done for a subset of 50 cases. An attempt was made to check for the comparison of napsin A with TTF1 in all 50 cases.
- Adenocarcinoma had a maximum incidence of 46%. Followed by squamous cell carcinoma accounting for 40% and small cell carcinoma accounting for 14% of the total cases.
- The peak incidence was noted in a age group of 51 to 60 years. The tumor seems to be distributed along the age group in no specific pattern.
- The incidence of lung cancers in males and females were 70% and 30 % respectively. Male to female ratio was 2.3:1
- Lung cancer was found to be more common in Right lobe with relative percentage of 56% followed by left lobe with little less frequency of 44%.
- The lung cancer seems to be predominant in upper lobe with relatively higher percentage of 66%.
- Most common presenting symptom was cough followed by chest pain.
- Lung cancers were common in smokers than non smaokers. The smokers will have two times the risk of developing lung cancers when compared to non smokers.
- On imaging they were commonly diagnosed as lung mass. Occasionally they mimic collapse, consolidation.

- TTF1 is a nuclear marker for adenocarcinoma. Napsin A is a cytoplasmic marker for adencarcinoma.
- TTF1 was found to be positive in all adenocarcinomas and in few small cell carcinomas. TTF1 was found to be consistently negative in all cases of squamous cell carcinoma.
- TTF1 sensitivity and specificity to adenocarcinoma were 100% and 88.86%
- TTF1 sensitivity for excluding small cell carcinoma was 57.14% and its specificity for excluding small cell carcinoma was 53.9%.
- TTF 1 sensitivity to excluding squamous cell carcinoma was 100% and its specificity to excluding squamous cell carcinoma was 88.67%.
- Napsin A was positive in all adenocarcinomas and invariably negative in almost all cases of small cell carcinomas and squamous cell carcinomas. So napsin A can be used as a specific marker for distinguishing adenocarcinoma from small cell carcinomas. With the Napsin A positivity we can exclude small cell carcinoma.
- Napsin A can be used as negative exclusion marker for small cell carcinoma.
- The sensitivity of napsin A in adenocarcinoma was 100% and its specificity was 100%.
- Napsin A sensitivity to exclude small cell carcinoma was 100% and its specificity to exclude small cell carcinoma was 52.49%.

- Napsin A sensitivity to exclude squamous cell carcinoma was 100% and its specificity to exclude squamous cell carcinoma was 76.67%.
- NAPSIN A IS AS SENSITIVE AS TTF1 & MORE SPECIFIC THAN TTF1 in the subtyping of lung adenocarcinoma
- NAPSIN A IS AS SENSITIVE AS TTF1 & LESS SPECIFIC THAN TTF1 in the subtyping of lung squamous cell carcinoma.
- NAPSIN A IS AS SPECIFIC AS TTF1 & MORE SENSITIVE THAN TTF1 in the subtyping of lung small cell carcinoma.

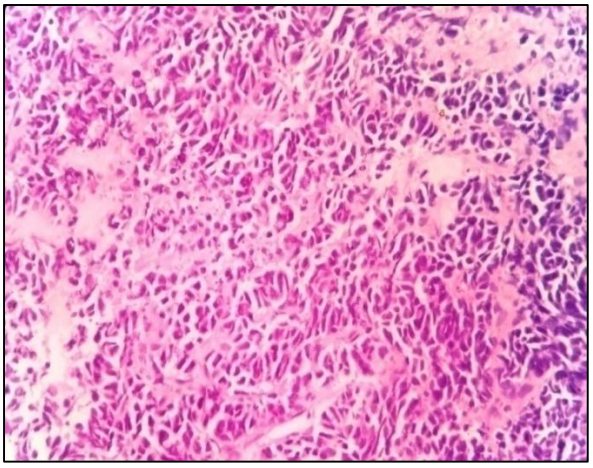
CONCLUSION

CONCLUSION

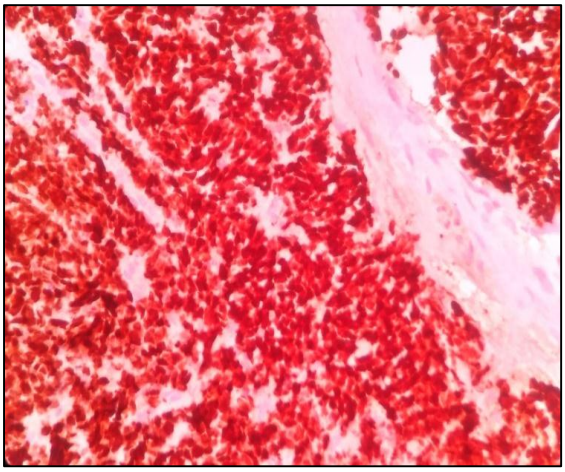
Increase in the target specific chemotherapeutic therapies required futher subcategorisation of NSCLCs.IHC can be used to achieve a greater diagnostic sensitivity and specificity than cytomorphology alone. A combination f napsin A and TTF1 is useful in the distinction of primary lung adenocarcinoma from primary lung squamous cell carcinoma and primary lung small cell carcinoma.Lung adenocarcinoma will have the IHC profile of napsinA + ve/ TTF1 + ve (or) Napsin A +ve / TTF 1 – ve ; lung squamous cell carcinoma will have IHC profile of Napsin A –ve / TTF1 –ve ; lung small cell carcinoma will have IHC profile of Napsin A – ve / TTF1 +ve . The combined uise of napsin A and TTF1 increases the sensitivity and specificity of identifying the specific subtype. Since Napsin A is negative in all small cell carcinoma this stain may prove to be a useful exclusionary marker in distinguishing pulmonary small cell carcinomq from other poorly differentiated lung carcinoma with similar morphology especially those with concomitant TTF 1 expression.

COLOR PLATES

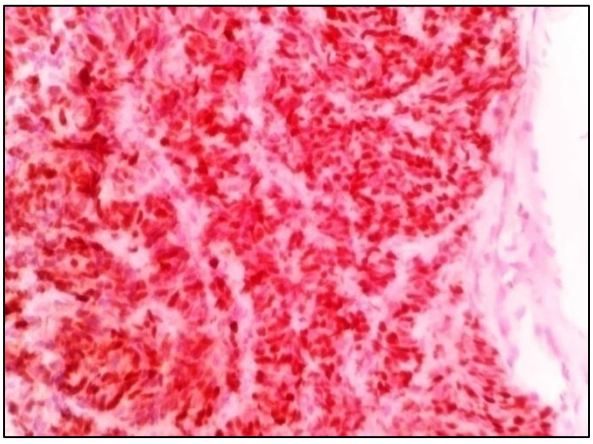
ADENOCARCINOMA:



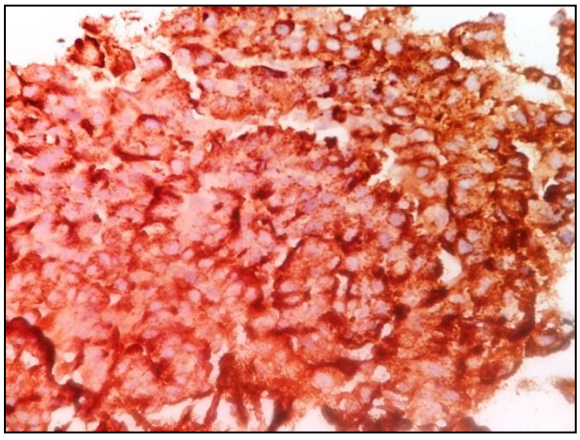
BX NO:3638/17: (10X, H&E)
Sheets of round to polygonal cells with moderate eosinophilic cytoplasm with round to oval pleomorphic hyperchromatic nuclei.



IHC NO: 424/17: (40X) :
TTF1 DIFFUSE STRONG POSITIVITY IN TUMOUR CELLS

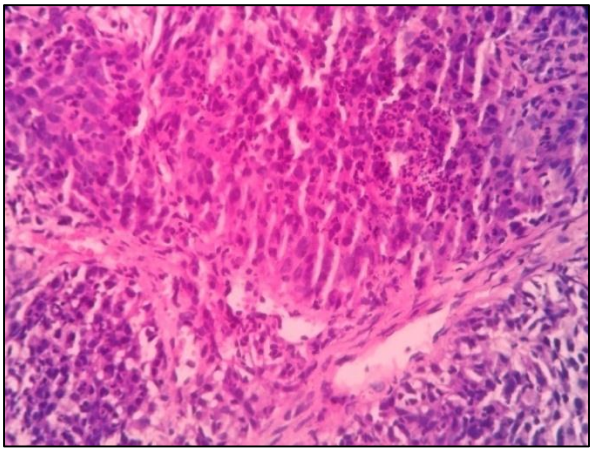


IHC NO: 424/17 (40 X) P63 DIFFUSE STRONG POSITIVITY

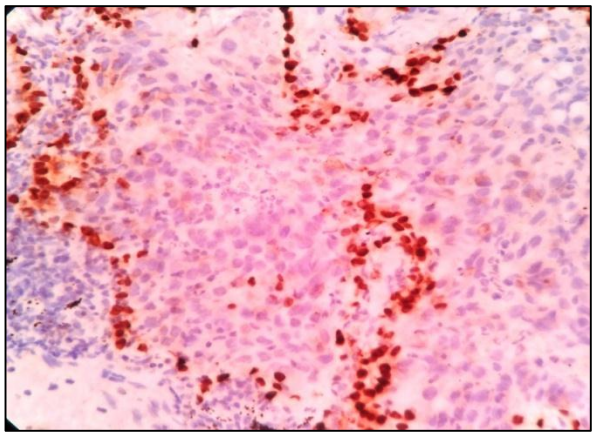


IHC NO: 424/17: (40 X) DIFFUSE STRONG POSITIVITY

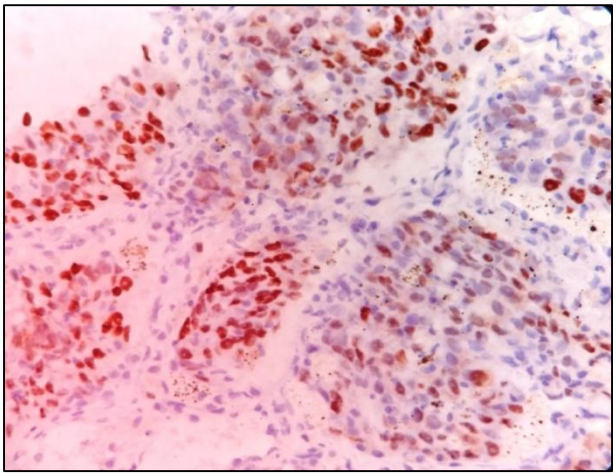
SQUAMOUS CELL CARCINOMA:



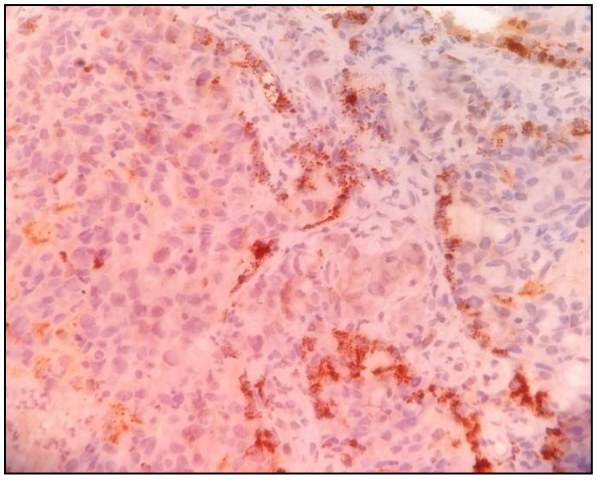
BX NO: 740/17: (40X: H&E) nests and sheets of round to polygonal cells with moderate cytoplasm with round dark staining nuclei.



IHC NO: 79/17: (40X) TTF1 NEGATIVE IN TUMOUR CELLS

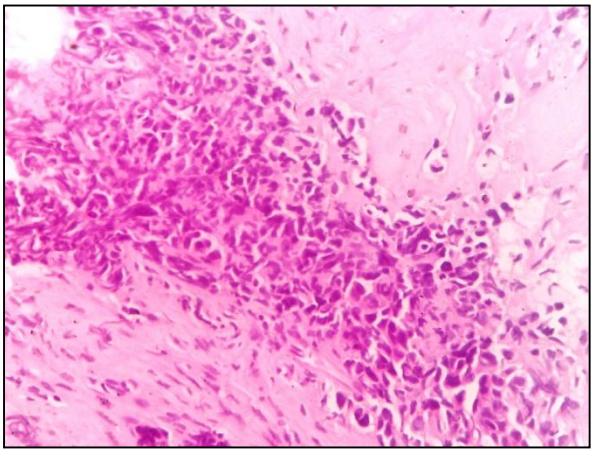


IHC NO: 79/17: (40 X) P63 POSITIVE IN TUMOUR CELLS

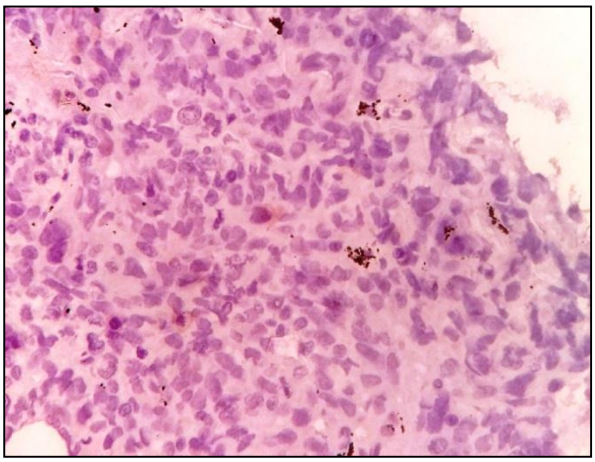


IHC NO: 79/17: (40 X) NAPSIN A NEGATIVE IN TUMOUR CELLS

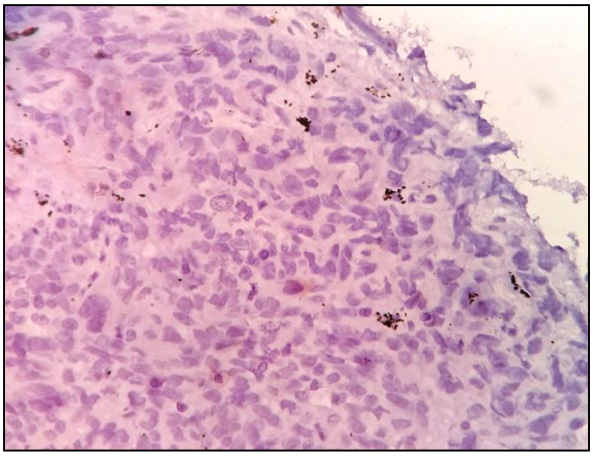
SMALL CELL CARCINOMA:



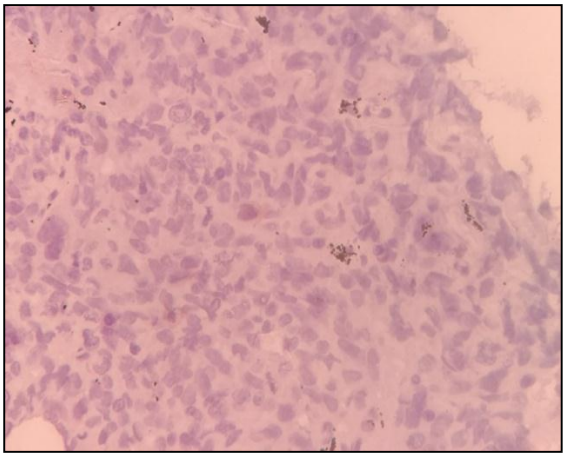
BX NO : 6112/16: (H&E: 40X) sheets of round to oval pleomorphic cell with irregular round hyperchromatic nuclei.



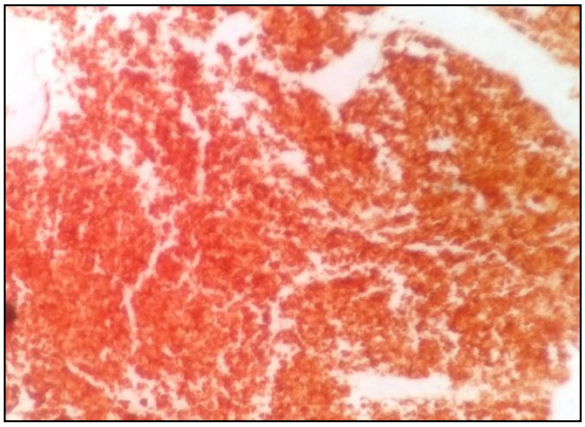
IHC NO: 616/16: (40X) TTF1 NEGATIVE IN TUMOUR CELLS.



NAPSIN A NEGATIVE IN TUMOR CELLS

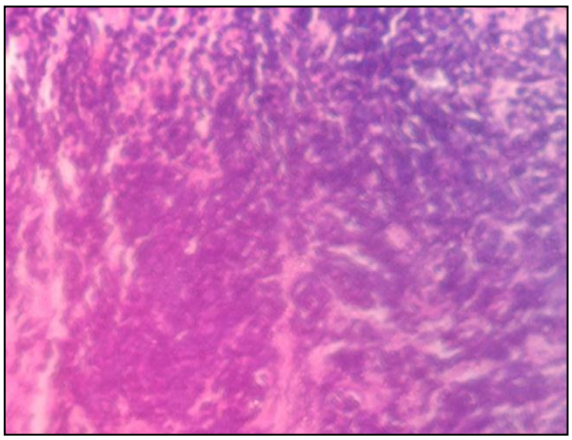


P 63 NEGATIVE IN TUMOR CELLS

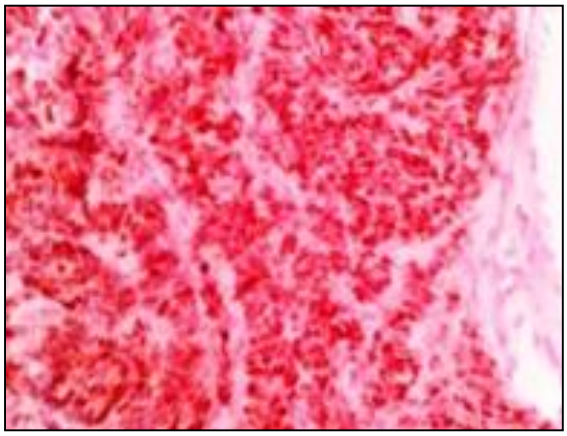


CHROMOGRANIN DIFFUSE STRONG POSITIVE IN TUMOUR CELLS

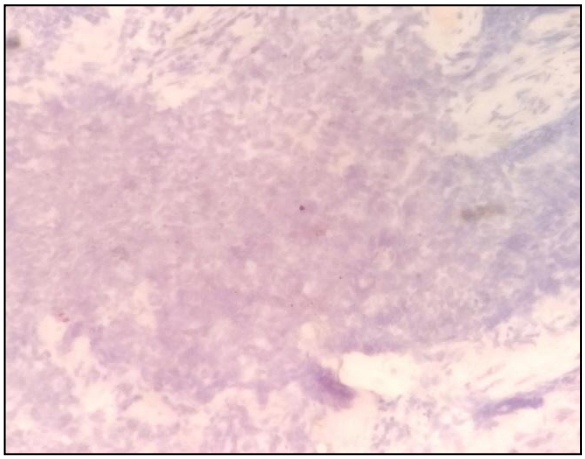
SMALL CELL CARCINOMA:



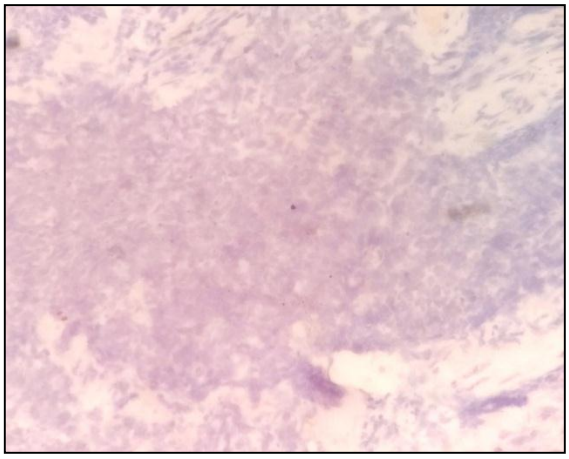
BX NO: 2430/17: (40 X, H&E) Sheets of round to oval cells withscant cytoplasm dark staining nuclei



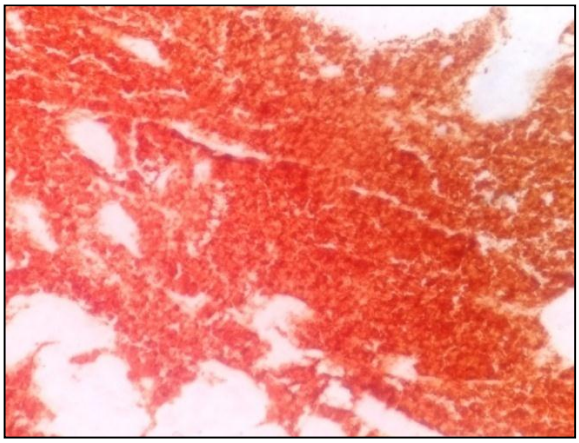
IHC': 312/17: (40 X) TTF1 POSITIVE IN TUMOUR CELLS



P63 NEGATIVE IN TUMOUR CELLS



NAPSIN A NEGATIVE IN TUMOUR CELLS



CHROMOGRANIN DIFFUSE STONG POSITIVE IN TUMOUR CELLS

ANNEXURES

ANNEXURE – 1

2015 WHO CLASSIFICATION OF LUNG EPITHELIAL TUMOURS:

Adenocarcinoma

- Lepidic adenocarcinomae
- Acinar adenocarcinoma
- Papillary adenocarcinoma
- Micropapillary adenocarcinomae
- Solid adenocarcinoma
- Invasive mucinous adenocarcinomae
- Mixed invasive mucinous and nonmucinous adenocarcinoma
- Colloid adenocarcinoma
- Fetal adenocarcinoma
- Enteric adenocarcinoma
- Minimally invasive adenocarcinoma
  - Nonmucinous / Mucinous

Preinvasive lesions

- Atypical adenomatous hyperplasia
- Adenocarcinoma in situe
  - Nonmucinous / Mucinous

Squamous cell carcinoma

Keratinizing squamous cell carcinoma

Nonkeratinizing squamous cell carcinoma

Basaloid squamous cell carcinomae

Preinvasive lesion

Squamous cell carcinoma in situ

Neuroendocrine tumors:

Small cell carcinoma

Combined small cell carcinoma

Large cell neuroendocrine carcinoma

Combined large cell NEC

Carcinoid tumors

Typical carcinoid tumor

Atypical carcinoid tumor

Preinvasive lesion

Diffuse idiopathic pulmonary NE hyperplasia

Large cell carcinoma

Adenosquamous carcinoma

Sarcomatoid carcinomas

Pleomorphic carcinoma

Spindle cell carcinoma

Giant cell carcinoma

Carcinosarcoma

Pulmonary blastoma

Other and Unclassified carcinomas

Lymphoepithelioma-like carcinoma

NUT carcinoma

Salivary gland-type tumors

Mucoepidermoid carcinoma

Adenoid cystic carcinoma

Epithelial-myoepithelial carcinoma

Pleomorphic adenoma

Papillomas

Squamous cell papilloma

Exophytic / Inverted

Glandular papilloma

Mixed squamous and glandular papilloma

Adenomas

Sclerosing pneumocytoma*e*

Alveolar adenoma

Papillary adenoma

Mucinous cystadenoma

Mucous gland adenoma

ANNEXURE – 2

PROFORMA:

CASE NO:

BIOPSY NO:

NAME:

AGE:

SEX:

IP NO:

CLINICAL DIAGNOSIS:

SYMPTOMS:

RISK FACTORS IF ANY:

CT/MRI FINDINGS:

FOB FINDINGS:

PROCEDURE DONE:

CYTOLOGY REPORT:

MICROSCOPY:

HISTOLOGICAL TYPE:

IHC PROFILE:

INFORMATION SHEET

Title : "Comparision Of Napsin A Versus Thyroid Transcription Factor-1 In The Typing Of Lung Carcinoma''

Your specimen has been accepted.

- We are conducting a study to compare utility of Napsin A with TTF-1 in the typing of lung carcinoma, Rajiv Gandhi government general hospital, Chennai and for that your specimen may be valuable to us.

- The purpose of this study is to compare the Utilization of Napsin A with TTF-1 in the typing of Lung carcinoma.

- We are selecting certain patients with lung carcinoma and we will 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 **: "Comparision Of Napsin A Versus Thyroid Transcription Factor -1 In The Typing Of Lung Carcinoma''**
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
"Study of Comparision Of Napsin A Versus Thyroid Transcription Factor -1 In The Typing Of Lung Carcinoma''

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the lung biopsy will be subjected to histopathological examination and special tests.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I 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_____

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BIBLIOGRAPHY

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MASTER CHART

BX NO	IHC NO	Age range	AGE	SEX	Symptoms	smoke	Side	Lobe	IMAGING	HPE DIAGNOSIS	TTF	NAPSIN	P63	CK5/6	NE marker	DIAGNOSIS
3870/15	316/15	61-70	68	m	No	Yes	R	UL	lung mass	Positive for malignancy	Negative	Negative		Positive		SqCC
4449/15	362/15	51-60	58	m	cough	yes	L	UL	collapse	Small cell carcinoma	Positive	Negative			Positive	SCC
8867/15	689/15	51-60	60	f	hemoptysis	no	R	LL	collapse	Squamous Cell carcinoma	Negative	Negative		Positive		SqCC
123/16	024/16	51-60	60	f	dyspnoea	no	L	ML	consolidation	Adenocarcinoma	Positive	Positive				AdCC
157/16	026/16	51-60	60	m	chest pain	yes	L	UL	lung mass	Squamous Cell carcinoma	Negative	Negative		Positive		SqCC
7198/16	721/16	61-70	61	m	No	yes	L	ML	lung mass	Squamous Cell carcinoma	Negative	Negative	Positive	Positive		SqCC
239/16	033/16	71-80	72	f	cough	no	R	UL	lung mass	Adenocarcinoma	Positive	Positive				AdCC
464/16	051/16	61-70	62	m	hemoptysis	yes	R	LL	collapse	Positive for malignancy	Positive	Positive		Negative		AdCC
642/16	061/16	41-50	45	m	dyspnoea	yes	R	ML	collapse	Squamous Cell carcinoma	Negative	Negative		Positive		SqCC
694/16	071/16	41-50	48	f	chest pain	no	R	LL	consolidation	Positive for malignancy	Negative	Negative		Positive		SqCC
926/16	090/16	61-70	70	f	cough	no	R	UL	lung mass	Non Small cell Lung Carcinoma	Positive	Positive	Positive	Negative		AdCC
1508/16	139/16	61-70	62	f	dyspnoea	no	L	LL	lung mass	Squamous Cell carcinoma	Negative	Negative		Positive		SqCC
1510/16	141/16	41-50	47	m	hemoptysis	yes	L	LL	lung mass	Adenocarcinoma	Positive	Positive				AdCC
1657/16	162/16	61-70	65	f	cough	no	R	UL	collapse	Non Small cell Lung Carcinoma	Positive	Positive				AdCC
1692/16	166/16	51-60	60	f	no	no	L	UL	lung mass	Adenocarcinoma	Positive	Positive		Negative		AdCC
1920/16	188/16	61-70	70	f	no	no	R	LL	lung mass	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC
2362/16	237/16	51-60	55	m	chest pain	yes	R	UL	collapse	Positive for malignancy	Negative	Negative			Positive	SCC
2732/16	274/16	51-60	59	m	dyspnoea	yes	R	UL	consolidation	Adenocarcinoma	Positive	Positive		Negative		AdCC
2788/16	275/16	51-60	60	m	cough	yes	R	UL	consolidation	Adenocarcinoma	Positive	Positive	Positive			AdCC
2977/16	293/16	41-50	42	m	hemoptysis	yes	R	UL	lung mass	Adenocarcinoma	Negative	Negative		Positive		SqCC

3413/16	330/16	51-60	55	m	hemoptysis	yes	L	ML	pleural effusion	Positive for malignancy	Negative	Negative		Positive		SqCC
3933/16	380/16	51-60	58	m	cough	yes	L	ML	lung mass	Positive for malignancy	Negative	Negative		Positive		SqCC
4454/16	444/16	71-80	77	m	dyspnoea	yes	R	UL	collapse	Adenocarcinoma	Positive	Positive		Negative		AdCC
4504/16	447/16	61-70	63	m	cough	yes	R	UL	lung mass	Positive for malignancy	Positive	Negative		Negative	Positive	SCC
4749/16	463/16	51-60	52	m	chest pain	yes	L	UL	lung mass	Positive for malignancy	Positive	Positive		Negative		AdCC
5173/16	505/16	51-60	55	m	no	no	R	UL	consolidation	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC
5513/16	528/16	51-60	55	m	no	yes	L	LL	lung mass	Adenocarcinoma	Positive	Positive	Positive			AdCC
5531/16	526/16	51-60	60	m	dyspnoea	no	R	UL	lung mass	Positive for malignancy	Positive	Positive	Positive			AdCC
6112/16	615/16	61-70	67	m	cough	yes	R	UL	pleural effusion	Small cell carcinoma	Negative	Negative			Positive	SCC
6113/16	616/16	51-60	60	f	chest pain	no	L	LL	lung mass	Positive for malignancy	Negative	Negative		Positive		SqCC
6299/16	656/16	71-80	73	m	hemoptysis	yes	R	UL	collapse	Positive for malignancy	Negative	Negative		Negative	Positive	SCC
6397/16	644/16	71-80	75	m	cough	yes	R	UL	lung mass	Squamous Cell carcinoma	Negative	Negative		Positive		SqCC
6865/16	679/16	61-70	65	m	chest pain	yes	L	LL	consolidation	Positive for malignancy	Negative	Negative		Positive		SqCC
7376/16	750/16	71-80	80	m	no	yes	R	UL	lung mass	Non Small cell Lung Carcinoma	Negative	Negative	Negative	Negative		NSCC
8133/16	824/16	51-60	55	f	no	no	L	LL	collapse	Non Small cell Lung Carcinoma	Negative	Negative	Positive			SqCC
10100/16	1032/16	51-60	60	m	dyspnoea	yes	R	UL	lung mass	Positive for malignancy	Negative	Negative	Positive			SqCC
50/17	012/17	41-50	50	f	chest pain	no	R	UL	collapse	Non Small cell Lung Carcinoma	Positive	Positive	Positive			AdCC
81/17	021/17	71-80	72	m	hemoptysis	yes	L	UL	collapse	Non Small cell Lung Carcinoma	Negative	Negative	Negative	Positive		SqCC
158/17	029/17	61-70	69	m	cough	yes	L	UL	lung mass	Non Small cell Lung Carcinoma	Positive	Positive	Positive			AdCC
740/17	079/17	61-70	65	f	cough	no	R	UL	lung mass	Non Small cell Lung Carcinoma	Negative	Negative		Positive		SqCC

839/17	120/17	61-70	62	m	dyspnoea	no	L	LL	consolidation	Small cell carcinoma	Negative	Negative		Positive	Negative	SqCC
1471/17	181/17	61-70	62	m	chest pain	yes	L	UL	consolidation	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC
1458/17	179/17	61-70	70	m	chest pain	yes	R	UL	lung mass	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC
1755/17	210/17	51-60	54	f	hemoptysis	no	L	LL	lung mass	Adenocarcinoma	Positive	Positive				AdCC
2299/17	280/17	31-40	40	f	cough	no	L	UL	pleural effusion	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC
2356/17	292/17	71-80	77	m	no	yes	L	UL	lung mass	Non Small cell Lung Carcinoma	Negative	Negative	Positive			SqCC
2430/17	312/17	61-70	65	m	cough	yes	R	UL	lung mass	Positive for malignancy	Positive	Negative		Negative	Positive	SCC
3207/17	388/17	61-70	68	m	dyspnoea	yes	R	UL	lung mass	Non Small cell Lung Carcinoma	Negative	Negative	Positive		Negative	SqCC
3283/17	390/17	41-50	50	m	chest pain	yes	L	UL	collapse	Non Small cell Lung Carcinoma	Positive	Positive	Negative			AdCC
3638/17	424/17	51-60	60	m	hemoptysis	no	R	UL	lung mass	Non Small cell Lung Carcinoma	Positive	Positive		Negative		AdCC