IMMUNOHISTOCHEMICAL STUDY OF PDL 1 EXPRESSION IN NON SMALL CELL LUNG CARCINOMA

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfilment of the regulations

for the award of the degree of

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BRANCH – III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI -600 003

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – TAMILNADU.

MAY 2020

CERTIFICATE

This is to certify that this dissertation entitled **"IMMUNOHISTOCHEMICAL STUDY OF PDL 1 EXPRESSION IN NON SMALL CELL LUNG CARCINOMA"** is the original work of **DR. VANI PRIYA .P,** in partial fulfillment of the requirement of M.D., (Branch III) in Pathology examination of the Tamilnadu DR.M.G.R Medical University to be held in May 2020.

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DECLARATION

I, **Dr. VANIPRIYA .P**, solemnly declare that the dissertation titled **"IMMUNOHISTOCHEMICAL STUDY OF PDL 1 EXPRESSION IN NON SMALL CELL 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.G. SELVAMBIGAI, MD, DCH.,** Professor of Pathology, Regional Institute of Ophthalmology, Madras Medical College. The dissertation is submitted to the Tamilnadu DR.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

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ACKNOWLEDGEMENT

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CERTIFICATE OF APPROVAL

To
Dr. P. VANI PRIYA
I Yr. PG in MD PATHOLOGY
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Dear Dr. P. VANI PRIYA,

The Institutional Ethics Committee has considered your request and approved your study titled **"IMMUNOHISTOCHEMICAL STUDY OF PDL1 EXPRESSION IN NON SMALL CELL LUNG CARCINOMA"** - **NO.10032018**

The following members of Ethics Committee were present in the meeting held on **27.03.2018**   conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar   :Chairperson
2. Prof.R.Jayanthi,MD.,FRCP(Glasg) Dean,MMC,Ch-3   : Deputy Chairperson
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13.Tmt.Arnold Saulina, MA.,MSW.,   :Social Scientist
14.Thiru K.Ranjith, Ch- 91   : Lay Person

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

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

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Sources included in the report:

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This is to certify that this dissertation work titled **"IMMUNOHISTOCHEMICAL STUDY OF PDL 1 EXPRESSION IN NON SMALL CELL LUNG CARCINOMA"** of the candidate **Dr. VANI PRIYA** with registration Number **201713010**for the award of **M.D PATHOLOGY** (Branch-III). I personally verified the urkund.com website for the purpose of Plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion and result shows **14%** of plagiarism in the dissertation.

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ABBREVIATIONS

AIS	-	Adenocarcinoma in situ
ALK	-	Anaplastic lymphoma kinase
BAC	-	Bronchoalveolar carcinoma
EGFR	-	Epidermal growth factor receptor
EWSR1	-	E wings sarcoma breakpoint region 1
GWA	-	Genome wide association study
IHC	-	Immunohistochemistry
MIA	-	Minimally invasive adenocarcinoma
NSCLC	-	Nonsmall cell lung carcinoma
PDL1	-	Programmed cell death ligand1
SNP	-	Single nucleotide polymorphisms
TTF 1	-	Thyroid transcription factor 1

CONTENTS

Introduction

INTRODUCTION

Lung cancer is one of the most frequently diagnosed cancer with high mortality rate. Incidence of lung cancer is also been increasing in the past two decades. The effect of smoking plays a major role in it. As with other cancers oncogenic mutations due to smoking related carcinogens results in neoplastic transformation. Cancers with Non smokers also occur with certain genetic mutations.

Mortality rates can be reduced if diagnosed early and targeting the molecular pathways. Not infrequently these tumors are identified with metastatic spread. Although much progress has recently been made for lung cancer such as low-dose spiral screening, minimally invasive techniques for diagnosis and treatment, advances in radiation therapy and molecularly targeted therapies, patients with lung cancer are still facing a relatively low 5-year survival rate.

Efforts to improve outcomes have not only led to a greater understanding of the etiology of lung cancer, but also the histologic and molecular characteristics of individual lung tumors.

Lung cancer and lung cancer–related deaths have been increasing in epidemic proportions throughout the world, with differences between countries largely explained by differences in smoking rates. A revolutionary change is the introduction of immunohistochemistry and genetic testing (EGFR mutation and ALK rearrangement) for many tumors, to guide clinicians in making personalized therapeutic decisions.

These changes impact not only the evaluation of tumors obtained as nonresection specimens in patients with advanced lung cancer, but also tumors obtained as resection specimens.

Aims and Objectives

AIMS AND OBJECTIVES

- To evaluate the expression of PDL 1 in Non small cell lung carcinoma.

- To correlate the immunohistochemical expression of these markers with various clinico pathological variables like age gender, family history, smoking alcohol, histological characteristics thereby aiding in prognosis and treatment.

Review of Literature

EPIDEMIOLOGY

In western countries the incidence of lung cancer in men increased in the 1980s, followed by decline, with similar patterns in women following 20 years later[1].In relation between racial and ethnic groups, particularly black men have mortality rates higher, than that of Asian Americans, the group with the lowest cancer-specific mortality[2] .

These disparities are due to discrepancy in cigarette smoking prevalence, and higher probability of diagnosis in late stage of diseases[3]. The incidence of smoking and lung cancer trends in UK is in analogue with US[4]. In women, incidence of lung cancer is decreased in US and UK, but rates peak in central and eastern Europe[5].

These differences are due to the usage of the tobacco and socioeconomic and educational differences, as well as diagnosis at end stages of disease, confer to difference in lung cancer trend and mortality within Europe[6].In Asia, Japan has highest measure and mortality rates from lung cancer, in equivalent to those of the US and Europe[7].

In emerging countries such as Brazil, Russia, India, China, and South Africa are recognized by their large and fast-growing economies where tobacco smoking peaked in the 1970s and lung cancer mortality in men highest in 1993 and continues to increase among women[8].

In India lung cancer incidence and mortality rates are lowest in the world[9]. The most common cancers in men are head and neck, gastric, and esophageal cancers, due to high usage of smokeless tobacco; in women the most common being cancers of cervix and breast[10].

Figure 1; Incidence of lung cancer with age at diagnosis
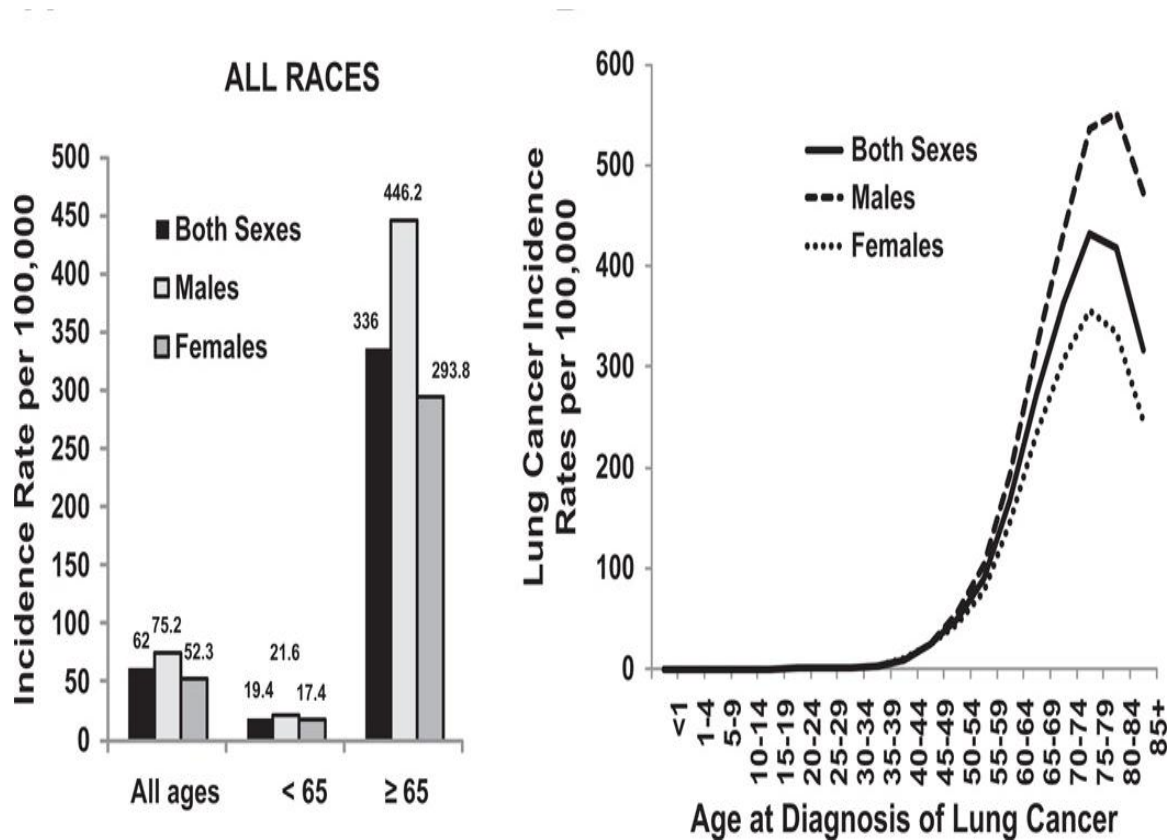
RISK FACTORS

1.FAMILY HISTORY

A linkage analysis spotted out a major susceptibility locus to chromosome 6q23–25[11] . Lung cancer risk also known to be increased within the framework of the Li–Fraumeni syndrome,which is due to germline mutation in the tumour-suppressor gene p53[12].

2.GENETIC POLYMORPHISMS

Current genome-wide association (GWA) studies have been useful to identify multiple genetic polymorphisms in lung cancer risk by studying a million tagging single-nucleotide polymorphisms (SNP) to identify common genetic variations.

GWA studies discuss only a portion of the overall genetic variations in lung cancer but the fact is only a minority of smokers develop cancer supports the hypothesis that genetic susceptibility play a major role in carcinogenesis[13].Three Most common loci identified are 15q25, 5p15 and 6p21 regions[14]. The 15q25 region contains six coding regions which are three cholinergic nicotine receptor genes (CHRNA3, CHRNA5, and CHRNB4), encoding nicotinic acetylcholine receptors in neuronal and other tissues.The locus in 5p15.33 represents a region that is TERT and CLPTM1L [15].

3.SMOKING

Tobacco smoke induces a carcinogenic effect on lung which was established in epidemiological studies done in early 1950s and has been supported by public health and regulatory authorities[16] . Passive smoking, is also identified as a known human carcinogen and is known to cause ~50,000 deaths annually. Passive smoking is a combination of two forms of smoke from burning tobacco: sidestream smoke, which comes from the end of a lighted source (cigarette, pipe, or cigar), that contains smaller particles which make their way into the cells and is rich in carcinogens, and the mainstream smoke which is exhaled by a smoker[17].

Figure2:Relation between nicotine addiction and lung cancer
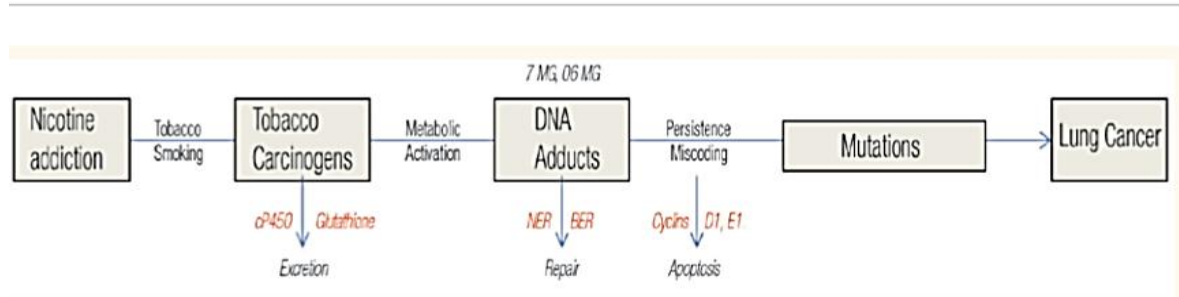
Around 20 potential carcinogens have been detected in a burning cigarette. The most common are the polycyclic aromatic hydrocarbons (PAH) and the tobacco specific N-nitrosamine 4-(methylnitrosamino) Asz-arenes, Dibenz(a,h)acridine, inorganic compounds like cadmium, chromium, nickel, arsenic, radioactive polonium (Po210) and organic compounds like butadiene. Nitrates are reduced to NH2– and NH3 while smoking[18].

Cigarette smoke also contains free radicals (FR) which initiate oxidative damage in animal models as well as humans, while catechol and hydroquinone play their roles in single strand DNA breaks caused by the release of FR[19].Smoking is strong association with squamous-cell carcinoma (SCC).currently due to manufactual change in production of cigarettes adenocarcinoma is more frequent than squamous cell carcinoma .

Low tar cigarettes and filter vents in cigarettes makes easy drawing of smoke and deeper inhalation so that it transports tobacco specific carcinogens toward the bronchoalveolar junction where adenocarcinoma mostly occur.. Secondly, blended reconstituted tobacco produces a higher concentration of N-nitrosamines[20].

Table 1: Difference between smokers and never smokers

	NEVER SMOKERS	**SMOKERS**
AETIOLOGY	Passive smoking Environmental exposure Occupationalexposure Genetic predisposition	Tobacco smoke
AGE	Relatively younger	Any
GENDER	Usually females	Either gender
HISTOLOGY	Usually adenocarcinoma	Any
CELL PATHWAYS	EGFR,P53,ROS 1	K RAS,P53

Smokers develop frequent side-effects during therapeutic courses of chemotherapy and radiotherapy (i.e. mucositis) and surgical complications. They also have poor post-surgical outcome . smoking also pave way to develop secondary cancers and chronic lung diseases, which will these patients unsuitable and vulnerable to further oncological interventions[21]. Retrospective studies reveal that non-smokers have greater benefit from TKI therapy compared to chronic smokers[22]. Hence smoking remains the most consistent agent for developing carcinoma and carries a definitive prognostic and predictive value.

4.DIET AND ALCOHOL

High intake of fried or red meat is known to increase the risk of lung cancer because of formation of nitrosamines during cooking[23]. Many studies established the risk of lung cancer with intake of either β-carotene or total carotenoids[24].

There is also evidence from recent studies that low levels of vitamin D are known to be associated with lung cancer risk[25]. There is strong association between alcohol consumption and tobacco smoking,however alcohol seems to a confounding factor in contribution to lung cancer[26].

5.CHRONIC INFECTIONS AND OTHER MEDICAL CONDITIONS

Chronic obstructive pulmonary disease and Tuberculosis are at increased risk for lung cancer[27]. Chlamydia pneumoniae infection consistently detected a positive association with lung cancer[28].

6. IONISING RADIATION

Radiation exposure has a strong correlation with development of lung cancer. This increased risk has been proved in atomic bomb survivors, and patients treated with radiotherapy [29]. Also smoking modifies the carcinogenic effect of radon. The lung cancer risk from radon and its end products comes from residential source rather than occupational exposure[30]

7. OCCUPATIONAL EXPOSURE

Occupational exposures play a major role in causation of lung cancer , and the risk of development of lung cancer is increased in workers employed in industries producing asbestos, silica, radon, heavy metals and polycyclic aromatic hydrocarbons[31].

ASBESTOS

Various forms of asbestos (chrysotile and amphiboles, including crocidolite, amosite and tremolite) are identified as carcinogenic to the human lung, although chrysotile due to its early clearance has lower effect in development of lung cancer[32].

METALS AND MIXED OCCUPATION EXPOSURES

Chromium compounds are known to increase the risk of lung cancer among workers employed in production of chromate ,chromate pigment, chromium platers and in production of ferrochromium . Also there is an increase in risk of lung cancer in manufacturers of nickel miners, smelters, electrolysis workers and high-nickel alloy. An increase in risk of lung cancer has also been established among people who exposed to drinking water containing Arsenic[33].

SILICA

An increase in risk of lung cancer has been consistently established in silicotic patients [34]. Studies in crystalline silica-exposed workers in foundries, pottery making, , diatomaceous earth mining,ceramics, brick making and stone cutting, develop silicosis are at increasd risk of development of lung cancer[35].

POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons are group of chemicals produced during combustion of organic material. The risk of lung cancer has been increased in several industries involving exposure to polycyclic aromatic hydrocarbons, such as aluminium production, coal gasification, coke production, iron and steel founding, tar distillation, roofing and chimney sweeping[36].

8. AIR POLLUTION

Indoor air pollution during coal burning in poorly ventilated houses is considered to be a major risk factor for lung cancer in non smoking women living in various regions of Asia[37].

OTHER RISK FACTORS

Oestrogen and progesterone receptors are normally expressed and in lung cancer cell lines, and oestradiol has a proliferative effect on the these type of cells[38].

GENETICS

Lung carcinomas arise from stem cells and progenitor cells which are capable of differentiation into one or various histologic cell types. These suggest that lung tumor cell differentiation is determined by the consequences of genetic transcriptional activation or repression events that reform embryonic lung development [39].

ADENOCARCINOMA

In evaluation of lung adenocarcinoma specimens it is found that there is focal regions of amplification and deletion included 14q13.3, 12q15, 8q24.21, 7p11.2, and 8q21.13 and also there is amplification of the transcription factor TTF-1 on chromosome 14q13.3. TTF-1 which encodes thyroid transcription factor 1, which belongs to member of the Nk-2 homeobox family that activates and binds the promoter of thyroid- and lung-specific genes[40].

SQUAMOUS CELL CARCINOMA

In Squamous cell carcinoma genomic alterations being loss of TP53 and loss of CDKN2A in vast majority of cases.Other highly prevalent alterations include mutations of NFE2L2/KEAP1/CUL3 , which activate a transcriptional program associated with response to oxidative stress, and mutations of the NOTCH1 gene, which is a critical regulator of squamous cell differentiation .Many SCC lung tumors display somatic alterations in one or more genes involved in PI3K/AKT signaling[41].

NEUROENDOCRINE TUMORS

This molecular distinction show two distinct clusters of small cell carcinoma and carcinoid tumors, with small cell carcinoma due to expression of proliferation markers such as MCM2, PCNA, MCM6, and thymidylate synthase[42].

CLINICAL FEATURES

Most aggressive neoplasm usually discovered in patients of elderly age group. With symptoms of few months in duration. However the outlook is still poor in spite of improvements in thoracic surgery, radiotherapy and chemotherapy. symptoms of metastasis include back pain, headache, hemiparesis and other CNS manifestations.

Table 2: clinical feaures with its pathologic basis

CLINICAL FEATURE	PATHOLOGIC BASIS
Cough	Involvement of central airways
Hemoptysis	Hemorrhage from tumor in airway
Chest pain	Extension of tumor into mediastinum
Pneumonia, abcess	Airway obstruction by tumor
Pleural effusion	Tumor spread to pleura
Hoarseness	Recurrent laryngeal nerve invasion
Dysphagia	Esophageal invasion
Diaphragm paralysis	Phrenic nerve invasion
SVC syndrome	SVC compression by tumor
Horner syndrome	Sympathetic ganglia invasion
Pericarditis	Pericardial involvement
Rib destruction	Chest wall involvement

DIAGNOSIS

1.IMAGING TESTS:

An X-RAY image of lungs may reveal an abnormal mass or nodule.A CT scan can reveal small lesions of lung which might not be detected on an X-RAY.

2.SPUTUM CYTLOGY

Sputum cytology under the microscope may reveal the presence of tumor cells.

3.TISSUE BIOPSY

The shift in epidemiology of lung cancer from central small cell and squamous Cell carcinoma to peripheral adenocarcinoma made an impact in traditional bronchoscopy as primary diagnostic tool in lung cancer. Radiological guided Transthoracic biopsy is increasingly employed in lung cancer diagnosis[43].

The Endobronchial ultrasound probe is inserted into the bronchoscope and advanced to various segments of the target lobe until the location of the nodule for sampling is determined. Traditional bronchoscopy techniques are used to perform a biopsy of the located lesion[44].

Table 3 Sensitivity of various diagnostic modalities

DIAGNOSTIC MODALITY	SENSITIVITY % CENTRAL LESION	SENSITIVITY % PERIPHERAL LESION
SPUTUM CYTOLOGY	71	49
BRONCHIAL WASH	47	43
BRONCHIAL BRUSH	56	54
TRANSTHORACIC BIOPSY	-	90
CRYOBIOPSY	95	74

HISTOLOGICAL DIAGNOSIS OF LUNG ADENOCARCINOMA

It is divided into two main histological groups: small cell lung carcinoma (SCLC, 15% of all lung cancers) and non-SCLC (NSCLC, 85% of all lung cancers). NSCLCs are further subcategorized into adenocarcinoma, squamous cell carcinoma (SqCC), and large cell carcinoma.Many major revisions were made in this classification to update on recent molecular pathology of lung cancer.

Adenocarcinoma defined as carcinoma with an acinar/tubular structure or mucin production, whereas Squamous Cell Carcinoma was defined as carcinoma with keratinization or intercellular bridges. If carcinoma lacking evidence of glandular differentiation then it is poorly differentiated carcinoma . The WHO classification was updated based on newly identified molecular profiles and targetable genetic alterations in lung cancer.

One of the great advances in this classification is that there are   therapeutic decisions are based on unique histological characteristics of the patient's tumor. This has lead to a new classification to classify Non small cell lung carcinoma into specific pathologic subtypes that is adenocarcinoma or   squamous cell carcinoma as this designates eligibility for   specific molecular testing and therapeutic strategies. Primary Adenocarcinoma was further   by confirmed by EGFR mutations and rearrangements of ALK and ROS1[45]

WHO CLASSIFICATION OF LUNG CANCER 2015

EPITHELIAL TUMORS

- Adenocarcinoma

- Lepidic adenocarcinoma

- Acinar adenocarcinoma

- Papillary adenocarcinoma

- Micropapillary adenocarcinoma

- Solid adenocarcinoma

- Invasive mucinous adenocarcinoma

- Mixed invasive mucinous and

- nonmucinous adenocarcinoma

- Colloid adenocarcinoma

- Fetal adenocarcinoma

- Enteric adenocarcinoma

- Minimally invasive adenocarcinoma

-       Nonmucinous

-       Mucinous

-    Preinvasive lesions

-     Atypical adenomatous hyperplasia

-     Adenocarcinoma in situ

-       Nonmucinous

-       Mucinous

-    Squamous cell carcinoma

-     Keratinizing squamous cell carcinoma

-     Nonkeratinizing squamous cell carcinoma

-     Basaloid squamous cell carcinoma

-     Preinvasive lesion

-       Squamous cell carcinoma in situ

-   Neuroendocrine tumors

-    Small cell carcinoma

-     Combined small cell carcinoma

-    Large cell neuroendocrine carcinoma

-     Combined large cell neuroendocrine carcinoma

-    Carcinoid tumors

-     Typical carcinoid tumor

-     Atypical carcinoid tumor

-    Preinvasive lesion

-     Diffuse idiopathic pulmonary neuroendocrine cell 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

- Alveolar adenoma

- Papillary adenoma

- Mucinous cystadenoma

- Mucous gland adenoma

Mesenchymal tumors

- Pulmonary hamartoma

- Chondroma

- PEComatous tumors

- Lymphangioleiomyomatosis

- PEComa, benign

- Clear cell tumor

- PEComa, malignant

- Congenital peribronchial myofibroblastic tumor Diffuse pulmonary lymphangiomatosis

- Inflammatory myofibroblastic tumor

- Epithelioid hemangioendothelioma

- Pleuropulmonary blastoma

- Synovial sarcoma

- Pulmonary artery intimal sarcoma

- Pulmonary myxoid sarcoma with *EWSR1–CREB1* translocation

- Myoepithelial tumors

- Myoepithelioma

- Myoepithelial carcinoma

Lymphohistiocytic tumors

- Extranodal marginal zone lymphomas of mucosa-associated

- Lymphoid tissue (MALT lymphoma) Diffuse large cell lymphoma

- Lymphomatoid granulomatosis

- Intravascular large B cell lymphoma

- Pulmonary Langerhans cell histiocytosis

- Erdheim–Chester disease

Tumors of ectopic origin

- Germ cell tumors

- Teratoma, mature

- Teratoma, immature

- Intrapulmonary thymoma

- Melanoma

- Meningioma, NOS

- Metastatic tumors

The 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart has just been updated with important changes from the 2004 WHO classification.

The most significant changes in this edition involve

1. use of immunohistochemistry in entire classification,

2. a new evolution in genetic studies to prognosticate and therapeutic strategies

3. a new classification for small biopsies

4. different approach to lung adenocarcinoma as proposed by the 2011 Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification,

5. restricting the diagnosis of large cell carcinoma only to resected tumors that lack any clear morphologic or immunohistochemical differentiation with reclassification of the remaining former large cell carcinoma subtypes into different categories,

6. reclassifying squamous cell carcinomas into keratinizing, nonkeratinizing, and basaloid subtypes with the nonkeratinizing tumors requiring immunohistochemistry proof of squamous differentiation,

7. grouping of neuroendocrine tumors together in one category,

8. adding NUT carcinoma,

9. changing the term sclerosing hemangioma to sclerosing pneumocytoma,

10. changing the name hamartoma to pulmonary hamartoma,

11. creating a group of PEComatous tumors that include

    (a) lymphangioleiomyomatosis,

    (b) PEComa, benign (with clear cell tumor as a variant) and.

    (c) PEComa, malignant,

12. introducing the entity pulmonary myxoid sarcoma with an EWSR1-CREB1 translocation,

13. adding the entities myoepithelioma and myoepithelial carcinomas, which can show EWSR1 gene rearrangements,

14. recognition of usefulness of WWTR1-CAMTA1 fusions in diagnosis of epithelioid hemangioendotheliomas,

15. adding Erdheim-Chester disease to the lymphoproliferative tumor, and

16. a group of tumors of ectopic origin to include germ cell tumors, intrapulmonary thymoma, melanoma and meningioma

LUNG CANCER CLASSIFICATION IN SMALL BIOPSIES

New criteria has been implemented in recent classification because two thirds of lung cancer patients are diagnosed in advanced stages, and they are confirmed in only small biopsies[46]. Non−small cell carcinomas that show no adenocarcinoma or squamous cell carcinoma morphology or any immunohistochemical markers are regarded as NSCC not otherwise specified (NOS) because of the possibility of metastatic carcinoma .

Table 4: Terminology and criteria or adenocarcinoma,squamous cell carcinoma in small biopsies and cytology compared with resected specimens.

Small Biopsy/Cytology Terminology	Morphology/Stains	2015 WHO Classification in Resection Specimens
Adenocarcinoma (describe identifiable patterns present)	Morphologic adenocarcinoma patterns clearly present	Adenocarcinoma predominant pattern: lepidic, acinar, papillary, solid, and micropapillary
• Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded) • Invasive mucinous adenocarcinoma (describe patterns present; use term mucinous adenocarcinoma with lepidic pattern if pure lepidic pattern) Adenocarcinoma with colloid features Adenocarcinoma with fetal features Adenocarcinoma with enteric features		• Minimally invasive adenocarcinoma, adenocarcinoma in situ, or an invasive adenocarcinoma with a lepidic component • Invasive mucinous adenocarcinoma • Colloid adenocarcinoma Fetal adenocarcinoma Enteric adenocarcinoma

NSCC, favor adenocarcinoma	Morphologic adenocarcinoma patterns not present but supported by special stains (i.e., TTF-1 positive)	Adenocarcinoma (solid pattern may be just one component of the tumor)
Squamous cell carcinoma	Morphologic squamous cell patterns clearly present	Squamous cell carcinoma
NSCC, favor squamous cell carcinoma[c]	Morphologic squamous cell patterns not present but supported by stains (i.e., p40-positive)	Squamous cell carcinoma (nonkeratinizing pattern may be a component of the tumor)
NSCC NOS	No clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern	Large cell carcinoma

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LUNG CANCER CLASSIFICATION IN RESECTION SPECIMEN

Major Changes in Adenocarcinoma Classification WHO classification for resected tumors including

1) discontinuing the terms bronchioloalveolar carcinoma (BAC)

2) the addition preinvasive lesion

3) addition of MIA,

4) classification of invasive adenocarcinomas according to the predominant subtype

5) use of the term "lepidic" for a noninvasive component (previously classified as BAC) present as part of an invasive adenocarcinoma;

6) introducing the term "invasive mucinous adenocarcinoma" for adenocarcinomas formerly classified as mucinous BAC, excluding tumors that meet criteria for AIS or MIA

7) discontinuing the subtypes of clear cell and signet ring adenocarcinoma and recognizing these as a feature when any amount is present, however small;

8) discontinuing the term mucinous cystadenocarcinoma and including these under the category of colloid adenocarcinoma[47].

Figure: 3A. Lepidic pattern of Adenocarcinoma *B.* Correlation with the computed tomography (CT) scan shows a 2.5-cm pure ground glass nodule with no solid component
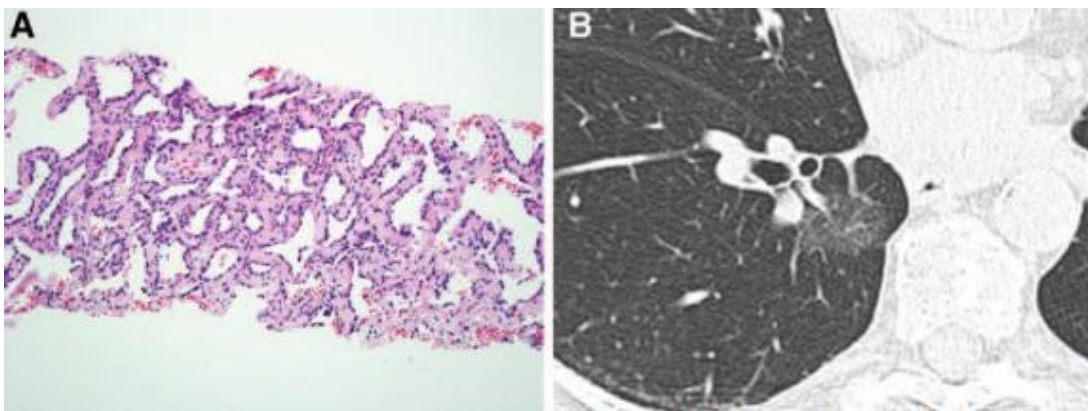
Figure 4:shows histology of Invasive Adenocarcinoma nos
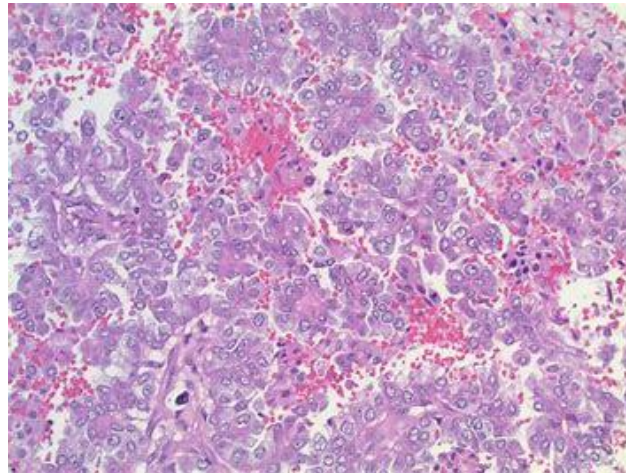
SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is subtyped into keratinizing, nonkeratinizing, and basaloid types. Tumors are classified as keratinizing subtype based on amount of keratinization is present and basaloid squamous cell carcinoma if this component is greater than 50% of the tumor, regardless of the presence of any keratinization. In tumors with 50% or less of a basaloid component, this can be acknowledged in the diagnosis "with basaloid features."1 There is no prognostic significance to keratinizing versus nonkeratinizing squamous carcinomas[48]. Some studies suggest a poorer prognosis for basaloid squamous cell carcinomas[76]. So in the absence of unequivocal keratinization, immunohistochemistry with positive squamous markers such as p40 or p63 is required to diagnose surgically resected nonkeratinizing squamous cell carcinoma. As with adenocarcinoma, clear cell change is also regarded as a cytologic feature that can occur in keratinizing or nonkeratinizing squamous cell carcinoma.

Figure 5 :histology of squamous cell carcinoma
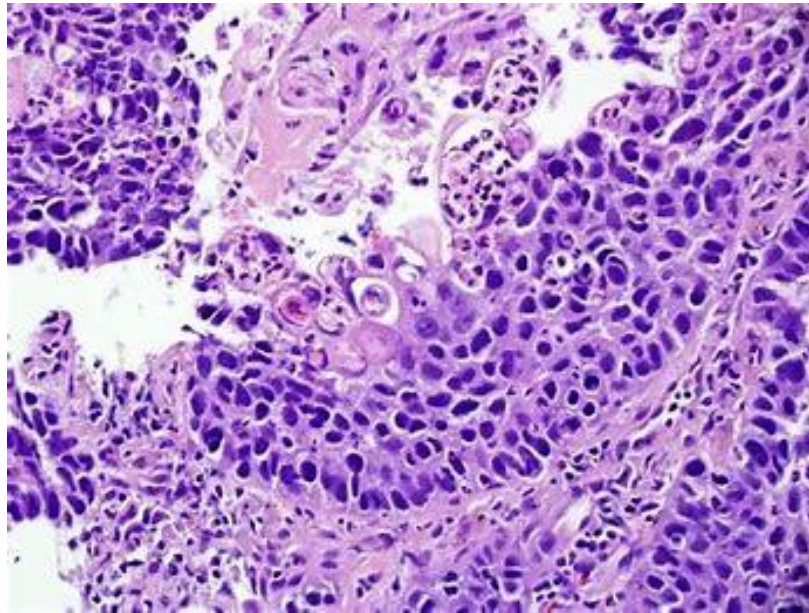
IMMUNOHISTOCHEMISTRY

Albert Coons et al in 1941 demonstrated antigens on tissue sections by using an antibody linked to fluorescent label.Nakane and Pierce et al in 1966 reported the use of secondary antibody that was conjugated with peroxidase enzyme.Sternberger in 1979 described peroxidase anti peroxidase method.Hsu et al in 1981 defined the method of avidin -biotin-peroxidase complex abbreviate as ABC method.

Steps:

1. 4 Micron thick sections were cut from formalin fixed paraffin embedded tissue blocks and transferred to positively charged glass slides. The glass slides were incubated at 37 degree celcius overnight

2. Deparaffinisation :

The slides were immersed in xylene for 15 minutes for 2 changes and immersed alcohol for 5 minutes for 2 changes .after which the slides will be kept in distilled water for 5 minutes

3. Antigen retrieval:

To unmask the antigenic determinants in fixed tissue   sections following methods are generally used:

Proteolytic enzyme digestion , microwave ovens, heating plate, pressure cookers, autoclaves, and water baths.

In our institute this was done by using pressure cooker with the slides immersed in Tris Buffer which is preheated for 10 minutes at 160degree celcius.After preheating the slides were placed inside the tris buffer under pressure for 15 minutes at 130 degree celcius and then the temperature is reduced by maintaining 60degree for 5 minutes and then gradually cooled to room temperature. The cool slides were then washed with distilled water for 5 mins x 2 changes

4. Peroxide application:

When using peroxidase antiperoxidase system in detection step, blocking of endogenous peroxidase activity is indispensable[104]. Diluted hydrogen peroxide as 3% is widely used for blocking endogenous peroxidase activity by washing the slides twice after the slides were washed with wash buffer

5. Application of primary antibody :

After placing the slides in wash buffer for 5 minutes the primary antibody PDL 1 each added to one slide and incubated at room temperature for 40 minutes. After 40 minutes the slides were washed in wash buffer for 5 mins for 2 changes.

6. Application of secondary antibody :

Then HRP conjugated polymer was added and washed in wash buffer(2changes for 2 minutes) after 12minutes.

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect method. In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are fluoro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex

or avidin biotin complex further increases the sensitivity of immunohistochemical stains.

7. Application of chromogen:

The sections were covered with Diamino benzidine (DAB) chromogen .(DAB was prepared by diluting 1 drop of DAB chromogen in 1 ml of DAB buffer). The slides were washed in distilled water after 5 minutes

8. Counter staining with hematoxylin and washed in running tap water for 15 minutes ,the slides were dried and mounted .

IMMUNOHISTOCHEMISTRY IN NON SMALL CELL LUNG CARCINOMA

The panel of markers for differentiating non small cell carcinoma include p63,CK5/6TTF 1,Napsin and CK 7.In terms of specific staining patterns, coarse granular cytoplasmic staining was considered positive for Napsin A. Nuclear staining was considered positive for TTF-1 and P63. Cytoplasmic staining was considered positive for CK7 and CK5/6. In Squamous Cell Carcinomas, TTF-1 and Napsin A could stain entrapped bronchial epithelial cells and alveolar macrophages other than tumor cells. Tumor cells were considered negative for TTF-1 and Napsin A. In Squamous Cell Carcinoma P63 and CK5/6 are commonly used markers.

Human TP63 gene is located on the chromosome 3q2729; and the expression of the gene produces the full length protein P63and the truncated protein P40.CK5/6 is expressed in neoplasms of epithelial origin ,including Squamous Cell Carcinoma, mesothelial carcinoma and urothelial carcinoma[49]. CK7, TTF-1 and Napsin A are the most commonly used primary lung Adenocarcinoma markers Although CK7 has been used for decades to identify lung ADCs, its suboptimal sensitivity and specificity [50].TTF-1 is a nuclear transcript factor that is expressed in epithelial cells of the lung and thyroid. In the lung, it regulates the expression of genes involved in production of surfactant. Napsin A is a relatively new marker for primary lung Adenocarcinoma[51].

Figure 6;:IHC panel of Non small cell lung carcinoma
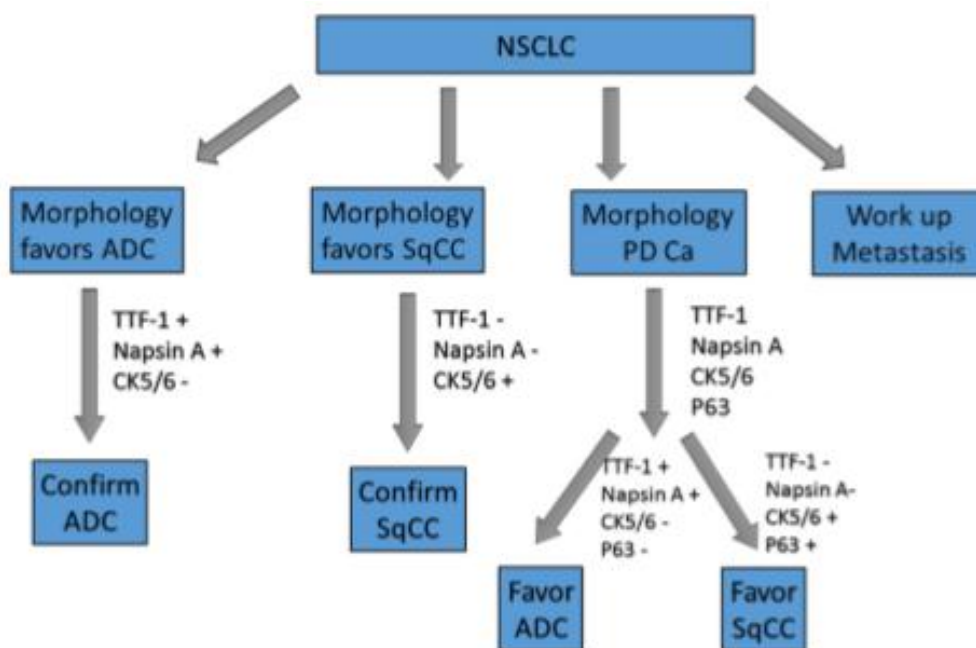
PROGRAMMED CELL DEATH LIGAND 1

Programmed death 1 (PD-1) and its ligands, PD-L1 and PD-L2, have emerged as critical inhibitory signaling pathways that regulate T cell response and maintain peripheral tolerance. PD-1 signaling inhibits alloreactive T cell activation, and can promote induced regulatory T cell development. Both programmed cell death ligand-1 (PD-L1) and PD-L2 are members of the B7 family and bind to PD-1. It can be expressed in various cells including T cells, epithelial cells, and endothelial cells.

Figure 7:shows PD1 and PDL 1receptors
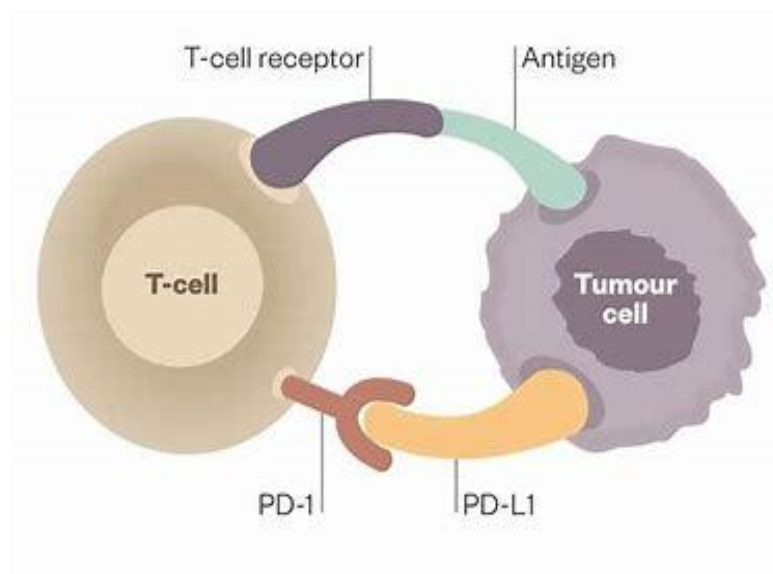
Although CTLA-4 works in the initial phase of T-cell recognition, the PD-L1 pathway plays a role in the latter phase of the immune response, such as within inflammatory tissues, to regulate T-cell function and prevent autoimmunity. In cancer tissues, PD-1 is upregulated on Tumor infiltrating Lymphocytes, while the ligand, PD-L1, is expressed on many cancer cell types[52].

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A major role of the PD-1:

PD-L pathway is the inhibition of T cell function by engagement of the PD-1 receptor on T cells by PD-L1 or PD-L2 on antigen presenting cells (APCs) There appears to be an intrinsic adaptive response, in that cancer cells express PD-L1 to escape from immune surveillance via ligation of PD-1-expressed Tumor infiltrating lymphocytes.PD-L1 expression may be present on dendritic cells, macrophages, mast cells, and T- and B-lymphocytes, as well as on endothelial and tumor cells .PD-L1 has two small hydrophilic regions for binding sites of IHC detection antibodies.

Figure 8 :Mechanism of PD1 and PDL1 pathway
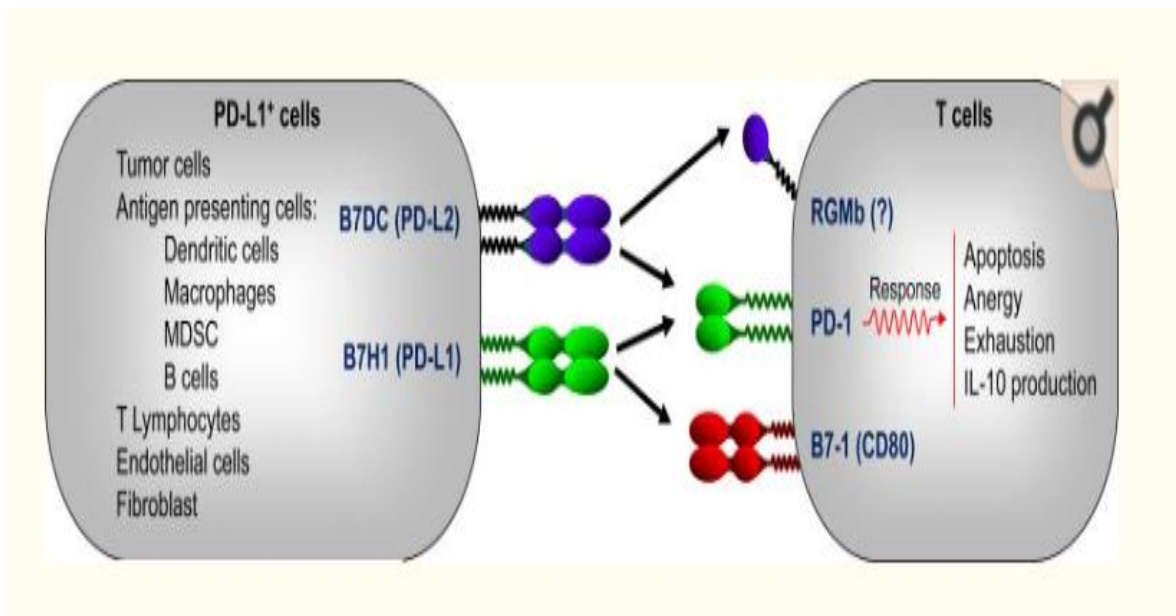
PDL1 BLOCKADE AS THERAPEAUTIC STRATEGY

The negative regulation of lymphocytes by PD-1 is mediated by the interaction with its ligands and B7-like proteins, PD-L1 and PD-L2. Many tumors have increased expression of PD-L1, including squamous cell carcinoma, colon adenocarcinoma, and breast adenocarcinoma. Transgenic expression of PD-L1 on tumors increase tumorigenesis and invasiveness in vivo; its overexpression also makes tumor cells less susceptible to specific CD8 T cell–mediated lysis in vitro. In mouse melanoma models, tumor growth is transiently abrogated in PD-1 knockout mice or with treatment with antibodies blocking the interaction between PD-L1 and its receptor PD-1. Initial studies have reported not only better tolerability than with prior immunotherapies but also more impressive clinical efficacy than would be expected from preclinical mouse models[53].

SMOKING EFFECTS AND PDL1

Smoking is considered a serious problem that leads to diseases particularly non-small cell lung cancer (NSCLC). Smoking cessation is critical for the prevention of lung cancers. PD-L1 expression was correlated with exposure to smokers and never smokers. In addition, higher expression levels on PD-L1 was found higher in smokers [54]. This is because exposure to tobacco produce more neoantigens resulting in higher PDL1 expression.furthermore the inflammatory response produced by smoking induces interferon gamma which will further produce PDL 1 expression[55]. smokers with Non small cell lung carcinoma have a better treatment response with anti PDL1 drugs than never smokers. Smoking is a

major risk factor in developing lung cancer. However, its potential for inducing chemotherapy sensitivity has not been adequately studied. Smoking status is also associated with mortality of lung cancer and in treatment effectiveness[54]. From an immunological perspective, it is being reported that smoking significantly associated with maturation and function of dendritic cells[55]

PD 1/PDL 1 IN IMMUNOTHERAPY

PD-1/PDL-1 signaling pathway plays an definitive role in tumor immune escape. PD-1 functions in the effector phase of T cell activation. It inhibits T cell immune activity and promotes tumor growth by binding to PDL-1. Therefore, blocking the binding of PD-1 and PDL-1 can reactivate the immune activity of T cells thereby enhancing the killing effect of the patient's immune system on tumor cells[56].

T cells that are activated produce PD-1, and inflammatory cytokines which will induce tumor cell proliferation.These cells produce PDL-1, and PD-1 on the surface of T cells and binds to PDL-1 on the surface of tumor cells, which will further inhibit T cell proliferation and differentiation, leading to recuction of T cell function and apoptosis . These effects cause tumor cells to escape the immune system, promoting the survival of tumor cells and creating a microenvironment of tumor immunity further adding to the tumor cell survival and progresson[57].

CTLA-4 is a key inhibitory receptor that affects T cell function, and is mainly expressed on the surface of activated CD4+ T cells, CD8+ T cells. CTLA-4 is involved in the negative regulation of T cell activation, inactivating T cells, and thus tumor cells survive and progress[58]. tumor cells express PD-L1 can inhibit t cell-mediated immune response and can progress to distant metastasis[59]. Therapies targeting the programmed cell death receptor PD-1 and its ligand PD-L1 have revealed promising effects.

In the presence of PD-1 inhibitors or PDL-1 inhibitors, PD-1 and PDL-1 cannot bind to produce inhibitory effects on T cells. Continuous activation of T cells destroys the immunosuppressive microenvironment for the survival of tumor cells and produces a strong immune effect on tumor cells, thereby killing them .

Materials and Methods

MATERIALS AND METHODS

In a study period of 2 years from June 2017 to June 2019 we performed both prospective and retrospective analysis of patients diagnosed to have biopsy proven.

Non small cell lung carcinoma in Institute of pathology ,Madras medical college, Rajiv Gandhi Government General Hospital, Chennai. In this study period we received 23,348 specimens for histopathological examination.Of the total specimens 42 cases of pneumonectomy specimens and 938 cases of small biopsies received. All 42 cases were subjected to histopathological examination and 36 cases were turned out to be Non small cell lung carcinoma 6 cases showed features of benign and non neoplastic conditions.

INCLUSION CRITERIA

All cases histologically diagnosed Non small cell lung carcinoma with age > 18 years .

EXCLUSION CRITERIA

1. Patients aged less than 18 years.

2. Lack of representative tumor tissue

3. Patient treated with prior chemotherapy

METHOD OF DATA COLLECTION

Detailed history of the cases regarding age,sex, family history, smoking alcohol, type of procedure done were obtained for all cases cases reported during study period from surgical pathology records. Representative sections were submitted for routine histopathological examination. The following clinical and pathologic parameters were evaluated Age, sex. smoking alcohol and histology. Among the 36cases 30 cases were selected randomly and analyzed for immunohistochemical expression of PDL 1.

IMMUNOHISTOCHEMICAL EVALUATION

Immunohistochemical analysis of PDL1 were performed in paraffin embedded tissue samples using super sensitive polymer HRP system based on non biotin polymer technology.4 micron thick sections were cutfrom formalin fixed paraffin embedded tissue samples and transferred onto positively charged slides heat induced antigen retrieval was done.The antigen was bound with mouse monoclonal antibody against PDL1 and then detected by adding secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidine substrate.

INTERPRETATION AND SCORING

The antibody treated slides were analyzed for the presence or absence of reaction, localization of staining pattern and intensity of staining.

EVALUATION OF PDL1 STAINING

Immunohistochemical analysis of PDL 1were performed in paraffin embedded tissue samples using super sensitive polymer HRP system based on non biotin polymer technology. 4 micron thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred onto positively charged slides.Heat induced antigen retrieval was done.The antigen was bound with mouse monoclonal antibody PDL 1 and then detected by adding secondary antibody conjugated with horse radish peroxidase -polymer and diaminobenzidine substrate

ANTIGEN	VENDOR	SPECIES (CLONE)	DILUTION	POSITIVE CONTROL
PDL 1	Pathnsitu	Mouse	Ready To Use	PLACENTA,TONSIL

The antibody treated slides were analyzed for the presence or absence of reaction, localization of staining pattern, percentage of cells stained and intensity of the reaction

EVALUATION OF PDL1 STAINING:

For assessing the positivity scoring system Rizviet al is followed. According to this system only nuclear staining pattern with appropriate staining of internal and external controls was considered positive (Retained expression)and the level of PDL 1 expression was assessed semi quantitatively by the intensity and percentage of cells stained on a scale of 0-3+.Cytoplasmic staining was considered nonspecific.

SCORE	LOCALIZATION	PERCENTAGE OF CELLS STAINED
Score <1%	Membranous	Indeterminate
Score 1-50%	Membranous	More than 10%
Score>50%	Membranous	More than 50%
negative	Nil	No cells stained

There is an entity called **INDETERMINATE** when less than 1 % tumor cell nuclei show positive staining which indicates further workup in that patient.

Since the immunohistochemical expression of these markers can even be focal lack of staining in a small biopsy sample may not be reliably interpreted as loss of protein in the entire tumor, thus limiting the utility of IHC in small biopsy specimens.

STATISTICAL ANALYSIS

The statistical analysis was performed with IBM SPSS statistical package for the social sciences version 20.an initial analysis of collected variables was performed.immunohistochemical analysis of PDL1 was analyzed with variables like age,gender,smoking,alcohol,histology.Pearson chi square test was used in analyzing these variables.Immunohistochemical expression of PDL1 was compared with histology and analyzed for statistical correlation.In the present study ,the P value below 0.05 is considered significant.

Observation & Results

OBSERVATION AND RESULTS

During the study period of 24 months from June 2017-june 2019 biopsy proven Non small cell lung cancer cases were subjected to PDL1 expression immunohistochemically

Table 5. Total number of Non small lung carcinoma diagnosed during study period

HISTOLOGIC TYPE	FREQUENCY	PERCENT
ADENOCARCINOMA	17	56.7
SQUAMOUS CELL CARCINOMA	13	43.3
Total	30	100.0

In our study among 30 cases the commonest histologic type is Adenocarcinoma NOS constituting about 56% in 17 cases followed by squamous cell carcinoma in 13 cases constituting 43%.other histologic subtypes like small cell carcinoma,sarcomatoid carcinoma were also reported in our institute.

AGE WISE INCIDENCE OF CASES

Table 6:Age wise incidence of Non small cell lung carcinoma

AGE	FREQUENCY	PERCENT
40 to 50 Years	4	13.3
51 to 60 Years	16	53.3
61 to 70 Years	10	33.3
Total	30	100.0

The highest incidence of Non small cell lung carcinoma were found in the age group of 51-60 years constituting about 53% in 16 cases , followed by 61-70 years constituting 33.3% in 10 cases.The youngest age of presentation of non small cell lung cancer was 46 years in this study.

AGE WISE INCIDENCE OF HISTOLOGIC SUBTYPES

Incidence of squamous cell carcinoma and Adenocarcinoma is highest in the age group of 51-60 years,followed by 61-70 years.there is no statistical correlation between age and histology of Non small cell lung carcinoma

Chart 1 : Age wise incidence of histologic subtypes
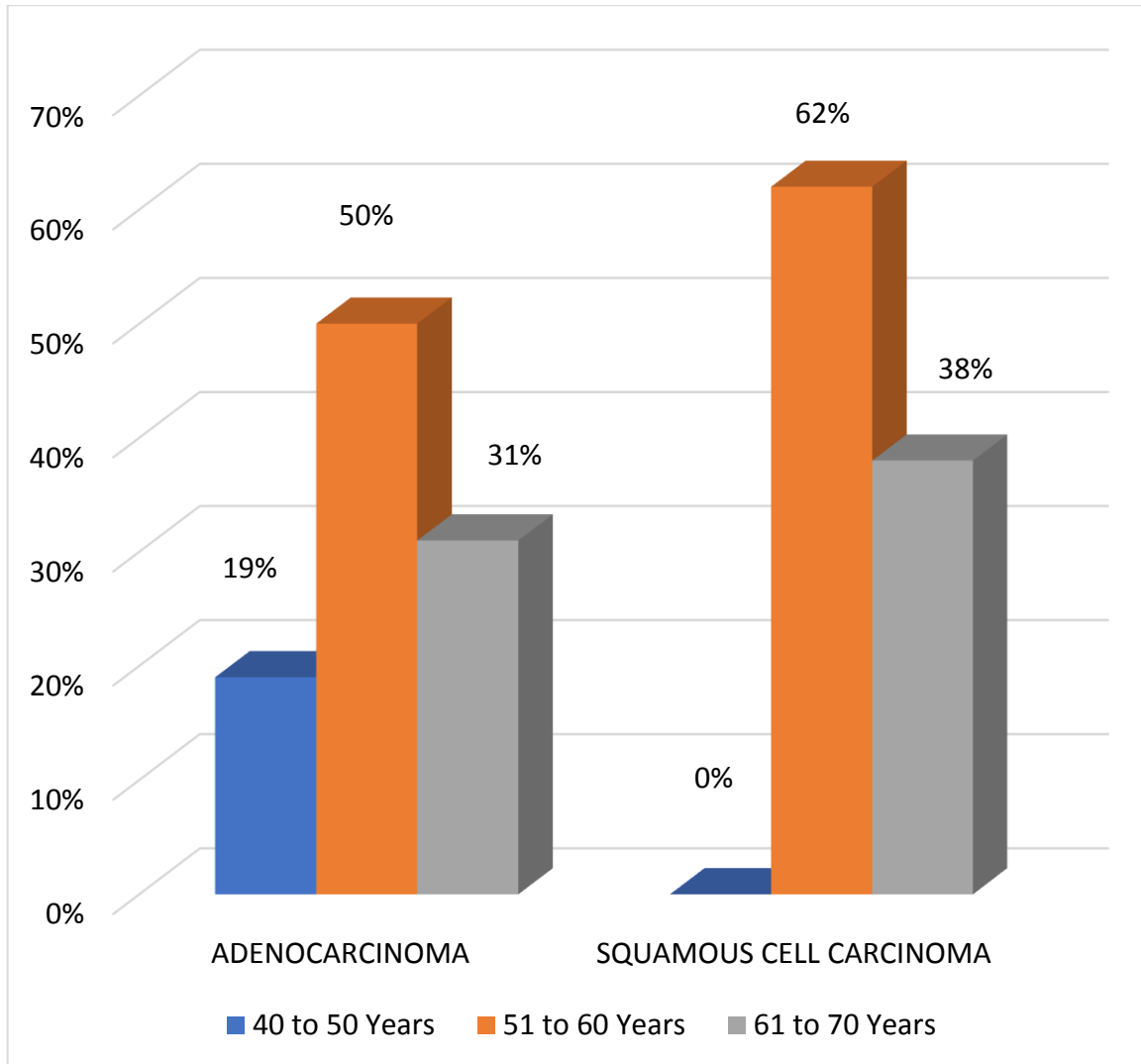
Pearson Chi-Square=2.719 p=0.257

GENDER WISE DISTRIBUTION OF CASES

In the present study males constituted about 22 cases with highest 73% followed by females 26% in 8 cases.

Table;7 Gender wise distribution of cases

SEX	FREQUENCY	PERCENT
FEMALE	8	26.7
MALE	22	73.3
Total	30	100.0

Char 2; gender wise distribution of cases
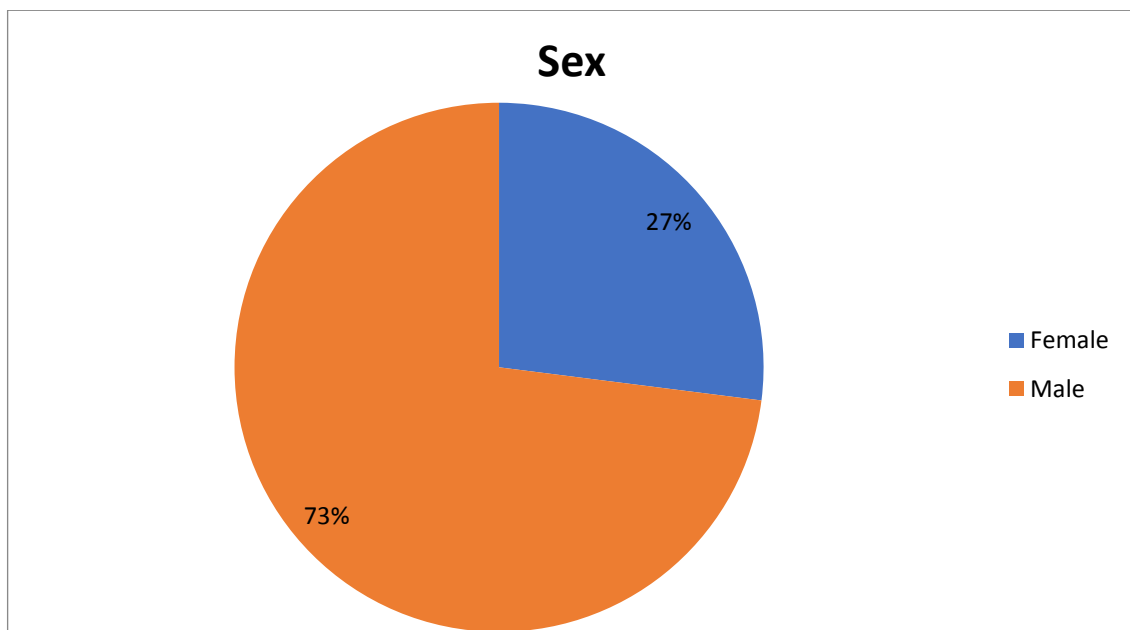
GENDER WISE DISTRIBUTION OF HISTOLOGIC SUBTYPES

In our study both Adenocarcinoma and Squamous cell carcinoma have highest incidence in males followed by females.There is no significant correlation between sex and histologic subtypes.

Chart 3: Gender wise distribution of histologic subtypes
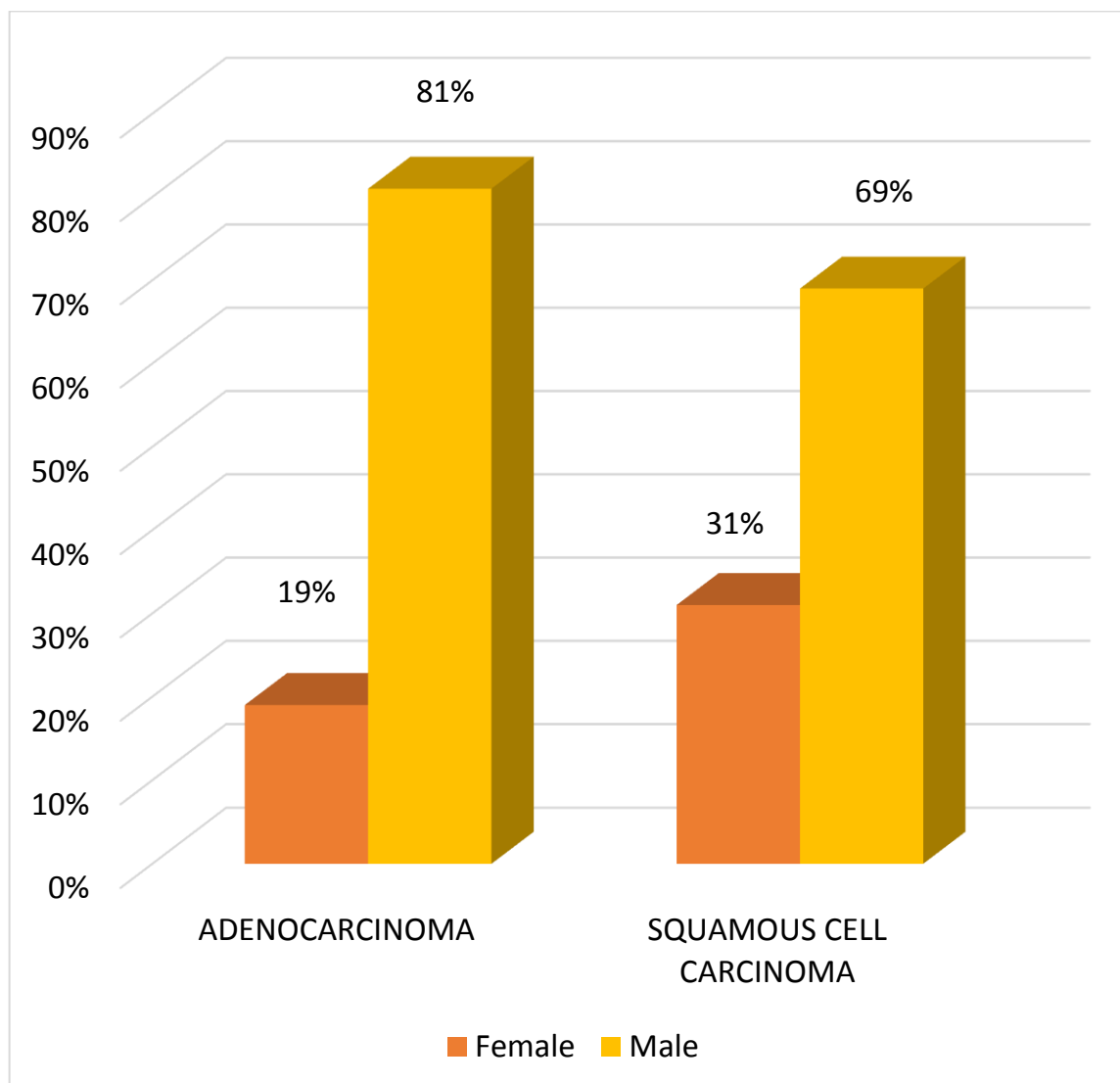
Pearson Chi-Square=0.566 p=0.452

RESULTS OF IMMUNOHISTOCHEMICAL STUDIES:

Correlation of PDL1 with age

PDL1 positivity of score 1-50% is highest in 61-70years of age constituting 40% in 4 cases ,followed by in 51-60years constituting 37.5% in 6 cases.PDL1 Positivity score of >50% highest in 51-60 years of age constituting12.5% in 2 cases followed by 61-70 years constituting 10%in 1 case.PDL1 negativity observed to be highest in 40-50 years constituting 75% followed by 50% in age group of 51-60 years.There was no statistical correlation between PDL 1 and age.

Chart 4: correlation of age with PDL 1
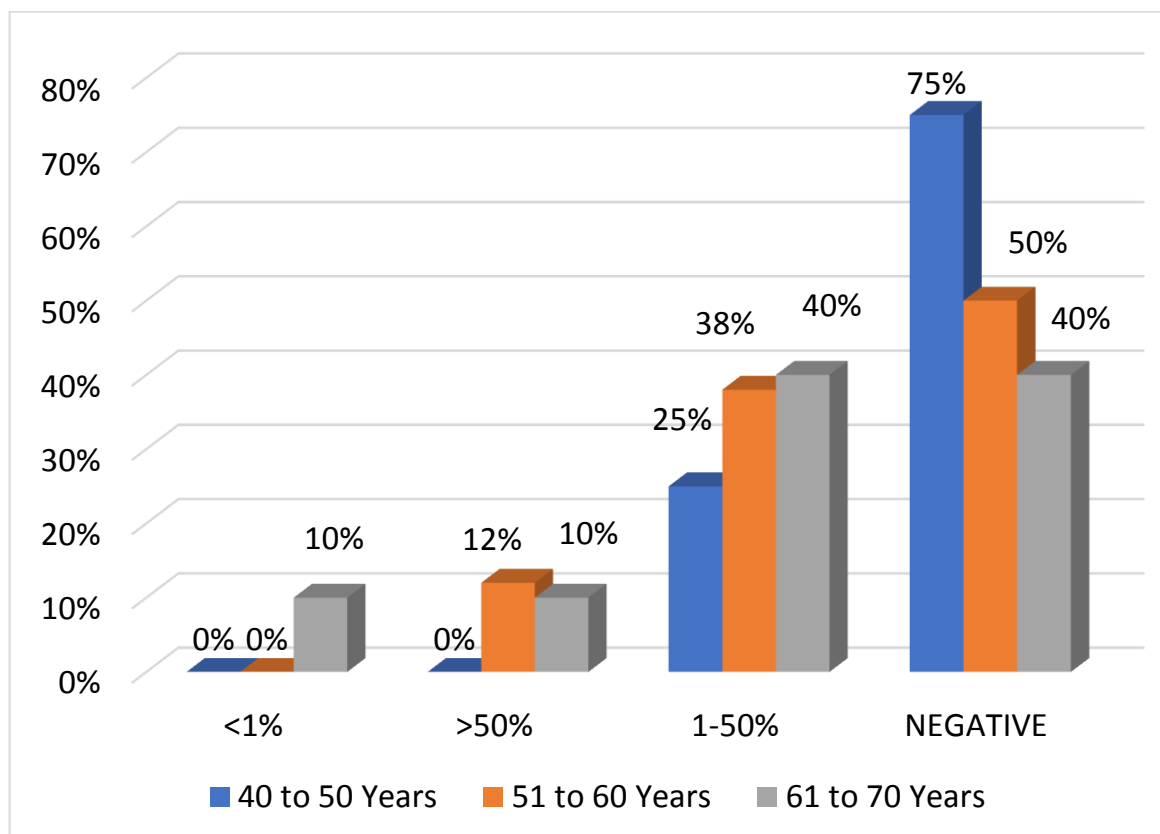
Pearson Chi-Square=3.382 P=0.760

CORRELATION OF PDL 1 WITH GENDER

PDL1 positivity of 1-50% is highest in females constituting 38% than males of about 36% and score >50%also being highest in females constituting 12%than males of about 9%.PDL1 negativity is eaual in both sex.There was no statistical correlation between PDL 1 and gender in our study.

Table:8 correlation between PDL1 with gender

			PDL1				Total
			<1%	**>50%**	**1-50%**	**NEGATIVE**	
Sex	F	Count	0	1	3	4	8
		% within sex	0.0%	12.5%	37.5%	50.0%	100.0%
	M	Count	1	2	8	11	22
		% within sex	4.5%	9.1%	36.4%	50.0%	100.0%
Total		Count	1	3	11	15	30
		% within sex	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=0.434   P=0.933

Chart 5 :correlation of PDL1 with gender
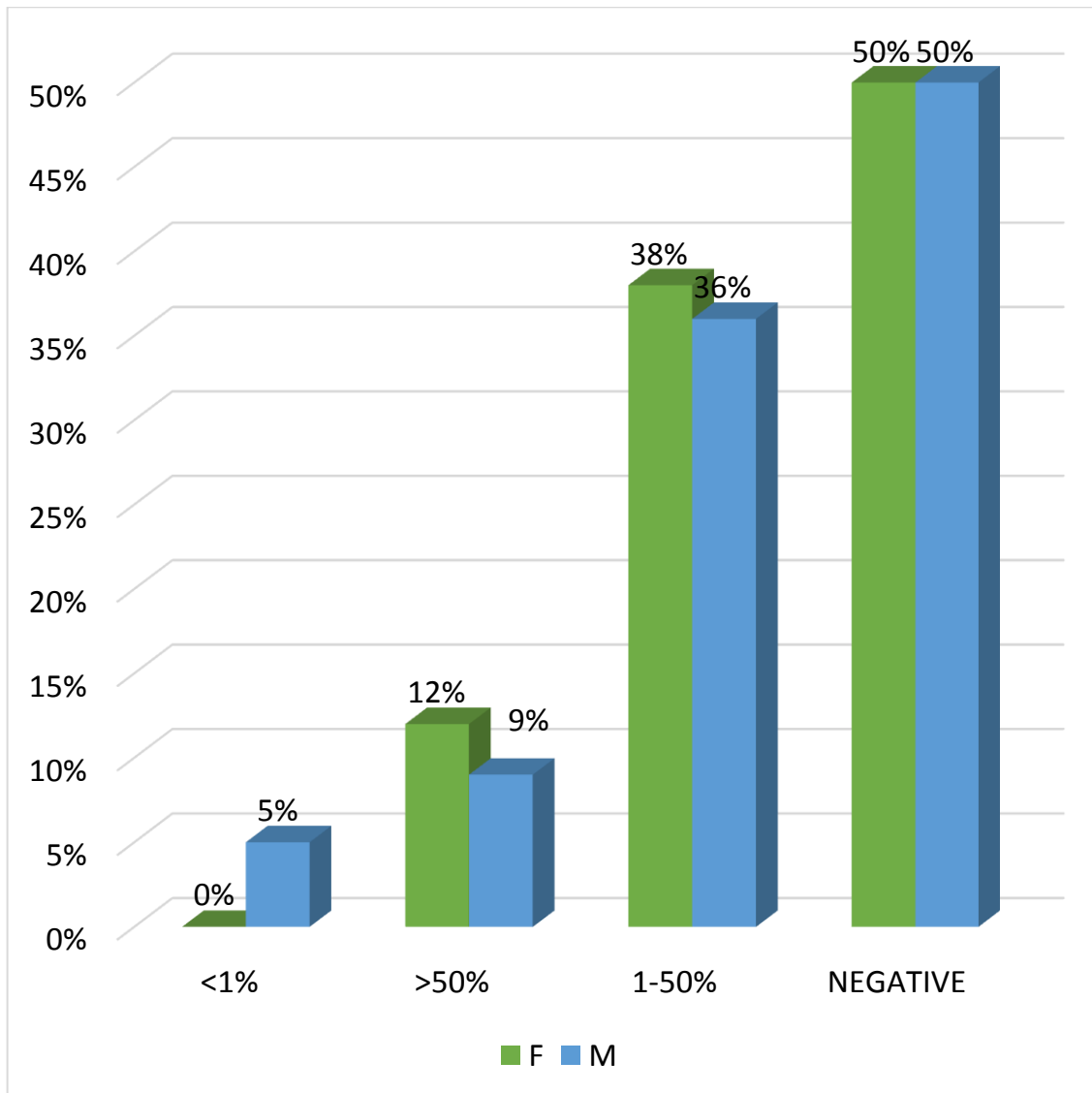
CORRELATION OF PDL1 WITH SMOKING

PDL1 positivityof 1-50% is highest in never smokers constituting 38% in never smokers than in smokers being 35%,and score of >50% is highest in smokers than in non smokers.PDL1 negativity is also highest in never smokers.There is no significant correlation between PDL 1 and smoking in our study because smoking causes overexpression of PDL 1 in adenocarcinoma ,and in case of squamous cell carcinoma smoking induces inflammatory cytokines,and carcinogens favoring PDL 1 expression.

Chart 6 : correlation of PDL 1 with smoking.
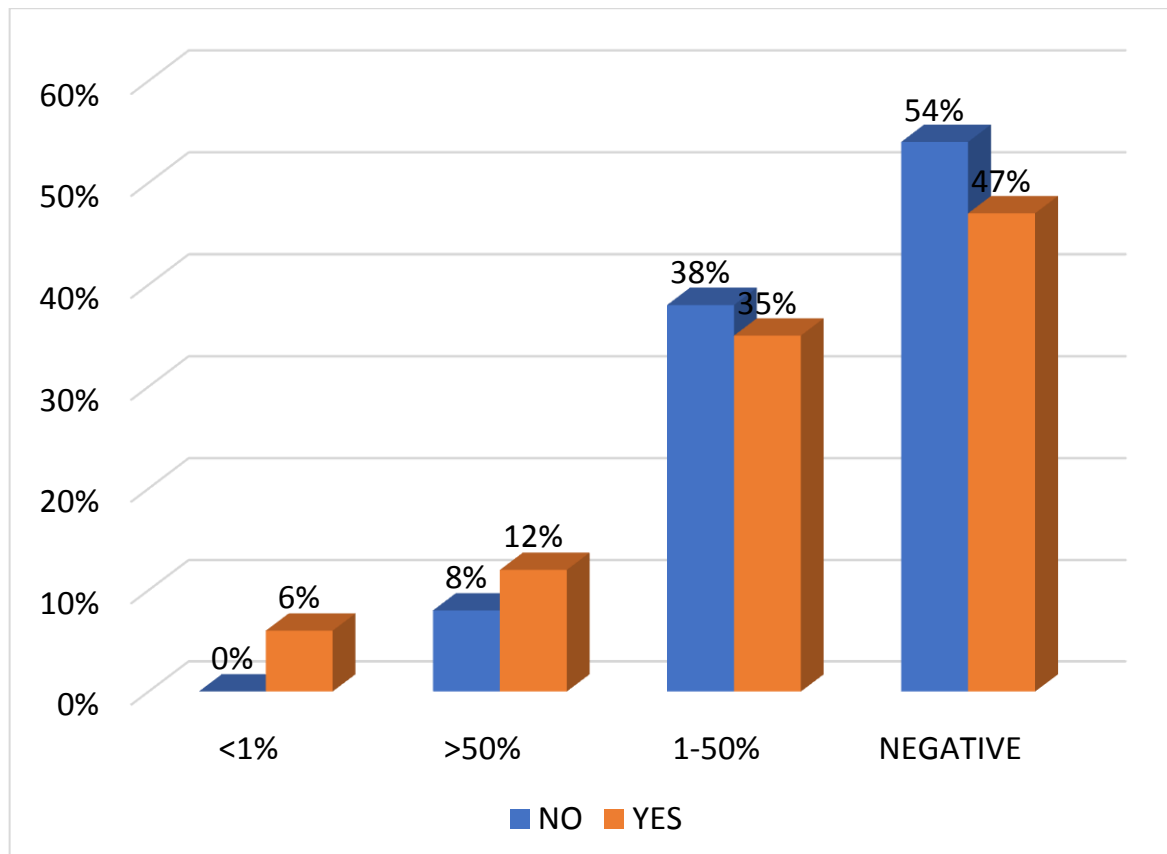
Pearson Chi-Square=0.975   P=0.807

CORRELATION OF PDL1 WITH ALCOHOL

History of alcoholism is higher in >50%PDL 1 expression than non alcoholic ,but PDL1 expression score 1-50% is highest in non alcoholic than alcoholic patients.There is no correlation of PDL 1 expression and alcohol in our study

Table 9:correlation between Alcohol and PDL 1 expression

Alcohol		PDL1_				Total
		<1%	>50%	1-50%	NEGATIVE	
NO	Count	0	1	5	7	13
	% within alcohol	0.0%	7.7%	38.5%	53.8%	100.0%
YES	Count	1	2	6	8	17
	% within alcohol	5.9%	11.8%	35.3%	47.1%	100.0%
Total	Count	1	3	11	15	30
	% within alcohol	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=0.975   P=0.807

Chart 7 : correlation between alcohol and PDL 1 expression
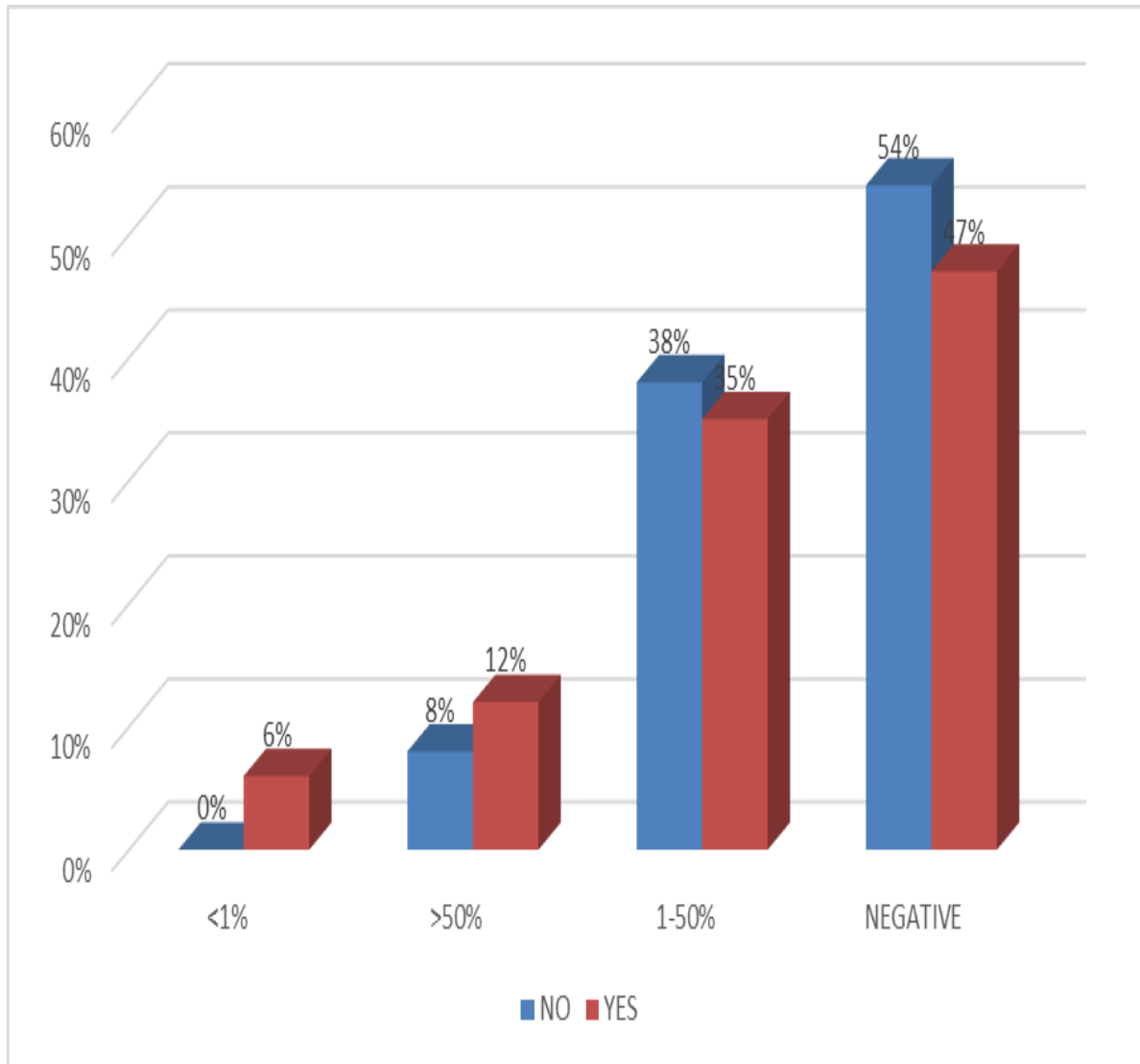
CORRELATION OF PDL1 WITH HISTOLOGY

PDL 1 score of >50%is highest in adenocarcinoma constituting 17.6% in 3 cases,1-50% highest in squamous cell carcinoma constituting 69% in 9 cases followed by adenocarcinoma constituting 11.8%in 2 cases.PDL1 score of negative being highest in adenocarcinoma being highest in 64.7%in 11 cases,followed by 30.8%in squamous cell carcinoma constituting 30.8% in 4 cases. There is a significant correlation between PDL 1 expression and histology in our study.

Table 10: correlation of PDL 1 with histology

HISTOLOGY		PDL1_				Total
		<1%	>50%	1-50%	NEGATIVE	
Adeno carcinoma	Count	1	3	2	11	17
	% within Histology	5.9%	17.6%	11.8%	64.7%	100.0%
Squamous cell carcinoma	Count	0	0	9	4	13
	% within Histology	0.0%	0.0%	69.2%	30.8%	100.0%
Total	Count	1	3	11	15	30
	% within Histology	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=10.832 * P=0.013

Chart 8 : correlation of PDL 1 with histologic subtypes
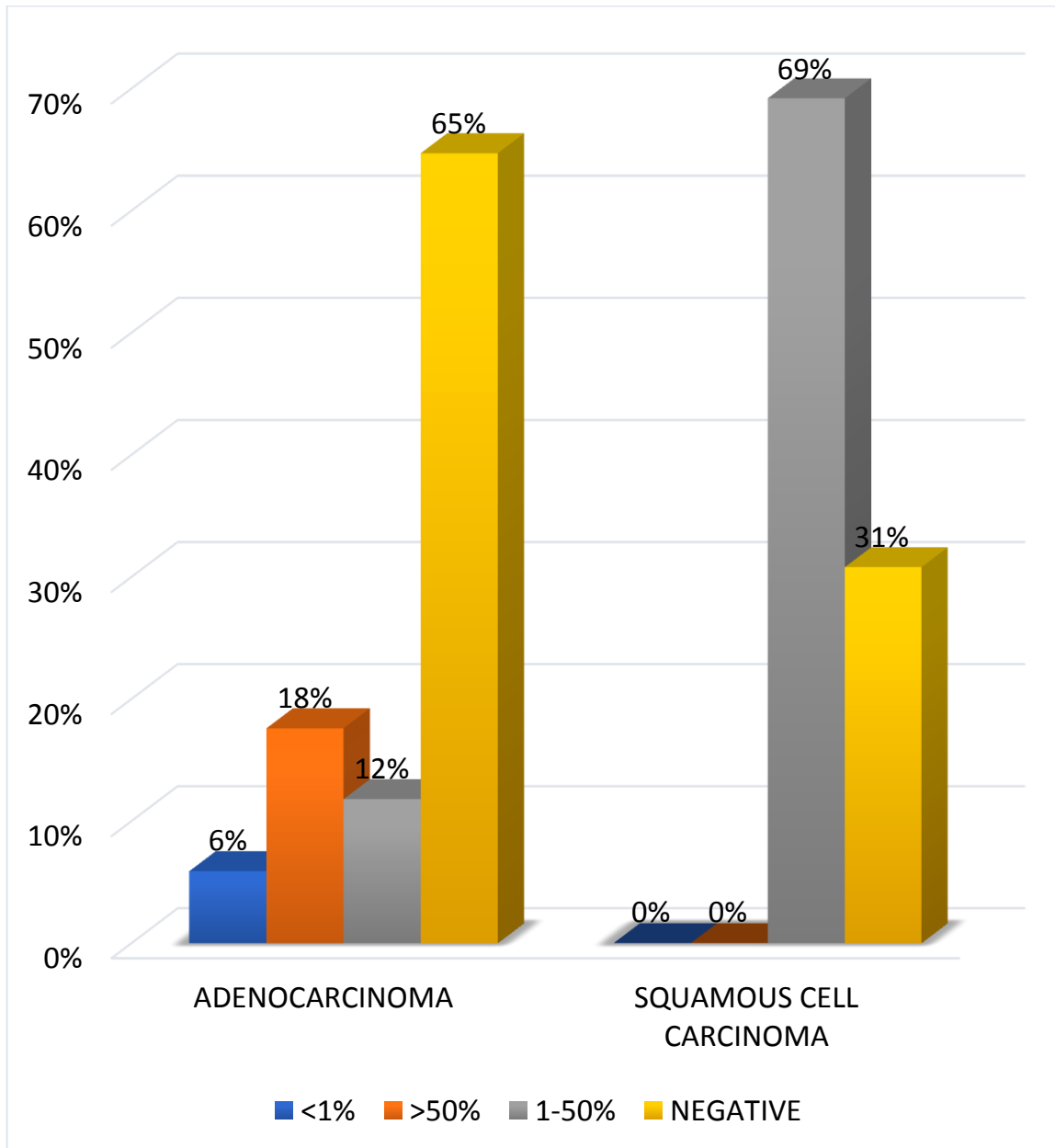
CORRELATION OF PDL1 WITH P63

PDL 1scoring >50%is highest in p63 negative cases constituting 17.6%in 3 cases ,and 1-50% scoring highest in p63 positive cases constituting 89.2% in 9 cases followed by p63 negative cases constituting 11.8% in 2 cases.PDL1 negative scoring is highest in p63 negative cases constituting 64.7% in 11 cases,followed by 30.8% in p63 positive cases of 4 patients. There is significant correlation between P63 expression and PDL 1 expression.

Table: 11; correlation of PDL 1with p63

p63		PDL1_				Total
		<1%	**>50%**	**1-50%**	**NEGATIVE**	
Negative	Count	1	3	2	11	17
	% within p63	5.9%	17.6%	11.8%	64.7%	100.0%
Positive	Count	0	0	9	4	13
	% within p63	0.0%	0.0%	69.2%	30.8%	100.0%
Total	Count	1	3	11	15	30
	% within p63	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=11.390* P=0.010

Chart 9 : correlation of PDL 1 with p63
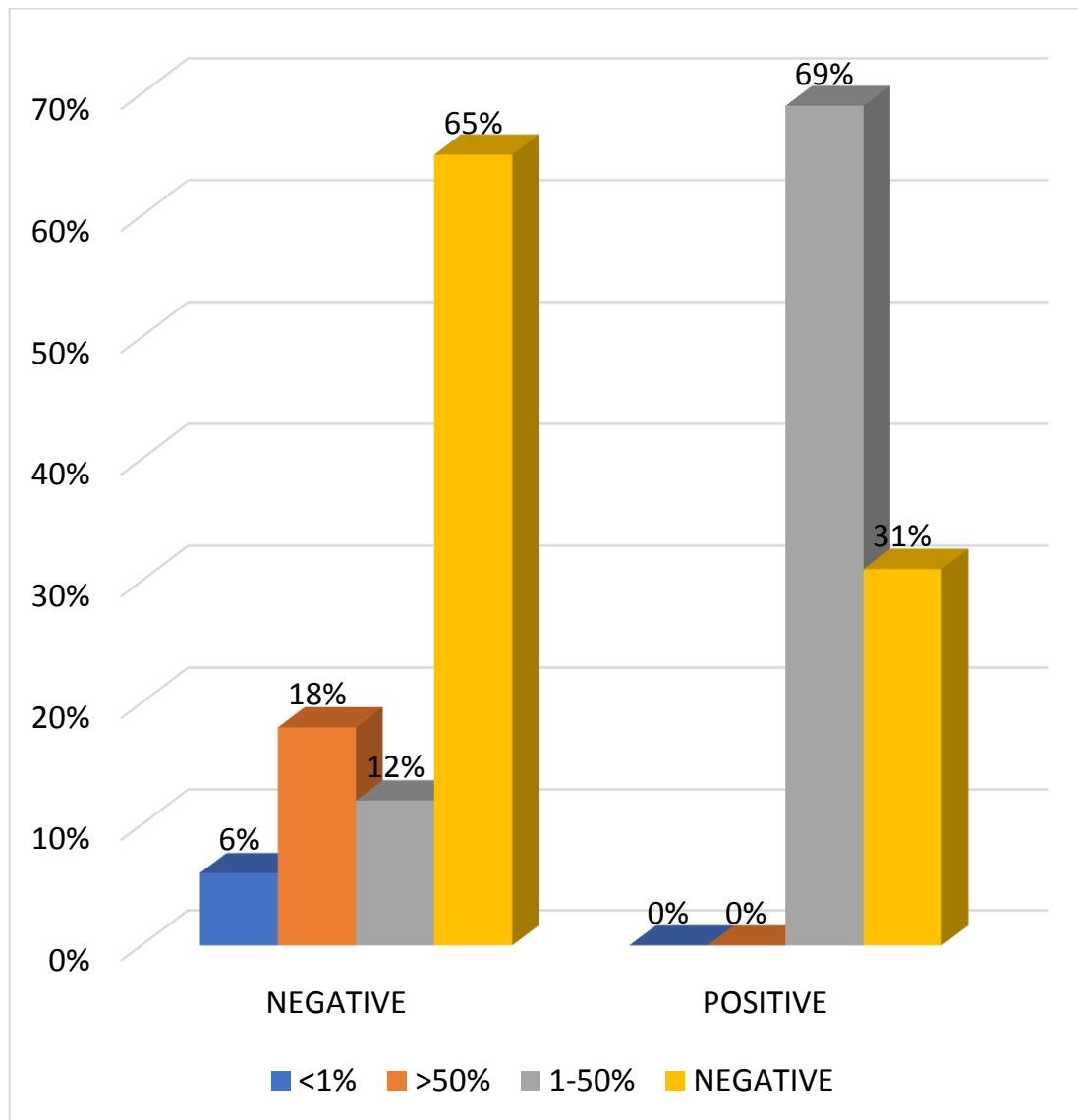
CORRELATION OF PDL1 WITH NAPSIN

PDL 1 expression of >50% is highest in Napsin positive cases constituting of 17.6%in 3 cases than negative cases .PDL 1 score of 1-50% is highest in Napsin negative cases constituting 69% in 9 cases followed by Napsin positive cases of 11.8% in 2 cses.There is a significant correlation between PDL 1 expression and napsin expression

Table 12: correlation of PDL 1 with Napsin.

NAPSIN		PDL1_				Total
		<1%	>50%	1-50%	NEGATIVE	
Negative	Count	0	0	9	4	13
	% within napsin	0.0%	0.0%	69.2%	30.8%	100.0%
Positive	Count	1	3	2	11	17
	% within napsin	5.9%	17.6%	11.8%	64.7%	100.0%
Total	Count	1	3	11	15	30
	% within napsin	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=11.390* P=0.010

Chart 10 : correlation of PDL 1 with Napsin
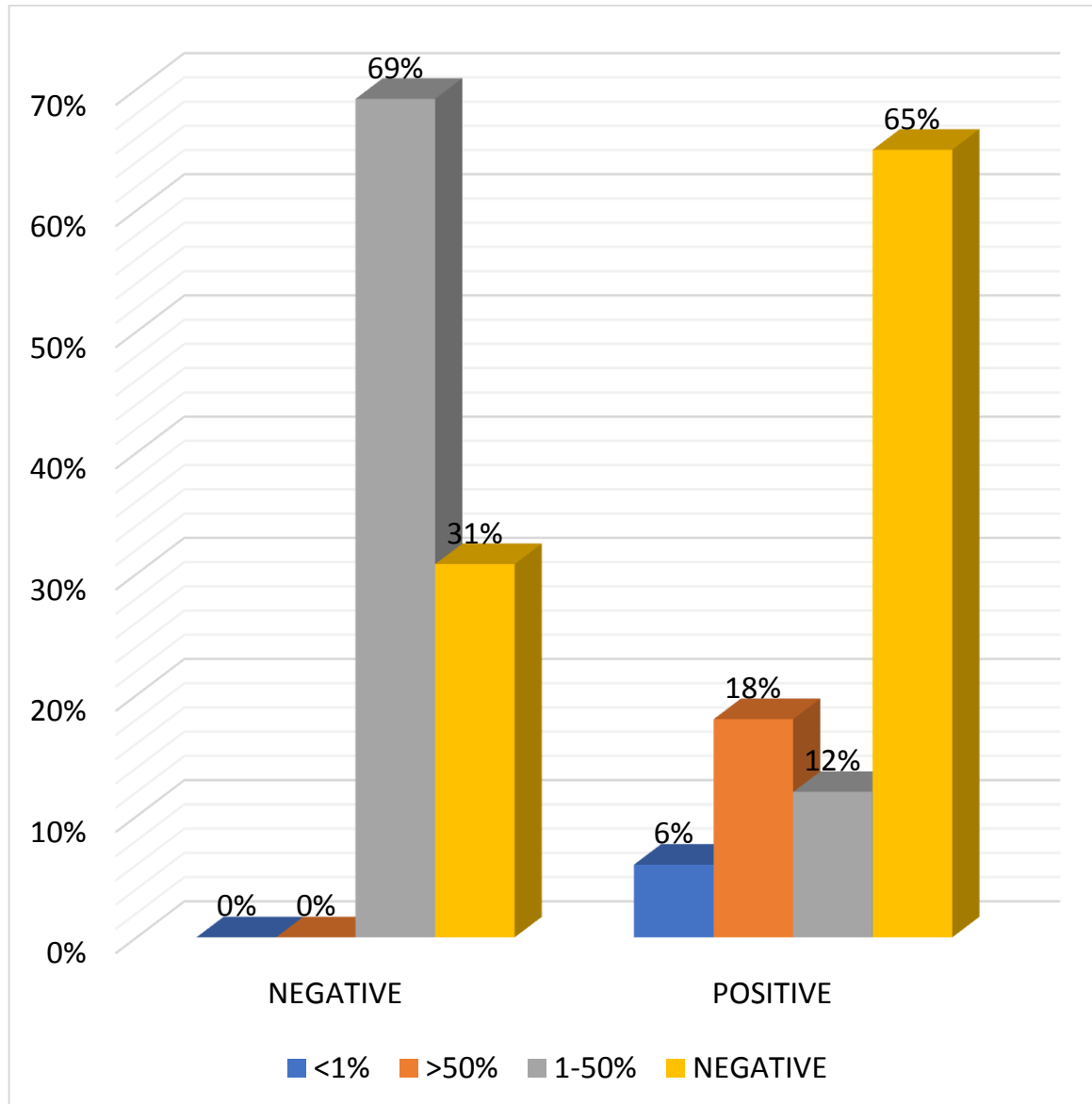
CORRELATION OF PDL1 WITH TTF 1

PDL 1 expression of > 50% is highest in TTF 1 positive cases constituting 17.6%in 3 cases,followed by TTF 1 negative cases. PDL1 score of 1-50%is highest in TTF 1 negative cases constituting 69.2% in 9 cases followed by TTF1 positive cases constituting 11.8%in 2 cases.There is significant correlation between TTF 1 expression and PDL 1 expression.

Table 13 : correlation of PDL 1 with TTF 1

TTF_1		PDL1_				Total
		<1%	>50%	1-50%	Negative	
Negative	Count	0	0	9	4	13
	% within TTF_1	0.0%	0.0%	69.2%	30.8%	100.0%
Positive	Count	1	3	2	11	17
	% within TTF_1	5.9%	17.6%	11.8%	64.7%	100.0%
Total	Count	1	3	11	15	30
	% within TTF_1	3.3%	10.0%	36.7%	50.0%	100.0%

Pearson Chi-Square=11.390* P=0.010

Chart 11 : correlation of PDL 1 wuth TTF 1
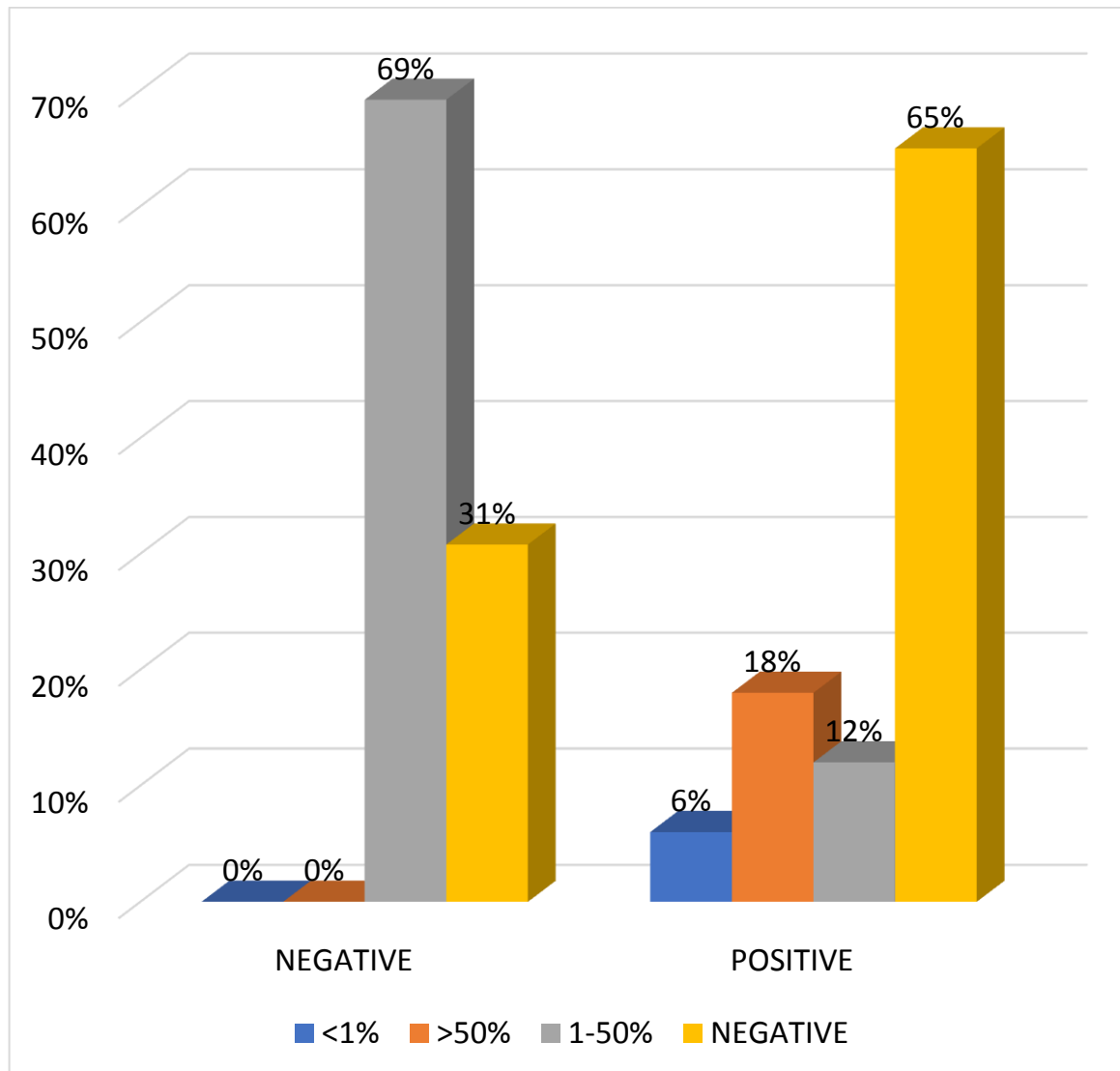
Discussion

DISCUSSION

Lung cancer is the leading cause of mortality rates amongst all malignancies worldwide. Non-small cell lung cancer (NSCLC) constitutes 80% - 85% of all diagnosed lung cancer cases and more than 70% of NSCLC are diagnosed in late stage of the disease[60]. PD-1 is a regulatory molecule on Tcells that decreases T cell activation. PD-1 is a 288-amino acid cell-surface protein, can bind two ligands, PD-L1 and PD-L2, which negatively control the immune response. Tumor cells express PD-L1 which binds to PD 1 of T cells and inhibit T cell-mediated immune response thereby progress to distant metastasis[61].

PD-L1 over-expression has been reported to be a poor prognostic marker in many human cancers [62]. Blockage of PD-1/PD-L1 interaction with monoclonal antibodies will replenish T cell activity in the tumor microenvironment resulting in an effective anti-tumor therapeutic effect activity in many malignancies [63].

The standard management of advanced NSCLC according is limited to radiotherapy, chemotherapy or both. Recently, targeted molecular therapies have been developed in place of traditional therapeutic methods for patients whose cancers express certain genetic alterations. attempts to provide immunotherapy in lung cancers were accelerated and now the development of therapeutics targeting PD-1 and PD-L1 has developed. an immunotherapy will be stated as the third line in the treatment of advanced lung cancer[64,65].

A consistent finding in many of the early studies has been higher response rates to checkpoint inhibitors in tumors found to express higher levels of PD-L1 measured by IHC. There are some reports that chemotherapy can induce PD-L1 expression, so that a tumor tests as PD-L1 negative in the diagnostic biopsy could be PD-L1 ''positive'' after chemotherapy, or at disease progression/relapse.

PD-L1 IHC is the best currently available biomarker for evaluation. PD-L1 expression in T Cells is heterogenic and dynamic because it is a pathophysiologically inducible factor, and it will be a continuous variable from zero through low to high levels. PD-L1 expression produces a median survival of about 9 months, whereas tumors with scoring 50% or more as PD-L1 positive, median survival was not reached with 26 months of follow-up[66].

There is also an increasing evidence that PD-L1 plays an important role in peripheral tolerance. Clinically, PD-L1 IHC 22C3 pharmDx is a qualitative IHC assay for NSCLC tissue helps to identify NSCLC patients .

In this present study Immunohistochemistry was done on 30 cases and evaluated the status of expression PDL1 and the Intensity in tumors thereby aiding in prognosis and immunotherapy.

In this study period we received 23,348 specimens for histopathological examination. Of the total specimens 42 cases of pneumonectomy specimens and 938 cases of small biopsies received. All 42 cases were subjected to histopathological examination and 36 cases were turned out to be Non small cell lung carcinoma 6 cases showed features of benign and non neoplastic conditions.

In our study among Non small cell lung carcinoma Adenocarcinoma constitute 56.7% Squamous cell carcinoma constitute 43.3%. According to Rashed et al study Adenocarcinoma constitute 60% and Squamous cell carcinoma 40% which correlate with our study.

AGE WISE INCIDENCE OF CASES

AGE	PRESENT STUDY	RIDGE et al
40 to 50 Years	13.3%	12%
51 to 60 Years	53.3%	58.5%
61 to 70 Years	33.3%	29.5%

In our present study more number of cases were seen in the 51-60 years age group..Mean age of presentation was 59.56 years.According to Bridge et al[121] age range was 57.45 years which correlate with our study.

GENDER WISE INCIDENCE OF CASES

In our study more number of cases were seen in men about 73.3% and females about 26.7%.According to Rashet he et al incidence was about 82%in males and 18% in women which correlate with our study.

SEX	PRESENT STUDY	RASHED HE et al
MALE	73.3%	82%
FEMALE	26.7%	18%

AGE WISE DISTRIBUTION OF CASES

In our study highest number of cases occur in 51-60 years for both squamous cell carcinoma and adenocarcinoma ,youngest age of presentation being 46 years.according to ou en zall study highest number of both squamous cell and adenocarcinoma occur in 61-70 years which does not correlate with our study .it may be due to reduced number of sample size.

GENDER WISE DISTRIBUTION OF CASES

In our study incidence of adenocarcinoma is highest of about 81.2% in males than squamous cell carcinoma being 69.2%,and in females incidence of squamous cell carcinoma being 30.7% and adenocarcinoma 23.5%.There was no correlation between sex and histology of non small cell lung carcinoma in our study,P value being 0.452.According to ou en zall the incidence of adenocarcinoma in male 52.2% and females 47.8%,ans squamous cell carcinoma in male 65.6% and females 34.4%.

INCIDENCE OF SMOKING IN NON SMALL CELL LUNG CANCER

In our study incidence of lung cancer in smokers is 56.7%,non smokers is 43.3%.according to Dela cruz et al study incidence of lung cancer is 25% in never smokers and 75% in smokers

Smoking	Present study	DELA CRUZ et al
NO	43.3	25%
YES	56.7	75%

INCIDENCE OF PDL1 EXPRESSION IN LUNG CANCER

In our study expression of PDL1 >50% is 10% and 1-50%is 36,7%,and negative cases being 53.3%.In edward et al study expression of PDL 1 > 50% is 50%,1-50% being 19.2% and negative cases being 16.7% which does not correlate with our study.Expression of PDL1 is heterogenic in tumor tissues.

PDL1	PRESENT STUDY	EDWARD ET AL
>50%	10%	50%
1-50%	36.7%	19.2%
NEGATIVE	53.3%	30.8%

INCIDENCE OF PDL1 INTENSITY IN LUNG CANCER

In our study the expression of PDL 1 intensity measured as strong constituting about 20%.weak being 46.7%,moderate being 33.3%.accorsing to Gustavo dix junquiera et al study weak intensity of about 84.1%,moderate intensity of about 15.2% and strong intensity of about 0.6% which does not correlate with our study because of heterogenic expression of PDL1 .

PDL1 INTENSITY	PRESENT STUDY	GUSTAVO DIX JUNQUEIRA PIN et al
,WEAK	46.7%	84.1%
MODERATE	33.3%	15.2%
STRONG	20%	0.6%

CORRELATION OF PDL1 EXPRESSION WITH HISTOLOGY

In our study PDL1 expression of >50% in adeno carcinoma is about 17.6% and 1-50% is about 11.8% whereas in squamous cell carcinoma score of >50% is 0% and 1-50% is about 69.2%.According to munari et al PDL 1 score of >50% is 10% and 1-50% is 34% in Adenocarcinoma and score of >50% is 8% and 1-50% is 24% in squamous cell carcinoma.there is significant statistical correlation between PDL1 scoring and histological characteristics of Non small cell lung carcinoma with p value of 0.013.

CORRELATION BETWEEN PDL1 AND P63

In our study PDL 1 expression is highest in p63 negative cases constituting 64.7%than p63 positive cases constituting 30.8%.According to Rashet he et al study there is no significant correlation between p63 and PDL1 expression since some adenocarcinoma overexpress p63.

LIMITATIONS OF THE STUDY

- Through these discussions, limitations of the present study was also noted.

- The cases were selected on the basis of Histopathological classification in a tertiary care centre and not a population bases study,which will not reflect the true prevalence of the general population

- Establishing the presence of PDL1 requires PCR based technology by examining DNA sequences of tumor tissue.They give more accurate results in identifying exact percentage of expression however the disadvantage being very expensive

- Different patterns of staining have caused much confusion in interpretation-Focal staining, Lack of positive internal control and cytoplasmic staining. With experience accurate interpretation by IHC staining is still easily achievable.

Summary

SUMMARY

This is a prospective and retrospective study conducted in the Institute of pathology, Madras medical College, Chennai during the period from June 2017 to June 2019.

Of the biopsy proven non small cell lung cancer cases Immunohistochemical analysis of PDL 1 were done in these 30 cases.Slides were evaluated and scoring was done and results were compared with other histopathologic parameters

- Highest incidence of lung carcinomas are seen in 61-70years age group

- The predominant histologic type is Adenocarcinoma NOS.

- Highest incidence is males

- No significant correlation between smokers and non smokers.

- Significant correlation between PDL 1expression and histologic subtypes

- There is a significant correlation between PDL 1 expression and P63

Conclusion

CONCLUSION

To conclude, a large proportion of advanced NSCLC cases have a positive PD-L1 immunoreactivity so they may be potentially responsive to PD-L1 immunotherapy. PD-L1 expression is significantly related to p53 protein expression. Current data suggest that PD-1 and its ligand PD-L1 are promising targets, and immunotherapy against these proteins have a great impact on overall survival and prognosis. PD-L1 IHC is the best currently available biomarker, although it is not optimal. The possibility of a choice approaches to PD-L1 IHC testing would bring significant challenges for both oncologists and pathologists. High expression of PD-L1 indicates the presence of an ongoing tumor microenvironment immune response influencing patient survival. Evaluating their expression in the tumor microenvironment assist treatment decision-making by allowing individualized risk stratification and aiding in the selection of patients for adjuvant treatment and choice of order and type of treatment.

CARCINOMA LUNG

Figure 10:Left pneumonectomy- Grey White Infiltrative mass

Figure 11: CT Chest shows soft tissue density 4x4x3cm in Left hilum with subcentimetric upper and lower paratracheal nodes
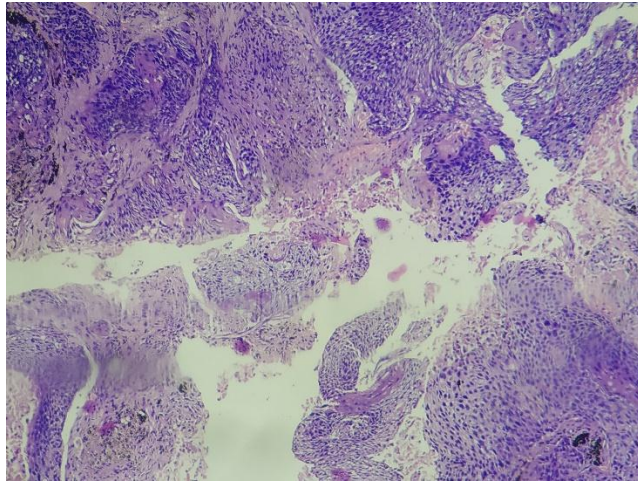
Figure 12:Bx 5568/18 Squamous cell carcinoma (100x)
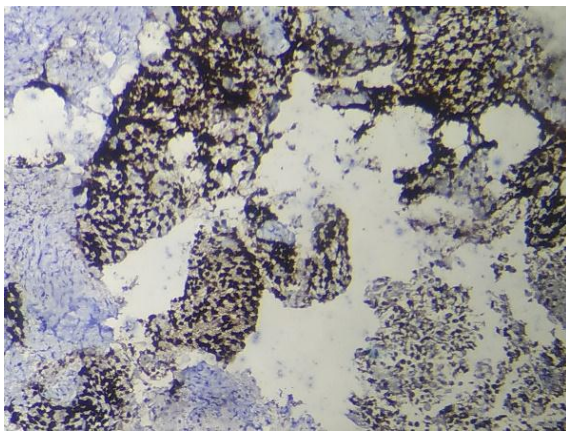
Figure 13:Bx 5568/18
P63 -Strong nuclear positivity(100x)
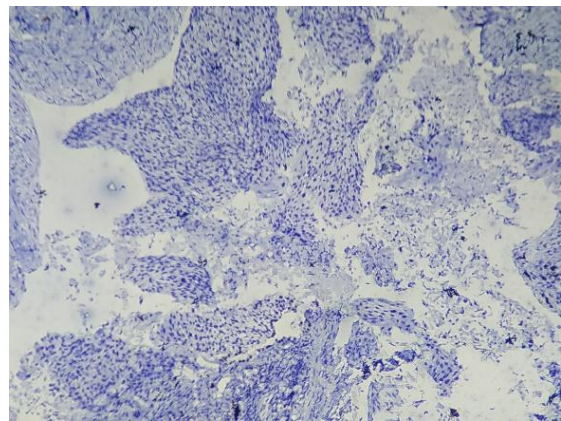
Figure 14:Bx 5568/18
TTF 1 – Negative in tumor cells
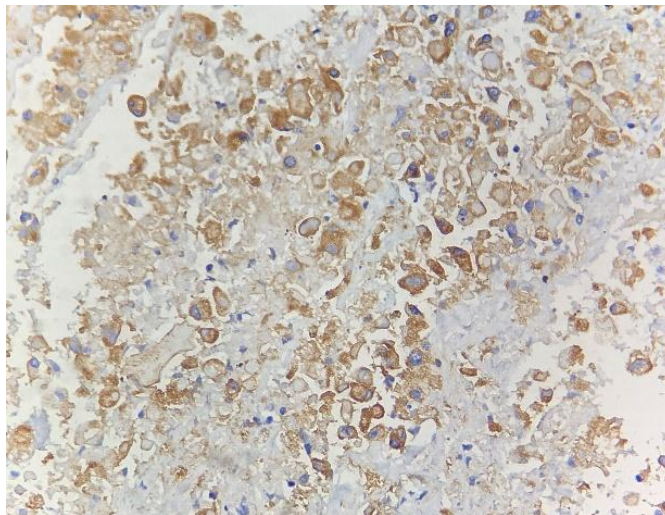
Figure 15: PDL 1 Membranous positivity score 1-50% with moderate
intensity(100x)
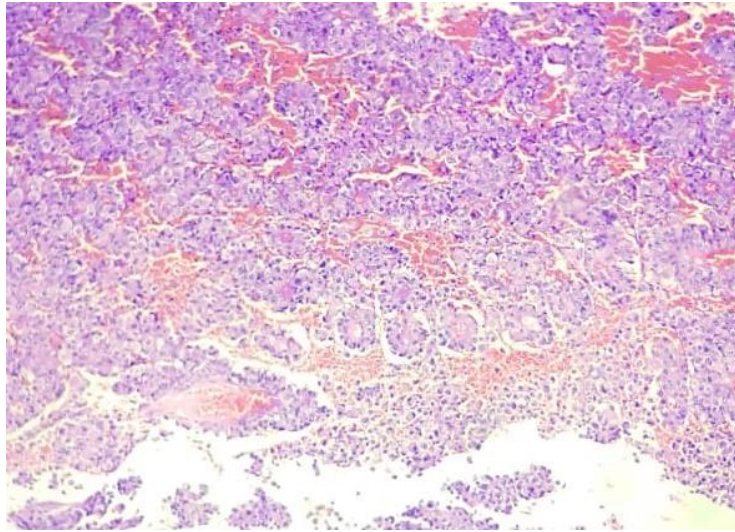
Figure 16: Bx 8279/18   Adenocarcinoma lung (100x)
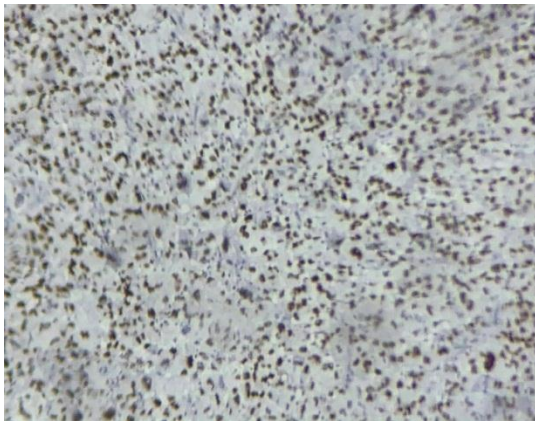
Figure 17:Bx 8279/18 TTF 1 Nuclear positivity (100X)
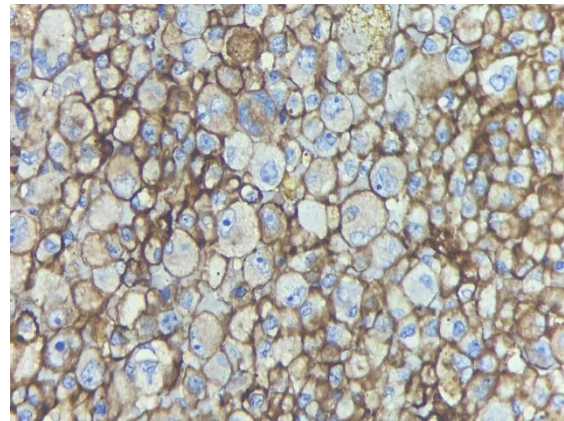
Figure 18: Bx 8279/18 PDL1 score >50% strong positive

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ANNEXURE 1

TNM CLASSIFICATION OF LUNG CARCINOMA

T (primary tumor)

Tx- primary tumor cannot be assessed ,or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

T0 No primary tumor

Tis Carcinoma in situ (squamous or adenocarcinoma)

T1 Tumor < 3 cm

T1mi Minimally invasive adenocarcinoma

T1a Superficial spreading tumor in central airways

T1a Tumor <1 cm

T1b Tumor>1 but<2 cm

T1c Tumor>2 but<3 cm

T2 Tumor>3cm but<5 cm or tumor involving: visceral pleura, main bronchus (not carina), atelectasis to hilum

T2a Tumor>3 but<4 cm

T2b Tumor>4 but<5 cm

 T3 Tumor>5 but<7 cm or invading chest wall, pericardium, phrenic nerve; or separate tumor nodule(s) in the same lobe

T4Tumortumorinvading:mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, esophagus, spine; or tumor nodule(s) in a different ipsilateral lobe

N (regional lymph nodes)

N0 No regional node metastasis

N1 Metastasis in ipsilateral pulmonary or hilar nodes

N2 Metastasis in ipsilateral mediastinal or subcarinal nodes

N3 Metastasis in contralateral mediastinal, hilar, or supraclavicular nodes

M (distant metastasis)

M0 No distant metastasis

M1a Malignant pleural or pericardial effusionz or pleural or pericardial nodules or separate tumor nodule(s) in a contralateral lobe

M1b Single extrathoracic metastasis

M1c Multiple extrathoracic metastases (1 or>1 organ)

INFORMATION SHEET

- We are conducting a study on Immunohistochemical study of PDL1 expression in Non small cell lung carcinoma among patients attending Government General Hospital, Chennai and for that your sample may be valuable to us.

- The purpose of this study is to identify PDL1 expression among NSCLC patients so that it helps in the targeted therapy and aids in prognosis .

- We are selecting certain certain cases if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in anyway 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

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு :

நுரையீரல் புற்றுநோய் திசுக்களில் உள்ள டியூமர் பட்டிங்கில் PDL1 வெளிப்பாடு மற்றும் நோயாக்கம் பற்றிய ஆராய்ச்சி.

ஆய்வாளர்　　　　　　:　　　**மரு. ப. வாணிபிரியா,**
　　　　　　　　　　　　　முதலாம் ஆண்டு,
　　　　　　　　　　　　　நோய்க்குறியியல் துறை,
　　　　　　　　　　　　　சென்னை மருத்துவக் கல்லூரி, சென்னை – 600003.

தங்களது திசு இங்கு பெற்றுக்கொள்ளப்பட்டது.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம் நுரையீரல் புற்றுநோய் திசுக்களில் உள்ள டியூமர் பட்டிங்கில் PDL1 வெளிப்பாடு மற்றும் நோயாக்கம் பற்றிய ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

நுரையீரல் புற்றுநோய் திசுக்களில் உள்ள டியூமர் பட்டிங்கில் PDL1 வெளிப்பாடு மற்றும் நோயாக்கம் பற்றி கண்டறிவதே இந்த ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய திசு சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த திசுப் பரிசோதனை முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு. ப. வாணிபிரியா, செல் : 9042763713

பங்கேற்பாளர் கையொப்பம்... இடம் :............ தேதி :...................

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் ....

ஆராய்ச்சியாளர் கையொப்பம்... இடம் :............ தேதி :...................

INFORMED CONSENT FORM

Title of the study: **"Immunohistochemical study of PDL1 expression in Non small cell lung carcinoma"**

Name of the Participant	:	DR.P.Vani Priya
Name of the Principal (Co-Investigator)	:	
Name of the Institution	:	MadrasMedicalCollege

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 **Immunohistochemical study of PDL1 expression in Non small cell 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 blood sample will be tested for various platelet parameters on automated blood cell analysers.
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 have informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. 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.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. 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_____

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு :

நுரையீரல் புற்றுநோய் திசுக்களில் உள்ள டியூமர் பட்டிங்கில் PDL1 வெளிப்பாடு மற்றும் நோயாக்கம் பற்றிய ஆராய்ச்சி

சென்னை மருத்துவக் கல்லூரி நோய்க்குறியியல் துறையில் மரு. மா. மெர்சி இவாஞ்சலின், அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள .... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் நுரையீரல் புற்றுநோய் திசுக்களில் உள்ள டியூமர் பட்டிங்கில் PDL1 வெளிப்பாடு மற்றும் நோயாக்கம் பற்றியஇந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்... இடம் :............ தேதி :....................

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் ....

ஆராய்ச்சியாளர் கையொப்பம்... இடம் :............ தேதி :....................

MASTER CHART

S.NO	BIOPSY NO	AGE	SEX	SMOKING	ALCOHOL	TTF 1	P63	NAPSIN	HISTOLOGIC SUBTYPE	PDL1 SCORE	INTENSITY
1	2625/18	63	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	1-50%	MODERATE
2	7772/18	50	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	1-50%	WEAK
3	10511/17	55	F	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	WEAK
4	2686/17	63	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	1-50%	WEAK
5	1780/18	66	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	MODERATE
6	6108/18	52	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	MODERATE
7	7133/18	56	F	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	NEGATIVE	0
8	6237/17	52	F	NO	NO	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
9	7091/18	67	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	NEGATIVE	0
10	8279/17	58	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	>50%	STRONG
11	4961/18	55	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
12	2881/18	57	F	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	MODERATE
13	8066/17	63	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	WEAK
14	6897/18	66	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	>50%	STRONG
15	8232/17	55	F	NO	NO	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	>50%	STRONG
16	5908/18	54	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	WEAK
17	7158/18	55	M	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	MODERATE
18	5562/17	68	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
19	5568/18	56	M	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	WEAK
20	6923/17	53	M	NO	NO	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
21	8014/18	48	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
22	2025/18	46	F	NO	NO	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
23	4961/18	53	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
24	6478/18	51	M	NO	NO	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
25	11639/18	62	M	YES	YES	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	NEGATIVE	0
26	11416/18	66	F	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	1-50%	MODERATE
27	2781/17	55	M	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	NEGATIVE	0
28	2825/17	67	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
29	2784/17	59	M	YES	YES	NEGATIVE	POSITIVE	POSITIVE	ADENOCARCINOMA	NEGATIVE	0
30	7518/18	46	F	NO	NO	POSITIVE	NEGATIVE	NEGATIVE	SQUAMOUS CELL CARCINOMA	INDETERMINATE	0