International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2022; 5(4): 95-99
Received: 16-07-2022
Accepted: 22-08-2022

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Role of histopathological and immunohistochemical technique in diagnosis of lung carcinoma

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DOI: https://doi.org/10.33545/pathol.2022.v5.i4b.498

Abstract

Background: Lung cancer is currently the most frequently diagnosed major cancer and the most common cause of cancer mortality worldwide. Most lung cancers are associated with a well-known Carcinogen i.e., Cigarette Smoking. Immunohistochemistry helps in the sub typing of the lung neoplasm into different categories which have a therapeutic and prognostic importance.

Materials and Methods: The present study is both retrospective and prospective study done at Pathology Department over a period of 1 year (October 2021 to September 2022). The present study was carried out on 50 patients presented with lung mass in radiological examination and undergone biopsy procedure and samples of the same were received at Histopathological section of Pathology Department, Shri M.P. Shah Govt. medical college, Jamnagar. Cases were diagnosed by histopathological examination and subtyping were done by Immunohistochemistry.

Aims and Objectives

- To determine the incidence of lung carcinoma with respect to age and sex of patients.
- To correlate between incidence and associated risk factors for lung carcinoma like smoking.
- To use Immunohistochemistry for subtyping of lung carcinoma.

Results: In present study, out of 50 cases, most common histological type of lung carcinoma was Adenocarcinoma (n=23 cases,46%) followed by Squamous cell Carcinoma (n=20 cases,40%) and Small Cell Carcinoma (n=7 cases, 14%). There was male preponderance (96%) and male to female ratio was 25:1. Right sided Lung was most commonly involved (60%) than Left sided Lung (40%).

Conclusion: As subtyping is important for different treatment modalities, the present study highlights the importance of histopathological and immunohistochemical study in diagnosis and subtyping of lung carcinoma. In this study we found that adenocarcinoma was most common subtype of lung carcinoma in Saurashtra region than other subtypes and majority of them were smokers.

Keywords: Lung carcinoma, immunohistochemistry, histopathological examination, smoking

Introduction

Lung carcinoma is currently the most frequently diagnosed major cancer and the most common cause of cancer mortality worldwide. According to GLOBOCAN India statistics 2018, there were an estimated 1.16 million new cancer cases and 7,84,821 cancer deaths. Among total new cancer cases, 5.9% were found to be of lung cancer which constitutes approximately 67,795 cases. Also, 8.1% cases were of death due to lung cancer which constitutes approximately 63,475 cases [1]. Each year, lung cancer kills more people than colon, breast, and prostate cancer combined. It is generally a disease of older adults, occurring most often between ages 55 and 84 years, with a peak incidence between 65 and 74 years. Only 2% of all cases occur before the age of 40 [2].

Because lung cancer is strongly linked to cigarette smoking, changes in smoking habits greatly influence lung cancer incidence and mortality as well as the prevalence of the various histologic types of lung cancer [2].

According to the last recommendations of the International Association for the Study of Lung Cancer, the American Thoracic Society, the European Respiratory Society, and the World Health Organization (WHO), novel therapeutic methods need typing of lung tumors into small cell carcinoma and NSCLC, and further subtyping of NSCLC into Adenocarcinoma and Squamous cell carcinoma even on cytological samples and small biopsies, whenever possible [3, 4, 5].

Immunohistochemistry helps in the sub typing of the lung neoplasm into different categories which have a therapeutic and prognostic importance.

Based on WHO recommendations for diagnosing by examining cytological samples and small biopsies, lung adenocarcinoma is an epithelial malignant Tumor morphologically with glandular differentiation, vacuolated cytoplasm, mucin production, enlarged nuclei, or specific immunohistochemical marker expression – napsin-A, thyroid transcription factor-1 (TTF-1) and CK 7 positivity after immunostaining [4, 6].

Squamous cell lung cancer is an epithelial malignant tumor morphologically with keratinization, dense cytoplasm, intercellular bridges and specific immunohistochemical marker expression – p40 or p63 and CK 5/6 positivity after immunostaining. NSCLC, not otherwise specified (NOS), includes cancers without either morphological characteristics specific for adenocarcinoma or squamous cell carcinoma or immunostaining positivity [4, 6].

Small Cell Carcinoma of lung is a neuro-endocrine Tumor, most aggressive of lung tumors, metastasizing widely and virtually always proving to be fatal. It comprised of relatively small cells with scant cytoplasm, ill-defined cell borders, finely granular nuclear chromatin (salt and pepper pattern) and absent or inconspicuous nucleoli and specific immunohistochemical marker expression- neuroendocrine markers such as chromogranin, synaptophysin and CD 56 [2].

Aim and objectives

- To determine the incidence of lung carcinoma with respect to age and sex of patients.
- To correlate between incidence and associated risk factors for lung carcinoma like smoking.
- To use Immunohistochemistry for subtyping of lung carcinoma.

Type of Study: Prospective & Retrospective study

Materials and Methods

- **Place of study:** The present study was carried out at the Department of Pathology, Shri M.P. Shah Govt. medical college, Jamnagar in collaboration with the department of TBCD in our hospital.
- **Design of study:** A diagnostic prospective & retrospective study.
- **Duration of study:** 1 year (October 2021 to September 2022)
- **Sample size:** Total 50 cases.
- **Sample types:** Biopsy specimens from lung mass with adequate material received in histopathology section of the pathology department.

Clinical work up, radiological investigations and detailed history of present complaint were taken from requisition forms in histopathology section.

Biopsy Specimen

The whole of the specimen was submitted for the histological processing and paraffin embedding. Histological paraffin sections, 3-5um thick were taken. H & E staining was performed and examined by light microscopy. The diagnosis was given according to morphology.

IHC Panel

IHC using relevant antibodies was done according to histomorphological features. Immunohistochemical studies were carried out with 3-5µ paraffin sections for Napsin A, TTF-1, CK-7, p63, CK 5/6, synaptophysin and chromogranin.

IHC staining will be performed using Peroxidase antiperoxidase method (PAP) using paraffin embedded blocks cut into 3-4um thick sections. The sections will be taken on poly L lysine coated glass slides. The secondary antibody kit of Biogenex Ltd will be used for the study. The primary monoclonal ready to use antibodies will be of DAKO, Thermofischer and Biogenex company. Tris-EDTA solution of high pH (8.4) will be used for the antigen retrieval, i.e Heat Induced Epitope Retrieval (HIER), in pressure cooker for up to one whistle.

Thereafter the slides will be brought down to room temperature and taken through the steps of the immunostaining protocol using Tris buffered saline (Annexure III) as the wash buffer. The peroxide block will be freshly prepared for each use and protein block will be used as per the company protocol from DAKO, Thermofischer and Biogenex for the use of primary antibodies. The sections will be covered with one to two drops of the primary antibody for incubation for 1 to 1.5 hours as per requirement. The incubation of TTF 1, Napsin A, CK 7, P 63 and CK 5/6 for 60 minutes and synaptophysin and Chromogranin A for 45 minutes was done in humidity chamber. The slides will be thoroughly washed with tris buffered saline in between each step. After this the polymer HRP provided in the kit will be used. Finally DAB chromogen will be used in the specific concentration as specified by the company. The slides will be counter stained by haematoxylin and the mounted by DPX. After drying the test slides will be examined under the light microscopy simultaneously with the positive control slides.

Negative external control slides will be treated similarly except that the primary antibody was omitted. The positive control section for respective IHC marker will be selected appropriately.

Assessment of IHC markers

- Tumor cells take up brown color.
- There could be nuclear and cytoplasmic expression.
- CK 5/6 and CK7 shows diffuse cytoplasmic positivity
- Napsin A, Synaptophysin and Chromogranin shows a granular cytoplasmic positivity.
- P 63 and TTF 1 shows nuclear positivity.

Once the slides will be ready, respective markers will all be viewed under microscope.

Inclusion Criteria

- Patients of any age groups.
- Both sexes.
- Clinical and Radiological investigation suggestive of lung tumors.
- Any biopsy material received for the histopathological diagnosis of lung Tumor which is sufficient for IHC.

Exclusion criteria:

- Lesions with extensive necrosis without viable tumor cells.
- Case with inconclusive diagnosis due to lack of adequate material will be excluded.

Result

The present study was conducted in the department of pathology with collaboration of the department of TB & Chest Diseases in our hospital.

Total 50 cases were studied, which include CT- guided biopsy specimen. The age range was 31 to 90 years and maximum cases (32%) were seen in the 61-70 years of age group. There was male preponderance (96%) and male to female ratio was 25:1. (Table no. 1)

In this study, right sided Lung was most commonly involved (60%) than Left sided Lung (40%). Upper lobe of lung was more commonly involved compare to Lower and Middle Lobe of lung.

Table 1: Age and Gender distribution of cases (n=50)

Age in years	Male	Female	Total
31-40	01	00	01 (02%)
41-50	10	00	10 (20%)
51-60	12	01	13 (26%)
61-70	15	01	16 (32%)
71-80	06	00	06 (12%)
81-90	04	00	04 (08%)
Total	48	02	50 (100%)

Table no. 1 shows that most cases belong to age group of 61 to 70 years. All cases were confirmed by appropriate immunohistochemistry markers; among them, 23 cases (46%) were positive for TTF, CK 7 and Napsin A which were categorized under Adenocarcinoma (Figure 1). Another 20 cases (40%) were positive for CK 5/6 and p63 which were categorized under Squamous Cell Carcinoma (Figure 2) and Remaining 7 cases (14%) were positive for TTF, Synaptophysin and Chromogranin which were categorized under Small Cell Carcinoma (Figure 3).

Table 2: Incidence of Lung Carcinoma according to age groups (n=50)

Age Group in years	Non-Small Cell Carcinoma		Small Cell Carcinoma	Total
	Squamous Cell Carcinoma	Adenocarcinoma		
31-40	00(00%)	01(4.34%)	00(00%)	01(02%)
41-50	06(30%)	04(17.4%)	00(00%)	10(20%)
51-60	04(20%)	08(34.8%)	01(14.3%)	13(26%)
61-70	06(30%)	05(21.7%)	05(35.7%)	16(32%)
71-80	03(15%)	03(13.04%)	00(00%)	06(12%)
81-90	01(05%)	02(8.7%)	01(14.3%)	04(08%)
Total	20(40%)	23(46%)	07(14%)	50(100%)

Table no. 2 shows, among 50 cases, most common histological type of lung carcinoma was Adenocarcinoma (n=23 cases, 46%) followed by Squamous cell Carcinoma (n=20 cases, 40%) and Small Cell Carcinoma (n=7 cases, 14%).

Table 3: Incidence of Lung Carcinoma amongst smokers and non-smokers (n=50)

	Squamous Cell Carcinoma	Adenocarcinoma	Small Cell Carcinoma	Total
Smokers	18 (90%)	19 (82.6%)	06 (85.7%)	43 (86%)
Non- smokers	02 (10%)	04 (17.4%)	01 (14.3%)	07 (14%)
Total	20	23	07	50

Table no.3 shows relation between lung carcinoma and habit of smoking. It was observed that majority of cases (86%) of all types of lung carcinoma were associated with habit of chronic smoking.

Discussion

Our study of 50 cases of lung carcinoma diagnosed over 1 year gives an idea about it's occurrence in Saurashtra region. This study included a biopsy specimen with different histopathological patterns and Immunohistochemistry was done for confirmation and subtyping of lung carcinoma.

Noronha *et al*. (2012) carried out study on total of 489 patients of which majority i.e., 380 (77.7%) were male and 109 (23.3%) were female[7]. Dattatreya *et al*. carried-out study on total of 446 of which majority i.e., 304 (68%) were males and 142 (32%) were females [8]. In our study, out of 50 patients, 48 were males (96%) and 2(4%) were females. All the studies conducted showed maximum incidence in male population.

In our study, the right lung to be more commonly involved (60%) than the left (40%) and so was seen by Mohan *et al*. (52.3%) [9] and Kumar *et al*. (65.79%) [10] in their studies.

In the present study, 86% of patients were smokers. A study from Kolkata at a tertiary medical care centre reported a high incidence of smoking of 81.2% [10].

In our study, 86% had NSCLC histological type, with adenocarcinoma being most commonly found in 46% of patients. In the study by Noronha *et al*. conducted at Tata Memorial Hospital on 489 patients, they showed 92% had NSCLC histology, with adenocarcinoma constituting 43.8% [7]. We found squamous cell carcinoma in 40% of the patients and small cell carcinoma in 14%, while Noronha *et al*. found squamous cell histology in 26.2% of the patients and small cell carcinoma was diagnosed in 8% of patients [7]. Similar findings were also reported by Dattatreya *et al*, they showed NSCLC was reported in 81.1% patients. Adenocarcinoma was the most common histological subtype (66%). Squamous cell carcinoma was present in 11.6% patients [8].

Immunophenotypes of the various histological types of lung cancer are stable and highly reproducible in most cases, with TTF1 and CK7 showed positivity in the glandular differentiation of adenocarcinoma and CK5/6 and p63 the keratinizing differentiation of squamous cell carcinoma [11, 12]. In our study, 46% cases were positive for TTF, CK 7 and Napsin A, categorized as Adenocarcinoma, similarly 40% cases were positive for P63 and CK 5/6 categorized as Squamous Cell Carcinoma and remaining 14% were positive for Synaptophysin and Chromogranin, categorized as Small Cell carcinoma of Lung.

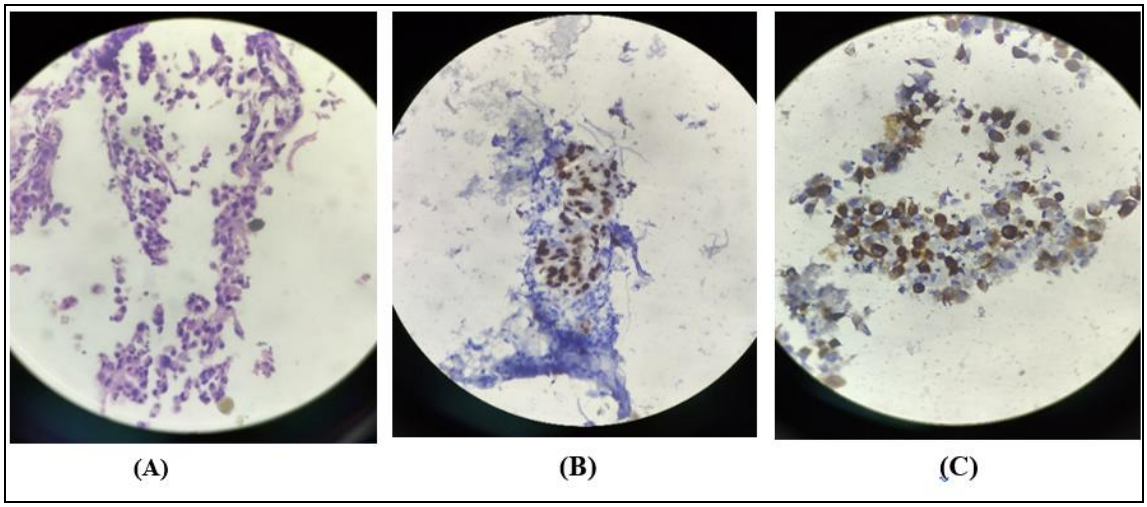

Fig 1: (A) Adenocarcinoma of Lung (H & E,40X) (B) Tumor cells showing nuclear positivity for IHC marker TTF-1(40X) (C) Tumor cells showing diffuse cytoplasmic positivity for IHC marker CK 7(40X)

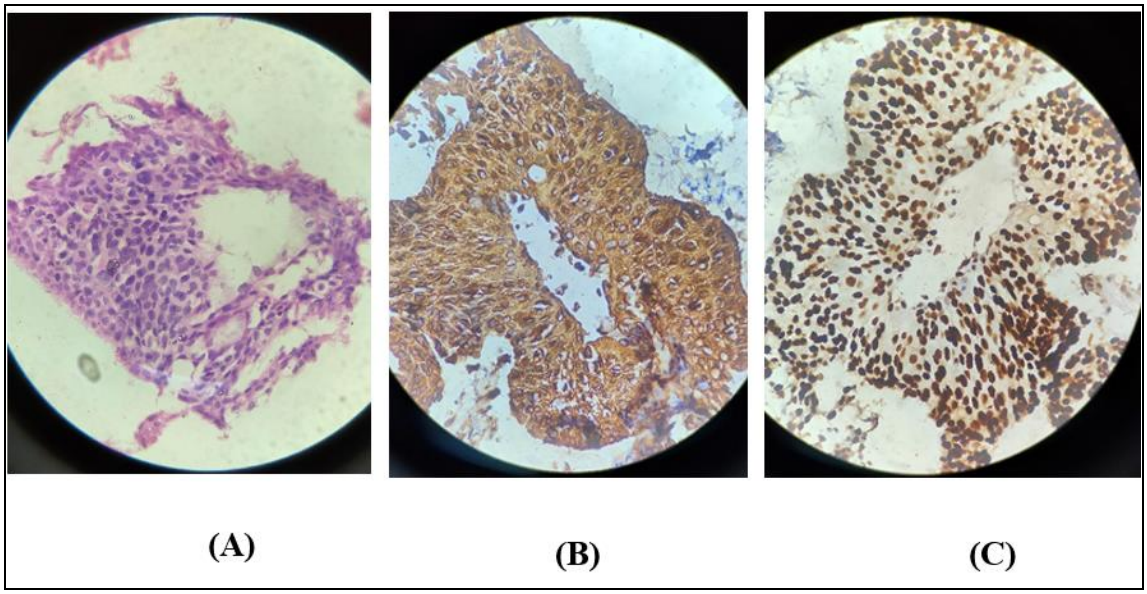

Fig 2: (A) Squamous Cell Carcinoma of Lung (H & E,40X) (B) Tumor cells showing diffuse cytoplasmic positivity for IHC marker CK 5/6(40X) (C)Tumor cells showing nuclear positivity for IHC marker p63(40X)

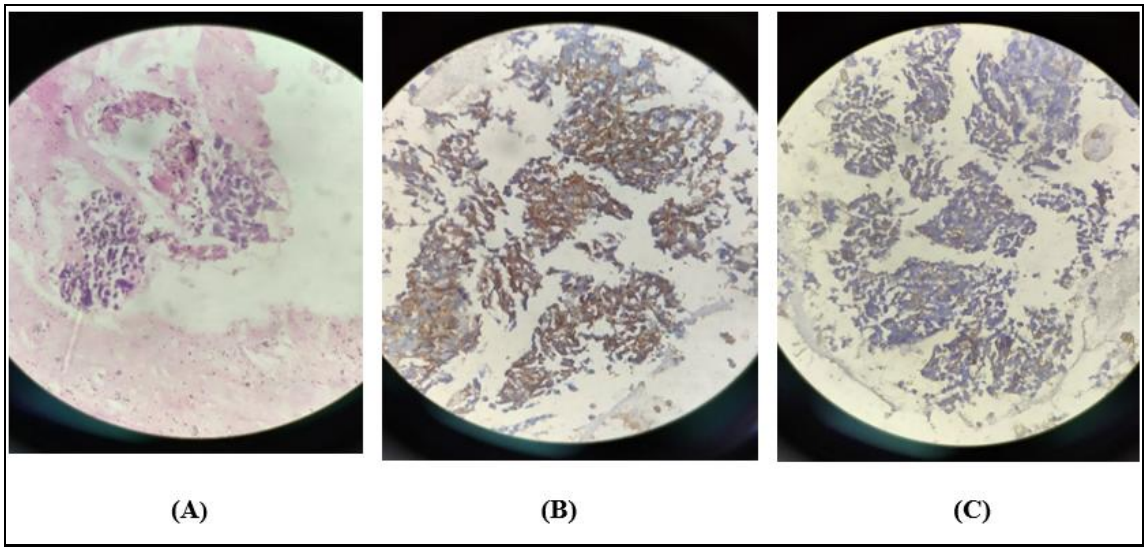

Fig 3: (A) Small Cell Carcinoma of Lung (H & E,40X) (B) Tumor cells showing granular cytoplasmic positivity for IHC marker Synaptophysin(40X) (C) Tumor cells showing granular cytoplasmic positivity for IHC marker Chromogranin(40X)

Conclusion

As subtyping is important for different treatment modalities, the present study highlights the importance of histopathological and immunohistochemical study in diagnosis and subtyping of lung carcinoma. In present study it is found that adenocarcinoma was most common subtype of lung carcinoma in Saurashtra region than other subtypes and majority of them were smokers.

Limitation: Our study had smaller sample size, so certain observation cannot be generalised & larger cohort study is required to support our findings.

Conflict of interest: None identified so far.

Source of funding: None

Acknowledgement: To all the patients and Dr Bharat Bhetariya Sir (Associate Professor, Pathology Department).

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How to Cite This Article
Thakor P, Bed V, Bhetariya B. Role of histopathological and immunohistochemical technique in diagnosis of lung carcinoma. International Journal of Clinical and Diagnostic Pathology. 2022;5(4):95-99.