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Study of Histopathological Spectrum and Immunohistochemical Profile of Primary Lung Carcinomas at a Tertiary Care Hospital in Rohilkhand Region of North India

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ABSTRACT

Lung carcinoma is the most frequently diagnosed carcinoma in the world and one of the leading causes of cancer mortality worldwide. It is more commonly affecting males than females. Lung biopsies obtained by percutaneous route under radiological guidance is simple, rapid, easily accessible and reliable technique for the diagnosis of lung mass. Immunohistochemistry (IHC) can aid in accurate histological subtyping, which further decides molecular tests and treatment plans of patients. This is a retrospective observational study that included 133 lung biopsies diagnosed as lung carcinoma from January 2019 to June 2020 in the Department of Pathology, Shri Ram Murti Smarak (SRMS) Institute of Medical sciences (IMS), Bareilly. IHC markers p40, TTF1, CK7, Synaptophysin, Chromogranin and CD56 were performed on representative paraffin embedded sections. Only primary lung cancers were included in the study. The incidence of lung carcinoma was high in male patients with M:F ratio is 3.9:1. Out of 133 cases IHC markers were performed on 84 cases (84/133, 63.15%). P40 was positive in all the cases (24/24, 100%) of squamous cell carcinoma. Out of 24 cases of adenocarcinoma, 16 cases (66.6%) showed strong nuclear positivity for TTF1, while 8 cases (33.3%) revealed weak staining. CK7 was strong positive in all the cases of adenocarcinoma. Neuroendocrine markers were positive in all cases of small cell carcinoma. Histopathology, being the gold standard was found to be self sufficient in diagnosing most of the cases. However, immunohistochemistry is very helpful especially in cases with unusual pattern. We concluded that use of p40 for squamous cell carcinoma, TTF1 and CK7 for adenocarcinoma and synaptophysin, chromogranin and CD56 for small cell carcinoma are reliable markers and when used in combination can prove effective to arrive at correct diagnosis.

OPEN ACCESS

Key Words
Lung carcinoma, Immunohistochemistry, p40, TTF1, CK7, Neuroendocrine markers

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INTRODUCTION

Lung carcinoma is the most frequently diagnosed carcinoma in the world and one of the leading causes of cancer mortality worldwide[1]. It is estimated that about 1-1.5 million people die due to lung carcinoma every year[2]. However, India has low incidence than western countries[3]. According to Globocan 2020 data, total number of new cases of lung carcinoma were 2206771 and mortality due to it was 1796144[4]. It is more commonly affecting males than females. There is a positive relationship between the occurrence of lung carcinoma and smoking which is also evidenced by statistical analysis and experimental data[1]. Other risk factors include increased exposure to high-dose ionizing radiation, heavy metals (like uranium, asbestos, arsenic and nickel) and polycyclic aromatic hydrocarbons[5]. Unlike many other carcinomas various genetic mutations have been observed in the lung carcinoma in which most frequently involved dominant oncogenes are c-myc, KRAS, EGFR, C-MET and C-KIT, while tumour suppressor genes include p53, RBI, p16(INK4a) and multiple loci on chr.3p[2]. Previously, lung carcinomas were classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Recently it is reclassified on the basis of various mutations which now considered as the basis of targeted therapy or immunotherapy such as epidermal growth factor receptor (EGFR), Anaplastic Lymphoma Kinase (ALK) rearrangements, ROS-1 translocation, or expression of programmed death receptor-1 (PD-1)/PD ligand-1[6,7]. Lung biopsies obtained by percutaneous route under radiological guidance is simple, rapid, easily accessible and reliable technique for the diagnosis of lung mass[8]. It's a big responsibility of histopathologists to correctly diagnose and classify lung carcinoma in small biopsies. However, immunohistochemistry (IHC) can aid in accurate histological subtyping, which further decides molecular tests and treatment plans of patients[3].

MATERIAL AND METHODS

This is a retrospective observational study that included all the lung biopsies diagnosed as lung carcinoma from January 2019 to June 2020 in the Department of Pathology, Shri Ram Murti Smarak (SRMS) Institute of Medical Sciences (IMS), Bareilly. The data of total 133 patients were retrieved from Lab Information System (LIS) who underwent either CT guided or bronchoscopically guided trucut lung biopsies. Demographic data like age, sex, clinical history etc. were recorded from the case file of patients. Patients are having clinical or radiological evidence of primary tumour other than lung, patients already on chemoradiation or immunotherapy were excluded from the study. Hematoxylin and Eosin (Hand E) stained slides and immunohistochemistry slides wherever done (on the basis of morphological features of tumour cells) were reviewed by two pathologists at different time. We used the following IHC markers p40, TTF1, CK7, Synaptophysin, Chromogranin and CD56. IHC markers were performed on representative paraffin embedded section according to streptoavidine-biotin immunoperoxidase technique.

RESULT AND DISCUSSIONS

Total 133 cases were included in the study out of which IHC markers were performed on 84 cases (84/133, 63.15%). The incidence of lung carcinoma was high in male patients with M:F ratio is 3.9:1 and age ranged from 31-83 years. Maximum cases of lung carcinoma were seen in the age-group of 51-70 years (59.8%) while only four cases belonged to <40 years of age (3.01%) (Table 1). All the subtypes of lung carcinoma were more prevalent in male patients (Squamous cell carcinoma- 90.90%, Adenocarcinoma-76.19%, Small cell carcinoma- 74.28%, Adenosquamous carcinoma- 100% and Non small cell carcinoma- 50%) while single case which showed both small cell and non small cell components was found in female patient. (Table 2). The most common histological type of lung carcinoma was squamous cell carcinoma comprising of (44/133, 33%) followed by adenocarcinoma (42/133, 31.5%), small cell carcinoma (35/133, 26.3%), adenosquamous carcinoma (5/133, 3.7%), lung carcinoma-unclassified (6/133, 4.5%) and small cell carcinoma with NSCC(1/133, 0.75%).

(Table 3) Shows out of the 44 cases of Squamous cell carcinoma, IHC was done on 24 cases. P40 was positive in all the cases (24/24, 100%). 3 out of 24 (12.5%) cases showed weak positivity for TTF1. None of the cases showed CK7 positivity. There were total 42 cases of Adenocarcinoma (Fig. 1), of which IHC was performed on 24 cases. Out of 24 cases, 16 cases (66.6%) showed strong nuclear positivity for TTF1 (Fig. 2), while 8 cases (33.3%) showed weak staining. In all the 24 cases, CK7 was also performed which revealed strong cytoplasmic staining in tumour cells (Fig. 3). Two cases showed patchy p40 staining. Histologically, 35 cases were reported as small cell carcinoma (Fig.4), of which 25 cases were subjected to IHC markers synaptophysin, chromogranin and CD56. 15 out of 25 cases (60%) showed strong positivity for synaptophysin (Fig.5) and 10 cases showed weak staining. Chromogranin was positive in 24 cases (96%). There were 100% (25/25) cases which showed diffuse and strong CD56 positivity (Fig.6). Five cases revealed both glandular pattern as well as cords and sheets of tumour cells. These tumour cells were positive for p40, CK7 and TTF1 in different tumour areas. So on the basis of morphology and IHC, these cases were classified as adenosquamous carcinoma. Single case showed component of both small cell carcinoma and non small

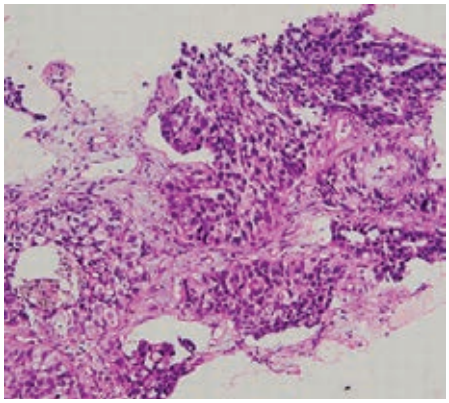

Fig. 1: Adenocarcinoma (H and E, 400X)

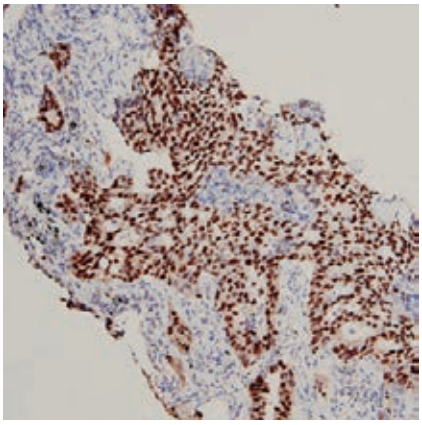

Fig. 2: Adenocarcinoma-Strong nuclear positivity of TTF1 in tumour cells (400X)

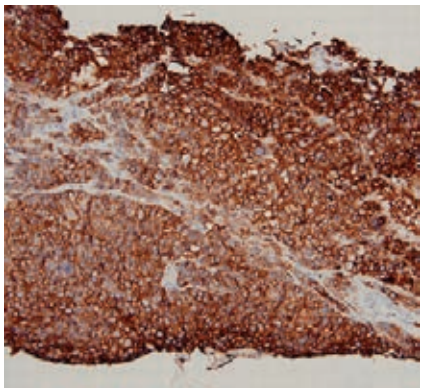

Fig. 3: Adenocarcinoma-Strong cytoplasmic positivity of CK7 in tumour cells (400X)

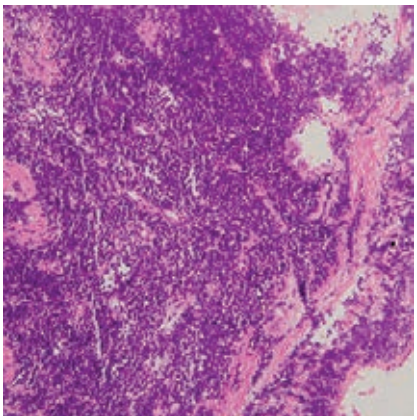

Fig 4. Small cell carcinoma (H and E, 100X)

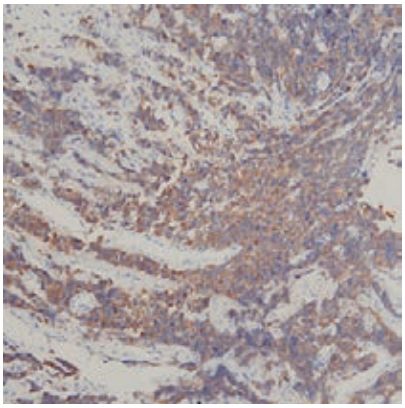

Fig. 5: Small cell carcinoma-Moderate cytoplasmic staining of Synaptophysin in tumour cells (100X)

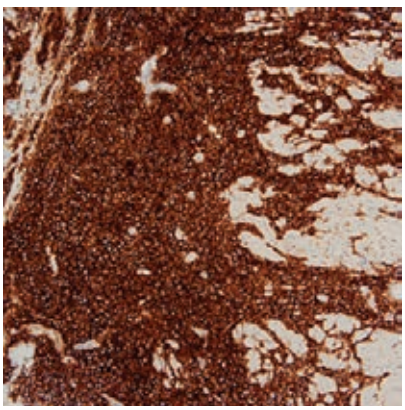

Fig. 6: Small cell carcinoma-Strong cytoplasmic staining of CD56 in tumour cells (100X)

cell carcinoma and it was positive for synaptophysin, CD56, TTF1 and CK7 on various foci of tumour cells, hence final diagnosis was given as small cell carcinoma with adenocarcinoma.

There were six cases, morphology of which is very confusing as tumour cells were scattered singly without forming any pattern and showing highly pleomorphic hyperchromatic nuclei, irregular nuclear membrane, prominent nucleoli and moderate to abundant cytoplasm. These tumour cells were negative for TTF1, p40, CK7 and synaptophysin. These cases were reported as poorly differentiated carcinoma-unclassified. Single case revealed spindle cell morphology and showed positivity for vimentin. The tissue was exhausted so further IHC panel could not be done and this case was reported as malignant spindle

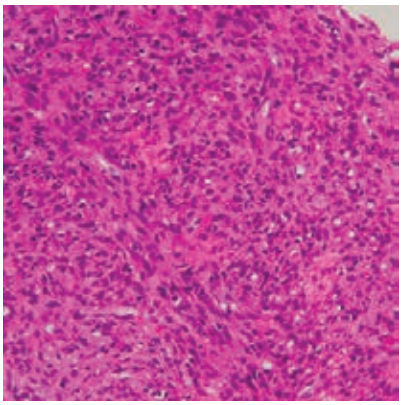

Fig. 7: Spindle cell neoplasm (H and E, 400X)

Fig. 8: Spindle cell neoplasm-Strong staining of Vimentin in tumour cells (100X)

cell neoplasm and molecular testing was advised. (Fig. 7,8). There is a rising trend of incidence of lung carcinoma in India and it is a major health problem causing millions of deaths every year worldwide[2]. Males are affected more than the females. In the present study total 133 cases were included. The male to female ratio is 3.9:1, which is very much similar to most of the Indian studies conducted previously[3,5,9,10]. CT guided percutaneous needle biopsy of the lung is safe, rapid, simple, reliable and commonly used procedure[8]. It is very helpful to differentiate between benign and malignant lesion and can also be used for molecular analysis and immunohistochemistry when required[3,5,8].

In our study incidence of squamous cell carcinoma (33%) and adenocarcinoma (31.5%) was almost equal. Several studies have shown that adenocarcinoma has surpassed squamous cell carcinoma as the most common histological subtype of lung carcinoma[2,6]. This change in pattern may be due to altered smoking habits as well as increased incidence in female patients. Most of the previous studies have described squamous cell carcinoma as the most common histologic variant[6,11,12]. However, recent studies have mentioned variable pattern of presentation[2,3,6,8]. We found that squamous cell carcinoma, adenocarcinoma and small cell carcinoma all are more common in males as compared to females, while Nirali *et al*[8]. found squamous cell carcinoma was more common in men and adenocarcinoma was found to be more common in females, which was consistent with studies conducted by Naik *et al.* and Kumar *et al*[13,14].

Immunohistochemistry was performed in 63.15% cases. We used p40, TTF1, CK7, Synaptophysin, Chromogranin and CD56 immunohistochemistry panel in different cases depending upon the morphology on Hand E stained sections. Out of 44 cases of squamous cell carcinoma, p40 was done on 24 cases. All the cases showed strong nuclear positivity our findings were similar to Walia *et al* [15]. There are various other markers for squamous cell carcinoma like CK 5/6, p63 etc. Many authors[3,7,8,9] used the combination of p63 and CK5/6 for squamous differentiation. p63 positivity is variable and ranges from 61-99% (Kargi *et al* – 61%, Alekhya *et al*-90%, sterlacci *et al*-99% and Yaman *et al*-87%). p40 was shown to be excellent marker for squamous differentiation[16,7,17,18]. Sensitivity of p40 and p63 is almost equal, but specificity of p40 is markedly high[3,19]. There were five cases of adenosquamous carcinoma in which squamous component was very well decorated by p40 staining.

Lung adenocarcinomas show positive nuclear staining for TTF1. As TTF1 is also positive in normal alveolar epithelium, so caution should be taken in diagnosing adenocarcinoma in small lung biopssies[3]. In our study, 66.7% of adenocarcinoma cells showed strong positivity while eight cases (33.3%) showed weak positivity. Our study was similar to Bhatti *et al*. Walia *et al*. Tan *et al*. and Withaus *et al*.[3,15,20,21]. TTF1 is a known marker for pulmonary adenocarcinoma, however, few cases of squamous cell carcinoma can also show weak staining pattern. In the present study 3 out of 24 cases (12.5%) of squamous cell carcinoma showed weak staining pattern for TTF 1 which is similar to alakhya *et al.* and Sterlacci *et al*[7,17]. Some poorly differentiated adenocarcinomas can be negative for TTF1, in such cases cocktail of Napsin A, TTF1 and CK7 is very helpful in diagnosing the cases correctly. One of the fact that we could not use extensive IHC panel in small biopsies for accurate subtyping of non small cell carcinoma, particularly when tissue has to be preserved for other ancillary testing[15].

There were six cases in our study which were negative for p40, TTF1, CK 7 and synaptophysin. We were not able to subtypify those cases hence reported as Non small cell carcinoma - Unclassified and molecular testing was advised. A single case revealed spindle cell morphology and showed positivity for vimentin. The tissue was exhausted so further IHC panel could not be done and this case was reported as malignant spindle cell neoplasm. Bhatti *et al*. also found five such cases which could not be classified even after extensive immunohistochemistry panel and

Table 1: Distribution of cases according to age groups

Age group	Adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma	Adeno squamous carcinoma	SCC+NSCC	NSCC-Unclassified	Total
≤30	00	01	00	00	00	00	01
31-40	06	03	00	00	00	01	10
41-50	08	09	05	00	00	02	24
51-60	09	11	12	03	01	01	37
61-70	11	13	13	02	00	01	40
≥70	08	07	05	00	00	01	21
Total	42	44	35	05	01	06	133

Table 2: Distribution of cases according to gender

Cancer type	Male	Female	Total
Adenocarcinoma	32 (76.19%)	10 (23.8%)	42
Squamous cell carcinoma	40 (90.9%)	04 (9.01%)	44
Small cell carcinoma	26 (74.28%)	09 (25.71%)	35
Adenosquamous carcinoma	05 (100%)	00	05
SCC+NSCC	00	01 (100%)	01
NSCC-Unclassified	03 (50%)	03 (50%)	06
Total	106 (79.69%)	27 (20.30%)	133

Table 3: Distribution of cases according to IHC profile

IHC profile	SCC(44)	Adenocarcinoma(42)	Small cell carcinoma(35)	Adeno +SCC (5)	SCC+NSCC(1)	Other(6)
P40	24/24	2/24	1/7	4/4	0/1	0/6
TTF1	3/24	24/24	5/6	4/4	1/1	0/6
CK7	0/8	24/24	0/4	4/4	1/1	0/6
Synaptophysin	-	-	25/25	-	1/1	0/6
Chromogranin	-	-	24/25	-	-	0/6
CD56	-	-	25/25	-	1/1	0/6

reported as Non small cell carcinoma-Unclassified[3]. Synaptophysin, chromogranin, Neuron Specific Enolase (NSE) and CD56 are established neuroendocrine markers. In our study, we found that 15 out of 25 cases (60%) showed strong positivity for synaptophysin and 10(40%) cases revealed weak staining pattern. 100% cases showed strong and diffuse positivity for CD56 and 96% cases revealed positivity for chromogranin. Our study is similar to Bhatti *et al*. who found 100% positivity for all neuroendocrine markers (synaptophysin, chromogranin, NSE and CD56)[3]. Nirali *et al*. used synaptophysin and chromogranin as a marker of small cell carcinoma which was found positive in all the cases[8].

CONCLUSION

The integration of newer therapeutic modalities requires the accurate and correct diagnosis of lung carcinoma. Imaging guided lung biopsy is a rapid, safe and reliable method which can also be done on outpatient to avoid unnecessary hospitalization. Histopathology, being the gold standard was found to be self sufficient in diagnosing most of the cases however, immunohistochemistry is very helpful especially in cases with unusual pattern. We concluded that use of p40 for squamous cell carcinoma, TTF1 and CK7 for adenocarcinoma and synaptophysin, chromogranin and CD56 for small cell carcinoma are reliable markers and when used in combination can prove effective to arrive at correct diagnosis. Small percentages of squamous cell carcinoma can also show weak positivity of TTF1, in such cases combination of Napsin A, CK7 and TTF1 should be used to avoid wrong typing of lung carcinoma. Thorough histological examination along with immunohistochemistry play an important role in the correct diagnosis as well as deciding the accurate therapeutic modalities.

Limitations: Our study has certain limitations like follow-up of the patients is not possible as the patient came in very advanced stage. Also, surgical specimens were not available for the final histopathological diagnosis. Small sample size was another limitation of our study. Further studies with lager sample size, proper patient follow up and confirmation of diagnosis with the help of molecular tools are required to validate the finding of present study.

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