Original Article

The Diagnostic value of TTF-1 and P63 Immunostaining in the Classification of Non Small cell Lung Carcinoma

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ABSTRACT

Aim/Objectives: The aim of the present study was to differentiate non small cell lung carcinomas (NSCLC), especially for distinguishing adenocarcinoma and squamous cell carcinoma by Immunohistochemistry

Materials and Methods: A prospective study of all the patients presented to department of Pulmonary Medicine, Chalmeda Anand Rao Institute of Medical Sciences, suspected of having lung carcinoma

Results: Among, 30 cases of Non Small Cell Lung Carcinoma (NSCLC), 21 cases were diagnosed as adenocarcinoma and 9 cases were squamous cell carcinoma. Patient age ranged from 41-80 years with peak incidence between 61-70 years (40%). Males are affected most commonly than females (63%). Weight loss (47%) and cough (43%) were the commonest presenting symptoms. 73% cases were associated with smoking. In the present study 100% cases diagnosed as adenocarcinoma on histomorphology showed TTF-1 positivity , 100% cases diagnosed as squamous cell carcinoma on histomorphology showed P63 positivity .

Conclusion: Analysis of a lung biopsy is very important as many lung tumors cannot be resected. To get a concrete diagnosis the use of molecular techniques as well as IHC in concert is very important. TTF1 and P63 are complementary for differentiating adenocarcinoma and squamous cell carcinoma and is now important because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type.

Keywords: Lung carcinoma, adenocarcinoma, squamous cell carcinoma, TTF-1, p63

INTRODUCTION

Lung cancer is the most common cancer worldwide. It is also the most common cause of death from cancer worldwide, responsible for over 1 million deaths annually, with a yearly growth in incidence. Tobacco smoking is the leading cause of lung cancer. [1]

Non small cell lung carcinomas (NSCLC) accounts for about 80-85% of all lung cancers. Adenocarcinoma (50%) and squamous cell carcinoma (30-35%) are the two major subtypes of non small cell lung carcinoma.[2]

The importance of differentiating between different lung tumors cannot be over emphasized. For e.g. the treatment modalities for squamous cell carcinoma and adenocarcinoma of the lung are very different. Many times it is very difficult to resect a lung tumor and to start treatment a diagnosis is required; this can prove problematic as some cases lack differentiation or do not show morphology that gives us a diagnosis. This is where immunohistochemistry comes into the picture; it can give us a great deal of clarity as to the nature of the lesion.

P63 estimation has been used to differentiate between primary lung cancers, and TTF-1 is considered the single most important Immunohistochemical marker in the

study of lung cancers. P63 has been shown to be a sensitive marker for squamous cell carcinoma.[3] and TTF-1 to be a sensitive marker for adenocarcinoma. Additionally p63 does not usually stain adenocarcinomas or neuroendocrine carcinomas such as small cell carcinoma. TTF-1 stains adenocarcinomas and small cell carcinomas but does not stain metastatic lesions nor does it stain squamous cell carcinomas. The correct application of these markers can avoid any misclassifications of tumors. Hence can be of great benefit to the patient. TTF-1 and p63 are both nuclear proteins, this avoids the problems inherent in detection of cytoplasmic markers in tumor cells with scant cytoplasm.

Immunohistochemistry with thyroid transcription factor-1 (TTF1) and P63 is highly effective in differentiating non small cell lung carcinomas (NSCLC) especially for distinguishing adenocarcinoma and squamous cell carcinoma. Accurate categorization of non small cell lung carcinomas will be done for therapeutic strategies.[4]

In short both p63 and TTF-1 together can give us invaluable data to diagnose a wide range of lung cancers.

MATERIALS AND METHODS

A prospective study was carried out at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar over a period of 1 year from February 2019 to January 2020.

Patients presenting to pulmonary medicine outpatient department and inpatient of our hospital were included in the present study. A pre-structured proforma was used to collect the baseline data.

Permission to conduct the study was obtained from Institute Ethics Committee (IEC), Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar.

Inclusion Criteria

- All biopsies of lung carcinoma which were reported as non small cell lung carcinoma on histopathology.

Exclusion Criteria

- Microscopically diagnosed cases of small cell lung carcinoma.
- Other metastatic tumors to the lung
- Patients not fit for bronchoscopy/USG/CT guided biopsy
- Inadequate biopsies

Procedure

The study was approved by the ethical committee of Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. The selected patients were briefed about the study and a informed, written consent was obtained. Patients suspected of having lung carcinoma who underwent biopsy (USG, CT and Brochoscopic guided biopsies) were included in the study. 10% formalin was used for fixation. Paraffin tissue blocks were prepared and stained with routine hematoxylin and eosin staining. Routine immunohistochemistry protocol followed with TTF-1 and P63.

RESULTS

The present study was conducted among 30 patients suspected of having lung carcinoma, attending medical outpatient department or admitted in wards of Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar.

Table 1: Age distribution of NSCLC cases

Age group	No. of cases	Percentage (%)
41-50	5	16.7%
51-60	6	20%
61-70	12	40%
71-80	7	23.3%

In our study, maximum age wise distribution (40%) were seen in age group 61-70 years followed by 71-80 years age group (23.3%) (Table 1).

Table 2: Gender distribution of NSCLC cases

Gender	No. of cases (%)
Male	19 (63%)
Female	11 (37%)

Among 30 cases, 63% were male and 37% cases were female (Table 2).

Table 3: Distribution of cases as per presenting symptoms

Symptoms	No. of cases	Percentage (%)
Weight loss	14	47%
Cough	13	43%
Weakness	12	40%
Dyspnea	10	33%
Hemoptysis	8	27%
Chest pain	7	23%
Fever	3	10%
Hoarseness of voice	3	10%
Wheeze	3	10%

Weight loss (47%), cough (43%) and weakness (40%) were the commonest presenting symptoms followed by dyspnea in 33% and hemoptysis in 27% cases (Table 3).

Table 4: Smoking history

Smoking history	Adenocarci-noma		Squamous Cell carcinoma		Total (30)
	No. of Cases	%	No. of Cases	%	No. of Cases (%)
Smokers	14	67%	8	89 %	22 (73%)
Non Smokers	7	33%	1	11%	8 (27%)

Overall 22 cases (73%) associated with smoking and 8 cases (27%) were seen in non smokers (Table 4).

Table 5: Distribution of cases

NSCLC type	No. of cases	Percentage (%)
Adenocarcinoma	21	70%
Squamous cell carcinoma	09	30%

Among, 30 cases of NSCLC cases, 70% were diagnosed as adenocarcinoma and 30% were squamous cell carcinoma (Table 5).

Table 6: Adenocarcinoma-Age and gender distribution

Age group	Male (%)	Female (%)	Total (%)
41-50	3 (25%)	1(11.1%)	4(19%)
51-60	1 (8.3%)	2 (22.2%)	3(14.3%)
61-70	7 (58.3%)	4 (44.4%)	11(52.4%)
71-80	1 (8.3%)	2 (22.2%)	3 (14.3%)
Total	12	9	21
Percentage	57%	43%	100%

Among, 21 cases of adenocarcinoma, 12 cases were male (57%) and 9 cases (43%) were female. Maximum patients in both the male (58.3%) and female patients (44.4%), were in the age group of 61-70 years (Table 6).

Table 7: Squamous cell carcinoma-Age and gender distribution

Age group	Male (%)	Female (%)	Total (%)
41-50	1 (11.1%)	0 (0%)	1 (14.3%)
51-60	2 (33.3%)	1 (50%)	3 (28.6%)
61-70	1 (11.1%)	0 (0%)	1 (14.3%)
71-80	3 (44.5%)	1 (50%)	4 (42.8%)
Total	7	2	9
Percentage	78%	22%	100%

Among, 9 cases of squamous cell carcinoma 7 cases were male (78%) and 2 cases (22%) were female. In the 7 male patients, 3 cases were in the age group of 71-80 years (Table 7).

Table 8: Immunohistochemical analysis with TTF 1 and P63

Type of NSCLC	TTF 1		P63	
	No. of Cases	%	No. of Cases	%
Adenocarcinoma (21)	21	100%	1	4.8%
Squamous cell carcinoma (9)	0	0	9	100%

Among, 21 cases of adenocarcinoma, 21 cases (100%) were positive for TTF-1 and squamous cell carcinoma cases were negative with TTF1. Among, 9 cases of squamous cell carcinoma, 9 cases (100%) were positive for P63 and among adenocarcinoma cases, 1 case (4.8%) was focal positive for P63. (Table 8)

DISCUSSION

Lung cancer is the most common cancer worldwide. It is also the most common cause of death from cancer worldwide, responsible for over 1 million deaths annually, with a yearly growth in incidence. Tobacco smoking is the leading cause of lung cancer. [1]

Symptoms of lung cancer include cough, dyspnea, hemoptysis, wheeze, stridor, loss of weight and loss of appetite.

Approximately 80% of the lung carcinomas are non- small cell lung cancers (NSCLC) and 20% are small cell lung cancers (SCLC). Worldwide squamous cell carcinomas are the most common histological type. All histological types of lung cancer are associated with smoking, but the link is stronger for squamous cell carcinomas and small cell carcinomas than with adenocarcinomas.

Adenocarcinoma is a malignant epithelial tumor with glandular differentiation and/ or mucin production. Squamous cell carcinoma (SCC) is a malignant epithelial tumor with keratinization and/or intercellular bridges.

Immunohistochemistry with Thyroid transcription factor-1 (TTF1) and P63 is highly effective in differentiating non small cell lung carcinomas (NSCLC) especially for distinguishing adenocarcinoma and squamous cell carcinoma. Accurate categorization of non small cell lung carcinomas will be done for therapeutic strategies. [4]

Table 9: Comparison of age distribution of present study with other studies

Study	Maximum no. of cases in which age group
Varma et al[4]	51-60 years (19 cases)
Akram et al[5]	51-60 years (126 cases)
Present study	61-70 years (12 cases)

The highest frequency of lung carcinomas occurred in the age group of 61-70 years with 12 cases (40%), followed by 71-80 years with 7 cases (23.3%). Worldwide most of the lung cancers are diagnosed in the 6th and 7th decades of life.

Table 9: Gender distribution

Study	Male: Female
E.A.Sinna et al[6]	2.3:1
Varma et al[4]	3:1
Gurda et al [7]	1.1:1
Present study	1.7:1

In present study, there was an overall predominance of male patients 19 cases (63%), whereas the female patients 11 cases (37%). Thus male to female ratio was 1.7:1 (Table 9).

Table 10: Comparison of smoking as an etiological Factor between present Study and other studies

Study	Smokers: Non smokers
Navinpandhi et al [8]	1.5:1
N.A. Khan et al [9]	7.6:1
Present study	2.7:1

In present study, 73% cases were associated with smoking and 8 cases (27%) were seen in non smokers. 89% of Squamous cell carcinomas and 67% of Adenocarcinomas were associated with smoking (Table 10).

The clinical symptoms among the patients studied showed that weight loss was the most common symptom being reported by 47% followed by coughin 43% which was similar to Noronha et al [10].

Table 11: Distribution of cases

Study	NSCLC cases
Noronha et al[10]	Adenocarcinoma-43.8%, Squamous cell carcinoma- 26.2%
Varma et al[4]	Adenocarcinoma-44.2%, Squamous cell carcinoma- 55.8%
Singh N et al[11]	Adenocarcinoma-26%, Squamous cell carcinoma- 34.8%
Prasad R et al[12]	Adenocarcinoma-18.5%, Squamous cell carcinoma- 46.5%
Present study	Adenocarcinoma-70%, Squamous cell carcinoma- 30%

Among 30 cases of NSCLC cases, 21 cases (70%) were diagnosed as adenocarcinoma and 9 cases (30%) were squamous cell carcinoma which was in concordance withNoronha et al[10] and discordance with Singh N et al[11] and Prasad R et al[12] (Table 11).

Table 12: Immunohistochemical analysis with TTF 1

Study	TTF1 staining in adenocarcinoma cases
Amin MB et al [13]	12 out of 15 (80%) of the cases of micropapillary lung adenocarcinoma were positive for TTF-1
Stenhouse G et al [14]	110 out of 128 (86%) of the pulmonary adenocarcinoma cases were positive for TTF-1, 14 showed only weak expression
Srodon M, Westra WH [15]	All 11 cases (100%)of metastatic lesions from a lung primary stained positive for TTF-1
Saad RS et al [16]	37 out of the 50 cases (74%) of pulmonary adenocarcinomas stained positive for TTF-1
Mukhopadhyay et al [17]	16 out of the 20 cases (80%) of pulmonary adenocarcinomas stained positive for TTF-1
Varma et al [4]	All 16 cases (100%) of pulmonary adenocarcinomas stained positive for TTF-1
Present study	All 21 cases (100%) of pulmonary adenocarcinomas stained positive for TTF-1

In the present study 100% cases diagnosed as adenocarcinoma on histomorphology showed TTF-1 positivity which closely correlates with the study by Varma et al [4] and Srodon M, Westra WH. [15] (Table 12).

Study	TTF1 staining in squamous cell carcinoma cases
Sturm N et al[18]	None of the 12 cases (0%) of pulmonary squamous cell carcinoma were positive for TTF-1
Tan D et al[19]	9 out of the 43 cases (21%)of pulmonary squamous cell carcinoma were positive for TTF-1
Gurda et al[7]	2 out of the 36 cases (56%)of pulmonary squamous cell carcinoma were positive for TTF-1
Varma et al[4]	None of the 22 cases (0%) of pulmonary squamous cell carcinoma were positive for TTF-1
Present study	None of the 13 cases (0%) of pulmonary squamous cell carcinoma were positive for TTF-1

In the present study none of the squamous cell carcinoma cases were positive for TTF-1 which was similar to Sturm N et al [18] and Varma et al. [4]

Immunohistochemical analysis with P63

Study	P63 staining in Squamous cell carcinoma cases
Kalhor N et al[20]	All 13 cases (100%) of pulmonary SCC expressed p63
Loo PS et al[21]	21 out of the 23 cases (91%)of pulmonary SCC expressed p63
Wu M, Szporn AH, Zhang D,et al[22]	All 4 cases (100%) of pulmonary SCC expressed p63
Mukhopadhyay et al[17]	All 15 cases (100%) of pulmonary SCC expressed p63
Present study	All 13 cases (100%) of pulmonary SCC expressed p63

In the present study, 100% cases diagnosed as squamous cell carcinoma on histomorphology showed P63 positivity which closely correlates with the study by Kalhor N et al [20], Wu M, Szporn AH, Zhang D, et al [22] and Mukhopadhyay et al. [17]

Study	P63 staining in adenocarcinoma cases
Wu M, Szporn AH, Zhang D,et al[22]	None of the 4 cases (0%) of pulmonary adenocarcinoma stained positive with p63
Kargi A et al[23]	None of the 10 cases (0%) of pulmonary adenocarcinoma stained positive with p63
Gurda et al[7]	11 out of 42 cases (26%) of pulmonary adenocarcinoma stained positive with p63
Mukhopadhyay et al[17]	2 out of 20 cases (10%) of pulmonary adenocarcinoma stained positive with p63
Present study	1 out of 21 cases (4.8%) of pulmonary adenocarcinoma stained positive with p63

In the present study shows 1 adenocarcinoma (4.8%) case were focally stained for P63.

CONCLUSION

Among, 30 cases of Non Small Cell Lung Carcinoma (NSCLC), 21 cases were diagnosed as adenocarcinoma and 9 cases were squamous cell carcinoma. In the present study 100% cases diagnosed as adenocarcinoma on histomorphology showed TTF-1 positivity, 100% cases diagnosed as squamous cell carcinoma on histomorphology showed P63 positivity. So both markers TTF-1 and p63 work well together to give us very important and reliable data so help us get a very important conclusive diagnosis. TTF1 and P63 are complementary for differentiating adenocarcinoma and squamous cell carcinoma and is now important because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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