ORIGINAL ARTICLE

Diagnostic Utility of TTF-1 and P40 Immunohistochemical Markers for Subtyping of Non-Small Cell Lung Carcinoma

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ABSTRACT

Background : Lung cancer is the leading cause of cancer-related mortality over worldwide. Although the pathological diagnosis of lung carcinoma is limited as only small specimen available for diagnosis, the availability of targeted therapies has created a need for precise subtyping of non-small cell lung carcinoma. Several recent studies have demonstrated that the use of immunohistochemical markers can be helpful in differentiating squamous cell carcinoma from adenocarcinoma not only on surgically resected specimen but also on small biopsy samples. **Material and Methods:** A cross-sectional study of one year duration including 50 cases of lung carcinomas on guided biopsies were first reported on Haematoxylin and Eosin sections and later subjected for IHC using relevant markers TTF-1 and p40. **Results:** In our study IHC with TTF-1 and p40 aided in subtyping of 35 (92.1%) cases of non-small cell lung carcinoma and this diagnostic accuracy was found to be statistically significant with p value <0.001. On statistical analysis, p40 showed 100% sensitivity and 85.7% specificity for squamous differentiation whereas TTF-1 showed sensitivity of 85.7% and specificity of 100% for adenocarcinoma. Out of 50 cases, after IHC, 29 (58%) were diagnosed as squamous cell carcinoma, 18 (36%) as adenocarcinoma, 3 (6%) as non-small cell lung carcinoma. **Conclusion:** The minimalist IHC based model of p40 and TTF-1 on biopsy samples were effective to correctly subtype most cases of non-small cell lung carcinoma and contribute in sparing material for molecular testing.

KEY WORDS: Non-small cell lung carcinoma, immunohistochemistry, squamous cell carcinoma.

Introduction

Lung cancer is the second most common and major cause of death worldwide.[1] Its generally a disease of elderly individuals, occurring most often between ages 55 and 84 years.[2] In India, lung cancer constitutes 6.9% of all new cancer cases and 9.3% of all cancer related deaths in both sexes.[3] Incidence of lung cancer in male is 1 in 68 and in female is 1 in 201.[3] Tobacco smoking is the most common cause of lung cancer and accounts for 80% cases and other causes are air pollution, industrial hazards like asbestos, arsenic, chromium, nickel, vinyl chloride which accounts for 20% cases.[1] There are various methods that are being used to diagnose various lung tumors but core lung biopsy has a major role in diagnosing lung tumors as it is safe, rapid and reliable technique.[4] A limitation of availability of adequate material in a small biopsy precludes the usage of an extensive panel of immunohistochemical markers to accurately subtype non-small cell lung carcinoma (NSCLC), especially when tissue needs to be saved for molecular studies. Hence, it has been proposed to use one specific marker each for squamous and glandular differentiation.[5] There are several biomarkers present in the lung like CK7, TTF-1, CK5, p40, p63, S100, Chromogranin, CD20 but TTF-1 and p40 are very reliable and specific markers used for distinguishing squamous cell carcinoma (SCC) from adenocarcinoma (ADC) and helps in providing more accurate diagnosis and sub-typing of NSCLC.

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p40 is an isoform of p63 and denote the non-transactivating domain (deltaNp63).[6,7] It is commonly present in basal layers of stratified epithelium and in some glandular epithelium.[6,7] p40 has been shown to be superior to the normally used antibody p63 (clone 4A4) for differentiation of squamous cells because p63 can be found in some cases of lung adenocarcinomas and even large cell lymphomas, which makes it less specific.[6]

TTF-1 is a homeodomain-containing transcription factor which is predominantly found in normal type II alveolar pneumocytes that has shown high sensitivity and specificity in the immunohistochemical labelling of lung adenocarcinomas.[8] TTF-1 is a nuclear stain and has long been used to help confirm origin from a lung adenocarcinoma at metastatic sites.

The present study was carried out to determine the effectiveness of a minimal panel of antibodies TTF-1 and p40 in sub-classification of NSCLC.

Material & Methods

This cross-sectional study was conducted in the Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, after Institutional Ethical Committee approval and included lung biopsies from suspected patients of primary lung cancer attending the Department of Respiratory Medicine and Oncosurgery. A total of 50 cases were taken. Biopsies containing inadequate tumour component on histopathology for immunohistochemical evaluation were excluded. All biopsies were processed using automated tissue processor and embedded in paraffin wax. 3-5μm thick sections were cut and stained with standard Haematoxylin and Eosin stain and extra sections were cut on Poly-l-lysine coated slides for IHC to prevent loss of tissue due to repeated sectioning of the block. The IHC (p40 with clone Polyclonal and TTF-1 with clone 8G7G3/1+NX2.1/690) staining were applied using rabbit and mouse monoclonal IgG antibodies after deparaffinization of the sections using xylene and then re-hydration in decreasing concentrations of alcohol.

Interpretation of immunohistochemistry

The results of IHC showed positive nuclear staining for TTF-1 and p40 and were recorded according to standardized guidelines. Immunoreactivity was recorded semi quantitatively on a scale from 0 to 3+. The percentage of positive cells and intensity was graded as 0 (0% positive cells), no staining; 1+ (1-25%), weak; 2+ (25-50%), moderate; 3+ (50-100%), strong.[5]

Statistical Analysis

Statistical analysis was done by using SPSS software (v.23.0). Chi-square test used to evaluate the significance of the correlation in the diagnosis of NSCLC before and after addition of IHC, with a threshold of less than 0.05 was considered to be statistically significant. The sensitivity and specificity of IHC markers was calculated and reported.

Results

Total 50 cases were included in the study. In our study, age range was 21-80 years with maximum number of cases (58%) seen in age group of 41-60 years. Out of 50 cases, 45 (90%) were male patients and 5 (10%) were female patients. (Figure 1) 36 (72%) cases were having history of smoking, weight loss was the most common symptom in our study being reported in 46 (92%) cases. Based on the histomorphological analysis, 7 (14%) cases were classified as ADC, 5 (10%) cases were classified as SCC and the remaining 38 (76%) cases were labelled as NSCLC. (Table 1) All the cases were subjected for IHC (p40 and TTF-1) after which, 18 (36%) cases were diagnosed as adenocarcinoma (TTF-1+/p40-), 29 (58%) as squamous cell carcinoma (p40+/TTF-1-) and 3 (6%) as non-small cell lung carcinoma (p40-/TTF-1-). This showed that there is reduction in 92.1% cases of non-small cell lung carcinoma after applying IHC. (Table-1) In terms of sensitivity and specificity, p40 showed 100% sensitivity and 85.7% specificity while TTF-1 showed sensitivity of 85.7% and specificity of 100%. (Table 2)

All histomorphologically identified SCC were p40 positive with 2+ to 3+ positivity. The staining intensity was strong in all cases. While, all histomorphologically identified ADC were TTF-1 positive except for 1 case which was negative to TTF-1 and was positive with p40. All cases showed 3+ positivity. The staining intensity was moderate to strong in all cases. Both the markers were negative in 3 (6%) cases, which remained NSCLC and could not be subtyped further.

According to above findings, IHC with two specific markers when used adjunctively with morphology was able to correctly classify 47 of the 50 (94%) cases of NSCLC ($P<0.001$). (Table 1)

Table 1: Distribution of cases according to histopathology and IHC marker (P40 and TTF-1) with subtype characterization

IHC Final diagnosis	Histopathological Diagnosis				Subtype characterization	p-value
	Squamous Cell carcinoma (n=5)	Adenocarcinoma (n=7)	Non-Small Cell Lung Carcinoma (n=38)	Total		
Squamous Cell Carcinoma (P40+/TTF1-)	5	1	23	29	Increment of 82.7%	< 0.001*
Adenocarcinoma (P40- / TTF1+)	0	6	12	18	Increment of 61.1%	
Non-Small Cell Lung Carcinoma (P40- / TTF1-)	0	0	3	3	Reduction of 92.1 %	
Total	5	7	38	50		

**statistically significant

Table 2: Sensitivity and Specificity of IHC markers in ADC and SCC

IHC Marker		No. of cases diagnosed as ADC on H&E	No. of cases diagnosed as SCC on H&E	
TTF-1	TTF-1 Positive	6 (TP)	0 (FP)	Sensitivity: 85.7% Specificity: 100% PPV: 100% NPV: 83.3%
	TTF-1 Negative	1 (FN)	5 (TN)	
P40	P40 Positive	1 (FP)	5 (TP)	Sensitivity: 100% Specificity: 85.7% PPV: 83.3% NPV: 100%
	P40 Negative	6 (TN)	1 (FN)	

TP- True Positive, FP- False Positive, TN- True Negative, FN- False Negative, PPV- Positive Predictive Value, NPV- Negative Predictive Value, H&E- Hematoxylin and Eosin

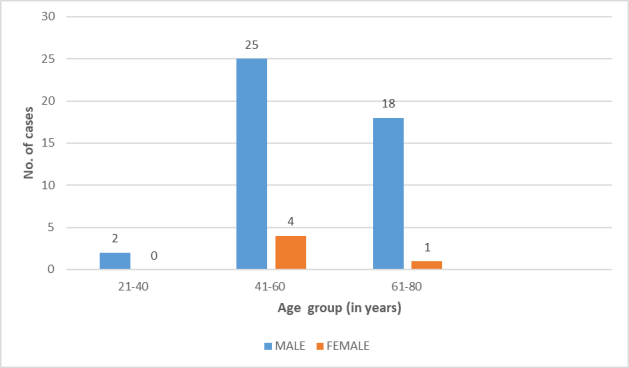

Figure 1: Distribution of cases according to age and gender

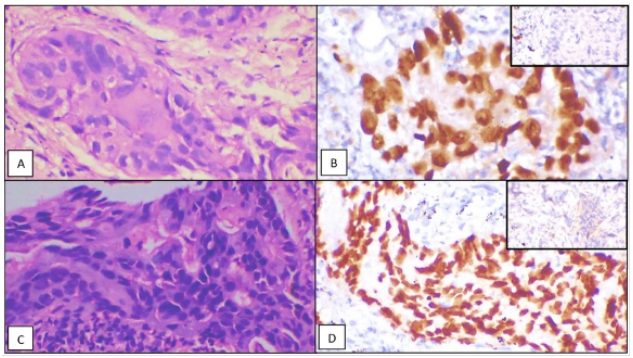

Figure 2: (A) Morphologically differentiated Squamous cell carcinoma with intercellular bridges and intracellular keratinization (H & E X400), (B) p40 immunostain shows 80% nuclear positivity and strong staining intensity of tumor cells. Inset shows negative TTF-1 stain (IHC X400), (C) Morphologically differentiated Adenocarcinoma with glandular formation (H & E X400), (D) TTF-1 immunostain shows 80% nuclear positivity and strong staining intensity in tumor cells. Inset shows negative p40 stain (IHC X400)

Discussion

Lung cancer is one of the most frequently diagnosed malignant neoplasms.[9] NSCLC, constituting tumors other than small cell carcinoma, accounts for 85% of all lung cancers but is heterogenous in nature, comprising lung adenocarcinoma and squamous cell carcinoma as the most frequent histologic subtypes.[10] Morphologically, adenocarcinoma is

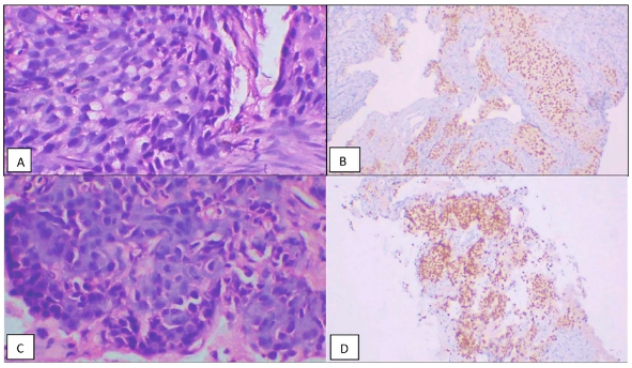

Figure 3: (A) Non-Small Cell LungCarcinoma without morphological differentiation (H & E X400), (B) P40immunostain shows 90% nuclear positivity of tumor cells (IHC X100), (C) NonSmall Cell Lung Carcinoma without morphological differentiation (H & EX400), (D) TTF-1 immunostain shows 80% nuclear positivity of tumor cells (IHCX100)

identified by glandular differentiation and mucin production. Squamous differentiation is recognized by keratin pearls, keratinization and intercellular bridges. However, distinguishing these two tumors can be challenging, particularly in cases of poorly differentiated tumors. In such cases, immunohistochemical markers (p40 and TTF-1) are recommended for precise histologic subtyping. p40 is used for squamous differentiation and found in basal layers of stratified epithelium and some glandular epithelium while TTF-1 is used for differentiation of adenocarcinoma and found in normal type II alveolar pneumocytes.

The present study included a total of 50 cases of non small cell lung carcinomas. The age distribution of patients in this study ranged from 21-80 years with a mean age of 56 years and suggesting a disease of elderly individuals. The youngest patient in our study was of 26 year which indicate a rising incidence in younger age group. 29 cases (58%) belonged to age groups 41-60 years followed by 19 cases (38%) in age group of 61-80 years and 2 cases (4%) in age group of 21-40 years. The finding were comparable with the study of Walia et al[5] who also found that median age of the patients was 58 years with an range of 50-65 years. The sex wise distribution of cases showed that males clearly dominate with 45 cases (90%) and females account for 5 cases (10%) with male to female ratio of 9:1. A similar finding was observed in the study of Muhammad et al[11] which showed male to female ratio as 5.2:1.

In our study, majority of population had a history of smoking accounting for 36 (72%) cases followed by 14 (28%) cases who were non-smoker. The finding were comparable with the study of Varma et al[12] and found that out of total 52 cases, 34 (65.38%) cases were associated by smoking and 18 (34.62%) cases were in non-smoker which suggest a strong association of smoking with lung carcinoma**.** Weight loss was the most common symptom in our study being reported in 46 (92%) cases followed by fever in 43 (86%) cases, breathlessness in 37 (74%) cases, chest pain in 22 (44%) cases, blood in sputum in 14 (28%) cases. Our findings were compared with the study done by Noronha et al[13] and found that most common presentation was weight loss accounted for 84.7% followed by cough in 76%, loss of appetite in 68.8%, chest pain in 51.3%, dyspnea in 33%.

The most common histopathological interpretation was non-small cell lung carcinoma (NSCLC) comprising of 38 (76%) cases followed by adenocarcinoma in 7 (14%) cases and squamous cell carcinoma in 5 (10%) cases. This was done on the basis of International Association for the Study of Lung Cancer/ American Thoracic Society/ European Respiratory Society (IASLC/ATS/ERS) classification criteria for reporting of small biopsies.[14]

The immunohistochemical study of lung biopsies is a very interesting with diverse endeavour. In this study two markers were selected TTF1 and p40, both have vast data derived from prior studies. The expression of both p40 and TTF1 were studied in lung carcinoma to classify non-small cell lung carcinoma and to achieve a better understanding of the diagnosis of various lung carcinomas which cannot be assessed through histopathological examination alone.

In present study, TTF-1 was positive in 6 (85.7%) cases of histologically diagnosed adenocarcinoma. Out of 38 cases diagnosed as NSCLC, TTF-1 showed positivity in 12 cases and diagnosed as adenocarcinoma. This took an tally of 18 cases and showed increment of 61.1% of subtype characterization as adenocarcinoma after applying IHC. (Table 1) Our findings were compared with the study done by Stenhouse G et al[15] and found similar results with increment of subtype characterization after applying IHC.

However, our study showed discordance in 1 case as on histopathological diagnosis it was given as adenocarcinoma but after applying immunohistochemical

markers (TTF-1 and P40), it showed negativity for TTF-1 and was positive for P40 and finally diagnosed as squamous cell carcinoma. Unlike to our findings, Srodon et al[16] showed 100% positivity to TTF-1 in histopathologically diagnosed adenocarcinoma.

In present study, p40 was positive in all 5 (100%) cases of histologically diagnosed squamous cell carcinoma. Out of 38 cases diagnosed histologically as NSCLC, p40 immunostain showed positivity in 23 cases and were further classified as squamous cell carcinoma. This took an tally of 29 cases and showed increment of 82.7% of subtype characterization as squamous cell carcinoma after applying IHC. (Table 1) Our findings were similar with the study done by Thamtam et al[17] and showed 100% positive stain for histomorphologically diagnosed squamous cell carcinoma. Unlike to our findings, Affandi et al[18] showed 96.8% positive stain for Squamous cell carcinoma.

P40 is a recent marker added to subclassify the NSCLC and can be considered as a single best marker for identifying SCC with 100% sensitivity and 85.7% specificity. (Table 2) A similar finding was observed in the study of Pelosi et al[19]. For Adenocarcinoma, TTF1 showed sensitivity of 85.7% and specificity of 100%. (Table 2) Our study was comparable with the study done by Rekhtman et al[20] and showed similar findings.

In our study, 3 cases were negative with both markers (TTF-1 and p40) which may be due to decreased sensitivity of TTF-1 and specificity of p40. These cases remained NSCLC and could not be subtyped further. Our findings were compared with the study done by Walia et al[5] and found similar results.

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. These two markers proved very helpful in diagnosing the cases of non-small cell lung carcinoma with certainty. The biopsy material is often very limited, so only a few markers can be used as opposed to resection specimens where a number of sections can be taken. So using the correct markers is very important.

Therefore, this study gives additional proof to the reliability and necessity of using these two makers in the diagnosis of a non-small cell lung carcinoma on a biopsy. Considering the limited tissue available, these markers are very reliable.

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