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Diagnostic Usefulness Of TTF-1 And P40 In Cases Of Non-Small Cell Lung Carcinoma.

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ABSTRACT

Histomorphology is not enough for subtyping of non-small cell lung carcinoma (NSLCC). Immunohistochemistry (IHC) is helpful in identification of different tumor subtypes. Two marker approach, one each for glandular and squamous cell differentiation maximizes the proportion of accurately subtyped NSCLC on small biopsy samples. To observe expression of TTF-1 and P40 in diagnosed cases of non-small cell lung carcinoma and to evaluate the effectiveness of TTF-1 and P40 immunostain in categorization of non-small cell lung carcinoma and thus guiding treatment protocol. 50 lung biopsies of primary lung carcinoma were prospectively studied. These were subtyped morphologically and then by IHC with p40 and TTF-1. The diagnosis of NSCLC before and after addition of IHC was evaluated. Out of 50 cases in our study, 36 cases were morphologically diagnosed as Adenocarcinoma, 12 cases diagnosed as Squamous cell carcinoma and 2 cases were diagnosed as Adenosquamous carcinoma. Following IHC with TTF-1 and P40 it shows, there is 14% decrease in Adenocarcinoma which resulted in 8% increase in Squamous cell carcinoma, 6% cases were diagnosed as Non adeno non squamous carcinoma. Adenosquamous carcinoma cases remain same. This study provides evidence on the diagnostic effectiveness of minimalist duo. It also shows that either positive or negative profiles are meaningful and diagnostically helpful. It is the best way to save time, material, financial resources, thus further molecular testing and targeted therapy can be initiated.

Keywords: Non-small cell lung carcinoma, p40, TTF-1.

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INTRODUCTION

Pulmonary cancer is the most commonly diagnosed cancer worldwide, and is the leading cause of cancer mortality in men [1]. Its incidence is increasing at a rate of 0.5% per year [2]. The incidence of lung cancer in India is about 5.6 per one lakh population and is the most common cancer related mortality in male. In India, lung cancer ranks second in males and sixth in females in terms of incidence [3]. In female the incidence of lung cancer in developed countries is quite high, in India it is low with a rising trend.[3] The increasing incidence is due to increase in smoking habit, environmental pollution, changing trend in life styles of people and at the same time due to increased availability of the diagnostic modalities to detect lung cancers.

Lung cancer is classified into Small cell carcinoma and Non-Small Cell Carcinoma. Non-small cell lung carcinoma accounts for 80% of all lung cancers and is comprised of Adenocarcinoma (ADC), Squamous Cell Carcinoma (SCC) and Large cell carcinoma [4]. A few of the less common types are adenosquamous, sarcomatoid, pleomorphic, carcinoid tumor, salivary gland type carcinoma, and unclassified carcinoma [5]. Adenocarcinomas are further subclassified into lepidic, acinar, papillary, solid variant and mucinous carcinoma. Squamous cell carcinomas are subtyped as keratinizing, non-keratinizing and basaloid variants. In our study we are concerned with typing Non small cell lung carcinoma into adenocarcinoma and squamous cell carcinoma by using immunohistochemistry marker TTF-1 and p40.

About 60% of non-small cell lung carcinomas present with locally advanced disease at the time of initial diagnosis [6]. Patients with advanced disease are usually inoperable, they often receive targeted molecular therapies. In such situations, a small biopsy and cytology preparations are often the only specimens available for histologic diagnosis and molecular testing [7]. This has led to major changes in the latest pathologic classification by the World Health Organization (WHO) with emphasis on tumor classification and strategic tissue management for handling non-resected specimens. The diagnosis of adenocarcinoma and squamous cell carcinoma can be made based on morphology alone in 50%–70% of non-resected specimens [8]. However, special stains and immunohistochemistry are often required in the setting of poorly differentiated tumors that do not show definite morphology by routine light microscopy. In 2004, the World Health Organization (WHO) first addressed cytology in its lung cancer classification system [9]. Cytomorphologic accuracy when diagnosing adenocarcinomas and Squamous cell carcinoma is reportedly 80% and 87%, respectively [10]. In difficult scenarios IHC can be used to achieve a greater diagnostic sensitivity and specificity than cytomorphology alone. A two-hit diagnostic model based on thyroid transcription factor-1 (TTF1), a very popular and sensitive detector of pulmonary adenocarcinoma [11-15] and p40, a relatively poorly known marker of squamous cell differentiation, deltaN isoforms of the p63 gene family of nuclear transcription factors, [16-18] could be helpful to better refine diagnoses. In the normal lung tissue, by IHC, deltaNp63-p40 is confined to the nonterminal branch of respiratory tree where it consistently decorates the basal layer of the bronchial/bronchiolar epithelium and the myoepithelial cells of the bronchial glands, P40 expression in cancer is almost always confined to the squamous cell carcinoma (SQC) domain, independently of the organ of origin.

Thyroid Transcription Factor-1 (TTF-1), a nuclear stain expressed in pulmonary alveolar lining cells and follicular cells of the thyroid, has long been shown to have diagnostic usefulness in assessing adenocarcinoma of lung origin [19]. TTF-1 reportedly stains 73% of adenocarcinoma and none of the Squamous Cell Carcinoma specimens in one series [20]. TTF-1 has been shown to be highly specific for lung adenocarcinoma. In a series by Hecht et al [21].

So, to correlate the expression of p40 and TTF-1 with the histological types of NSCLC cases to guide the personalized targeted therapy for the patients who are positive for p40 and TTF-1 expression, we intended to perform the present study.

MATERIAL AND METHODS

This study was conducted in the Department of Pathology, of a tertiary care hospital of Eastern India and included 50 consecutive lung biopsies from patients of suspected primary lung cancer attending the department of Pulmonary Medicine. Tissue specimens are obtained by computed tomography guided Tru-cut biopsies and bronchoscopic biopsies. All biopsies were stained by standard hematoxylin and eosin stain and extra sections were cut for IHC. The tumours were initially evaluated by

histomorphological examination. Small cell carcinomas, other neuroendocrine tumours and large cell neuroendocrine carcinomas were excluded. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all patients.

Histopathology

All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. 5 µm thick sections from formalin fixed paraffin embedded blocks were cut and stained with hematoxylin and eosin for histopathological diagnosis of tumor type and subtype.

Immunohistochemistry (IHC)

For IHC staining, 3µm thick sections from formalin fixed paraffin embedded tissues were taken on Poly-L Lysine coated slides. IHC was done using TTF-1 (mouse anti-human TTF-1 monoclonal antibody, clone 8G7G3/1 from Spring Bioscience Pleasanton, California) in 1:50 dilution and p40 (PC373 anti-p40 rabbit polyclonal antibody, from Calbiochem, Darmstadt, Germany) in 1:3000 dilutions and the steps mentioned in the kit supplied were followed.

Scoring of Immunohistochemistry

Immunoreactivity was rendered semi- quantitatively on a scale from 0 to 3+, which was calculated as follows: percentage positivity of cells was graded as **0**, **1** (1-25%), **2** (25-50%) and **3** (50-100%) [22]. Care was taken not to interpret entrapped normal bronchial epithelium or pneumocytes as positive for tumour cell staining.

Data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.

RESULTS

In this study a total 50 cases of non-small cell lung carcinoma were studied. Mean age of our study population was 52.08 ± 12.03 (mean ± SD) years with age range between 32 to 78 years. There were 36 (72%) male patients and 14 (28%) female patients. Majority of the patients were smokers (60%). Adenocarcinoma was the predominant histological variant in non-smokers. Out of 50 cases in our study, 36 (72.0%) cases were morphologically diagnosed as Adenocarcinoma (Fig 1), 12 (24.0%) cases diagnosed as Squamous cell carcinoma (Fig 2) and 2 (4.0%) cases were diagnosed as Adenosquamous carcinoma (Fig 3).

After performing IHC with TTF-1 and P40 it shows, 29 (58%) cases are Adenocarcinoma, 16 (32%) cases are squamous cell carcinoma, 4 (4%) cases are Adenosquamous and 3 (6%) cases are Non adeno Non squamous (Large cell) (Fig 4).

There is 14% decrease in Adenocarcinoma, 8% increase in squamous cell carcinoma, 6% cases diagnosed as non adeno non squamous carcinoma and Adenosquamous carcinoma cases remain same (Table 1). This increment and decrement are statistically significant with a p-value <0.0001. After doing IHC we can approach more accurately towards confirmation of diagnosis. So IHC with this minimal panel is a significant step in diagonosis of non-small cell lung carcinoma (Table 2).

Change in diagnosis occurs mainly in the solid variant of adenocarcinoma and pseudo glandular pattern of squamous cell carcinoma. After performing IHC out of 9 cases of solid variant of adenocarcinoma 5 case became squamous cell carcinoma and 4 remain adenocarcinoma. Out of 3 cases of squamous cell carcinoma with pseudo glandular pattern after IHC 1 became positive for TTF-1 (Fig. 6) and diagnosed as adenocarcinoma and 2 cases became positive for P40 (Fig. 5) and remain as squamous cell carcinoma (Table 3).

Association of Tumor type (morphologically diagnosed) and respective Diagnosis after IHC was statistically significant (p<0.0001).

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Table 1: Distribution of lesions under study before IHC and after IHC

Diagnosis before IHC	Diagnosis after IHC			
	Adenocarcinoma (TTF-1 +/P40-)	Squamous cell carcinoma (TTF-1-ve/p40+ve)	Adenosquamous carcinoma (TTF-1+ve/p40+ve)	Non adeno non squamous carcinoma [Large cell carcinoma] (TTF-1-ve/p40-ve)
Adenocarcinoma (36)	28	05	00	03
Squamous cell carcinoma (12)	01	11	00	00
Adeno squamous carcinoma(02)	00	00	02	00
Total (50)	29	16	02	03
P value	<0.0001			

Table 2: Utility of IHC in diagnosis of non-small cell lung carcinoma

Tumor type	Diagnosis before IHC Number (Percentage)	Diagnosis after IHC Number (Percentage)	Percentage increase / decrease
Adenocarcinoma	36 (72%)	29 (58%)	14% decrease
Squamous cell carcinoma	12 (24%)	16 (32%)	08% increase
Adenosquamous carcinoma	02 (04%)	02 (04%)	Same
Non adeno non squamous carcinoma	00 (00%)	03 (06%)	06% increase
P value	0.0760		<0.0001

Table 3: Changes in morphological variants of non-small cell lung carcinoma revealed by IHC

Diagnosis before IHC	Diagnosis after IHC	
	Squamous cell carcinoma (p40 +ve)	Adenocarcinoma (TTF-1 +ve)
Solid variant of adenocarcinoma (09)	05	04
Squamous cell carcinoma with pseudoglandular pattern (03)	02	01
Total(12)	07	05
P value	0.0645	

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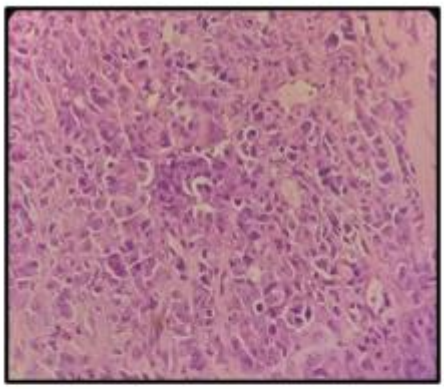

Figure 1: Solid Variant of Adeno Carcinoma (H&E, 400X)

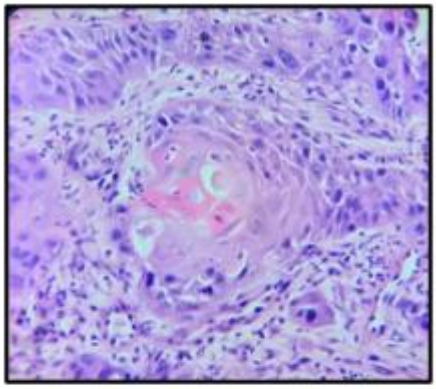

Figure 2: Squamous Cell Carcinoma (H&E, 400X)

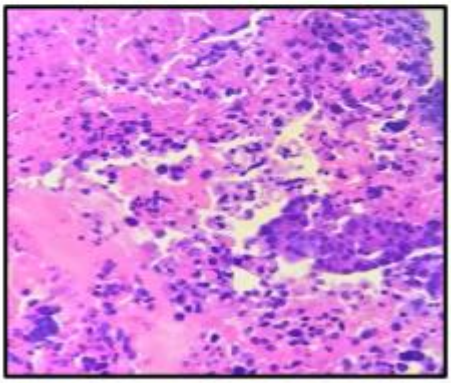

Figure 3: Adenosquamous Carcinoma (H&E,400X)

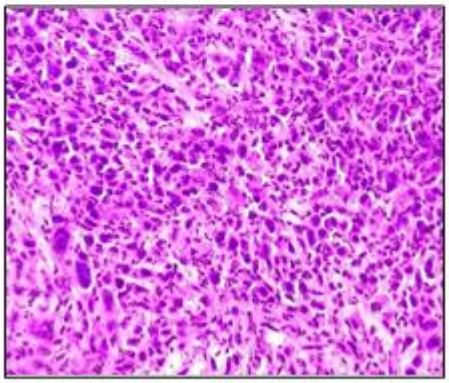

Figure 4: Large Cell Carcinoma [Non adeno non squamous carcinoma] (H&E,400X)

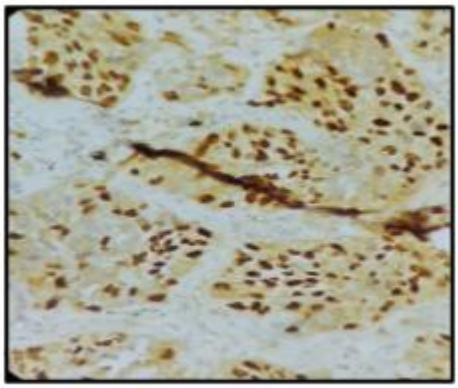

Figure 5: P40 Score 3+ (400X)

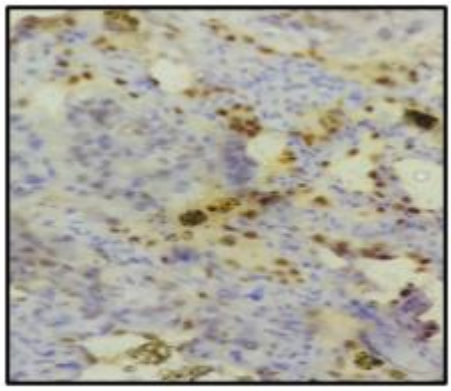

Figure 6: TTF-1 Score 3+ (400X)

DISCUSSION

In the present study, the age of the patients ranged from 32-78 years with the mean age of 52.08 ± 12.03 (mean ± sd which is consistent with study of Malik PS et al [23], Doval DC et al [24] and Kaur H et al [25].

In this study, there were 36 (72%) male patients and 14 (28%) female patients with male: female ratio 2.57:1 Doval DC et al.[24] in their study found similar gender ratio.

Out of 50 study subjects, 30 (60%) were smokers and 20 (40%) were never smokers and smoker to never smoker ratio was 1.5:1. Only 9 out of 36 male patients were never smoker whereas only 4 out of 14 female patients was smoker.

Till now it is a hard challenge for pathologist to come into more specific and accurate diagnosis by morphology alone from small biopsy or cytology [26]. A remarkable shift of traditional morphology related approach on small biopsy has recently been incorporated with minimal IHC algorithm approach in accurately subtyping NSCLC [27]. As available tissues for molecular diagnosis are small in quantity we select two hit diagnostic model based on TTF-1 which is very popular sensitive detector of lung adenocarcinoma and P40 expression which almost always positive for squamous cell carcinoma of lung.

p40 is equivalent to p63 in terms of sensitivity but is superior in terms of specificity as it is negative in p63 positive ADC [28]. p40 was positive in all morphologically differentiated SQC in our study and negative in all ADC. p40 also decorates the squamous component of adenosquamous carcinoma [29]. There were two cases of adenosquamous carcinoma in our study all of which were focally positive for p40 defining the squamous component. These adenosquamous carcinoma cases were diagnosed in retrospect after IHC revealed focal p40 positivity.

TTF-1 is an established marker for lung adenocarcinoma. However, its recorded sensitivity varies from 70 to 96 per cent [26,30]. In our study 3 cases were diagnosed as squamous cell carcinoma with pseudoglandular pattern among which on IHC one case turns to be TTF-1 positive and finally diagnosed as adenocarcinoma.

We observed that out of 36 adenocarcinoma diagnosed before IHC there are 28 (77.8%) cases positive for TTF-1 and negative for P40 and finally diagnosed as adenocarcinoma, 5 (13,8%) cases positive for P40 and negative for TTF-1 and diagnosed as squamous cell carcinoma as well as 3 (8.33%) cases are negative for both p40 and TTF-1 and diagnosed as non adeno non squamous carcinoma which on further ancillary investigations were diagnosed as large cell carcinoma. It is a significant observation of our study.

After doing IHC we can approach more accurately toward confirmation of diagnosis. So IHC performed with this minimal panel is a significant step in diagnosis of non-small cell lung carcinoma.

In this study we found that solid variant of adenocarcinoma and pseudoglandular pattern of squamous cell carcinoma having morphological resemblance are difficult to diagnose accurately on light microscopy. In these cases IHC with TTF-1 and p40 plays an important role to differentiate them as adenocarcinoma or squamous cell carcinoma accurately. Out of 9 cases of solid variant of adenocarcinoma diagnosed morphologically, after IHC showed 5 cases are positive for P40 and negative for TTF-1 and finally diagnosed as squamous cell carcinoma other 4 cases showed TTF-1 positive and p40 negative and remain diagnosed as adenocarcinoma.

Out of 3 cases of morphologically diagnosed pseudoglandular pattern of squamous cell carcinoma after performing IHC showed that 1 case positive for TTF-1 and negative for P40 and finally diagnosed as adenocarcinoma other 2 cases showed P40 positive and TTF-1 negative and finally diagnosed as squamous cell carcinoma.

CONCLUSION

Based on the finding of this study, it may be concluded that the expression of TTF-1 and P40 has immense importance in accurate diagnosis in non-small cell lung carcinoma especially solid variant of adenocarcinoma and pseudoglandular pattern of squamous cell carcinoma.

This study provides evidence on the diagnostic effectiveness of minimalist duo. It also shows that either positive or negative profiles are meaningful and diagnostically helpful. It is the best way to save time, material, financial resources, thus further molecular testing and targeted therapy can be initiated.

Limitation

It was a time bound study with limited number of cases and without any scope of follow up . A wider study with more number of cases for follow up and comparing the diagnosis confirmed by IHC with histopathological diagnosis of lobectomy specimens will be definitely of more help for better interpretation of efficacy of these two IHC marker. Our centre with little scope for lobectomy can depend on IHC as an alternative.

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