Original Research Article

Role of immunohistochemistry in the subtyping of non small cell lung carcinoma

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A R T I C L E   I N F O

A B S T R A C T

Introduction: Worldwide, lung carcinoma is the most common cancer in terms of number of cases and deaths. Lung carcinomas are broadly divided into small cell carcinoma and non-small cell lung carcinoma (NSCLC). In recent years availability of targeted therapies necessitated subtyping the NSCLC to improve the survival and quality of life. NSCLC can be subtyped by routine Haematoxylin and Eosin (H&E) stained section slides alone, poorly differentiated tumors are difficult to segregate morphologically, especially in guided biopsies, necessitating ancillary techniques like immunohistochemistry (IHC).
Materials and Methods: A prospective study of two years duration (2017 and 2018) during which 100 cases of NSCLC on guided biopsies were first reported on Haematoxylin and Eosin sections and later subjected for IHC using relevant markers like CK5/6, CK7, TTF-1, Napsin-A, P63, P40, Synaptophysin and Chromogranin.
Results: Out of 100 cases, after IHC, 49 were diagnosed as adenocarcinoma and 51 as squamous cell carcinoma. In adenocarcinoma positivity for CK7, TTF-1, and Napsin-A was 95%, 60% and 45% respectively. In squamous cell carcinoma positivity for CK5/6, P63 and P40 was 76%, 82% and 88% respectively.
Conclusion: P40 and TTF-1 are more specific markers in our study to differentiate squamous cell carcinoma and adenocarcinoma.

1. Introduction

Lung cancers account for more than 1.3 million deaths per year making it one of the leading causes of cancer-related deaths worldwide. Since the past two decades adenocarcinoma is observed to be the most common type of lung cancer compared to squamous cell carcinoma. Primary lung carcinoma have been classified into Small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), which includes Adenocarcinoma (50-70%), Squamous cell carcinoma (20-30%), and other less common subtypes (<5%). In the recent years molecular studies of lung cancers have led to the development of targeted therapies. Hence, it is necessary to subclassify NSCLC accurately. [1,2]

Most patients of lung cancer are diagnosed at late stage and small biopsies remains the main stay for accurate diagnosis. [3] The current 2015 updated World Health Organization classification of lung cancers also emphasizes the importance of accurate subclassification of lung cancers and the critical role of immunohistochemical (IHC) markers. It also recommends use of minimal tumor tissue for morphological diagnosis, IHC and molecular studies. [4,5] The purpose of present study is to subclassify NSCLC with IHC markers like CK5/6, CK7, TTF-1, Napsin-A, P63 and P40 and evaluate the specificity of markers in diagnosis.

2. Material and Methods

In this prospective study, 100 NSCLC patients who had been diagnosed and treated at our institute in the duration of 2017

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to 2018 were included. The detailed clinical history such as patient's age, habits like smoking or chewing, disease stage, histopathological findings, treatment offered and disease status was retrieved. All the 100 cases of these NSCLC were first reported on Haematoxylin and Eosin sections and were later subjected for IHC using markers like CK5/6, CK7, TTF-1, Napsin-A, P63 and P40.

Immunohistochemical localization was performed on formalin fixed paraffin embedded (FFPE) tissue blocks containing primary tumor evaluated by Hematoxylin and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA).

2.1. Statistical analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Mean, standard error (SE) of mean and median were calculated and Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between the two parameters.

3. Results

Total 100 cases were included in the study. Age range was 40-80 years. Mean age was 60 years. In our study 94% were male patients and 6% were female patients. 56 cases of NSCLC were in the age ≤ 60 years, out of which 32 (57%) cases were of adenocarcinoma and 24 (43%) cases were of squamous cell carcinoma. 44 cases were in the age > 60 years, out of which 17 (57%) cases were of adenocarcinoma and 27 (61%) cases were of Squamous cell carcinoma. In our study Adenocarcinoma was more common in less than 60 years of age whereas squamous cell carcinoma was more common in more than 60 years of age.

52 patients had habit of smoking and 4 patients had habit of tobacco chewing and 38 patients had none habits. Out of 52 smokers 29 (56%) were of adenocarcinoma and 23 (44%) were of squamous cell carcinoma while all 4 tobacco chewers showed squamous cell carcinoma.

In 20 out of 100 cases definite diagnosis was made on basis of histomorphology and out of these 20 cases 16 were diagnosed as adenocarcinoma and 4 as squamous cell carcinoma. Rest all the cases on H & E were either diagnosed as NSCLC or poorly differentiated carcinoma. All the cases were subjected for IHC and after IHC, 49 were diagnosed as adenocarcinoma and 51 as squamous cell carcinoma.

P40 and Napsin-A were done in all cases. TTF-1, CK7, CK5/6 and P63 were not done in 2, 11, 17 and 23 cases due to limited material.

The individual IHC marker positivity among the adenocarcinoma cases (n=49) is given in Table 1. Out of 49 cases, 41 (95%) cases were positive for CK7, 29 (60%) cases were positive for TTF-1 and 22 (45%) cases were positive for Napsin-A.

The individual IHC marker positivity among the squamous cell carcinoma cases (n=51) is given in Table 2. Out of 51 cases, 45 (88%) cases were positive for P40, 37 (82%) cases were positive for P63 and 34 (76%) cases were positive for CK5/6. Out of 51 cases, 7 (14%) cases show Napsin-A positivity along with other squamous markers in squamous cell carcinoma which on IHC was reported as Adenosquamous carcinoma cannot be ruled out.

4. Discussion

4.1. IHC in squamous cell carcinoma

P40 expression was observed in 88% of squamous cell carcinoma cases. P63 showed a positivity of 82% similar to Gurda et al (92%) and Alekhya M. et al (90%) as given in Table 3. p40 is equivalent to p63 in terms of sensitivity but is superior in terms of specificity.[3] CK5/6 was positive in 76% of cases almost similar to Zhao et al (81.25%).[6] p40 has recently entered the diagnostic milieu of NSCLCs with only a few studies evaluating its role. In our study it was also a specific marker for squamous cell carcinoma. All these studies considered p40 a single best marker for identifying SQC.[7] In our study, 14% of squamous cell carcinomas were found positive for Napsin-A. All such cases on IHC were reported as adenosquamous carcinoma cannot be ruled out.

4.2. IHC in adenocarcinoma

TTF-1 and Napsin-A expression was observed in 60% and 45% of adenocarcinoma cases respectively. TTF-1 expression in studies done by Kargi et al (40%),[8] Sainz et al (63%),[9] Bishop JA et al (73%)[10] and Burnnstrom et al (92%)[11] was variable. The reason for above variability in positivity for TTF-1 could be because TTF-1 will be more positive in better differentiated adenocarcinoma.[2] Unlike our findings, Gurda et al, Alekhya M. et al and Burnnstrom et al[11] observed Napsin-A positivity of 92%, 79% and 92% respectively as given in Table 4. Ye J et al, observed that Napsin-A and TTF-1 is a strong indication that an adenocarcinoma originated from lung.[12] Bishop JA et al found that Napsin-A positivity decreased with more poorly differentiated lung tumors.[10] CK7 showed a positivity of 95% similar to the findings of Gurda et al (93%) and Alekhya M. et al (95%). Although TTF-1 had a higher sensitivity, Napsin-A was useful as a surrogate marker when encountering a poorly differentiated lung Adenocarcinoma or an unknown primary tumor.

5. Conclusion

P40 is more specific and sensitive for squamous cell carcinoma than P63. Napsin-A was positive in 45% and TTF-1 was positive in 60% in our study which was low expression as sensitivity of these markers can decrease

Table 1: IHC results in Adenocarcinoma (n=49)

	TTF-1	**CK7**	**Napsin-A**	**CK5/6**	**P63**	**P40**
Positive n(%)	29 (60%)	41 (95%)	22 (45%)	1 (3%)	6 (19%)	7 (14%)
Negative n(%)	19 (40%)	2 (5%)	27 (55%)	37 (97%)	26 (81%)	42 (86%)

**Table 2:** IHC results in Squamous cell carcinoma (n=51)

	TTF-1	**CK7**	**Napsin-A**	**CK5/6**	**P63**	**P40**
Positive n(%)	2(4%)	25 (54%)	7 (14%)	34 (76%)	37 (82%)	45 (88%)
Negative n(%)	48 (96%)	21 (46%)	44 (86%)	11 (24%)	8(18%)	6 (12%)

Table 3: Comparative study showing positivity of IHC markers in Squamous cell carcinoma

Study	**TTF-1**	**CK7**	**Napsin-A**	**CK5/6**	**P63**	**P40**
Alekhya. M et al	7%	9%	0%	84%	90%	
Gurda et al	4%	50%	0%	100%	92%	
Present study	4%	54%	14%	76%	82%	88%
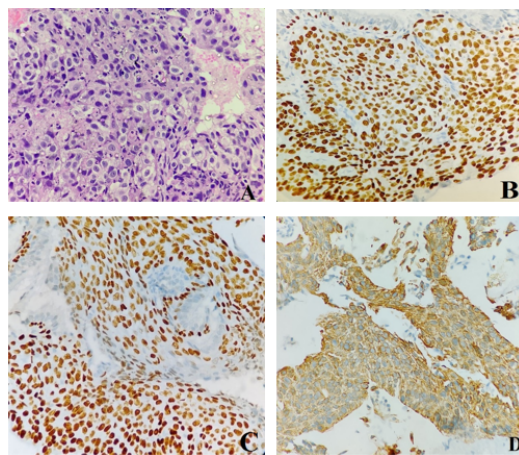
Fig. 1: IHC markers in squamous cell carcinoma; **A):** H&E 40X; **B):** P40 positive; **C):** P63 positive; **D):** CK5/6 positive

Table 4: Comparative study showing positivity of IHC markers in Adenocarcinoma

Study	**TTF-1**	**CK7**	**Napsin-A**	**CK5/6**	**P63**	**P40**
Alekhya. M et al	75%	95%	79%	15%	9%	
Gurda et al	85%	93%	92%	22%	22%	
Present study	60%	95%	45%	3%	19%	14%
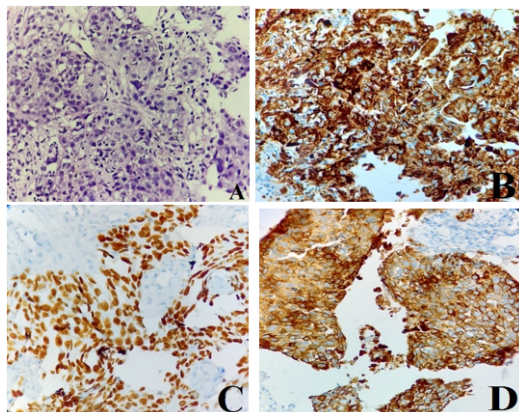
Fig. 2: IHC markers in Adenocarcinoma; **A):** H&E 40X; **B):** Napsin-A positive; **C):** TTF-1 positive; **D):** CK7 positive

with loss of differentiation. P40 and TTF-1 are more specific markers in our study to differentiate squamous cell carcinoma and adenocarcinoma. Also, P40 and TTF-1 are useful when limited material is available for diagnosis.

6. Source of Funding

None.

7. Conflict of Interest

None.

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