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Clinicomorphological and Immunohistochemical Profile of Lung Carcinoma Specimens Received at a Tertiary Care Centre

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ABSTRACT

Introduction: *Lung cancer is the major cause of mortality worldwide and its incidence is rising in India. Sub classifying non small cell carcinoma is vital for prognosis and treatment of the patients. Several immunohistochemical markers have been studied to serve this purpose. In small samples, TTF-1 and p63 are sufficient to further classify the non small cell lung carcinoma. The present study aimed to study morphology and imunohistochemical profile of various lung carcinoma.*

Methods: *A total of 65 cases of lung carcinoma were studied in a span of 18 months. All benign conditions were excluded from the study. Hematoxylin and eosin stained sections were studied and immunohistochemical panel was applied on the selected cases.*

Results: *54 cases were of non small cell lung carcinoma, 10 were of neuroendocrine tumours and 1 was a rare case of pulmonary blastoma. Maximum cases were found to be in the age group of 51-60 years. Squamous cell carcinoma constituted 47.69% of cases followed by adenocarcinoma accounting for 32.30% of cases. TTF-1 was 88.89% sensitive in detecting adenocarcinoma and p63 was positive in 80% of squamous cell carcinoma. Chromogranin was 88.88% sensitive for neuroendocrine tumours.*

Discussion: *TTF-1, p63, CK7, CK5/6 help in differentiating poorly differentiated tumours and hence serve as a prognostic marker in lung carcinomas.*

Keywords: *Lung carcinoma, immunohistochemistry, targeted therapy.*

INTRODUCTION

Lung cancer is the leading cause of mortality worldwide. In India lung cancer constitutes 6.9% of all new cases of cancer and 9.3% of all cancer related deaths are due to lung cancer. The incidence is maximum in Mizoram in both males and females[1]. Over last two decades, adenocarcinoma has replaced squamous cell carcinoma as the most common entity of lung cancer[2,3,4,5,6]. As per the previous Indian literature, squamous cell carcinoma was the most common carcinoma of lung [7,8] however recent studies have shown the changing trends in India [9,10]. Sub classification of non small cell lung carcinoma has become essential in the modern era as new targeted therapies are available which have different

effects in different histologic subtypes e.g. Bevacizumab, a monoclonal antibody against VEGF is associated with increased risk of hemorrhage in squamous cell carcinoma. Pemetrexed is also contraindicated in squamous cell carcinoma. Gefitinib, tyrosine kinase inhibitor, is first line therapy for adenocarcinoma with EGFR mutation.

The present study aimed to study morphology of lung cancer, and its classification into various subtypes on morphological grounds. And in conditions when subtyping of lung carcinoma was not possible on routine histopathology, immunohistochemical study was done for exact categorization of tumour.

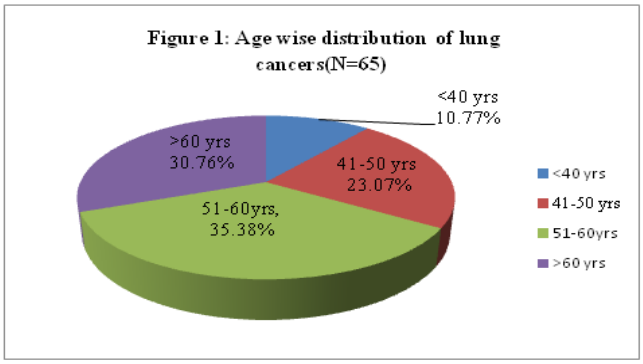
MATERIALS AND METHODS

The present study was done on properly labelled biopsy specimens, which included bronchial biopsies, trucut biopsies, transbronchial biopsies, pleural biopsies, lobectomy specimen and pneumonectomy specimens that were well preserved in 10% formalin, sent with patient's history and proper clinical details on requisition form in department of pathology at SMS Medical College. The study duration was 1.5 years (from March 2015 to September 2016).

Following adequate fixation for about 12-24 hours, the tissues were submitted for routine processing, following which the paraffin embedded serial sections of 3-4 micron thickness were obtained. These were stained with Hematoxylin and Eosin stain and immunohistochemistry was applied when and where required. In this study TTF-1, CK5/6,CK7 and p63 immunomarkers were applied as per the requirement. Immunohistochemical staining was performed using Biocare monoclonal TTF-1 mouse antibody; clone(8G7G3/1)diluted in phosphate buffer saline(PBS), biocare monoclonal p63 mouse antibody; clone (4A4)diluted in PBS, biocare monoclonal CK5/6 mouse antibody; clone(CK5/6.007)diluted in PBS and biocare monoclonal CK7 mouse antibody; clone(OV-TL 12/30) diluted in PBS. However, other markers like Napsin A, CK20 and neuroendocrine markers were also applied on cases as per the need.

RESULTS

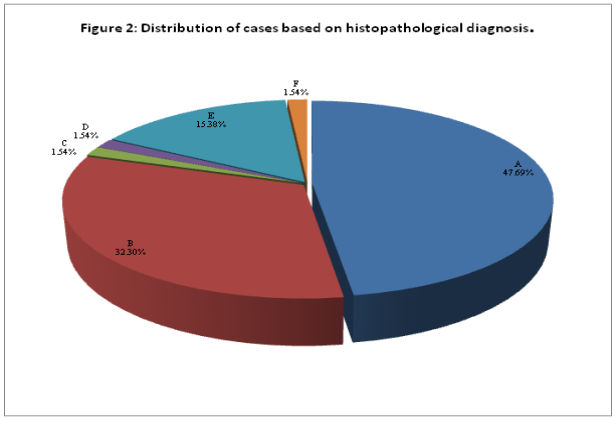
During the period of 18 months, a total of 65 lung biopsies were studied. Out of 65 cases studied, 54 were non small cell lung carcinoma, 10 were neuroendocrine tumours and 1 was of pulmonary blastoma. The highest number of cases were in the age group of 51-60 years (23 cases) which constituted 35.385%.Mean age was 55.66 years. Median age was 58 years. (figure 1).



Out of 65 cases, 54 were males (83.07%) and 11 were females (16.93%).

Amongst these 65 cases, 34 were smokers (52.31%) and 31 were non smokers (47.69%).

Squamous cell carcinoma accounted for maximum number of cases (31) constituting 47.69% of cases followed by adenocarcinoma(21 cases) accounting for 32.30% of cases.(figure 2)



A: squamous cell carcinoma
B: adenocarcinoma
C: large cell anaplastic carcinoma
D:pulmonary blastoma
E: neuroendocrine tumours
F: adenosquamous carcinoma

Squamous cell carcinoma was most commonly associated with smoking i.e. 22 out of 31 cases of squamous cell carcinoma were smokers(70.96%) . Immunohistochemistry was applied in 36 cases where exact subtyping of lung carcinoma was not possible on Hematoxylin and Eosin stained sections only. A panel of TTF-1 , p63, CK5/6, CK7 was applied on the cases which were reported on hematoxylin and eosin stained sections as poorly differentiated non small cell lung carcinoma. However in few cases (n=2), the tissue was very tiny and hence only TTF-1 and p63 markers were used to subtype the same.

Out of 54 non small cell carcinoma cases, 25 cases showed features of squamous cell carcinoma on routine hematoxylin and eosin stained sections. 3 cases were showing clear cut features of adenocarcinoma. Immunohistochemistry was done in 26 cases. 5 cases were diagnosed as squamous cell carcinoma on immunohisto-chemistry and 19 cases were diagnosed as adenocarcinoma after immunohistochemical studies .One case did not show features of squamous cell carcinoma or adenocarcinoma on hematoxylin and eosin stained sections study and immunohistochemistry and was labelled as large cell anaplastic carcinoma. One case showed immunohistochemical positivity for both adeno and squamous cell carcinoma and was classified as adenosquamous carcinoma.

One case was a rare case of pulmonary blastoma showing blastematous and mesenchymal components both morphologically and immunohistochemically. All 10 cases of neuroendocrine tumours were subjected to immunohistochemistry where CD56, NSE, synaptophysin , chromogranin ,CK in addition to CK5/6 ,p63,CK7 and TTF-1 were applied with MIB and napsin A in few cases. Sensitivity of p63 was found to be 80% for detection of squamous cell carcinoma.CK5/6 was applied on 2 cases of squamous cell carcinoma only and one showed positivity.

TTF-1 was applied on 32 cases. Out of 18 cases of adenocarcinoma, 16 showed strong nuclear positivity . None of the squamous cell carcinoma show positivity for TTF-1.However out of 6 neuroendocrine tumours, 1 showed TTF-1 positivity. Sensitivity of TTF-1 was 88.89% for diagnosing adenocarcinoma.

CK7 was applied on 22 cases. Out of 17 cases of adenocarcinoma, 15 showed diffuse membranous positivity. Sensitivity of CK7 was observed to be 88.23%.

Napsin A was applied on 17 cases. All squamous cell carcinoma(n=2) and neuroendocrine tumours(n=6) were negative for Napsin A and out of 9 cases of adenocarcinoma, 4 show cytoplasmic positivity.Sensitivity of Napsin A was 44.44% for adenocarcinoma. Out of 10 neuroendocrine tumours, NSE was applied in 8 cases and chromogranin was applied on 9 cases. NSE was 100% sensitive in diagnosing neuroendocrine tumours. Sensitivity of chromogranin for neuroendocrine tumours was 88.88%. (table 1- 9)

Representative histomorphological and immune-ohistochemical images are shown in figure 3-6.

Table 1: p63 in different subtypes of lung carcinoma

Type of cancer	p63+	p63 -
Squamous cell carcinoma(n=5)	4	1
Adenocarcinoma(n=17)	0	17
Large cell anaplastic carcinoma(n=1)	0	1
Pulmonary blastoma(n=0)	0	0
Neuroendocrine carcinoma(n=9)	0	9
Adenosquamous carcinoma(n=1)	1	1
Total	5	28

Table 2: CK5/6 in different subtypes of lung cancer

Type of cancer	CK5/6+	CK5/6 -
Squamous cell carcinoma(n=2)	1	1
Adenocarcinoma (n=15)	0	15
Large cell anaplastic carcinoma(n=1)	0	1
Pulmonary blastoma(n=0)	0	0
Neuroendocrine tumour(n=3)	0	3
Adenosquamous carcinoma(n=2)	1	0
Total(n=22)	2	20

Table 3: TTF-1 in different subtypes of lung cancer

Type of cancer	TTF-1+	TTF-1-
Squamous cell carcinoma (n=5)	0	5
Adenocarcinoma(n=18)	16	2
Large cell adenocarcinoma(n=1)	0	1
Small cell carcinoma(n=7)	1	6
Adenosquamous cell carcinoma (n=1)	1	0
Total (n=32)	18	14

Table 4: CK7 in different subtypes of lung cancer

Type of cancer	CK7+	CK-
Squamous cell carcinoma(n=1)	0	1
Adenocarcinoma(n=17)	15	2
Large cell anaplastic carcinoma(n=1)	0	1
Small cell carcinoma(n=3)	0	3
Total(n=22)	15	7

Table 5: Napsin A expression in different subtypes of lung carcinoma

Type of carcinoma	Napsin A-	Napsin A+
Squamous cell carcinoma(n=2)	2	0
Adenocarcinoma (n=9)	5	4
Neuroendocrine tumours(n=6)	6	0
Total(n=17)	13	4

Table 6: EGFR positivity in adenocarcinoma

Total cases	EGFR positivity	Percentage
10	8	80%

Table 7: NSE in neuroendocrine tumours

Type of cancer	NSE+	NSE-
Neuroendocrine tumours(n=8)	8	0

Table 8: chromogranin in neuroendocrine tumours

Type of cancer	Chromogranin+	Chromogranin -
Neuroendocrine tumours (n=9)	8	1

Table 9: The sensitivity of different markers used in the study:

Marker	Sensitivity
TTF-1	88.89%
P63	80%
CK5/6	50%
CK7	88.23%
Napsin A	44.44%

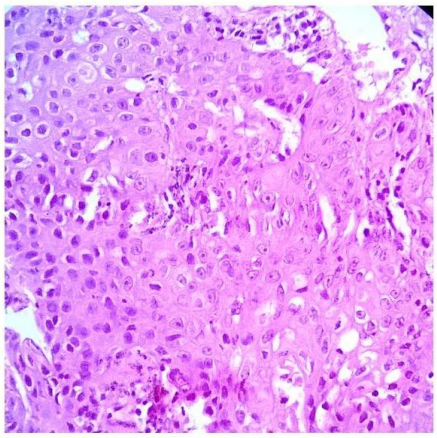


Figure 3: H&E stained sections showing squamous cell carcinoma(400x)

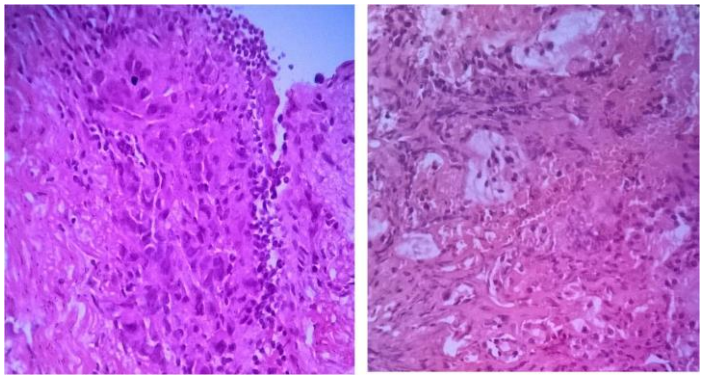


Figure 4(a): H&E stained sections showing adenocarcinoma; extracellular mucin is shown in right Figure.(100x).

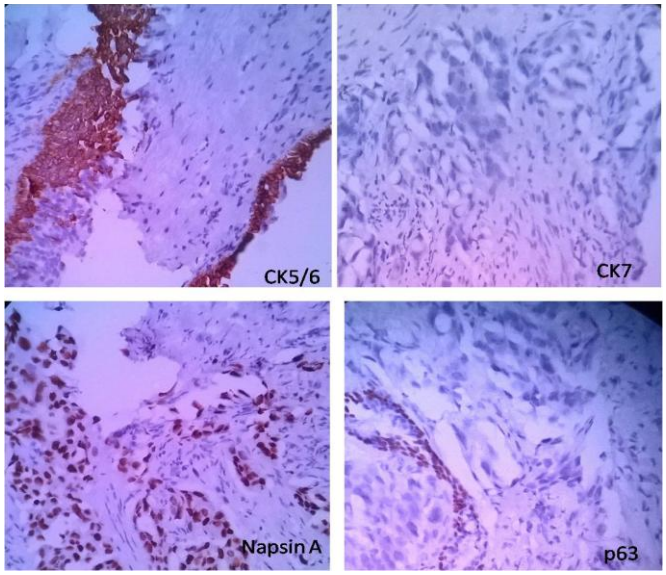


Figure4(b): (400x) showing:
Membranous positivity for CK5/6(top left) ;
Negative for CK7(top right);
Strong nuclear positivity for Napsin A(bottom left) and

Negative for p63.Positive internal control is shown.(bottom right)

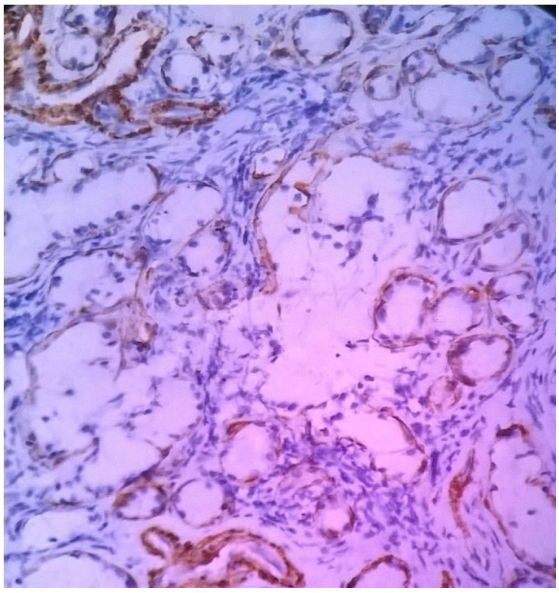


Figure4(c): showing diffuse nuclear positivity for TTF-1 (400x)

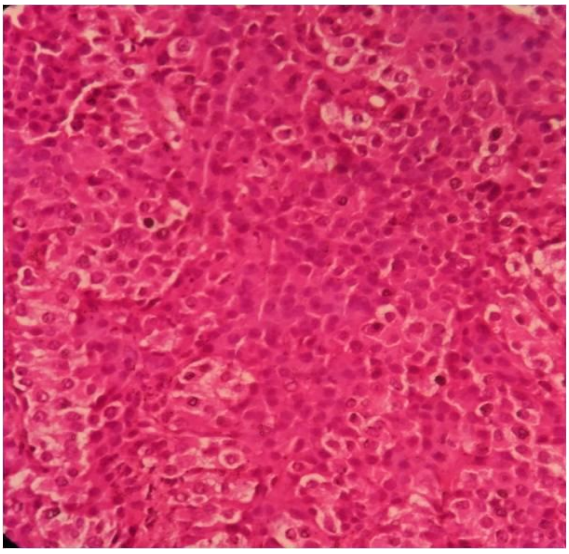


Figure 5(a): H&E stained sections showing neuroendocrine carcinoma(400x).

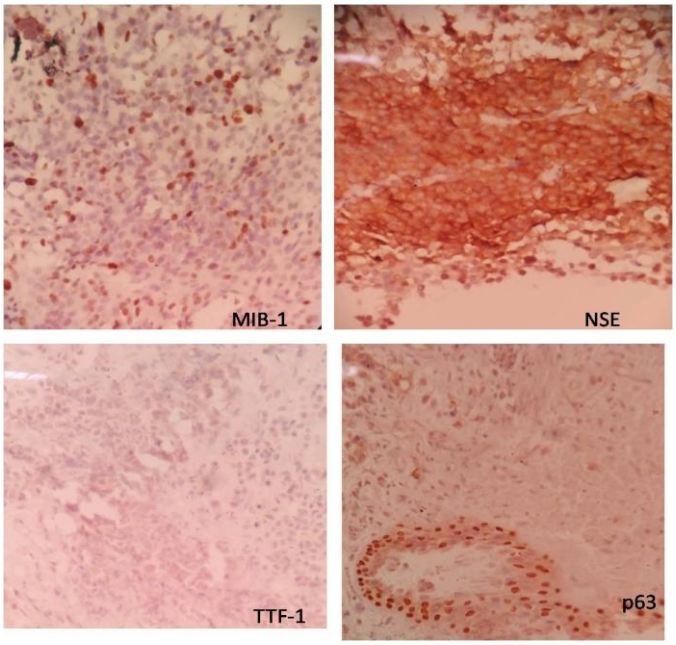


Figure 5(b): showing
MIB -1 index of 18% (top left)(100x);
Diffuse membranous staining for NSE(top right)(100x);
Negative for TTF-1(bottom left)(100x);
Negative for p63(bottom right); however the image shows positive internal control(100x):

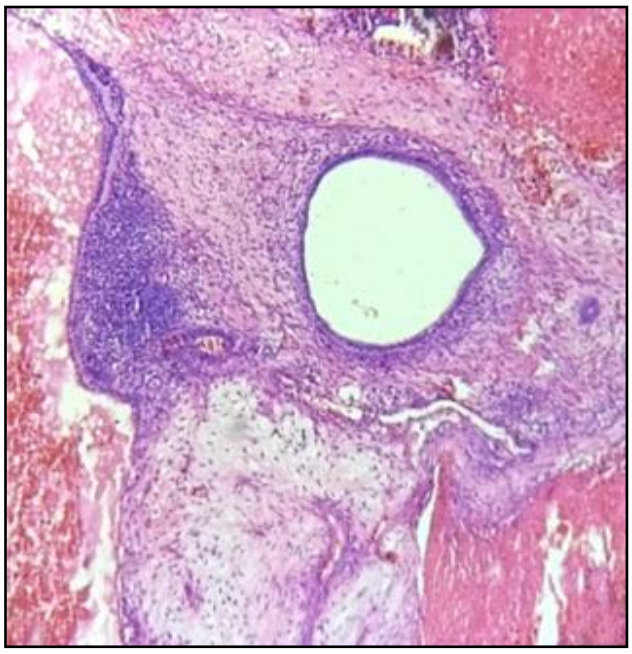


Figure 6(a): Pulmonary blastoma: H&E stained sections showing blastemal component in a myxoid background and cartilaginous differentiation.(100x)

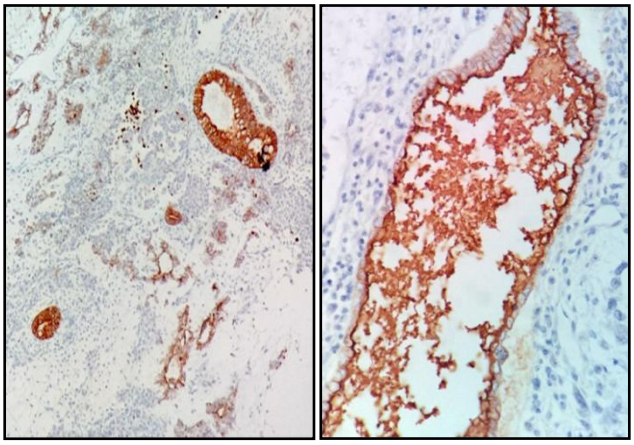


Figure6(b): Pulmonary blastoma showing immunopositivity for Cytokeratin (left); Carcinoembryonic antigen(right)

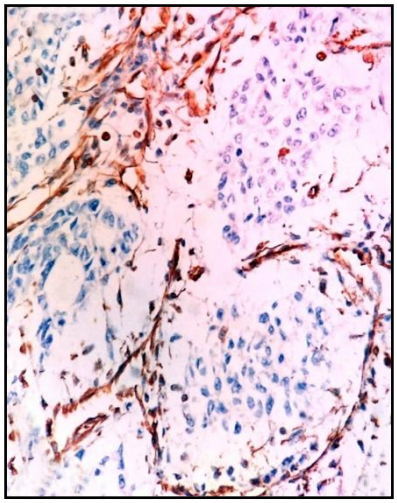


Figure 6(c) : Pulmonary blastoma showing immunopositivity for vimentin

DISCUSSION

The incidence of lung carcinoma is rising in India. Smoking remains the main etiological agent however the recent studies and researches have well elaborated the rising incidence of lung cancers in non smokers. The incidence of adenocarcinoma is increasing and adenocarcinoma is currently the most common hitopathological subtype of lung cancer worldwide. Precise subcategorization of non small cell lung carcinoma plays an important role in treatment and prognosis of the patients. The World Health Organization has enumerated guidelines for classification of lung cancer in resection specimens but majority of the cases are diagnosed at advanced stages and are unresectable, hence a new classification has been proposed for small biopsies and cytology specimens. This new classification is based on morphological, immunohistochemical studies and molecular studies.

In the present study squamous cell carcinoma constitutes 47.69% of the total lung carcinoma and adenocarcinoma constitutes 32.30% unlike other studies showing adenocarcinoma to be the most frequent lung carcinoma in the present. However the present study correlates with the study of Rawat J et al[11], Singh et al[8] and Behera et al[7]. Various studies show rising trend of adenocarcinoma and over the last two decades, adenocarcinoma has replaced squamous cell carcinoma as the most common lung cancer.[2,3,4,5,6]. However this variation from other studies may be attributed to small study population in the present study and also the biopsy of every affected individual was not received at our institution probably. This could also be attributed to the increased diagnosis of adenocarcinoma on cytology specimens and the therapy started thereafter on basis of cytology specimens leading to less number of biopsies of adenocarcinoma probably.

However it should be emphasized that the immunohistochemistry is neither 100% specific nor 100% sensitive. Despite it, a high degree of accuracy has been established in subtyping of non small cell lung carcinoma by applying a panel of immunohistochemical markers including TTF-1,CK7, p63 and CK5/6. Napsin A further aids in refining the diagnosis. TTF-1 is a protein encoded by NKX2-1 gene on chromosome 14q that regulates transcription of genes specific for thyroid, lung and central nervous system. It is highly specific for adenocarcinoma. Napsin A in combination with TTF- 1 act as potential evidence of pulmonary origin of the tumour. Adenocarcinoma difficult to diagnose over routine light microscopy are differentiated from squamous cell carcinoma by being positive for TTF-1 and CK7 and negative for p63 and CK5/6. The present study showed 16 out of 18 adenocarcinoma show intense nuclear TTF-1

positivity and the sensitivity of TTF-1 in diagnosing adenocarcinoma came out to be 88.89% which is consistent with study of Wei Zhao et al[12] and Mukhopadhyay S et al[13] . The present study showed 4 cases out of 9 adenocarcinoma cases revealed Napsin A positivity (44.44%) and all cases positive for Napsin A were also positive for TTF-1. Tumour protein p63 is encoded by TP63 gene , member of p53 family of transcription factors. It is located on chromosome 3q and expressed in normal respiratory epithelium of central air conducting system. In small biopsy specimen when it is extremely difficult to appreciate squamous differentiation ,p63 and CK5/6 positivity along with negative staining for TTF-1 and CK 7 support squamous differentiation.

Sensitivity of p63was calculated as 80% consistent with study of Jefferson Terry et al[14]. Squamous cell carcinoma of lung are CK5/6 positive whereas adenocarcinoma of lung are CK7+ and CK20-. In the present study CK 5/6 was applied only on 2 squamous cell carcinoma and one show diffuse cytoplasmic positivity i.e in 50% of the cases. The results vary slightly from other studies due to low number of cases where CK5/6 is used.

The present study showed sensitivity of CK7 to be 88.23%. The present study showed that sensitivity of chromogranin was 88.88% consistent with results of international workshop[15]. Shanmugapriya S et al stated that a immunohistochemical panel of p63, TTF-1 , CK5/6 and CK7 help in subtyping the non small cell lung carcinoma.[16]

The present study is in in correlation with other studies and demonstrates well that the above panel of CK5/6 ,CK7 ,p63 and TTF-1 is sufffiient to subtype non small cell lung carcinoma. When the tissue is very scant and the whole panel can not be applied , only TTF-1 and p63 will do the job.

CONCLUSION

In the era of targeted therapy, an accurate subclassification of non small cell lung carcinoma is necessary. Morphology is the gold standard however a panel of immunohistochemistry including TTF-1, p63, CK7, CK5/6 help in differentiating poorly differentiated tumours.

DECLARATIONS

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Conflict of interest: none

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