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Diagnostic Utility of Cell Block of Bronchial Brush Tip Washing in the Diagnosis of Carcinoma Lung and Application of Cytokeratin 5/6, TTF-1 & CD 56 Expression for Subclassification

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ABSTRACT

Background: Lung cancer is one of the most common malignancies worldwide with high mortality. Furthermore being one of the most insidious and aggressive neoplasm in the realm of oncology, it's timely diagnosis and accurate sub-classification becomes pre-requisite for administering appropriate and timely target therapy. In the present study, cell block from brush tip washings were prepared and immunoreactivity assessed for cytokeratin5/6, TTF-1 and CD56 with aim to diagnose and sub-classify carcinoma lung. **Methods:** The present study was conducted on 25 specimens of brushtip washings from suspected cases of carcinoma lung. Bronchoscopic investigation of pulmonary lesions was performed and routine brush smears were made and these brush tip were processed into cell block. Immunohistochemical staining for marker CK5/6, CD56 & TTF 1 was done and evaluated. **Results**: Brush smear cytology finding was mostly benign seen in 12 (48%) followed by SCC seen in 4 (16%), ADC in 3 (12%). Cell block microscopy showed SCC in 11 (44%), ADC in 5 (20%), small cell carcinoma in 3 (12%) and carcinoma in 3 (12%). It was inadequate in 3 (12%). The difference was significant (P< 0.05). Sensitivity and specificity of brush smear cytology in diagnosing lesion was 33.3 % and 52.6% respectively.Whereas for cell block microscopy in diagnosing lesion sensitivity was 91.67% and specificity 86.6%. The overall sensitivity of IHC CK 5/6 in diagnosing SCC was 100% and specificity was 52.4%. CD56, TTF1 were negative in these cases. CD56 showed 100% sensitivity in diagnosing small cell carcinoma with specificity being 24.6%, The overall sensitivity of IHC TTF 1 in diagnosing ADC was 100% & for small cell carcinoma was 40%. **Conclusion:** Cell block preparation is a simple method that increases diagnostic yield of flexible bronchoscopy, is cost effective & hence can be routinely used. IHC panel consisting CK 5/6, CD 56 and TTF 1 has more diagnostic value in precise subtyping of different types of lung carcinoma in adjunction to routine H&E staining.

Keywords: Brush smear, Cell block, Lung cancer.

INTRODUCTION

Lung cancer is one of the most common malignancies worldwide with high mortality, accounting for 13% of all new cancer cases and 19% of cancer related deaths worldwide. In India, 6.9% of all new cancer cases and 9.3% of all cancer related deaths are due to lung cancer. In India, estimated new cases of lung cancer during 2016 are 1.14 lakh (83,000 in males and 31,000 in females).[1] Although tobacco smoking remains the most important risk factor for development of lung cancer, association of indoor/outdoor air pollution, occupational exposures like asbestos and genetic factors with development of this disease has been identified especially amongst non-smokers.[2]

Bronchoscopy is a safe and effective means of diagnosing bronchogenic carcinoma. Approximately 70% of lung cancers are un-resectable as patients present in advanced stages and so cytology specimens continue to remain the primary method of diagnosis for the majority of lung cancer patients.[3]

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In bronchial brushing cells are taken from the airway mucosa or bronchial lesions through catheter-based brush under direct visualization or fluoroscopic guidance. It is used to find dysplastic or neoplastic changes. Cell block from Brush tip washings (BTW) is a recent modality which utilizes the cells that remain on the bronchoscope cytology brush following smearing onto cytology slides.[4]
Immunohistochemistry (IHC) is valuable for confirming the origin of malignancy and recent studies have demonstrated that the non-small-cell lung cancer (NSCLC) subtype determines the choice of systemic therapy in patients with advanced disease.[5,6] Most studies recommend an antibody panel comprising a combination of cytokeratin 5/6 and TTF-1 which are specific for squamous cell carcinoma and adenomacarcinoma respectively. CD 56 is also useful marker for detection of small cell carcinoma. In the present study, cell block from brush tip washings were prepared and immunoreactivity assessed for cytokeratin5/6, TTF-1 and CD56 with aim to diagnose and sub-classify carcinoma lung.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Department of Chest & TB and Department of Radiotherapy & Oncology, Government Medical College, Amritsar. It included 25 specimens of brushtip washings from suspected cases of carcinoma lung.
Bronchoscopic investigation of pulmonary lesions was performed with intravenous sedation and topical lignocaine 2%. The procedure was performed using a standard video-bronchoscope. After the lesion was located, sampling instruments were passed down the sheath and brushings collected and smeared onto slides. Once the smears are made, the brush tip will be rinsed with NAFS.

Cell block technique

The specimen collected was subjected to centrifugation at 2000 rpm for 15min. The supernatant was discarded and 3-4 drops of thrombin was added to the sediment & mixed, 2-3 drops of plasma will be then added to the mixture which will be allowed to clot. The sediment containing the cell button of the fluid sample was scooped out on to the filter paper and was processed along with other routine histopathological specimens to obtain paraffin sections and stained with Haematoxylin and Eosin.

IHC staining was also done. 3-5 µm sections were cut, mounted on freshly prepared 0.01% poly-L-lysine coated slides. Slides will be dried overnight at 37˚C, dewaxed in xylene and hydrated. The IHC score is calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score).

RESULTS

Table 1: Brush smear cytology in patients

Cytology	Number	Percentage	P value
Acellular	1	4	0.001
Benign	12	48	
Inflammatory	1	4	
Atypical	1	4	
Carcinoma	2	8	
SCC	4	16	
ADC	3	12	
Small cell carcinoma	1	4	
Total	25	100	

[Table 1] shows that brush smear cytology finding was benign seen in 12 (48%) followed by SCC seen in 4 (16%), ADC in 3 (12%). The difference was highly significant (P< 0.05).

Table 2: Cell block microscopy

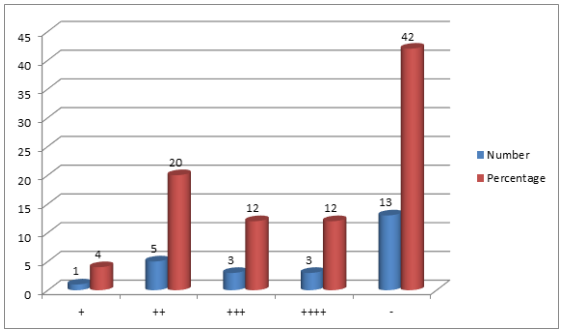
Cytology	Number	Percentage	P value
SCC	11	44	0.0012
ADC	5	20	
Small cell carcinoma	3	12	
Carcinoma	3	12	
Inadequate smear	3	12	
Total	25	100	

[Table 2] shows that cell block microscopy showed SCC in 11 (44%), Adeno Ca in 5 (20%), small cell carcinoma in 3 (12%) and carcinoma in 3 (12%). It was inadequate in 3 (12%). The difference was significant (P< 0.05).

Table 3: Comparison between brush smear microscopy and Cell block microscopy

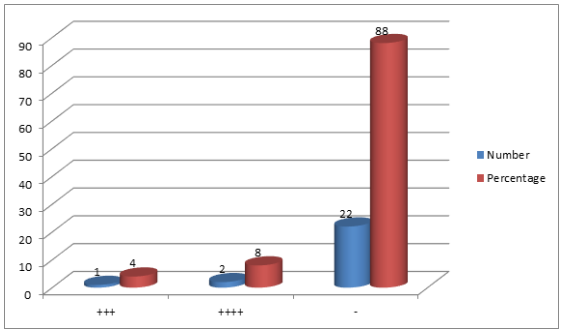
Diagnosis	Brush smear cytology No. of cases	Cell Block Microscopy No. of Cases
Acellular	1	03
Benign or inflammatory	13	00
Atypical	1	00
Carcinoma	2	03
SCC	4	11
ADC	3	05
Small cell carcinoma	1	03

[Table 3] shows that sensitivity of brush smear cytology in diagnosing SCC was 33.3 %, for ADC was 60%, for small cell carcinoma was 33.3%. Specificity was 52.6%, positive predictive was 48% and negative predictive value was 85%. Sensitivity of cell block microscopy in diagnosing SCC was 91.67%, for ADC was 100%, for small cell carcinoma was 100%. Specificity was 86.6%, positive predictive value was 68.2% and negative predictive value was 78%.



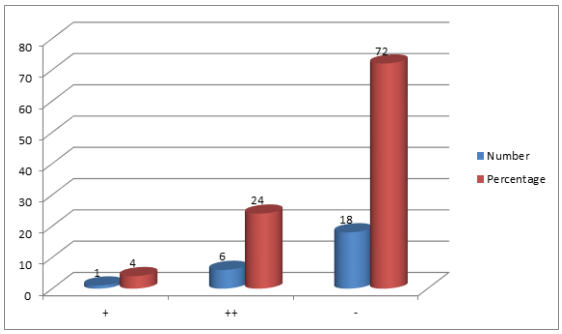
Graph 1: Assessment of IHC CK 5/6

Graph I shows that IHC was + in 1 (4%), ++ in 5 (20%), +++ in 3 (12%) and ++++ in 3 (12%) It was – in 13 (42%). Chi- square test showed significant difference (P< 0.05).



Graph 2: Assessment of IHC CD 56

Graph II shows that IHC CD 56 was +++ in 1 (4%) and ++++ in 2 (8%) males. It was – in 22 (88%). Chi- square test showed significant difference (P< 0.05).



Graph 3: Assessment of IHC TTF 1

Graph III shows that IHC TTF 1 was + in 1 (4%) and ++ in 6 (24%). It was – in 18 (72%). Chi- square test showed significant difference (P< 0.05).

Table 4: Efficacy of CK 5/6, CD 56 and IHC TTF 1 in diagnosing lesions

Cytology	Final diagnosis	CK 5/6	CD 56	IHC TTF 1	P value
SCC	12	12	0	0	0.001
ADC	5	0	0	5	
Small cell carcinoma	3	0	3	2	
Total	20	12	3	7	

[Table 4] shows that overall sensitivity of IHC CK 5/6 in diagnosing lesions was 56%. Sensitivity for SCC was 100%, for ADC, small cell carcinoma was 0%. Specificity, positive predictive value and negative predictive value was 52.4%, 58.2% and 52% respectively. Overall sensitivity of IHC CD 56 in diagnosing lesions was 28%. Sensitivity for SCC, ADC was 0% and for small cell carcinoma was 100%. Specificity, positive predictive value and negative predictive value was 24.6%,66.2% and 54.6% respectively. Overall sensitivity of IHC TTF 1 in diagnosing lesions was 28%. Sensitivity for SCC, ADC and small cell carcinoma 0%,100% and 40% respecively. Specificity, positive predictive value and negative predictive value was 24.6%, 66.2% and 54.6% respectively.

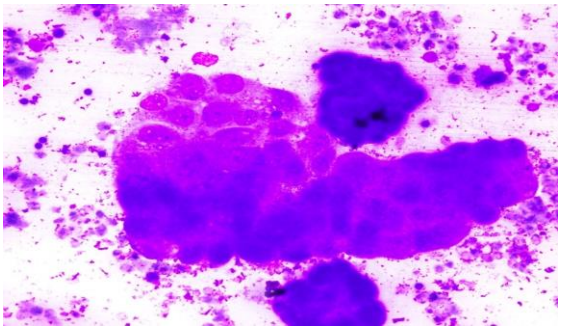


Figure 1: Photomicrograph Showing Adenocarcinoma Lung in Brush Smear (GIEMSA, 400X)

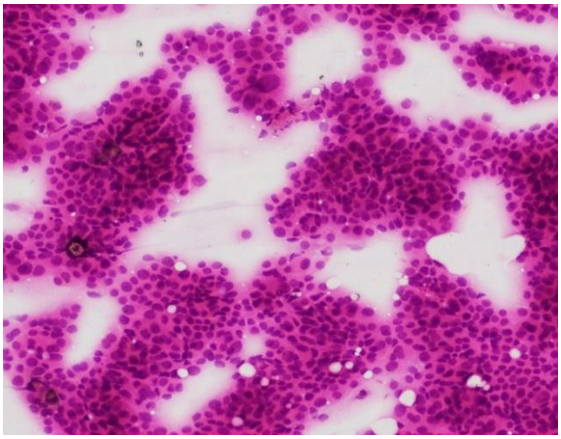


Figure 2: Photomicrograph Showing Acinar Pattern Adenocarcinoma Lung In Cell Block(H&E, 400x)

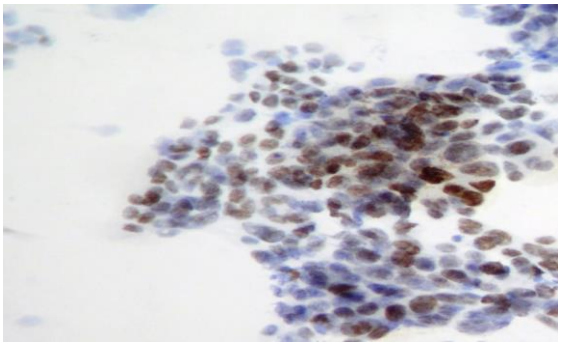


Figure 3: Photomicrograph showing immunoreactivity for ttf-1 in adenocarcinoma lung (ihc, 400x)

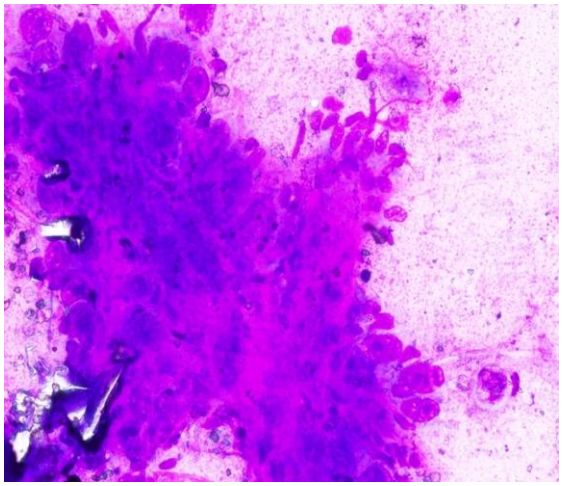


Figure 4: Photomicrograph showing highly atypical squamous cells in brush smear(giemsa,400x)

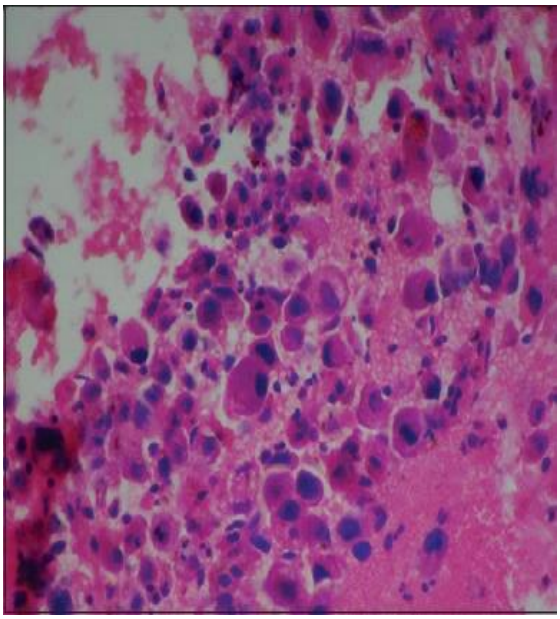


Figure 5: Photomicrograph Showing Atypical Squamous Cells In Cell Block (H&E, 400X)

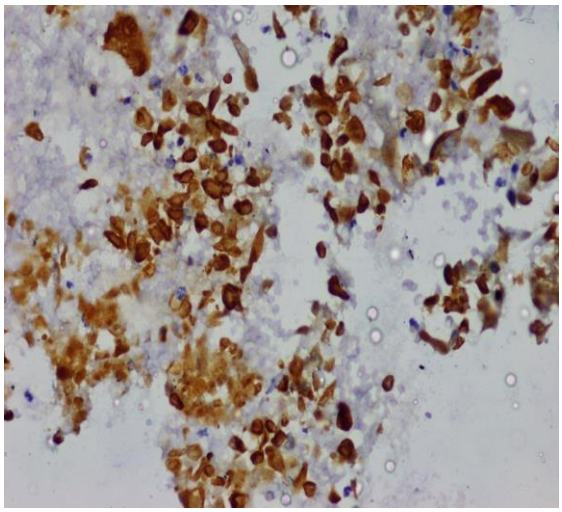


Figure 6: Photomicrograph Showing Immunoreactivity For Ck5/6 In Squamous Cell Carcinoma Lung (Ihc,400X)

DISCUSSION

Worldwide, 1.8 million patients were diagnosed with lung cancer in 2012 that caused an estimated 1.6 million deaths. In the United States, there are approximately 225000 new cases of lung cancer and over 160000 deaths annually.[7,8] The 2015 World Health Organization (WHO) classification recognizes four major types histologically: Adenocarcinoma (including bronchioalveolar carcinoma), squamous cell carcinoma, large cell carcinoma and small cell carcinoma.[9]

In present study, out of 25 patients, there were 21 (84%) males and 4 (16%) females. In a study by Kakodkar et al,[10] there were 77 males (74%) and 27 were females (25.96%).

The age of the patients ranged from 35 to 78 years with mean age in males was 56.04± 2.4 years and in females was 57.5± 3.5 years. Jafarian et al,[11] found that mean age of the patients was 60 years; ranged from 35 to 81. In present study, on brush smear cytology, 12(48%) cases were diagnosed as benign followed by SCC in 4 (16%), ADC in 3 (12%). Calabretto ML,[12] found that of the 201 cases considered, 200 were primary lung neoplasms (103 squamous cell carcinomas, 60 adenocarcinomas, 23 small cell lung carcinomas, 4 large cell carcinomas, 3 adenosquamous carcinomas, 2 non-Hodgkin lymphomas, and 5 malignant epithelial cells NOS) and 1 case was a metastatic clear-cell renal adenocarcinoma.

In present study, cell block smear was adequate in 22 (88%) and inadequate in 3 (12%). We found that cell block microscopy showed SCC in 11 (44%), ADC in 5 (20%), small cell carcinoma in 3 (12%) and carcinoma in 3 (12%). It was inadequate in 3 (12%).

In present study we observed that out of 12 SCC, cell block microscopy gave 11 positive results. It was positive in all 5 cases of ADC and all 3 small cell carcinoma. The overall sensitivity of cell block microscopy in diagnosing lesions was 84%.

Santoshpawar et al,[13] found that out of total 75 samples, 42 were given positive for malignancy on conventional smear. Cell blocks were positive for 48 malignancies and negative for 2 malignancies. Sensitivity and specificity of cell block method in diagnosis lung malignancies were 96% and 92.59% respectively as compared to conventional smear method. In present study, out of 12 SCC, IHC CK 5/6 gave 12 positive results. We can suggest that in squamous cell carcinoma cases, IHC CK 5/6 is a useful marker and it shows high sensitivity and specificity.

Kargi A et al,[14] found that p63 and CK 5/6 seem to be useful for differentiating AC and SCLC from SCC with 100% specificity and 82% sensitivity, 89% specificity and 79% sensitivity, respectively.

We observed that out of 12 SCC, IHC CD 56 gave no positive results. It was all negative results in cases

of adenocarcinoma, inadequate smears whereas all 3 small cell carcinoma showed positivity for CD 56. Viberti et al,[15] found that CD56 was positive in 8/10 of Small cell carcinoma, 1/10 of ADC, and 2/16 of SCC in their study. In a study by Jafarian et al,[11] CD56 was positive in 18/20 of small cell carcinoma and negative in ADC and SCC. Therefore, CD56 with 90% sensitivity, 100% specificity, 100% PPV, and 95.2% NPV can differentiate small cell carcinoma from non –small cell carcinoma. It could serve as a potential diagnostic marker of small cell carcinoma having high sensitivity and specificity.

In present study, out of 12 SCC, IHC TTF 1 gave no positive results. It was all positive in 5 cases of ADC, 2 positive out of 3 small cell carcinoma. Jafarian AH et al,[11] found that all adenocarcinoma were positive for CK7 and most of them (90%) were positive for TTF-1. Most of small cell lung carcinomas were positive for TTF-1 (85%), and CD56 (90%). All squamous cell carcinomas (SCCs) were negative for TTF-1.

Gurda GT et al,[16] found that the immunostaining patterns of TTF-1 was correlated with the histological diagnosis of the tumor. In 72 primary ADCs, TTF-1 showed a sensitivity and specificity of 84.5% and 92.0% respectively. In 131 metastatic ADCs, TTF-1 showed a specificity of 87.5%. We can suggest that IHC TTF 1 is an effective and useful marker in ADC cases.

CONCLUSION

We can conclude that cell block preparation is a simple method that increases diagnostic yield of flexible bronchoscopy, is cost effective & hence can be routinely used. IHC panel consisting CK 5/6, CD 56 and TTF 1 has more diagnostic value in precise subtyping of different types of lung carcinoma in adjunction to routine H&E staining.

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