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Original Article

Comparison of bronchoalveolar lavage fluid cytology, cell block preparation, and bronchial biopsy in the diagnosis of lung lesions.

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ABSTRACT

Introduction: Lung carcinoma is the leading cause of cancer-related mortality. Histopathological diagnosis of bronchial tissue biopsy is considered the gold standard for the diagnosis of lung tumors. The study aims to determine the spectrum of lung lesions and compare the diagnosis by cytological study of bronchoalveolar lavage (BAL) fluid, histopathological study of cell block preparation, with histopathological study of bronchial biopsy in suspected lung lesions.

Material and methods: The study was carried out in the Pathology department, Gujarat Medical Education and Research Society (GMERS) Medical College and Hospital, Sola, Ahmedabad, Gujarat, for a period of 3 years. Comparison of diagnoses made by cytological study of BAL fluid, histopathological study of cell block preparation, and histopathological study of bronchial biopsy in suspected lung lesions was done, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of each method were calculated.

Results: Out of 72 cases which were included in the study, a diagnosis of malignancy was given in 42(58.33%) cases by histopathological examination of lung biopsy, the cytological diagnosis of malignancy was given in 19(26.39%) cases in BAL fluid, and histopathological diagnosis of malignancy was given in 25 (34.72%) cases in cell block. The maximum number of cases was found in the age group of 51-60 years and in males.

Conclusion: The gold standard method for the diagnosis of lung lesions is histopathological examination. Though the BAL fluid and cell block method was inferior to bronchial biopsy in diagnosing lung lesions, BAL fluid is more effective in peripheral lung lesions.

Keywords: Bronchial biopsy, Bronchoalveolar lavage fluid, Carcinoma, Cell block preparation, Lung lesion

INTRODUCTION

The respiratory tract serves the dual purpose of supplying oxygen to and removing carbon dioxide from the circulating blood. Patients with diseases of the respiratory system generally present because of symptoms, an abnormality on a chest radiograph, or both.[1] Non-neoplastic lung diseases include emphysema, acute respiratory distress syndrome (ARDS), interstitial lung disease, occupational lung disease, drug hypersensitivity reactions, asthma, etc. Neoplastic lung disease - lung carcinoma is the leading cause of cancer-related mortality all over the world. It occurs most often in the age group of 40-70 years.[1] In India, ~63,000 new lung cancer cases are being reported each year.[2] A 5-year survival rate of lung cancer in India is 17.7%. The 5 year survival rate for lung malignancies is 55% for cases detected when the disease is still localized. Only 16% of cases are diagnosed at an

early stage. More than half of people with lung malignancies die within 1 year of diagnosis.[2] Smoking is considered to be the cause of 85% of deaths due to lung cancer.[3]

For the earliest diagnosis, different modalities are available, which include radiology, brush cytology, bronchial wash, bronchoalveolar lavage (BAL), FNAC, and bronchial biopsy.[4] More peripheral lesions that cannot be visualized, in which cytology played a more crucial role, with bronchial brushing and washing/ BAL samples being obtained from the relevant lobar segments. The sensitivity of BAL varies between 14-76% in various studies reported.[5] BAL can provide diagnostic information in cases of primary and metastatic lung cancer. BAL has also been used to investigate the pathogenesis of such diverse lung conditions as emphysema, ARDS, occupational lung disease, drug hypersensitivity reactions, and asthma.[6,7] BAL is an easily performed and well-tolerated procedure that is used in the routine assessment of patients for lung lesions. It also helps as a tampon to stop any bleeding that may have occurred as a result of a biopsy.[8] The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The cell block technique is one of the oldest methods for the evaluation of body cavity fluids.[9] The main advantages of the cell block technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.[10]

The diagnostic sensitivity of bronchial biopsy in diagnosing lung malignancies ranges from 65-83%.[11,12] Though histopathological diagnosis of bronchial tissue biopsy is considered the gold standard for the diagnosis of lung tumors, it has certain drawbacks. It is an invasive procedure and more expertise is required. The yield is higher in patients with endoscopically visible tumors than in those with tumors not visible endoscopically. The diagnostic ratio of bronchoscopies is lower for peripheral lesions.[13] The study aims to know the spectrum of lung lesions and compare the diagnosis by cytological study of BAL fluid and histopathological study of cell block preparation with histopathological study of bronchial biopsy in suspected lung lesions.

MATERIAL AND METHODS

This retrospective study was conducted at the Department of Pathology, GMERS Medical College, Sola, Ahmedabad, Gujarat, India, for a period of 3 years from 1st June 2019 to 31st May 2022. Patient's consent was taken for the bronchoscopy procedure by the chest physician of tuberculosis and the chest department. With the help of a flexible fiber optic bronchoscope, BAL fluid was collected, and then a bronchial biopsy was taken from the suspected lung lesion.

BAL fluid was collected by a chest physician at the tuberculosis and chest department. This was received in the cytology laboratory of the pathology department with a properly labelled container along with the cytology request form.

The BAL specimen was divided into two equal parts in different test tubes. One test tube with BAL fluid was centrifuged at 3000 rpm for 10 min, and from the sedimented cells, a cytological smear was prepared on a glass slide, fixed with methanol, and stained with hematoxylin and eosin stain. The remaining test tube with BAL fluid mixed with an equal amount of isopropyl alcohol was centrifuged at 1500 rpm for 15-20 min. Discard supernatant and then sediment the cell button, filtered by filter paper, and the filtrate is used for cell block preparation.

The bronchial biopsy specimen was obtained by a chest physician in the tuberculosis and chest department. This specimen was received in a properly labelled 10% formalin container along with a histopathology request form in the histopathology laboratory of the pathology department. The lung biopsy specimen was fixed in 10% neutral formalin in the histopathology laboratory. This biopsy material and BAL fluid material were processed for routine paraffin section. The tissue cassette was put into the tissue processor, and then the next morning, a paraffin block was made. 5 micron thin sections were cut by microtome and stained with Hematoxylin and Eosin stain.

Inclusion criteria

All patients whose adequate quantity of BAL fluid and lung biopsy material was obtained were included in this study.

Exclusion criteria

The inadequate samples for reporting all 3 techniques were excluded.

The diagnosis made by cytological study of BAL fluid and histopathological study of cell block and biopsy specimen was entered in the specially designed proforma, and cytological and histopathological comparisons were done.

RESULT

Table 1: Age and sex distribution of cases.

Age group (years)	Male (%)	Female (%)	Total (%)
31-40	3 (4.16)	5 (6.94)	8 (11.11)
41-50	10 (13.89)	2 (2.78)	12 (16.67)
51-60	22 (30.56)	5 (6.94)	27 (37.5)
61-70	11 (15.28)	7 (9.72)	18 (25)
71-80	6 (8.34)	1 (1.39)	7 (9.72)
Total	52 (72.22)	20 (27.78)	72 (100)

It can be seen from Table 1 that out of 72 patients, 52 (72.22%) were male and 20 (27.78%) were female. The maximum number of cases, 27 (37.5%), were in the age group of 51-60 years.

Table 2: Cytological diagnosis of bronchoalveolar lavage fluid & histological diagnosis of cell block

Cytological diagnosis of bronchoalveolar lavage fluid (%)				**Histological diagnosis of the cell block (%)**		
	Male	Female	Total	Male	Female	Total
Malignant lesion	10 (13.89)	9 (12.5)	19 (26.39)	14 (19.44)	11 (15.28)	25 (34.72)
Non-malignant lesion	42 (58.33)	11 (15.28)	53 (73.61)	38 (52.78)	9 (12.5)	47 (65.28)
Total	52 (72.22)	20 (27.78)	72 (100)	52 (72.22)	20 (27.78)	72 (100)

It can be seen from Table 2 that out of 72 BAL fluid smears, the diagnosis of malignancy was given in 19 (26.39%) cases and negative for malignancy in 53 (73.61%) cases. Out of 72 cell block smears studied, the diagnosis of malignancy was given in 25 (34.72%) cases, and the other 47 (65.28%) cases were given a diagnosis of negative for malignancy.

Table 4: Statistical analysis of bronchoalveolar lavage fluid

Bronchoalveolar lavage test results	**Diagnosis**	
	Diseased	**Not diseased**
Positive	True positive- 19	False positive- 0
Negative	False negative- 23	True negative- 30

Table 3: Histopathological diagnosis of lung biopsy

Histopathological diagnosis of lung biopsy		**Male (%)**	**Female (%)**	**Total (%)**	
Malignant	Squamous cell carcinoma	18 (25)	4 (5.56)	22 (30.56)	42 (58.33)
	Adenocarcinoma	9 (12.5)	8 (11.11)	17 (23.61)	
	Small cell carcinoma	2 (2.78)	1 (1.39)	3 (4.17)	
Non malignant	Squamous cell metaplasia	1 (1.39)	0	1 (1.39)	30 (41.67)
	Nonspecific inflammation	22 (30.56)	7 (9.72)	29 (40.28)	
Total		52 (72.22)	20 (27.78)	72 (100)	

It can be seen from Table 3 that out of 72 biopsy cases studied, the diagnosis of malignancy was given in 42 (58.34%) cases and the diagnosis of non-malignancy was given in 30 (41.67%) cases. Out of 42 malignant cases, 22 (30.56%) cases were SCC, 17 (23.61%) cases were adenocarcinoma, and 3 (4.17%) cases were small cell carcinoma. Out of 30 non-malignant cases, 1 (1.39%) case shows squamous cell metaplasia, and 29 (40.28%) cases show nonspecific inflammation.

Out of 72 cases, a confirmed diagnosis of malignancy was given in 42 (58.33%) cases by histological examination of lung biopsy, the cytological diagnosis of malignancy was made in 19 cases in BAL, and a histological diagnosis of malignancy was given in 25 (34.72%) cases in cell block.

Histopathological examination of lung biopsy was taken as the gold standard method for diagnosis in the present study.

It can be seen from Table 2 and Table 4 that out of 72 BAL fluid smears, 19 cases were diagnosed as malignancy, which were also confirmed by histopathology, so they are considered true positive cases, and 30 cases were diagnosed as negative for malignancy, which were confirmed by histopathology, so they are considered true negative cases.

Sensitivity = TP/ (TP+FN) X 100 = 19/(19+23) X 100 = 45.23%

Specificity= TN/ (FP+TN) X 100 = 30/(0+30) X 100 = 100%

Positive predictive value (PPV) = TP/ TP+FP X 100 = 19/19+0 X 100 = 100%

Negative predictive value (NPV) = TN / FN+TN X100 = 30/ (23+30) X100 = 56.60%

Accuracy = TP+TN / TP+FP+FN+TN X 100 = 19+30/19+0+23+30 X 100 = 68.05%

It can be seen from Table 2 and Table 5 that out of 72 cell block smears, 25 cases were diagnosed as malignancy, which were also confirmed by histopathology, so they are considered as true positive cases, and 30 cases were diagnosed as negative for malignancy, which were confirmed by histopathology, so they are considered as true negative cases.

Table 5: Statistical analysis of cell block preparation

CB test results	**Diagnosis**	
	Diseased	**Not diseased**
Positive	True positive- 25	False positive- 0
Negative	False negative- 17	True negative- 30

Sensitivity = TP/ (TP+FN) X 100 = 25/(25+17) X 100 = 59.52%

Specificity= TN/ (FP+TN) X 100 = 30/(0+30) X 100 = 100%

Positive predictive value (PPV) = TP/ TP+FP X 100 = 25/25+0 X 100 = 100%

Negative predictive value (NPV) = TN / FN+TN X100 = 30/ (17+30)= 63.83%

Accuracy = TP+TN / TP+FP+FN+TN X 100 = 25+30/25+0+17+30 X 100 = 76.39%

Table 6: Statistical correlation of diagnosis obtained by bronchoalveolar lavage fluid cytological examination and cell block histological examination in the present study:

Statistics	bronchoalveolar lavage fluid	Cell block preparation (%)
Sensitivity	45.23	59.52
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	56.60	63.83
Accuracy	68.05	76.39

It can be seen from Table 6 that sensitivity, NPV, and diagnostic accuracy are higher in cell block than in BAL fluid. Specificity and PPV are the same in BAL fluid and cell block.

DISCUSSION

Cytology and histopathology are valuable tools in the diagnosis of lung malignancies and other lung-related diseases. In the present study, the BAL fluid cytological diagnosis and Cell block histopathological diagnosis were correlated with the bronchial biopsy diagnosis.

For this study, we have taken 72 cases of BAL fluid, cell block, and corresponding lung biopsy of each patient, which were received in the department of Pathology, GMERS Medical College and Hospital, Sola, Ahmedabad, Gujarat, India, during the study period from 1st June 2019 to 31st May 2022.

It can be seen from Table 1 that out of 72 cases, 52 (72.22%) were males and 20 (27.78%) were females. The maximum number of cases was found in the age group of 51-60 years, comprising a total 37.5% of the study population, while the minimum number of cases was in the age group of 71-80 years, comprising a total 9.72% of the study population. Similar findings were observed in a study done by Bhat et al.[14], which shows that the maximum number of cases were found in the age group of 61-70 years, comprising a total 32.03% of the study population.

Table 7: Comparison of diagnoses obtained by bronchoalveolar lavage fluid cytological examination in the present study with other studies

Statistics	Present study (%)	Bhat *et al.*[14] (%)
Sensitivity	45.23	35.5
Specificity	100	78.16
Positive predictive value	100	89.70
Negative predictive value	56.60	18.46
Diagnostic accuracy	68.05	42.23

It can be seen from Table 7 that sensitivity, specificity, PPV, NPV and diagnostic accuracy of BAL in present study is 45.23%, 100%, 100%, 56.60%, and 68.05%, while study done by Bhat *et al.*[14] shows 35.5%, 78.16%, 89.70%, 18.46%, and 42.23%, respectively. Specificity, NPV, and diagnostic accuracy of the present study were significantly higher than those of the study done by Bhat *et al.*[14] This may be because the study population sample size is twelve times smaller in the present study as compared to the study done by Bhat *et al.*[14]

Table 8: Comparison of diagnostic accuracy of bronchoalveolar lavage fluid cytological examination with other studies

Study	Diagnostic accuracy (%)
Linder J *et al.*[5]	68.6
Pirozynski M[15]	64.8
De Gracia J *et al.*[6]	43.6
Debeljek A *et al.*[7]	27.9
Wongsurakiat *et al.*[16]	46.7
Present study	68.05

It can be seen from Table 8 that the diagnostic accuracy of BAL fluid cytological examination in the present study is 68.05%, while it ranges from 27.9% to 68.6% in foreign studies. It is because of variation in sample size, sample collection, and processing method.

Table 9: Comparison of diagnoses obtained by Cell Block histological examination in the present study with other studies:

Statistics	Present study (%)	Kakodkar *et al.*[17] (%)
Sensitivity	59.52	50
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	63.83	20.7
Diagnostic accuracy	76.39	54.28

It can be seen from Table 9 that sensitivity, specificity, PPV, NPV, and diagnostic accuracy of cell block in present study is 59.52%, 100%, 100%, 63.83%, and 76.39%, while the study done by Kakodkar *et al.*[17] shows 50%, 100%, 100%, 20.7%, and 54.28%, respectively. NPV and diagnostic accuracy of the present study were significantly more than the study done by Kakodkar *et al.*[17] This may be because of the study population's geographical variation in the prevalence of lung lesions and sample size.

Table 10: Comparison of diagnostic accuracy of cell block histopathological examination with other studies:

Study	Diagnostic accuracy (%)
Castro-Villabón D *et al.*[18] 2014	81.6
Kulkarni *et al.*[19] 2009	94
Present study	76.39

It can be seen from Table 10 that the diagnostic accuracy of cell block histopathological examination in the present study is 76.39%. Similar findings were seen in studies like Castro-Villabón D *et al.*[18] 2014 and Kulkarni *et al.*[19] 2009, which show diagnostic accuracy 81.6% and 94%, respectively.

LIMITATIONS

The present study has a few limitations. First, the sample size of 72 cases is relatively small compared to larger multicentric studies, which may impact the statistical power of the diagnostic comparisons. Second, being a retrospective, single-center study, the findings may be influenced by local geographical prevalence and institutional protocols. Finally, the diagnostic yield of BAL fluid and bronchial biopsy is often dependent on the operator's expertise and the specific anatomical location of the lung lesions.

CONCLUSION

The gold standard method for the diagnosis of lung lesions is histopathological examination. Though BAL fluid and cell block method were inferior to bronchial biopsy in diagnosing lung lesions, it was effective for peripheral lung lesions and when the patient was at risk of hemorrhage. The BAL procedure is relatively easy to perform, less complicated than a biopsy procedure. The BAL fluid obtaining procedure does not require special training, and a less experienced physician can also perform the procedure. So, BAL fluid and cell block method have a valuable role and are a useful screening method in the diagnosis of lung lesions.

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