VOLUME - 11, ISSUE - 01, JANUARY - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

CORROBORATION OF FINE NEEDLE ASPIRATION CYTOLOGY FINDINGS WITH CELL BLOCK STUDY OF MALIGNANT LUNG LESIONS USING IMMUNOSTAINS (TTF1, NAPSIN A, P40 AND CHROMOGRANIN A).

Dr. Maharshi Debnath	Pgt 3rd Year Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Gayatri Devi Pukhrambam	Associate Professor, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Laishram Deepak Singh	Assistant Professor, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Sushma Khuraijam	Professor And Head of Department, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur
Dr. Babina Sarangthem*	Associate Professor, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur. *Corresponding Author

ABSTRACT Background: Malignancy of the lung has been rising steadily worldwide and exact sub typing has become a necessity for targeted therapy. FNAC alone has its limitations in sub categorization of lung malignancy because of lack of architecture. Cell block preparation from FNAC material with immunohistochemistry panel would supplement the FNAC finding. Objectives: To determine the utility of cell block study using immunostains (TTF 1, Napsin A, p40 and Chromogranin A) in corroborating the findings of FNAC in the study of malignant lung lesions. Materials and methods: A hospital based cross sectional study was done on the FNAC sample from lung masses followed by cell block study and immunohistochemical staining for (CHROMOGRANIN A, NAPSIN A, p40, TTF1). Data was analyzed using IBM SPSS statistics version 21 Software. Results: A total of 32 (54.2%) cases were found malignant and 27 (47.3%) cases were found benign. A total 59 cell block were prepared from 32 cases positive for malignancy (PFM) and 27 cases negative for malignancy(NFM). After cell block and immunohistochemical studies, 2 benign lesion were recategorized as malignant and 3 malignant cases recategorized as benign lesion. There was a substantial agreement found between FNAC diagnosis and IHC diagnosis as in both cases the kappa value (coefficient) were found to be 0.747 (between 0.61 - 0.80). Conclusion: The cell block from the aspirated material revealed further architectural and immunohistochemical features which not only confirmed the FNAC diagnosis but helped to recategorize 2 benign and 3 malignant lesion.

KEYWORDS : Adenocarcinoma(ADC), Squamous cell carcinoma(SCC), Small cell lung carcinoma(SCLC), Non small cell lung carcinoma(NSCLC), Negative for malignancy(NFM), Positive for malignancy(PFM), Fine needle aspiration cytology(FNAC), Cell block(CB).

INTRODUCTION

Malignancies of the lungs have been rising steadily and are one of the dreaded visceral malignancies. In India, approximately 63,000 new lung cancer cases are reported each year.[1] In the previous WHO classification of lung cancer, diagnosis was based mainly on light microscopy using routine haematoxylin and eosin stain. In the 2015 World Health Organization classification, immunohistochemistry technique has been recommended along with cytomorphological features of FNAC specimen for specific diagnosis. Cell block plays an important role in differential diagnosis of lung cancer subtypes.

Immunohistochemistry is recommended for all NSCLC that cannot be classified as SCC or ADC based on morphology alone because of the advancement in the treatment of different types of lung cancer in the form of personalized therapy.[2] Most tumors can be classified using a single adenocarcinoma marker (e.g., TTF1 or mucin or NAPSIN A) and a single squamous marker (e.g. p40 or p63)2. Non□small cell lung carcinomas (NSCLC) that show no clear Adenocarcinoma or squamous cell carcinoma morphology or negative for Immunohistochemical markers are regarded as NSCLC not otherwise specified (NOS) . The application of Immunohistochemistry increases the refinement of diagnosis so that a diagnosis of NSCC NOS can be avoided in up to 90% of cases and helps in differentiating metastatic tumors from primary lung tumors.[2]

The present study envisages in evaluating the usefulness of cell block study along with a short panel of immunostains (TTF1, NAPSIN A, p40, CHROMOGRANIN A) in corroborating the findings of FNAC in the study of malignant lung lesions.

OBJECTIVES

To determine the utility of cell block study using immunostains (TTF 1, Napsin A, p40 and Chromogranin A) in corroborating the findings of FNAC in the study of malignant lung lesions.

MATERIALS AND METHODS

The study was a hospital based cross sectional study conducted in the department of Pathology and Respiratory Medicine of Regional Institute of Medical Sciences, Imphal, Manipur from September 2018 to August 2020. A total of 59 cases from both the outpatient department and inpatient department with lung mass were included in the study. Samples were collected by CT guided FNAC in patients having undiagnosed intrathoracic mass. A 22 gauze spinal needle was used for aspiration after applying local anaesthesia (2% lidocaine). From the aspirated material, smears was made, air dried and then it was fixed with 95% methyl alcohol and stained with routine May Grunwald Giemsa (MGG) and Papanicolaou (PAP) stain . Call block was prepared from aspirated material by adding ethanol and formalin. The prepared cell block was subjected to tissue processing as routine histopathological specimens and 3 μm thickness sections were cut. Immunostain was done for chromogranin A, napsin A, TTF1, p40. Monoclonal mouse antihuman monoclonal antibodies p40-clone ZR8, TTF1 clone SPT24, napsin A clone BS10, chromogranin clone LK2H10 and rabbit antihuman monoclonal antibodies p40-clone ZR8 by master diagnostica were used. Data were collected, recorded, analyzed and descriptive statistics and

Significance testing were carried out using IBM SPSS statistics version 21 Software. Chi square test was applied and p value less than 0.05 was considered statistically significant. Ethical approval was obtained from the research ethics board, RIMS, Imphal with reference number a/206/reb-comm(sp)/rims/440/58/2018 dated 30th Jan 2019.

RESULTS

The most common age group involved by malignant cases was 51 to 60 years with a mean age of 57.08 years. Of the 12 adenocarcinoma (ADC) cases, most common age group Involved was 61-70 years (5 cases, 41.7%) and out of 13 cases of squamous cell carcinoma (SCC) (Figure 1,2,3), 4 cases (30.8%) also involved the 61-70 age group. Out of the 59 cases, 32 (54.2%) cases were found to be malignant and 27 (47.3%) cases were found to be benign. Among the 32 (54.2%) malignant cases, 19 (59.3%) were male and 13 (40.6%) were female patients. Out of 13 SCC cases, 10 (76.9%) were males and 3 (23.1%) were females. In the 12 ADC cases, 5 (41.7%) were males and 7 (58.3%) were female patients. Out of 12 ADC cases, 5 cases (41.7%) and among 6 NSCLC cases, 3 (50.0%) found in left lower lobe of lung. Among 13 SCC cases, 5 (38.5%) cases found in right upper lobe of lung.

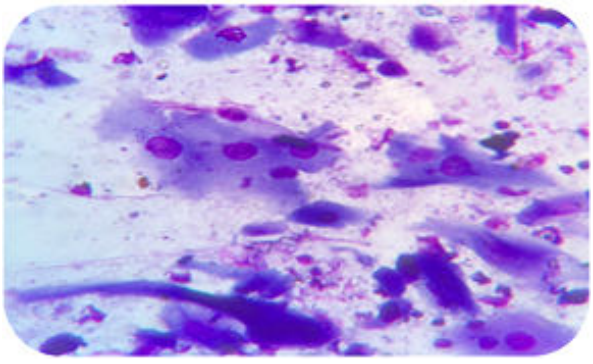

Figure 1: Photomicrograph of FNAC smear of Squamous cell carcinoma showing malignant Squamous cell with pleomorphic nuclei seen in background of necrosis. (Giemsa stain, 400x).

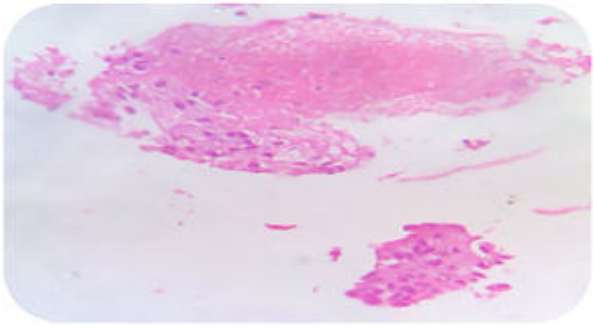

Figure 2: Photomicrograph of histopathology of cell block from squamous cell carcinoma (H&E stain, 400x).

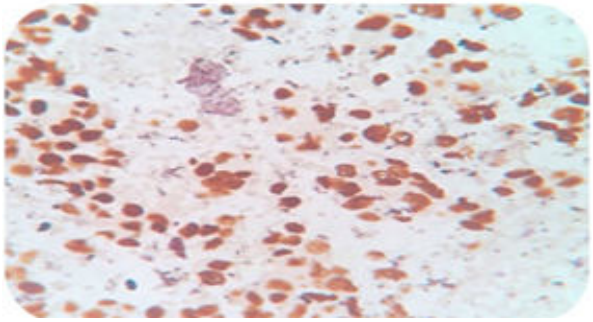

Figure 3: Photomicrograph of cell block from FNAC of Squamous cell carcinoma showing strong nuclear positivity of p40. (IHC stain, 400X)

Out of 32 malignant cases 03 were found negative for malignancy by cell block study and out of 27 NFM cases in FNAC study, 02 cases found to be PFM by CB. Considering CB study as the standard, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of CT guided FNAC of lung mass was 93.5 %, 89.4 %, 90.6 %, 92.6 % and 91.5 % respectively as shown in table 1.

Table 1: Association of cytological diagnosis and cell block diagnosis (N=59).

		Cell block diagnosis		Total, n(%)
		PFM, n(%)	NFM, n(%)	
Cytology Diagnosis	PFM *	29(96.7)	3(10.7)	32(54.2)
	NFM **	2(6.5)	25(89.3)	27(45.8)
Total		31(100.0)	28(100.0)	59(100.0)

*Sensitivity = (29/31) x 100 % = 93.5 %, Specificity=(25/28) x 100 % = 89.3 %, Positive predictive value= (29/32) x 100 % = 90.6 %, Negative predictive value= (25/27) x 100 % =92.6 %, Diagnostic accuracy=(54/59) x 100 % = 91.5 %, *PFM: Positive for malignancy, **NFM: Negative for malignancy.*

Out of the 13 CB for SCC, 12 cases showed positivity for p40 and one case was negative for p40 so diagnosed as NFM. Out of 12 ADC (figure 7,8) cases 10 cases show positive for both Napsin A, TTF1 as shown in figure and one case showing showed positivity for TTF1 only. Hence, in all diagnosis of ADC is retained and one case showed negativity for both TTF1 and Napsin A so diagnosed as NFM.

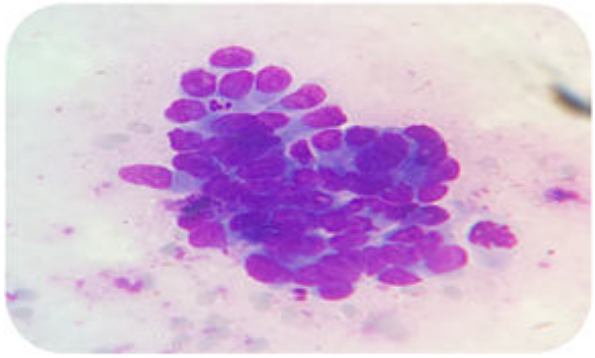

Figure 7: Photomicrograph of FNAC smear of adenocarcinoma of lung showing clusters of cells with pleomorphic nuclei with some vague glandular formation (Giemsa stain, 1000x).

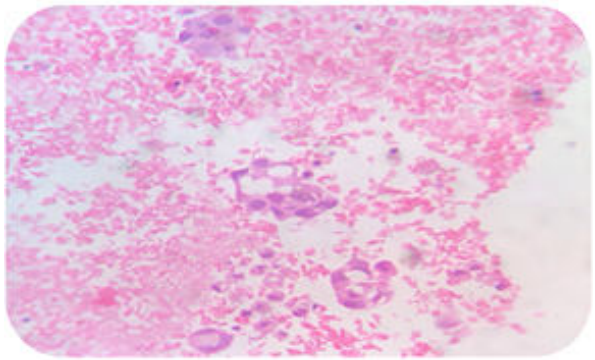

Figure 8: Photomicrograph of histopathology of cell block from of adenocarcinoma having few malignant acinar glands (H&E stain, 400X).

Out of 6 NSCLC cases 2/6 (33.3 %) cases show positivity for p40 and reclassified as SCC and 2/6 (33.3 %) cases show positivity for TTF1 and NAPSIN A (Figure 9,10) and reclassified as ADC but remaining 2/6 (33.3 %) cases show no reactivity to any one of the four antibody and remain as the type NSCLC nos.

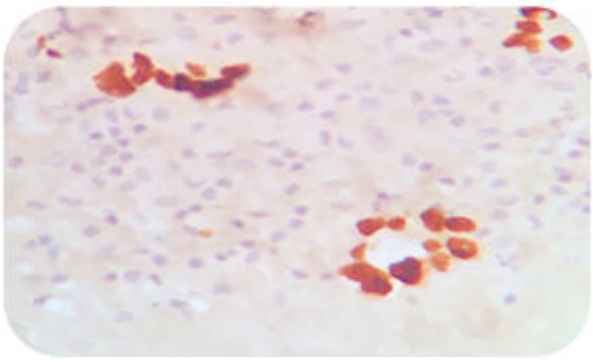

Figure 9: Photomicrograph of cell block from FNAC material from Adenocarcinoma lung showing TTF1 nuclear positivity of (IHC stain,400x).

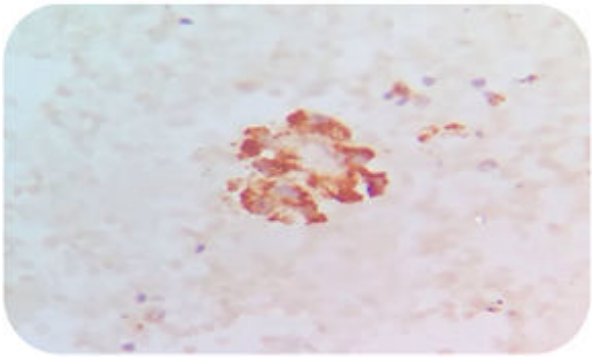

Figure 10: Photomicrograph of cell block from FNAC material from Adenocarcinoma lung showing Napsin A cytoplasmic positivity of (IHC stain, 400x).

One case was diagnosed as small cell carcinoma on cytomorphology alone which show positive reaction to chromogranin A (Figure 4,5,6).

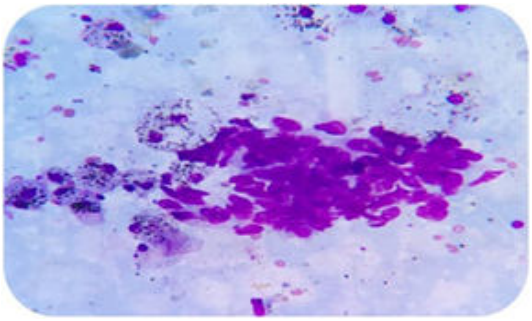

Figure 4: Photomicrograph of FNAC smear of small cell carcinoma showing nuclear moulding and some hemosiderin laden macrophage in background (Giemsa stain, 400x).

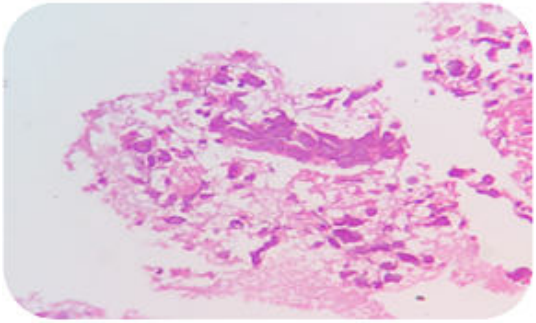

Figure 5: Photomicrograph of histopathology from cell block of small cell carcinoma (H&E stain, 400x).

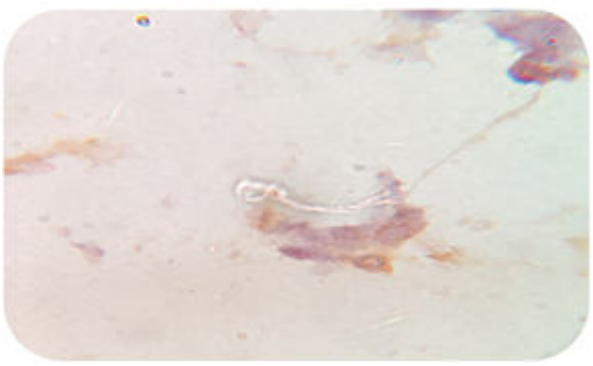

Figure 6: Photomicrograph of cell block from Small cell carcinoma showing cytoplasmic granule positivity for Chromogranin A (IHC stain, 400x).

Concordance between FNAC diagnosis and cell block with Immunohistochemistry diagnosis for SCC and ADC evaluated by kappa statistics after using a valid chi square test and a significant association was found with p value of 0.001 as shown in table 2 and table 3 and table 4.

Table 2: Concordance and disconcordance between FNAC diagnosis and cell block diagnosis(N=32).

Diagnosis	FNAC Diagnosis, n(%)	Cell Block Diagnosis, n(%)	Concordance Between Cell Block And FNAC, n(%)	Disconcordance Between Cell Block And FNAC, n(%)
SCC	13(40.6)	12(41.4)	12(41.4)	1(7.7)
ADC	12(37.5)	12(41.4)	12(41.4)	0(0.0)
NSCLC	6(18.7)	4(13.8)	4(13.8)	2(33.3)
SCLC	1(3.1)	1(3.5)	1(3.5)	0(0.0)
Total	32(100.0)	29(100.0)	29(100.0)	03(9.4)

Table 3: Concordance and disconcordance between FNAC diagnosis and IHC diagnosis (N=26).

Diagnosis	FNAC Diagnosis, n(%)	IHC Diagnosis, n(%)	Concordance Between IHC And FNAC, n(%)	Disconcordance Between IHC And FNAC, n(%)
SCC	13(50.0)	12(50.0)	12(50.0)	1(7.7)
ADC	12(46.1)	11(45.8)	11(45.8)	1(8.3)
SCLC	1(3.8)	1(4.1)	1(4.2)	0(0.0)
Total	26(100.0)	24(100.0)	24(100.0)	02(7.7)

Table 4: Final diagnosis of lung malignancies after using cell block and IHC technique (N=32).

Name	Number of cases by FNAC, n(%)	Final diagnosis after using IHC and cell block, n(%)
ADC	12(37.5)	14(43.7)
SCC	13(40.6)	15(46.9)
SCLC	01(3.1)	01(3.1)
NSCLC NOS	06(18.7)	02(6.2)
Total	32(100.0)	32(100.0)

There was a substantial agreement found between FNAC diagnosis and IHC diagnosis as in both cases the kappa value (coefficient) was found to be 0.747 (between 0.61 - 0.80) as shown in table 5 and table 6.

Table 5: Showing concordance and kappa value (coefficient) between FNAC diagnosis and cell block with immunohistochemical diagnosis of ADC (N=32).

Adenocarcinoma (ADC)		Immunohistochemistry		Total n (%)	* kappa value (k coefficient) 0.747
		ADC, n(%)	NON ADC, n(%)		
FNAC ADC	ADC	11(78.6)	1(5.6)	12(37.5)	
	NON ADC	3(21.4)	17(94.4)	20(62.5)	
Total		14(100.0)	18(100.0)	32(100.0)	

**kappa value (K coefficient) was calculated by applying kappa statistics.*

Table 6: Showing concordance and kappa value (coefficient) between FNAC diagnosis and cell block with immuno histochemical diagnosis of SCC (N=32).

Squamous cell carcinoma (SCC)		IHC of SCC		Total, n(%)	*kappa value (k coefficient) 0.747
		NON SCC, n(%)	SCC, n(%)		
FNAC Diagnosis	NON SCC	16(94.1)	3(20.0)	19(59.4)	
	SCC	1(5.9)	12(80.0)	13(40.6)	
Total	17(100)	15(100)	32(100		

**kappa value (k coefficient) was calculated by applying kappa statistics.*

Pneumothorax (1/59, 1.6 %) and transient pleuritic chest pain (2/59, 3.3%) were complications encountered during the study period. None of the cases required active management.

DISCUSSION

Lung cancer is a major cause of morbidity and mortality throughout the World. The accurate pathological diagnosis of lung cancer is crucial for selecting personalized treatment and targeted therapy. Specific antibodies for immuno histochemistry panel help to increase the diagnostic accuracy. Cell block study is simple, inexpensive and help in architectural analysis and immunohistochemical evaluation. Males are found to be more commonly affected (57.6%) in our study as compared to females. Similar finding were also reported by Bandopadhay A et al[6] (78.9%) and other workers.[8,4,10] This may be due to more recruitment of male patients in the study as carcinoma of lung is more common in males. Out of 12 ADC cases, 7 (58.3%) were seen in female and among 13 SCC case 10 (76.9%) seen involving male patients, similar findings were reported by Sterlacci W et al[8] and some other Indian workers.[9,11] Most common age group presenting with lung mass in our study is 51-60 age group comprising of 10 cases (10/32, 31.2%). The findings reported by Saha A et al[7] (23/57, 49.5%), Ahmed S et al[5] (23/58, 39.6%), Mondal SK et al[8] (60/124, 48.3%) are also consistent with our study. Out of 59 cases, majority 31/59 (52.7%) of the lesion are in the right lung as also reported by other workers (Mondal SK et al[8] 72/124 (58.0%) and Nalinimohan C et al[11] (85/123, 69.1%) also reported analogous finding). The most common site involved by malignant lesion is the right upper lobe of lung (27.1%), similar finding are also reported by Noronha V et al. In our study, smoker was 61.0% and non smoker was 39.0%. Makde MM et al[4] has reported homogenous number of smoker and non smoker 64.0%, 36.0% respectively. Among the 32 malignant cases in our study, 13 (40.6%) cases were SCC, 12 (37.5%) ADC cases were ADC, 06 (18.7%) were NSCLC and 01(3.1%) was SCLC, comparable to other studies by Ahmed S et al[5] and other workers.[6,14] While comparing the FNA considering CB as standard, the sensitivity, specificity, PPV, NPV and diagnostic accuracy in our study was 93.5%, 89.3%,90.6%, 92.6% and 91.5% respectively. The study conducted by Makde MM et al[4] showed sensitivity and specificity was 89.0% and 75.0% respectively, Saumya TM et al[3] showed sensitivity and specificity by FNAC as 96.4%, 100.0% and diagnostic accuracy 87.7% interchangeable to our study. The study by Modi MB et al[12] showed results comparable to our study with sensitivity and PPV value of 91.5% and 94.7% but specificity and NPV was low 72.5% and 61.5%. Exact subtyping of NSCLC based on IHC is essential for specific treatment regimens and for evidence based medicine.[2] The concordance/agreement between the FNAC and IHC in diagnosis of ADC and SCC in our study was substantial with kappa value 0.747 in both the situation. In the study conducted by Sawmya TM et al[3] the agreement between FNAC and IHC in the diagnosis of ADC and SCC was perfect (k = 1.0), but analogous to our study the substantial concordance shown by study by Nizzoli R et al[13] with k value 0.755. Among the 06 cases diagnosed on FNAC as NSCLC due to lack of definite cytomorphological features of ADC and SCC, on further evaluation by cell block and IHC studies, the NSCLC NOS case become 02/06 showing 66.7% reduction as in the study conducted by Patel TS et al[14] where reduction in the number of NSCLC NOS case from 14 to 2 (85.71 reduction) was seen. Though we could make a significant reduction in the NSCLC category into specific subtypes as ADC and SCC still we were unable to categorize the remaining 02 cases of NSCLC into specific subtypes due to non-availability of extended diagnostic markers. Further molecular study to identify specific mutations could not be done as molecular studies are not available in our institute. In spite of the short duration of the study and a smaller number of sample size the present study, would be a harbinger attempt for further larger studies with extended IHC panels and molecular markers in lung malignancies.

CONCLUSION

In the present study the cell block preparation from the same aspirated material revealed further architectural and other supplementary features which made re-categorization of 2 benign lesions as malignant and 3 malignant lesions as benign possible. The cell block study further facilitated the calculation of sensitivity, specificity, PPV, NPV and diagnostic accuracy of FNAC as 93.5%, 89.5%, 90.6%, 92.6% and 91.5% respectively. Application of IHC using four antibody panel of TTF1, Napsin A, p40 and Chromogranin A on the cell block preparation further enhanced the specific sub typing of the NSCLC cases. The combination of cell block study with IHC not only confirmed but also strengthened the FNAC diagnoses as substantial agreement was found between FNAC and IHC diagnoses with kappa value of 0.747 for both ADC and SCC category. A 66.6% reduction in NSCLC-NOS category was also achieved at the end of the study.

Conflict Of Interest

There are no conflicts of interest.

REFERENCES:

1) Noronha V, Dikshit R, Raut N, Joshi A, Pramesh CS, George K, et al. Epidemiology of lung cancer in India: focus on the differences between non-smokers and smokers: a single-centre experience. Indian J Cancer 2012 Jan-Mar;49(1):74-81.
2) Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 world health organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 2015 Sep;10(9):1243-60.
3) Sowmya TM, Balu S, Malathi M. Study of cytomorphology and immunohistochemistry on cell block for categorization of primary lung tumors. Int JClin Diag Pathol 2020;3(2):6-14.
4) Makde MM, Umap P, Munje R. FNAC, cell block and core needle biopsy in diagnosis of lung masses: a necessity or choice? Int J Res Med Sci 2017 Nov;5(11):4951-8.
5) Ahmed S, Ahamad MSU. Computed Tomography Guided Fine Needle Aspiration Cytology of Lung Lesions. JCMCTA 2009; 20(1):50-2.
6) Bandyopadhyay A, Laha R, Das TK, Sen S, Mangal S, Mitra PK. CT guided fine needle aspiration cytology of thoracic mass lesions. Indian J Pathol Microbiol 2007 Jan; 50(1):51-5.
7) Saha A, Kumar K, Choudhuri MK. Computed tomography – guided fine needle aspiration cytology of thorasic mass lesion: A study of 57 cases. J Cytol 2009 Apr;26(2):55-9.
8) Sterlacci W, Savic S, Schmid T, Oberaigner W, Fiegl M, Auberger J et al. Tissue-Sparing Application of the Newly Proposed IASLC/ATS/ERS Classification of Adenocarcinoma of the Lung Shows Practical Diagnostic and Prognostic Impact. Am J Clin Pathol 2012 Jun;137(6):946-56.
9) Mondal SK, Nag D, Das R, Mandal PK, Biswas PK, Osta M, et al. Computed tomogram guided fine needle aspiration cytology of lung mass with histological correlation: A study in Eastern India. South Asian J Can 2013 Jan;2(1):14-8.
10) Naqvi H, Edhi MM, AslamHM, Faridi N. Spectrum of intra-thoracic lesion detected by computed tomography guided fine needle aspiration biopsy. Int Arch Med 2013 Feb;6(1):4-8.
11) Nalinimohan C, Jayashankar E. Cytological evaluation of lung mass FNAC under computed tomography guidance: a study of 123 cases. Ind J Path Oncol 2017 Jul;4(3):376-9.
12) Modi MB, Rathva MR, Shah NR, Trivedi M, Patel H. Role of FNAC in lung carcinoma and its histo-cytological correlation. J Lung Pulm Respir Res 2016 Jun;3(4):90-4.
13) Nizzoli R, Tiseo M, Gelsomino F, Bartolotti M, Majori M, Ferrari L, et al. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. J Thorac Oncol 2011 Mar;6(3):489-93.
14) Patel TS, Shah MG, Gandhi JS, Patel P. A ccuracy of cytology in sub typing non small cell lung carcinomas. Diagn Cytopathol 2017 Jul;45(7):598-603.