



Original Article

CORRELATION OF CYTOLOGY WITH CLINICOPATHOLOGICAL PARAMETERS IN CASES OF NON-SMALL CELL LUNG CANCERS

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ABSTRACT

Introduction: Lung cancer is one of the most common causes of cancer related mortality. Various methods for cytological diagnosis like fine needle aspiration (FNA) along with exfoliative cytology by bronchial brushings play a pivotal role in the diagnosis as well as categorization of Non small cell lung carcinoma (NSCLC); especially in advanced stages and cases where biopsy may not be feasible.

Aims & Objectives: The aim of the study was diagnosis and subclassification of Non small cell lung carcinoma (NSCLC) from cytosmears of bronchial brushings and Fine needle aspiration cytology (FNAC) samples followed by histopathological and clinicopathological correlation.

Materials and Methods: The present study is both prospective & retrospective study done in Department of Pathology at Santhiram medical college & general hospital, Nandyal, Ap for a period of Two and half years from Jan 2023 to Aug 2025 through data collected from hospital records A total of 46 cases were included in the study group. Categorization of lung cancer was done based upon the 2015 World Health Organization (WHO) criteria of cytodagnosis. Histological correlation was done wherever available. Correlation with clinicopathological parameters was also done.

Results: Total 46 patients were included in the study. The age range of was 32-81 years with a mean age of 59.2 years. Male to female ratio was 2.06:1. Smokers were affected in 26 cases (56.52%). The incidence of Adenocarcinoma (ADC) was commonest in our study comprising of 15 cases (32.61%). This was followed by squamous cell carcinoma (SCC) in 11 cases (23.91%), NSCLC-favours ADC in 8 cases (17.39%), NSCLC-NOS in 4 cases (8.70%), suspicious of malignancy in 3 cases (6.52%) and 2 cases (4.35%) each of NSCLC favours SCC & atypical and one case (2.17%) of ADC-lepidic pattern. Cyto-histological correlation was possible in 25 core biopsy cases with 68% concordance.

Conclusion: Cytological techniques are safer, economical and provide quick results. But a comprehensive clinicopathological approach is needed for labelling a correct diagnosis.

Keywords: Cytology, cytosmears, lung carcinoma, smokers.

INTRODUCTION

Carcinoma of lung is the most common cancer worldwide and also the most common cause of cancer related mortality in developed countries with increasing incidence in developing countries.¹ It is more common in males than females but this trend is decreasing nowadays.² The most common age range is 40-70 years with peak incidence in 50-60 years. Only few cases present before the age of 40 yrs.³ Annually it accounts for 1-5 million deaths worldwide and 2.5 million deaths in developing countries.⁴ The overall 5 year survival rate is approximately 15% in developed countries and 5% in developing countries.⁵ Every year approximately 63,000 new cases are reported in India.⁶ There has been significant

rise in the incidence in metropolitan cities of India due to industrial hazards and air pollution.⁷ The incidence and types of lung cancer differ according to geographic region and ethnicity.

Etiology of lung cancer is multifactorial. The foremost and leading cause is tobacco smoking. It also reflects the prevalence and pattern of smoking.⁸ 80% of lung cancer occurs in active smokers or who have recently stopped smoking. Passive smoking increases risk of cancer twice than non-smokers. Other factors are industrial hazards, air pollution and genetics involving mutation of Tp53, EGFR, RB and KRAS genes.⁹

Previous categorization of lung carcinoma into small cell and non small cell type is now replaced by more detailed and specific diagnosis- Adenocarcinoma (ADC), Squamous cell carcinoma (SCC), Large cell carcinoma (LCC), Non small cell lung carcinoma - Not otherwise specified (NSCLC- NOS) and others according to revised WHO (World health organisation) classification of lung tumors in 2015.⁴

More than 70 percent cases of lung cancer present clinically in advanced stage and are unresectable. Only small biopsy and cytology samples are available for diagnosis and further management.⁴ Various methods for harvesting small biopsies and cytology samples are fine needle aspiration cytology, bronchial brush cytology, bronchioalveolar lavage fluid, sputum analysis, effusion fluid analysis with or without cell blocks along with small needle biopsies.^{4,10} These methods are cheap & less traumatic with decrease risk of haemorrhage allowing rapid on site evaluation and more so the procedures can be done multiple times if sample is not adequate.

AIMS & OBJECTIVES

- 1) Diagnosis and subclassification of Non small cell lung carcinoma (NSCLC) from cytosmears.
- 2) Cyto-histomorphological correlation wherever available.
- 3) Correlation with clinico-pathological parameters.

MATERIALS AND METHODS

Case selection

This was both prospective and retrospective study conducted in the Department of Pathology, Santhiram Institute of Medical Sciences & general hospital, Nandyal, Andhra Pradesh over a period of two and half years from Jan 2023 to Aug 2025. This was approved by institutional ethics committee (IEC). Informed consent before the procedure and staining was taken from all the patients.

Total 57 cases were selected initially for cytodiagnosis. Eleven cases were rejected because of low cellularity, obscuring factors and processing artifacts. So total 46 cases of NSCLC diagnosed cytologically were included in the study group showing mass lesion radiologically. Out of these cases, 10 cases were archived smears. The cytosmears included were bronchial brush smears, bronchoscopic guided Transbronchial needle aspiration (TBNA) & CT guided Transthoracic needle aspiration (TTNA) smears showing malignant cells. In our study correlation between cytology & histomorphology was possible in 25 cases. Patient's clinical data (i.e. age, sex, smoking status, clinical history and radiological findings) and other relevant data of all the cases were also collected and recorded.

Inclusion criteria

- 1) Lung masses suspicious of malignancy radiologically.
- 2) Bronchial brush smears, TBNA & TTNA smears showing malignant cells.
- 3) Core needle biopsy samples where ever available.

Exclusion criteria

- 1) Cytosmears showing scanty cellularity with few tumor cells.
- 2) Obscuring factors like extensive areas of haemorrhage with tumor diathesis.
- 3) Crushing or drying artefacts in the smear.

Methods

All cases were selected as per radiological and cytological evidence of malignancy. Bronchial brush cytosmears & TBNA smears from Department of Pulmonary Medicine and cytosmears done by CT guided TTNA from Department of Radiodiagnosis were obtained. Bronchial brush smears were obtained with help of Olympus Fiberoptic flexible bronchoscope after sedation of the patient. With the inbuilt brush in the bronchoscope, the Pulmonologist took samples from the surface of tumor sites or from suspicious areas of mucosa from desired segment of bronchi. This brush was subsequently rubbed on uncoated glass slides to prepare smears. Then smears were immediately fixed in 95% alcohol for two minutes in coplin jars and the same were sent to the Department of Pathology, Santhiram Institute of Medical Sciences & general hospital, Nandyal, Andhra Pradesh. CT guided TTNA samples were obtained using 20 Gauge needle attached to 10ml syringe holder. These smears were also fixed in 95% alcohol. All the smears (bronchial brushings, TBNA & TTNA) were stained with Hematoxylin & Eosin (H & E) and Papanicolaou (PAP) stains followed by DPX mounting. CT

guided core needle biopsy samples were obtained using 20 gauge automated cutting biopsy needle. The needle biopsy samples were received in 10% buffered formalin. These formalin fixed samples were processed by automated tissue processor for 16 hours. Paraffin embedded tissue blocks were made. 3 to 4 micrometer thick sections were taken by Leica microtome. The histopathology slides were stained with H&E. These cases were correlated with cytological diagnosis wherever possible. Cytodiagnosis was done based on 2015 WHO classification of lung tumors in small biopsy and cytology samples.¹¹ All the cases were correlated with clinicoradiological parameters.

Certain cytomorphological clues point toward broadly categorizing lung carcinoma into NSCLC or SCLC. In SCLC the cells have high nucleocytoplasmic ratio with nuclear molding showing salt and pepper chromatin with inconspicuous nucleoli and scant delicate cytoplasm. Crushing artifacts and apoptotic bodies are also seen.^{12,13} In NSCLC- favouring SCC, the cells are polygonal to spindloid having orangeophilic cytoplasm with distinct cell borders, intercellular bridges and hyperchromatic nuclei.^{12,13} In NSCLC- favouring ADC, cells are round to oval arranged in 3-dimensional clusters, glandular pattern and some showing papillary fragments. Cells show indistinct cell borders, intracytoplasmic mucin with vesicular opened up chromatin showing prominent nucleoli.¹⁴⁻¹⁷ In ADC lepidic pattern the tumor cells are arranged in balls with absence of nuclear moulding and papillary pattern of arrangement. The cells may have cytoplasmic microvilli along with vacuolation and round to oval nuclei having granular chromatin and inconspicuous nucleoli.¹¹

OBSERVATIONS & RESULTS

Total 46 cases were included in the study group, of which the age range was 32-81 years with mean age of 59.2 years. The age group of 51-60yrs and 61 -70 years had the highest number of cases, 16 cases (34.78%) in each group. Lowest number of cases was in age group of 81-90 years followed by 31-40 years. (Table 1)

Table 1: Distribution of cases according to age range (N=46)

Age range	Number	%
31-40	2	4.35
41-50	8	17.39
51-60	16	34.78
61-70	16	34.78
71-80	3	6.52
81-90	1	2.17
Total cases	46	100%

In our study the overall incidence of lung cancer was more common in males comprising of 31 cases (67.39%) as compared to females, 15 cases (32.61%). Male to female ratio was found to be 2.06:1. (Table 2) Smokers were more affected which were 26 cases (56.52%) than non-smokers, 20 cases (43.48%). The smoker to nonsmoker ratio was 1.3 to 1. (Table 3)

Table 2: Distribution of cases according to gender (N=46)

Sex	Number	%
Male	31	67.31
Female	15	32.69
Total cases	46	100

Right lung was less affected than left side. 31 cases (67.39%) had involvement of left lung and 15 cases (32.61%) had involvement of right lung. (Table 4) Right upper lobe was affected in 25 cases (54.35%) in our study. In 24 cases (52.37%) mass was detected in central location and in 22 cases (47.38%) it was in peripheral location. (Table 5)

Table 3: Distribution of cases according to smoking status (N=46)

Smoking status	No of cases	%
Smoker	26	56.52
Non smoker	20	43.48
Total cases	46	100

Table 4: Distribution of cases according to side of lung involved on radiology (N=46)

Side	No of cases	%
Right lung	15	32.61
Left lung	31	67.39
Total cases	46	100

Table 5: Distribution of cases according to site of involvement in radiology (N=46)

Site	No of cases	%
Central	24	52.17
Peripheral	22	47.83
Total Cases	46	100

Out of 46 number of cases, 35 cases (76.09%) were bronchial brush smears followed by 6 cases (13.04%) of TTNA and 5 cases were of TBNA (10.87%) smears. (Table 6)

Table 6: Distribution of cases according to cytological procedures (N=46)

Type of procedure	No of cases	%
Bronchial brush smears	35	76.09
TBNA smears	5	10.87
TTNA smears	6	13.04

Most common cases were ADC (Figure 1) in our study comprising of 15 cases (32.69%) followed by 11 cases (23.91%) of SCC (Figure 2), 8 cases (17.39%) of NSCLC-favours ADC, 4 cases (8.70%) of NSCLC-NOS, 3 cases (6.52%) of suspicious of malignancy, 2 cases (4.35%) each of NSCLC-favours SCC & atypical cells and one case (2.17%) of ADC-lepidic pattern. (Table 7)

Table 7: Distribution according to cytomorphological diagnosis (N=46)

Cytodiagnosis	Number	%
Adenocarcinoma	15	32.61
Squamous cell carcinoma	11	23.91
NSCLC-favours Adenocarcinoma	8	17.39
NSCLC-favours Squamous cell carcinoma	2	4.35
NSCLC-Not otherwise specified	4	8.70
Suspicious of malignancy	3	6.52
Atypical cells	2	4.35
Adenocarcinoma-lepidic pattern	1	2.17
Total cases	46	100%

In our study correlation between cytology and histomorphology was possible in 25 cases. Concordance was present in 17 cases (68%). 7 (28%) of each diagnosed as SCC and ADC in cytology showed similar results in histology. In this group, one case each which was diagnosed as NSCLC-favours SCC, NSCLC-favours ADC & NSCLC-NOS cytomorphologically, were diagnosed as SCC, ADC-lepidic pattern & NSCLC-NOS respectively histopathologically. Out of the rest 10 cases, 6 cases (24%) which were diagnosed NSCLC-ADC cytomorphologically, 2 cases were given SCC, 3 cases no opinion possible and one case was diagnosed as ADC in histology. The remaining 4 cases (16%) out of 10 cases, diagnosed as NSCLC-NOS, suspicious of malignancy, ADC, NSCLC-favours SCC cytologically, in three cases no opinion was given in histology and in one case which was diagnosed as NSCLC-NOS in cytology was reported same in histology. Thus, in our study cyto-histo concordance was present in 17/25 (68%) cases. (Table 8)

No opinion was possible in six cases of histopathological study. So, we evaluated cyto-histopathology correlation in remaining 19 cases. Out of 10 cases of ADC in cytodiagnosis, 8 cases were same in histology but two cases came out to be SCC in histology. All the 7 cases of SCC in cytology were diagnosed as same in histology. 2 cases of NSCLC-NOS in cytology were same in histodiagnosis. Detailed analysis showed the diagnostic accuracy of cytology for classification of NSCLC was 89.47% (17/19) in our study. (Table 10).

DISCUSSION

Lung cancer poses a great diagnostic and therapeutic problem worldwide. It is the most common cause of cancer related mortality in developed countries with an increasing trend in developing countries.¹ Previously lung cancer was broadly divided into SCLC and NSCLC. With introduction of newer personalized treatment modalities, targeted therapies & molecular analysis, new WHO classification of lung cancer was introduced in 2015 which took into consideration of small biopsy and cytology samples.¹¹ Presently according to WHO 2015 classification of lung cancer, it has become important for subtyping of NSCLC lung into ADC, SCC, LCC, NSCLC-NOS & others.¹⁰ This new updated classification gave better understanding of tumors and created a good harmony between pathologist and clinician by avoiding confusing terminologies.¹¹ Also it has benefited patients to receive therapy with lower side effects, higher therapeutic response and gives further knowledge of mutational analysis (EGFR mutation and ALK rearrangement).⁹ Many older entities were

removed and newer ones were introduced.

Nowadays studies are more focused on specific diagnosis based on cytology rather than histology as most of the cases present in advanced stages and only small biopsy and cytology samples are available for examination. With less manipulation, more material is preserved for molecular testing with higher efficacy in cytology. Cytomorphologically with routine stains like H&E and Pap, many of the cases have been diagnosed correctly and also correlated with clinicoradiological findings. The chromatin and DNA preservation is better in cytology than histology which also helps in different molecular diagnostic methods of study.¹⁸

A clear distinction between NSCLC and SCLC is important as bears treatment implications. Sometimes in cytosmear analysis of lung malignancies, sampling bias and tumor heterogeneity becomes the cause for misdiagnosis of ADC as SCC. This may be due lack of recognition of keratinised cells. The artifactual vacuolisation, pseudoacinar structure, poor staining or smearing technique error and tumor necrosis also poses to erroneous diagnosis. Poorly differentiated carcinoma with pleomorphic cells in sheets and necrotic background also implies inability to subtype NSCLC. Radiological correlation also gives clue to the diagnoses of lung malignancy. It is important to know the relationship between the radiological location and subtypes of NSCLC, as subtyping is important for the new modality of treatment.

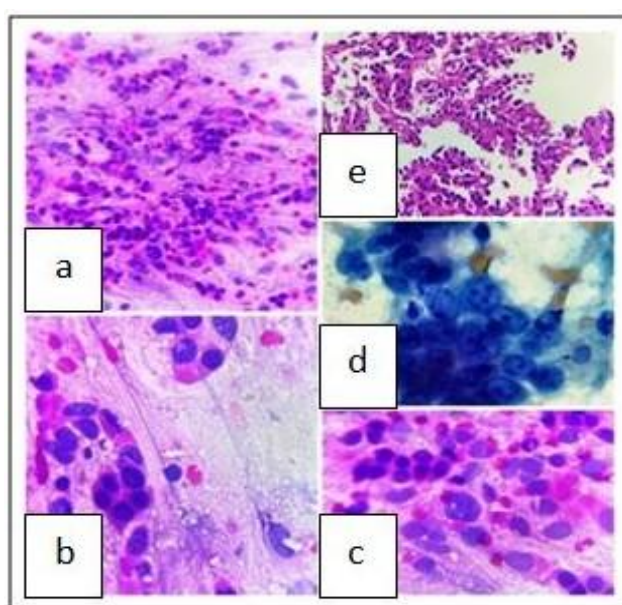


Fig. 1: (ADC): **a:** Cytosmear showing dyschesive clusters of pleomorphic atypical cells (H&E 100X); **b:** Cytosmear shows tumor cells arranged in glandular pattern (H&E 200X); **c:** Cytosmear shows pleomorphic tumor cells having high N:C ratio, hyperchromatic nuclei scattered singly(H&E 400X) **d:** Pap stained smear showing tumor cells arranged in vague acinar pattern & loose clusters (400X); **e:** Section showing tumor cells arranged in papillary pattern (H&E 200X)

In this study we have included 57 cases showing mass lesion in lungs radiologically. Total 46 out of 57 cases of NSCLC were taken into study group and rest 11 samples were rejected due to above mentioned criteria. Out of 46 cases, 35 cases (76.09%) were bronchial brush smear, 6 cases (13.04%) & 5 cases (10.87%) were smears of TTNA and TBNA.

The age range was from 32-81 yrs of age with mean age of 59.2 yrs. Rohtagi et al.¹⁹ and Raiza et al.²⁰ in their study also revealed most cases in 5th and 6th decades of life. The overall incidence of lung cancer was more common in males 31 cases (67.39%) as compared to females 15 cases (32.61%) with M:F ratio of 2.06:1. This ratio was comparable to study by Sinna EA et al.²¹ & Pavani et al.²² where sex ratio was 2.3:1.

Smokers were more affected which were in 26 cases (56.52%) than in non-smokers in 20 cases (43.48%). It was comparable to study by Rohtagi S et al.¹⁹ & Gupta et al.²³ which also showed that smokers are affected more than non-smokers.

Table 8: Comparison of cytomorphological diagnosis with other studies (N=46)

Authors	Year of study	Sample size	ADC cases (%)	SCC cases (%)
Nizzoli et al.	2011	186	56	44
Sinna EA et al.	2013	40	27.5	30
Van Zyl et al.	2019	271	74.0	19.6

Roh et al.	2015	13	46.15	7.69
Present case	2018	46	34.78	23.91

Table 9: Correlation between cytomorphological and histomorphological diagnosis (N=25)

Cytomorphological diagnosis	Histopathological diagnosis	Number of cases	
ADC	ADC	6	
SCC	SCC	6	
NSCLC-ADC	ADC-Lepidic Pattern	1	
NSCLC-SCC	SCC	1	Concordance in 17/25 cases (68%)
NSCLC-NOS	NSCLC-NOS	2	
	SCC	2	
NSCLC-ADC	No Opinion	3	
	ADC	1	
Suspicious of Malignancy		1	
ADC	No Opinion	1	
NSCLC-SCC		1	

Table 10: Statistical analysis for diagnostic accuracy of cytology in cyto-histopathological correlation (N=18)

Cytodiagnosis	ADC	Histodiagnosis SCC	NOS
ADC	8	2	0
SCC	0	7	0
NSCLC-NOS	0	0	2

In our study, 31 cases (67.39%) cases had involvement of left lung and 15 cases (32.61%) had involvement of right lung. But Gupta et al.²³ found right lung was affected more than left which was discordant with our study. In our study mass was detected in central location in 24 cases (52.37%) and in peripheral location in 22 cases (47.38%) radiologically. Our study was concordant with study by Gupta et al.²³ & Rohtagi et al.¹⁹ which showed mass in central location in 75% and 61.22% cases respectively.

Most common symptom in our study was cough in 35 cases (76.09%). In study by Gupta et al.,²³ Devesa SS et al.²⁴ & Brevet M et al.²⁵ cough was also the most common symptom.

Out of 46 cases, most common cases were ADC comprising of 16 cases (34.78%). The most common age group for ADC was 61-70 years, with male to female ratio is 1.6: 1.0. This was similar to the study done by Atta I S et al.,²⁶ and Alekhya et al.²⁷ ADC was common in peripheral location with involvement of right upper lobe in our study which was concordant with study by Rawat et al.²⁷ and data followed by WHO lung cancer 2015 statistics.¹¹

SCC was less common type of primary lung cancer after ADC comprising of 11 cases (23.91%). This matches study with Gruda et al.²⁸ and Van Zyl A et al.^{28,29} The average age for SCC was 59.7 years with a range of 50 years to 72 years. Maximum number of cases was between 51-60 years.

In our study correlation between cytology and histomorphology was possible in 25 cases. Concordance was present in 17 cases (68%). Detailed analysis shows the diagnostic accuracy of cytology for classification of NSCLC was 89.47% (17/19) in our study. (Table 10)

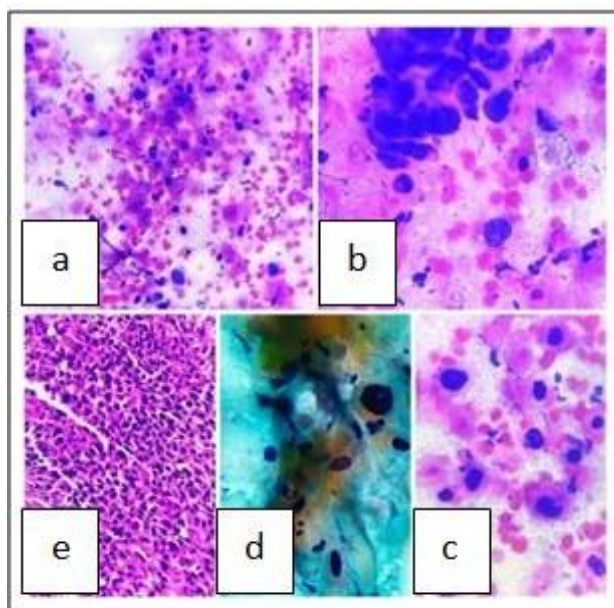


Fig. 2: (SCC): **a:** Cytosmear showing dyscohesive clusters of pleomorphic atypical cells (H&E 100X); **b:** Cytosmear shows tumor cells arranged in loose clusters (H&E 200X); **c:** Cytosmear shows scattered pleomorphic tumor cells having high N:C ratio, hyperchromatic nuclei with moderate amount of dense cytoplasm (H&E 400X); **d:** Pap stained smear showing dyskeratotic cells, tadpole cells & spindloid cells (400X); **e:** Lung biopsy of SCC (H&E 200X).

CONCLUSION

Early diagnosis of lung cancer plays a pivotal role in reducing the death rate. Cytology can play an excellent role in initial evaluation of lung cancer by giving an early diagnosis. Subclassification of NSCLC can be difficult in some cases on cytomorphology alone. Further ancillary investigations like Immunocytochemistry in cytology samples and Immunohistochemistry on cell block and biopsy samples can be useful in categorization of lung cancer in core biopsy or cytology samples.

Limitation of our Study

In our study, there was crushing artefacts along with obscuring factors like haemorrhage and necrosis for which we had to reject 11 cases. In some cases we had discordant results in cyto-histo correlation, which may be due to sampling site error during procedures. Sometimes if samples are collected from solid areas of ADC, these cases may be misdiagnosed as SCC cytologically, which we faced in 2 cases which were given as NSCLC-ADC in cytology came out to be SCC histologically. In these cases ICC plays an important role for conclusive opinion. So, further prospective study with larger sample size along with other molecular markers is necessary to validate our approach.

Source of Funding

The researchers did not receive any grant from outside funding agencies. This was a self funding study.

Ethical Approval

This study was conducted with approval from the clinical department and as per the approval of Institutional Ethics committee. Informed consent was taken from all the patients before the tests and procedures.

Conflicts of Interest

The authors declare that they have no conflict of interest

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