Original Article

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Pathology Section

Clinicopathological and Immunohistochemical Analysis of Lung Cancer Biopsies at a Tertiary Care Centre in Central Gujarat, India

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ABSTRACT

Introduction: Lung biopsy is a reliable and highly accurate tool for diagnosing and subtyping lung lesions. Two-thirds of lung cancer patients present with advanced disease, and small biopsies remain the main diagnostic tool. Histopathological and immunohistochemical examination of lung biopsies plays an important role in accurately diagnosing lung malignancies.

Aim: The aim of present study was to analyse the clinicopathological and immunohistochemical features of lung cancer.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology, Medical College, Baroda, over a period of two years and three months, from March 2019 to May 2021. A total of 53 cases of malignant lung cancer biopsies were included in this study. Clinical parameters, such as patient age, complaints, smoking history, clinical presentation, radiological findings, histopathology reports, and IHC findings, were retrieved. All cases underwent histopathological examination, and tumour typing was done according to the WHO classification 2021 of lung tumours. Immunohistochemistry (IHC) markers like CK5/6, CK7, TTF1, P63, synaptophysin, chromogranin, and pankeratin were used for further subtyping. Data analysis was done by Microsoft Excel 2019 spreadsheet.

Results: A total of 53 cases of malignant lung cancer were included in this study. Of these, 38 (71.69%) cases were male, and 15 (28.30%) cases were female. 43 (81.13%) cases were over 50 years old, with a mean age of 61 years. After IHC, adenocarcinoma was seen in 27 cases (50.9%), followed by Squamous Cell Carcinoma (SCC) in 19 cases (35.8%), five cases of small cell carcinoma, one case of large cell neuroendocrine carcinoma, and one case of Non Small Cell Carcinoma-Not Otherwise Specified (NSCC-NOS). In adenocarcinoma, positivity for CK7, TTF1, and p63 was 100%, 74.07%, and 16.6%, respectively. In squamous cell carcinoma, positivity for CK5/6 and p63 was 100% and 78.94%, respectively.

Conclusion: In the present study, the majority of cases were males. Adenocarcinoma was the most common subtype of Non Small Cell Lung Cancer (NSCLC). A panel of IHC markers is helpful in differentiating NSCLC into adenocarcinoma and squamous cell carcinoma on small true-cut lung biopsies. CK5/6 and p63 aid in detecting squamous cell carcinoma, while CK7 and TTF-1 help in detecting adenocarcinoma. The recommended immunohistochemical profile for NSCLC includes TTF1, CK5/6, CK7, and p63.

Keywords: Immunohistochemical markers, Lung biopsy, Non small cell carcinoma

INTRODUCTION

Lung cancer is the second most commonly diagnosed and the leading cause of cancer deaths in 2020. Lung cancer is the leading cause of morbidity and mortality in men, whereas in women, it ranks third [1]. The prevalence of lung cancer in India appears to be increasing compared to the Western world. According to the Indian Council of Medical Research Cancer registry, 57,795 new cases were reported in 2010. The number of cases is expected to rise sharply to 81,219 cases among males and 30,109 in females by 2025 [2,3]. Lung biopsy is reliable with high accuracy for the diagnosis and subtyping of lung lesions. Histopathological and immunohistochemical examination of lung biopsy plays an important role in making a correct and accurate diagnosis of lung malignancy [4]. In the 2015 WHO classification, there was more emphasis on using immunohistochemical markers for the accurate classification of lung cancers [5,6]. In the recent 2021 WHO classification, there is an emphasis on advances in molecular pathology across all tumour types and recommends routine testing of lung tumour biopsies for the presence of driver mutations/fusions in the EGFR, ALK, ROS1, BRAF, V600E, NTRK1-3, RET, KRAS, and MET genes with PD-L1 by immunohistochemistry [7]. The availability of targeted therapies has created a need for precise subtyping of NSCC. Differentiating between adenocarcinoma and squamous cell carcinoma is now important because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type. By using immunohistochemistry, we can minimise the rate of NSCC-not otherwise specified diagnoses in small biopsy specimens.

Two-thirds of lung cancer patients present in advanced stages, and small biopsy remains the main tool for diagnosis [8]. On histology, according to guidelines for small biopsy, lung cancers are broadly classified into small cell and NSCC. The main histological subtypes of NSCC are squamous cell carcinoma, adenocarcinoma, and NSCC-NOS type. CK5/6, p63, and p40 are commonly used IHC markers for squamous cell carcinoma, while TTF1, Napsin A, and CK7 are used for adenocarcinoma. Synaptophysin, chromogranin A, and pankeratin are IHC markers used for small cell carcinoma of the lung [9]. The aim of the present study is to perform a clinicopathological analysis of lung cancers on true-cut biopsy samples, first based on histology and then with IHC using different relevant markers such as CK5/6, CK7, TTF1, P63, synaptophysin, chromogranin A, and pankeratin. Through the present study, the authors aim to recommend an optimal panel of IHC markers for the precise diagnosis of lung cancers.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, Medical College, Baroda, over a period of two years

and three months from March 2019 to May 2021. A total of 53 cases of lung cancer biopsies were included in this study. Detailed clinical history, such as patients' age, complaints, smoking history, clinical presentation, and other investigations, was retrieved. Cases diagnosed as NSCC, adenocarcinoma, squamous cell carcinoma, and small cell carcinoma were included in the study, while cases diagnosed as lymphoma, sarcoma, improperly fixed biopsy samples, non-neoplastic lesions, biopsy containing only necrotic tissue, or absence of tumour tissue in the biopsy specimen were excluded.

Grossing of all lung biopsy specimens submitted was done with precision. After proper tissue processing, sections of 3 to 4 µm were obtained from the submitted biopsy specimens. The sections were stained routinely with Haematoxylin and Eosin Stain (H&E). All cases underwent histopathological examination, and the typing of tumours was done according to the WHO classification 2021 of lung tumours [7].

The distinction between adenocarcinoma and squamous cell carcinoma is based on standard morphological criteria. For squamous cell carcinoma, keratinisation, pearl formation, and intercellular bridges are important criteria, while for adenocarcinoma, glandular differentiation with lepidic, papillary, micropapillary, solid growth pattern, or intracellular mucin are important. Sometimes, this distinction may be difficult on small lung biopsies where defining glandular or squamous features are subtle or focal. These cases were classified as NSCC-NOS. For small cell carcinoma, morphological features include small to medium-sized cells, nuclear features like finely dispersed chromatin, molding, indistinct nucleoli, a high mitotic rate, and the presence of crushed artifact [6,10].

IHC markers like CK5/6, CK7, TTF1, P63, synaptophysin, chromogranin, and pankeratin were used for further subtyping. Primary antibodies from the Biogenix company were used, including EP67 and EP24 for CK5/6, OVTL12/30 for CK7, SP141 for TTF1, 4A4 for p63, synapto88 for synaptophysin, LK2H10 for chromogranin A, and AE1/AE3 for pankeratin. Known external positive controls were kept for each marker, prepared from squamous cell carcinoma of oral mucosa for CK5/6, p63, and pankeratin, lung adenocarcinoma for TTF1, gastric adenocarcinoma for CK7, and neuroendocrine tumour for synaptophysin and chromogranin A. Negative controls were done by excluding the primary antibody and replacing it with Phosphate-buffered Saline (PBS). CK5/6, CK7, and pankeratin show cytoplasmic positivity. TTF1 and p63 show nuclear staining positivity, while synaptophysin and chromogranin A give granular cytoplasmic staining.

A 3-4 µm thick section was taken from a representative paraffin-embedded block. IHC for various markers was performed on poly-L-lysine-coated slides. The slides were placed on a hot plate (65ºC) for 30 to 45 minutes for deparaffinisation. Antigen retrieval was done using the Heat induced epitope retrieval (HIER) method with a microwave at 900 W for 20 minutes, followed by 540 W for 20 minutes. Citrate buffer solution at pH 9 was used for antigen retrieval. The slides were allowed to cool at room temperature for 45 minutes and then washed with phosphate buffer in a humidifier box. Peroxidase block and power block were applied for 10 minutes. One drop of primary antibody was applied to the tissue and incubated for one hour for cytoplasmic antibodies and 1.5 hours for nuclear antibodies. Then a polymer Horseradish Peroxidase (HRP) as secondary antibody was applied to the tissue for 30 minutes, followed by phosphate buffer wash. Freshly made 3,3′-Diaminobenzidine (DAB) solution was applied to the tissue for 10 minutes, followed by phosphate buffer and washed with distilled water. The sections were then stained with hematoxylin and mounted with DPX solution.

The lung biopsies were scored as negative (no reactivity) in tumour cells versus focal positivity (labeling in the minority of cells) and diffuse positivity (labeling in the majority of cells) without exact quantification [10]. For non-small cell lung carcinoma, TTF1, CK7, CK5/6, and p63 were used to differentiate between adenocarcinoma and squamous cell carcinoma, and for small cell carcinoma, synaptophysin, chromogranin A, and pankeratin were used. IHC interpretation was done according to the expression of IHC markers shown in [Table/Fig-1].

STATISTICAL ANALYSIS

Each case's data was entered into a Microsoft Excel 2019 spreadsheet and analysed.

RESULTS

A total of 53 cases were included in this study. Of these, 38 (71.69%) cases were male, and 15 (28.30%) cases were female. 43 (81.13%) cases were over 50 years old. The mean age was 61 years. In terms of age distribution, the maximum number of cases was 18 (33.96%) in the 61-70 years age group, followed by 16 (30.18%) cases in the 51-60 years age group. There were 9 (16.98%) cases in the 71-90 years age group, 7 (13.2%) cases in the 41-50 years age group, and 3 (5.66%) cases in the 31-40 years age group [Table/Fig-2]. In the present study, chest pain (28 cases) and breathlessness (27 cases) were the most common symptoms, followed by cough (16 cases) and fever (9 cases).

Haemoptysis was present in three cases, hoarseness of voice in two cases, and weight loss in four cases. Out of 53 cases, 36 (67.9%) cases were associated with smoking, and 17 (32.0%) cases were seen in non smokers [Table/Fig-2]. In CECT findings, the right lung was most commonly involved in 29 (54.71%) cases, the left lung was involved in 17 (32.02%) cases, and bilateral lung involvement was seen in 7 (13.2%) cases [Table/Fig-2]. 31 (58.50%) cases presented with a mass lesion, followed by combined presentation in 15 (28.30%) cases, 4 (7.54%) cases with pleural effusion, and 3 (5.66%) cases with collapse with consolidation [Table/Fig-2]. 15 (28.3%) cases presented with metastasis to other organs such as the liver, adrenal glands, vertebrae, brain, and opposite lung.

The most common malignancy was adenocarcinoma in 27 cases (50.9%), followed by squamous cell carcinoma in 19 cases (35.8%). Squamous cell carcinoma was most commonly seen in males, with 17 cases, whereas only two female patients had squamous cell carcinoma. Adenocarcinoma was the most common subtype in female patients, with 13 out of 15 females having adenocarcinoma. All cases of small cell carcinoma were seen in male patients [Table/Fig-2]. On H&E diagnosis, 35 cases were diagnosed as NSCC, seven cases were adenocarcinoma, six cases were squamous cell carcinoma, and 5 cases were small cell carcinoma [Table/Fig-3]. The final diagnosis was given based on the positivity of IHC markers shown in [Table/Fig-1]. Out of the 35 cases initially diagnosed as

IHC Diagnosis	TTF1	CK7	CK5/6	p63	Synaptophysin	Chromogranin A
SCC	Negative	Negative (focal positivity may be present)	Positive	Positive	Negative	Negative
Adenocarcinoma	Positive (some cases may be negative)	Positive	Negative (focal positivity may be present)	Variable (-/+)	Negative	Negative
Small cell Carcinoma	Positive (some cases may be negative)	Negative	Negative	Negative	Positive	Positive

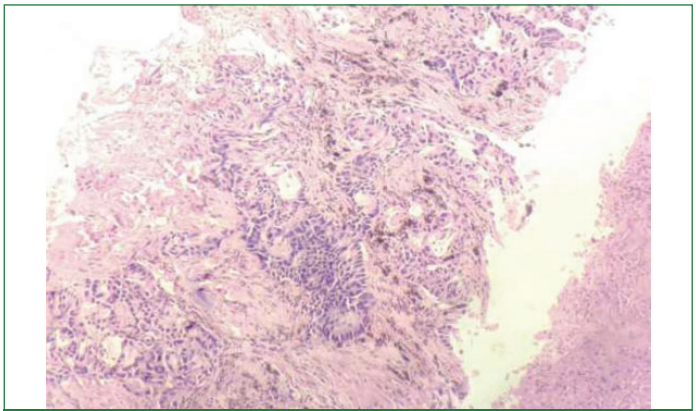
[Table/Fig-1]: Different IHC markers interpretation and Diagnosis according to expression of IHC markers.

Parameters		Total No of Cases=53	Squamous Cell Carcinoma (n=19)	Adenocarcinoma (n=27)	Small cell carcinoma (n=05)	Large cell Carcinoma (n=01)	Non small cell carcinoma (n=01)
Age (in years)	31 to 40	3 (5.6%)	00	03 (11.11%)	00	00	00
	41 to 50	7 (13.2%)	02 (10.5%)	02 (7.40%)	02 (40.0%)	00	01 (100%)
	51 to 60	16 (30.18)	06 (31.5%)	08 (29.62%)	01 (20.0%)	01 (100%)	00
	61 to 70	18 (33.96%)	08 (42.10%)	08 (29.62%)	02 (40.0%)	00	00
	71 to 80	8 (15.09%)	02 (10.5%)	06 (22.22%)	00	00	00
	81 to 90	1 (1.8%)	01 (5.2%)	00	00	00	00
Sex	Males	38 (71.69%)	17(89.47%)	14 (51.85%)	05 (100%)	01 (100%)	01 (100%)
	Females	15 (28.30%)	02 (10.53%)	13 (48.15%)	00	00	00
History of smoking	Non smokers	17 (32.07%)	02 (10.52%)	12 (44.44%)	01 (20.0%)	01 (100%)	01 (100%)
	Smokers	36 (67.92%)	17 (89.47%)	15 (55.55%)	04 (80.0%)	00	00
Symptoms	Chest pain	28 (52.83%)	07 (36.8%)	16 (59.26%)	03 (60.0%)	01 (100.0%)	01 (100%)
	Breathlessness	27 (50.94%)	08 (42.10%)	15 (55.55%)	04 (80.0%)	00	01 (100%)
	Cough	16 (30.18%)	08 (42.10%)	06 (22.22%)	01 (20.0%)	01 (100%)	00
	Fever	9 (16.98%)	02 (10.52%)	05 (18.52%)	02 (40.0%)	00	00
	Anorexia	7 (13.20%)	03 (15.78%)	03 (11.11%)	01 (20.0%)	00	00
	Weight loss	4 (7.54%)	02 (10.52%)	02 (7.40%)	00	00	00
	Haemoptysis	3 (5.66%)	03 (15.78%)	00	00		
	Hoarseness	2 (3.77%)	01 (5.3%)	01 (3.70%)	00	00	00
Site of lung cancer	Right lung	29 (54.71%)	12 (63.15%)	14 (51.85%)	02 (40.0%)	00	01 (100%)
	Left lung	17 (32.07%)	03 (15.78%)	10 (37.03%)	03 (60.0%)	01 (100%)	00
	Both lungs	O7 (13.20%)	04 (21.05%)	03 (11.11%)	00	00	00
Radiological presentation	Mass	31(58.50%)	15 (78.94%)	11 (40.74%)	03 (60.0%)	01 (100%)	01 (100%)
	Pleural effusion	04 (7.54%)	00	04 (14.81%)	00	00	00
	Collapse	03 (5.66%)	00	03 (11.11%)	00	00	00
	Combined	15 (28.30%)	04 (21.05%)	09 (33.33%)	02 (40.0%)	00	00

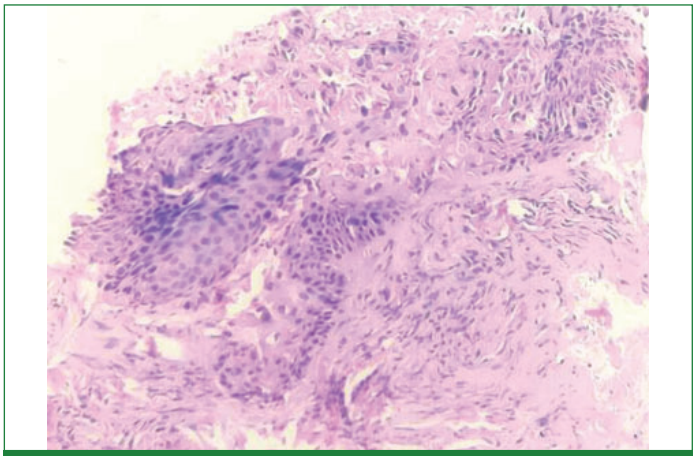
[Table/Fig-2]: Analysis of clinical parameters with histological typing of tumour.

Diagnosis on Histopathology	Diagnosis on IHC					Total No. of cases
	Adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma	Large cell neuroendocrine carcinoma	Non Small cell carcinoma (NOS)	
Non small cell carcinoma (NOS)	18	15	00	01	01	35
Squamous Cell carcinoma	02	04	00	00	00	06
Adenocarcinoma	07	00	00	00	00	07
Small cell carcinoma	00	00	05	00	00	05
Total no. of cases	27	19	05	01	01	53

[Table/Fig-3]: Diagnosis on histology and final diagnosis on IHC.



[Table/Fig-4]: Adenocarcinoma in tru-cut biopsy (H&E stain 100 X).



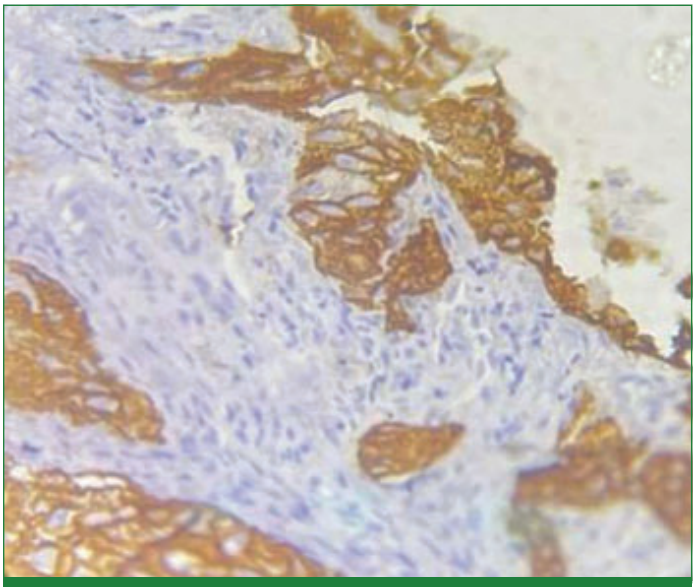
[Table/Fig-5]: Squamous cell carcinoma in tru-cut lung biopsy (H&E Stain 400x).

NSCC, 18 cases were diagnosed as adenocarcinoma [Table/Fig-4]. Fifteen cases were diagnosed as squamous cell carcinoma [Table/Fig-5]. One case was diagnosed as large cell neuroendocrine carcinoma, and one case was NSCC (NOS) type. All seven cases of adenocarcinoma on histology were confirmed as ADC on IHC. Out of the six cases of squamous cell carcinoma, four cases were confirmed as squamous cell carcinoma on IHC, while two cases were diagnosed as adenocarcinoma on IHC [Table/Fig-3].

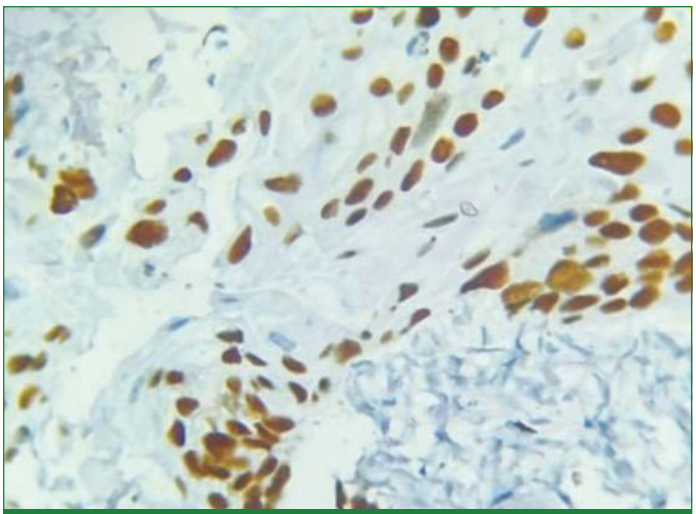
The positivity of different IHC markers among the squamous cell carcinoma cases (n=19) is shown in [Table/Fig-6]. Out of the 19 cases of squamous cell carcinoma, 15 cases were positive for CK5/6 and p63 and negative for TTF1 [Table/Fig-7,8]. Four cases showed focal positivity for CK7. All 27 cases of adenocarcinoma showed positivity for CK7 [Table/Fig-9], and nine cases showed focal positivity for CK5/6. The positivity of different IHC markers among the

IHC Markers	Squamous cell carcinoma (n=19)		Adenocarcinoma (n=27)		Small cell carcinoma (n=05)		Large cell neuroendocrine carcinoma (n=01)		Non small cell carcinoma NOS (n=01)	
	No. of positive cases (%)	No. of negative cases (%)	No. of positive cases (%)	No. of negative cases (%)	No. of positive cases (%)	No. of negative cases (%)	No. of positive cases (%)	No. of negative cases (%)	No. of positive cases (%)	No. of negative cases (%)
CK 7	4 (21.05)	15 (78.94)	27 (100)	00 (00)	-	-	-	-	-	01 (100)
CK 5/6	19 (100)	00 (00)	09 (33.3)	18 (66.66)	-	-	-	-	-	01 (100)
P63	15 (78.94)	04 (21.05)	02 (16.6)	10 (83.33)	-	-	-	-	-	01 (100)
TTF 1	00 (00)	15 (100)	20 (74.07)	07 (25.92)	03 (100)	-	-	01 (100)	-	01 (100)
Synaptophysin	-	-	-	-	04 (80)	01 (20)	01 (100)	00 (00)	-	01 (100)
Chromogranin A	-	-	-	-	04 (80)	01 (20)	01 (100)	00 (00)	-	01 (100)
Pankeratin					03 (75) (perinuclear dot like positivity)	01 (25%)	-	-	01 (100)	00 (00)

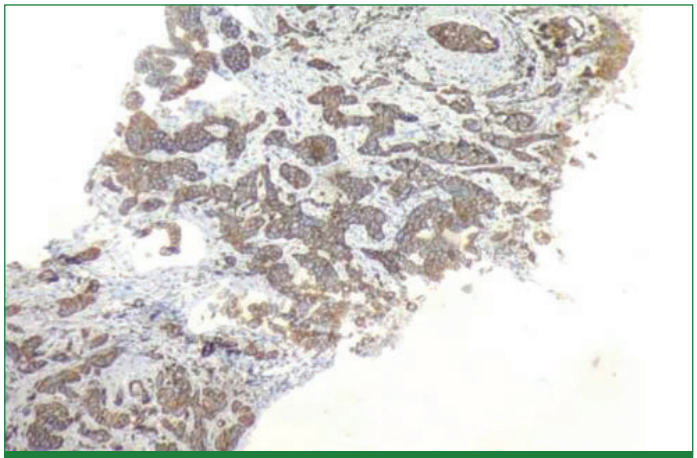
[Table/Fig-6]: IHC markers according to diagnosis.



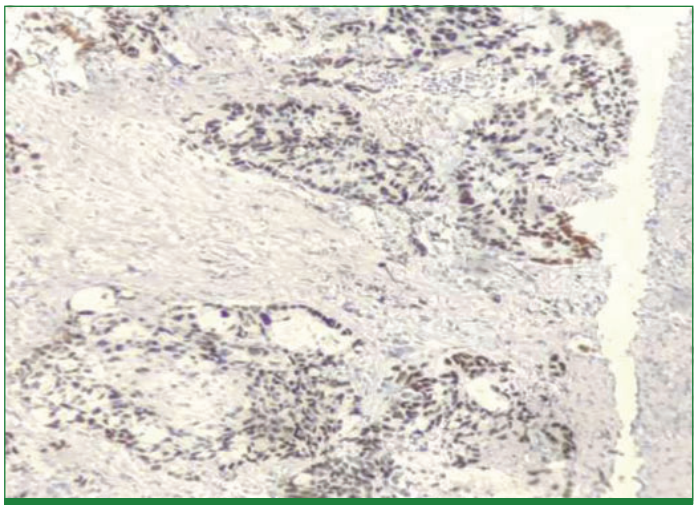
[Table/Fig-7]: CK5/6 cytoplasmic positivity in squamous cell carcinoma (IHC Stain 400x).



[Table/Fig-8]: p63 nuclear positivity in squamous cell carcinoma (IHC stain-400X).



[Table/Fig-9]: CK7 cytoplasmic positivity in adenocarcinoma (IHC stain 100x).



[Table/Fig-10]: TTF1 nuclear positivity in adenocarcinoma (IHC Stain 100x).

adenocarcinoma cases (n=27) showed that 20 cases were positive for TTF1 [Table/Fig-10]. Two cases showed positivity for p63.

DISCUSSION

The lungs are involved by a wide variety of inflammatory, infectious, and neoplastic pathological conditions and are almost always involved secondarily as a terminal event of cardiovascular diseases [11]. Lung biopsy is widely used for the diagnosis and management of diverse pulmonary diseases. The principal methods for obtaining lung tissue for histomorphology, immunohistochemical, and molecular studies are transbronchial lung biopsy, open lung biopsy, and CT-guided core needle biopsy [12]. In the present study, 38 (71.69%) cases were male, and 15 (28.30%) cases were female, resulting in a sex ratio of 2.53:1. Pandhi N et al., reported a male-to-female ratio of 2.7:1 in their study, Dhandapani S et al., reported 3.9:1, and Noronha V et al., reported 3.5:1 [13-15]. The majority of lung cancer patients presented after the age of 50. In the present study, 16 (30.18%) cases were between 51 to 60 years old, and 18 (33.96%) cases were between 61 to 70 years old. Varma A et al., reported 36.5% of cases in the 51 to 60 years age group and 30.7% of cases in the 61 to 70 years age group [16].

In the present study, the most common symptoms were chest pain in 52.83% of patients, followed by breathlessness in 50.94% of patients. Sarfraz S et al., reported chest pain in 49.25% of cases and breathlessness in 37.31% of cases, while Kumar M et al., reported chest pain in 68.2% of cases and breathlessness in 70.19% of cases [17,18]. Other clinical symptoms included cough in 30.18% of patients, weight loss in 7.54% of patients, and hemoptysis in 5.66% of patients. In the present study, 67.9% of cases were associated

with smokers, and 32.0% of cases were seen in non-smokers, which was comparable to Varma et al., findings of 65.38% [16]. In the present study, 89.47% of cases of squamous cell carcinoma and 80% of cases of small cell carcinoma were associated with smoking, which is similar to the study conducted by Mandal SK et al., where 91.9% of squamous cell carcinoma cases and 77.6% of small cell carcinoma cases were associated with smoking [19]. Additionally, 55.55% of cases of adenocarcinoma were associated with smoking in the present study. Mandal SK et al., reported 56.4% of cases, and Noronha V et al., reported 35.5% of cases, where adenocarcinoma was associated with smoking [15,19]. Sharma CP et al., and Mandal SK et al., found that the most common location of lung cancer was the right lung [19,20]. In the present study, it was observed that in 54.71% of cases of lung cancers, the right lung was involved, and in 32.07% of cases, the left lung was involved. These findings are comparable to the study conducted by Sharma CP et al., and Mandal SK et al., where they observed that in 54.2% and 60.3% of cases, the right lung was involved, and in 38.3% and 39.1% of cases, the left lung was involved, respectively [19,20].

The most common radiological presentation seen in the present study was a mass lesion (58.50%), followed by combined (28.3%), pleural effusion (7.54%), and collapse/consolidation (5.66%) cases. Mandal SK et al., reported mass lesion in 70.04% of cases, combined in 19.82% of cases, pleural effusion in 3.08% of cases, and collapse/consolidation in 6.6% of cases, while Rawat J et al., reported 40.31%, 8.37%, 4.43%, and 40.89%, respectively [19,21]. Metastasis was seen in 28.3% of cases, while Mandal SK et al., reported it as 32.5% [19].

In the present study, the majority of cases were adenocarcinoma (27 cases, 50.97%), followed by squamous cell carcinoma (19 cases, 35.84%), small cell carcinoma (5 cases, 9.43%), large cell neuroendocrine carcinoma (1 case, 1.88%), and NSCC NOS type (1 case, 1.88%). Noronha et al., found the cases of adenocarcinoma, squamous cell carcinoma, and small cell carcinoma to be 43.6%, 26.2%, and 8%, respectively, while Mandal SK et al., reported 52.63%, 22.8%, and 14.9%, respectively [15,22]. There has been a shift in the pathological distribution of NSCC and an increase in the incidence of adenocarcinoma, making it the predominant histological subtype of NSCC [15,22-24].

In the present study, TTF1 was positive in 20 cases (74.07%) of adenocarcinoma, which is similar to the study conducted by Alekhya M et al., (74%), Rathwa MR et al., (74%), and Bishop JA et al., (73%) [25-27]. Studies done by Mukhopadhyay S et al., (80%), Zhao W et al., (80%), Gurda GT et al., (84.5%), and Brunnström H et al., (92%) reported a higher percentage of positivity for TTF1 [28-31]. The variability in the expression of TTF1 is because it is better expressed in well and moderately differentiated adenocarcinoma and less expressed in poorly differentiated adenocarcinoma [30,32].

CK7 positivity was observed in all cases of adenocarcinoma (100%). Similar findings were noted by Zhao W et al., (100%), Mukhopadhyay S et al., (100%), and Brunnström H et al., (100%) [28,29,31]. Twenty cases of adenocarcinoma showed positivity for both CK7 and TTF1, while seven cases showed positivity only for CK7. A similar finding was also seen in the study conducted by Alekhya M et al., [25]. CK7 could diagnose ADCs with 100% sensitivity but low specificity. According to Gurda GT et al., in metastatic ADCs, CK7 showed better sensitivity than TTF-1 but less specificity [30].

Focal positivity of CK 5/6 was seen in 9 (33.3%) cases of adenocarcinoma. Out of these nine cases, five were positive for CK7 and TTF1, and the other four were CK7 positive but TTF1 negative. Alekhya M et al., Gurda GT et al., and Kargi A et al., also found focal positivity of CK5/6 in a subset of adenocarcinoma [25,30,33]. We found p63 focal positivity in two cases of adenocarcinoma out of 12 cases. Both of these cases were TTF1 and CK7 positive. P63 shows weak and focal expression in adenocarcinoma compared to strong and diffuse expression in squamous cell carcinoma [28].

CK5/6 positivity was observed in 19 (100%) cases of squamous cell carcinoma. Gurda GT et al., Brunnström H et al., and Sterlacci W et al., reported 100%, 98%, and 99.2% respectively, which is comparable to our study [30,31,34]. CK5/6 sensitivity was reported from 75% to 100% in various studies [30,31,34]. Diffuse positivity of p63 was noted in 15 (78.94%) cases of squamous cell carcinoma, and it was negative in 4 (21.05%) cases. Rathwa MR et al., and Kargi A et al., revealed 82.05% and 61% positivity, respectively [26,33]. Gurda GT et al., reported 91.75%, Brunnström H et al., reported 97%, and Sterlacci et al., reported 99.2% positivity for p63 [28-31,34]. Mukhopadhyay S et al., and Zhao W et al., reported 100% positivity for p63 [28,29,31,34]. All cases diagnosed as squamous cell carcinoma were negative for TTF1. Mukhopadyay et al., Zhao W et al., and Downey P et al., did not find positivity for TTF1 in squamous cell carcinoma, which is similar to our study [28,29,35]. According to Rekhtman et al., this TTF-1/p63 profile overlaps between adenocarcinoma and squamous cell carcinoma. This profile is seen in only a small subset of both tumour types, and with the additional application of CK5/6, these cases can be differentiated [10]. Five cases of small cell carcinoma were diagnosed on histology. Out of these five cases, four (80%) showed positivity for synaptophysin, and four (80%) showed positivity for chromogranin A. Bhatti V et al., reported 100% positivity for both synaptophysin and chromogranin A, and Gkika E et al., observed 80% and 63% positivity, respectively [36,37]. Perinuclear dot-like positivity with pankeratin was observed in three cases of small cell carcinoma of the lung [10]. TTF1 was applied to three cases, and all three cases (100%) were positive, which is comparable to Gkika E et al., who observed 90% positivity, and Bhatti V et al., who observed 89% positivity [36,37].

One case in our study was diagnosed as large cell neuroendocrine carcinoma, which was positive for synaptophysin and chromogranin A and negative for TTF1, CK7, CK5/6, and CD45. One case in our study was reported as non-small cell carcinoma NOS type, in which all markers were negative except pankeratin.

An accurate classification of lung tumours in small biopsy specimens is difficult due to scanty tumour cells, lack of a characteristic architectural pattern, differentiation, and heterogeneity of the tumour. Under these circumstances, the use of appropriate immunohistochemistry markers plays an invaluable role in the subclassification of NSCLC, that is, adenocarcinoma and squamous cell carcinoma [4]. The present study highlight the importance of IHC markers for the confirmative diagnosis of lung cancers on biopsy specimens, and the authors recommend the optimal panel of IHC markers CK5/6, CK7, TTF1, and p63 for NSCLC to differentiate between adenocarcinoma and squamous cell carcinoma. In the present study, CK5/6 showed 100% positivity, while p63 showed 78.94% positivity in squamous cell carcinoma, and CK7 showed 100% positivity, while TTF1 showed 74.07% positivity in adenocarcinoma cases. Synaptophysin, chromogranin A, and pankeratin are IHC markers used for the diagnosis of small cell carcinoma of the lung.

In lung cancer, targeted therapies depend on the accurate histological subclassification of the tumour. Recently, the therapeutic strategy has changed from traditional tumour stage-based approaches to histomorphology, immunohistochemistry, and genetic mutation-guided targeted therapies. The discovery of Epidermal Growth Factor Receptor (EGFR) gene mutations and tyrosine kinase inhibitors in adenocarcinoma highlights the critical role of accurate classification of the tumour. The EGFR inhibitors gefitinib and erlotinib are effective in adenocarcinoma. The antivascular endothelial growth factor agent bevacizumab is contraindicated in squamous cell carcinoma due to a higher

incidence of pulmonary hemorrhage than in non-squamous carcinoma. There has not been a major discovery in the molecular characterisation of lung squamous cell carcinoma. Therapeutically targetable driver mutations remain elusive for patients with squamous cell carcinoma, but they may benefit from anti-programmed cell death protein-1/PD-L1 therapy, alone or in combination with chemotherapy [7,10,28].

Limitation(s)

The sample size of our study was small. Follow-up of every patient with regards to clinical improvement and autopsy findings of resected lung specimens for final histopathology reports were not available. Hence, sensitivity, specificity, positive predictive value, and negative predictive value could not be calculated in the present study. We had a limited number of IHC markers available, so the evaluation of ALK, ROS1, and PD-L1 was not possible.

CONCLUSION(S)

In the present study, adenocarcinoma is the most common subtype of NSCLC. The right lung was the most common location for lung cancer. In the past, squamous cell carcinoma was the most common subtype of lung cancer in India. Similar to the global trend, a pathological shift to adenocarcinoma has occurred in India. A panel of IHC markers is helpful in differentiating NSCLC into adenocarcinoma and squamous cell carcinoma on small true-cut lung biopsies, which can influence the personalised treatment of the patient. CK5/6 and p63 help in detecting squamous cell carcinoma, while CK7 and TTF-1 help in detecting adenocarcinoma. The immunohistochemical profile recommended for NSCLC is TTF1, CK5/6, CK7, and p63.

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