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Immunohistochemical profile and molecular markers of lung carcinoma in tertiary care medical center - A retrospective observational study

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ABSTRACT

Background: Lung carcinoma is one of the most prevalent malignancies globally, with high morbidity and mortality rates due to late-stage diagnosis and aggressive disease progression. Understanding immunohistochemical and molecular profiles is essential for improving diagnostic precision and guiding personalized therapy. **Aims and Objective:** This study aimed to evaluate the immunohistochemical profiles and molecular markers of lung carcinoma at a tertiary care center. **Materials and Methods:** This retrospective observational study included 67 patients diagnosed with lung carcinoma between June 2019 and June 2024. Data on patient demographics, histopathological subtypes, immunohistochemical markers (thyroid transcription factor-1 [TTF1], cytokeratin 7 [CK7], p63, and others), and molecular markers (epidermal growth factor receptor [EGFR], anaplastic lymphoma kinase [ALK], and ROS1) were analyzed. Statistical analyses were performed using SPSS v26. **Results:** Non-small-cell lung carcinoma (NSCLC) adenocarcinoma was the predominant subtype (85.1%), followed by squamous cell carcinoma (11.9%). TTF1 and CK7 were positive in 82.1% and 40.3% of cases, respectively, whereas p63 was positive in 17.9%, primarily in squamous carcinoma. EGFR mutations were detected in 52.5% of cases, highlighting their therapeutic relevance. ALK and ROS1 rearrangements were absent in all the tested cases. **Conclusion:** This study emphasizes the predominance of NSCLC adenocarcinoma and the diagnostic importance of TTF1 and CK7 expression. Molecular profiling is critical for the effective management of lung cancer with targeted therapies.

Key words: Lung carcinoma; Thyroid transcription factor-1; Cytokeratin 7; Epidermal growth factor receptor mutations; Immunohistochemistry

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INTRODUCTION

Lung carcinoma remains a global health issue and one of the leading causes of cancer-related deaths worldwide. Recent global cancer statistics reveal that lung cancer accounts for approximately 18% of all cancer-related mortalities, highlighting its devastating impact on public health.[1] Even with advances in both diagnostic tools and therapeutic strategies, late-stage detection and the vicious nature of this disease result in a lower survival rate.[2]

Accurate histopathological diagnosis and molecular characterization will be crucial for optimizing the management of lung cancer. The identification of adenocarcinoma and squamous cell carcinoma is considered to be pivotal in the knowledge of tumor biology, guiding

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treatment choices.[3] Further, the incorporation of molecular markers, such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), has dramatically altered the treatment strategies, and allowed targeted therapies along with a customized approach.[4]

Immunohistochemistry (IHC) continues to be a key component in the diagnosis and management of lung carcinoma. Key markers such as thyroid transcription factor-1 (TTF1) and p63 assist in differentiating adenocarcinoma from squamous cell carcinoma, while biomarkers like cytokeratin 7 (CK7) and molecular targets such as ROS1 guide treatment strategies.[5] By facilitating accurate subtyping and identifying actionable mutations, IHC and molecular diagnostics play a major role in improving outcomes for lung cancer patients.[6]

The World Health Organization (WHO) classification of lung cancer was recently updated in 2015 to incorporate newly identified molecular profiles and targetable genetic alterations. Notably, the current WHO classification for lung adenocarcinoma largely adopts the 2011 classification established by the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society.

For various reasons, investigation of the immunohistochemical and molecular characteristics of lung carcinoma in tertiary settings is very important. First, the prevalence of specific subtypes and molecular markers varies among populations depending on genetics, environment, and lifestyle factors. This will set localized data and validate diagnostic and therapeutic protocols to make them more relevant and effective.[1,7]

Our study intended to find out biomarker expression patterns that may help predict treatment response and prognosis. For example, knowledge of the rate of EGFR mutations and ALK rearrangements in a given population could maximize the utilization of targeted therapies.[8] Additionally, IHC markers like CK7 distinguish primary lung tumors from metastatic lesions, an important factor in clinical decision-making.[9]

Our study was specifically designed to evaluate the immunohistochemical profiles and molecular markers in lung carcinoma.Focus on patient demographics, tumor characteristics, and biomarker prevalence would assist in increasing the accuracy of diagnosis and evolving guidelines of therapy in the management of lung carcinoma.

Aims and objectives

To study the immunohistochemical profiles and molecular markers of lung carcinoma cases diagnosed and treated at a tertiary care medical center and to analyze their distribution with histopathological subtypes and demographic characteristics.

MATERIALS AND METHODS

This was a retrospective observational analysis undertaken for 5 years from June 2019 to June 2024 tertiary care health center among 67 cases of lung carcinoma diagnosed based on histopathological and cytological investigations.

Inclusion criteria were patients who were aged 18 years and above with histopathologically proven lung carcinoma and confirmed through cytology, whereas primary tumors from other parts of the body excluded from the study.

Patient records, including the medical and histopathological documents, were sourced for demographic details like age, gender, and IP numbers, in addition to their histopathological profiles like subtypes of tumor, namely, squamous cell carcinoma, and adenocarcinoma, and markers such as TTF1, p63, CK7, and p53 as immunohistochemical and EGFR mutations, ALK rearrangement, and ROS1 rearrangements as molecular markers.

Immunohistochemical staining was conducted on tumor samples to analyze marker expression, and molecular marker testing was performed using fluorescence *in situ* hybridization and polymerase chain reaction where applicable. The collected data were entered into a Microsoft Excel spreadsheet and data were presented in frequency and percentage.

RESULTS

The age distribution of the patients revealed that the majority were aged between 61 and 70 years (29.9%), followed by those aged 51–60 years (25.4%). A smaller proportion of cases was observed in younger patients aged <40 years (10.4%) and older patients aged >71 years (20.9%). The study population comprised 35 females (52.2%) and 32 males (47.8%).

Histopathological analysis revealed that non-small-cell lung carcinoma (NSCLC) adenocarcinoma was the most common subtype, accounting for 57 cases (85.1%). NSCLC squamous carcinoma was identified in eight cases (11.9%), and adeno-squamous carcinoma was observed in two cases (3%) (Figure 6).

Immunohistochemical analysis revealed TTF1 positivity in 55 patients (82.1%). CK7, another marker frequently used to identify adenocarcinomas, was detected in

27 cases (40.3%). Napsin A, a specific marker for lung adenocarcinoma, was positive in 8 cases (11.9%). Markers indicative of squamous histology, such as p63, were positive in 12 cases (17.9%). CK20 positivity was rare, observed in only 1 case (1.5%), as was p53 positivity (1.5%). Vimentin, a marker of epithelial-mesenchymal transition, was positive in 5 cases (7.5%).

Molecular studies demonstrated the absence of ALK rearrangements, with all 40 cases testing negative for ALK (100%). ROS1 rearrangements were negative in all 40 patients (100%). Regarding EGFR mutations, 18 cases (45%) were negative, whereas 21 cases (52.5%) were positive (Table 1 and Figures 1-5).

DISCUSSION

Our study provides valuable insights into the histopathological, immunohistochemical, and molecular profiles of lung carcinoma in a tertiary care setting, focusing on key biomarkers such as TTF1, CK7, p63, and EGFR. Our study identified NSCLC adenocarcinoma as the predominant subtype (85.1%), followed by squamous carcinoma (11.9%) and adenosquamous carcinoma (3%). Immunohistochemically, TTF1 positivity was observed in 82.1% of cases, highlighting its diagnostic value in adenocarcinoma, whereas CK7 positivity was noted in 40.3% of cases, supporting its role in the diagnosis of adenocarcinoma. p63 expression was observed in 17.9% of the cases, consistent with its association with squamous histology. Molecular analysis revealed EGFR positivity in 52.5% of the cases, while ALK and ROS1 rearrangements were absent in all cases tested.

Our findings share similarities with prior studies and reiterate the necessity of using specific immunohistochemical markers such as TTF1 and p63 in diagnosing lung carcinoma. Kentaro Inamura et al. (2018) demonstrated that a solid carcinoma lacking glandular structures or mucin production yet exhibiting immunohistochemical positivity for "adenocarcinoma markers" such as TTF-1 (NKX2-1) and/or Napsin A, is classified as adenocarcinoma. Likewise, a solid carcinoma that lacks keratinization or intercellular bridges but shows immunohistochemical positivity for "SqCC markers," TP63 (p63), is classified as SqCC.[10] For instance, Shankar et al., illustrated the expression of TTF1 in 55% of adenocarcinoma cases, while it is predominantly used in diagnosing squamous cell carcinoma through the marker p63. These markers were highly valuable in the subtyping of poorly differentiated NSCLC, emphasizing their diagnostic significance.[11] Similarly, Reis-Filho et al., illustrated TTF1's specificity to primary lung adenocarcinomas with 61.53% sensitivity and

Table 1: Demographic distribution of the study participants

Category	Frequency (%)
Age	
<40	7 (10.4)
41–50	9 (13.4)
51–60	17 (25.4)
61–70	20 (29.9)
>71	14 (20.9)
Sex	
Female	35 (52.2)
Male	32 (47.8)
Histopathology	
Adenosquamous	2 (3)
NSCLC adeno	57 (85.1)
NSCLC squamous	8 (11.9)
Immunohistochemistry	
CK20	1 (1.5)
CK7	27 (40.3)
Napsin A	8 (11.9)
P53	1 (1.5)
P63	12 (17.9)
TTF1	55 (82.1)
Vimentin	5 (7.5)
Molecular study	
ALK (-ve)	40 (100)
ROS (-ve)	40 (100)
ROS1 (+ve)	1 (2.5)
EGFR (-ve)	18 (45)
EGFR (+ve)	21 (52.5)

EGFR: Epidermal growth factor receptor, ALK: Anaplastic lymphoma kinase, TTF1: Thyroid transcription factor-1, CK7: Cytokeratin 7, NSCLC: Non-small-cell lung carcinoma

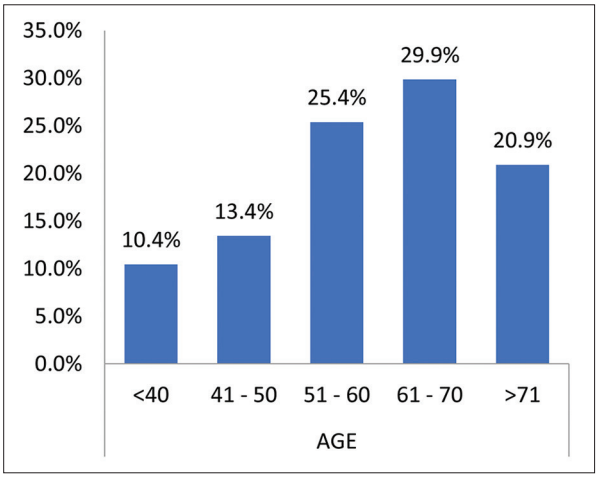

Figure 1: Demographic profile according to age distribution

100% specificity in differentiating primary from metastatic adenocarcinomas.[12] Schilsky et al., observed TTF1 positivity to be related to a longer period of overall survival in stage IV adenocarcinoma, and thus, it emphasizes its prognostic value.[13]

EGFR mutations are vital in guiding targeted therapies, such as those illustrated by studies of improved outcomes with EGFR tyrosine kinase inhibitors (TKIs) gefitinib

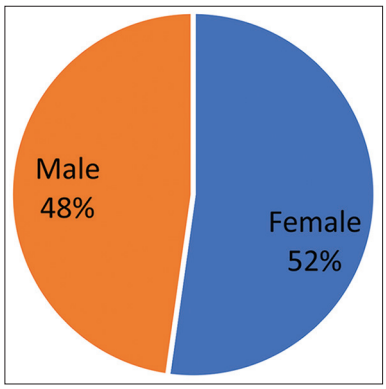

Figure 2: Demographic profile according gender distribution

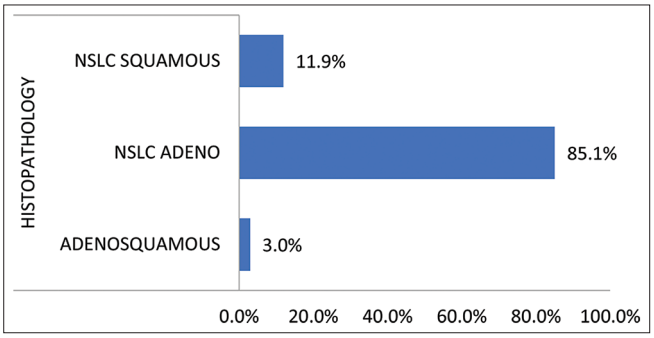

Figure 3: Histopathological distribution of non-small cell lung carcinoma

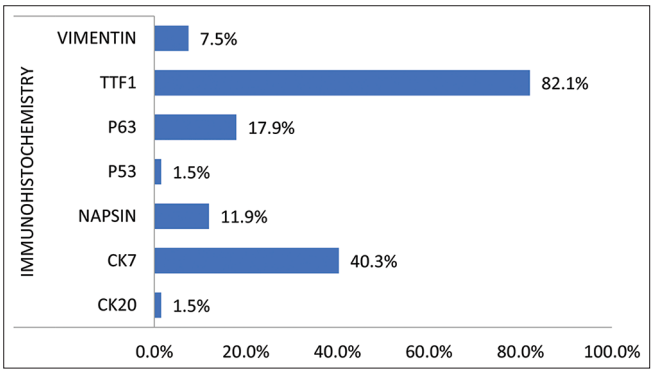

Figure 4: Immunohistochemical staining of lung carcinoma

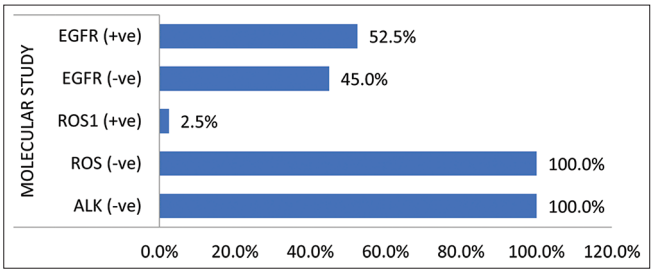

Figure 5: Molecular markers distribution of lung carcinoma

and erlotinib.[14] Zhang et al., estimated the overall EGFR mutation prevalence in NSCLC cases across the world as 32.3%, more frequently occurring at 38.4% in China than in Europe at 14.1%. It signifies the geographic diversity

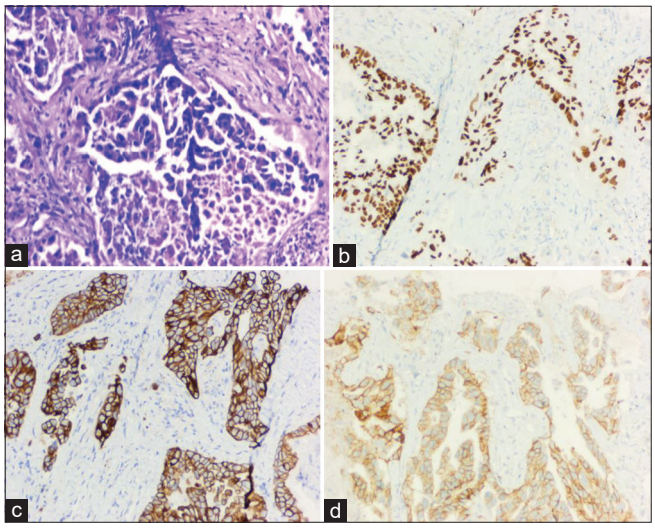

Figure 6: (a) H and E staining showing morphological characteristics of adenocarcinoma, (b) Immunohistochemical staining showing thyroid transcription factor-1 positivity, (c) Immunohistochemical staining showing cytokeratin 7 positivity, (d) Adenocarcinoma with epidermal growth factor receptor

in the incidence of mutation.[15] EGFR mutation rate, as reported by Midha et al., for the Asia-Pacific region, was found to be 47% and had predominantly adenocarcinoma histology that is in accord with the incidence rates seen within our study group.[16]

Mostly, the cases lack p53 and are CK20 positive; this goes well with their little role in diagnosing lung carcinoma, as reported by Myong in 2003, where he found TTF1 to be superior to the markers in the differentiation of adenocarcinoma from squamous carcinoma.[17] The lack of ALK and ROS1 rearrangements in our group is worth noticing, especially against studies like Bi, where it was reported that ALK rearrangement occurred in 3–7% of NSCLC cases.[18] This really calls for more research to reveal population-specific trends in molecular alteration. Besides, the relatively high rate of EGFR mutations observed in our study supports the validity of the use of EGFR testing in guiding therapy but rather underlines the need for standardized testing protocols to ensure comprehensive molecular profiling.

The comprehensive analysis of both immunohistochemical and molecular markers helped characterize lung carcinoma subtypes in detail. Accurate methods of data collection and a five-year study period also helped the reliability of the findings. The study is a retrospective one and, therefore introduces certain inherent biases like incomplete data and variability in sample quality. Another point is that this cohort lacked ALK and ROS1 rearrangements, diverging from the global studies. Larger multicenter studies are thus needed to investigate geographic variability.

Further large multicenter cohorts should be used to validate these findings and to address potential geographic and demographic variability. The mechanism by which ALK and ROS1 rearrangements are absent in this cohort could help elucidate the regional differences in lung cancer biology. The introduction of next-generation sequencing in future prospective studies might help in advancing the molecular characteristics of lung carcinoma and in revealing new therapeutic targets, such as mesenchymal-epithelial transition factor receptor exon 14 skipping mutations and rearrangements during transfection. Moreover, investigations into the prognosis with emerging markers like Napsin A interplay between molecular alterations and immunohistochemical profiles may define the diagnostic and therapeutic strategies better.

The results of our study emphasize the high frequency of NSCLC adenocarcinoma, the value of TTF1 and CK7 in diagnosis, and the utility of EGFR mutations in choosing targeted therapies. These findings, therefore, put emphasis on incorporating IHC and molecular testing into routine clinical practice to ensure optimal patient outcomes.

Limitations of the study

The study's limitations include its retrospective design lack of ALK/ROS1 positivity, and limited sample size. Larger, multicenter studies with advanced molecular techniques are needed to validate findings and explore regional variations in lung cancer biomarkers.

CONCLUSION

This study reveals that NSCLC adenocarcinomas are the most common types of lung carcinomas, with TTF1 and CK7 being good diagnostic markers. The presence of EGFR mutations was found in 52.5% of the cases, thus indicating their importance in the prescription of TKI therapies. ALK and ROS1 rearrangements were not found, indicating the existence of geographic or genetic variability. The results of our study emphasize the need for immunohistochemical and molecular profiling for diagnosis and treatment. Further research should focus on regional molecular patterns and novel biomarkers to enhance management modalities for lung cancer.

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Authors' Contributions:
BHT- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article**; GKS-** Concept, design, data collection clinical protocol, manuscript preparation, editing, and manuscript revision**; PRS-** Design of study, statistical analysis and interpretation; **NJN**- Review manuscript.

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