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IMMUNOHISTOCHEMISTRY BASED CHARACTERIZATION OF NON- SMALL CELL LUNG CARCINOMA IN A TERTIARY CARE CENTRE OF EASTERN INDIA.

Oncology

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ABSTRACT

INTRODUCTION- Lung malignancies are the leading cause of cancer-related mortality in industrialized countries. Lung malignancies are traditionally classified as Non-Small Cell Lung Carcinoma(NSCLC) and Small Cell Lung Carcinoma. The NSCLCs are the most common type and is further subclassified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. **MATERIAL AND METHODS-** This is a cross-sectional study done between 2019 and 2021 at the tertiary cancer centre at Jharkhand. A total of 100 diagnosed cases of Non-small cell lung cancer by histopathological examination of lung biopsy are included in this study. Followed by immunohistochemistry with p63, TTF-1, Napsin A, and CK 5/6. **RESULTS-** Out of 100 cases, 60 cases were of adenocarcinoma and 40 cases of squamous cell carcinoma. Among those 60 cases of adenocarcinoma, Napsin A was positive in 85% cases and TTF-1 was positive in 93% cases, CK5/6 and p63 positivity was seen in 7 % and 8% cases respectively. Out of 40 cases of squamous cell carcinoma, 92% cases were immunoreactive for CK5/6 while p63 was positive in 85% cases, 25% cases were also positive for TTF-1. **DISCUSSION-** In our study, Napsin A was more specific than TTF-1 regarding the diagnosis of lung adenocarcinoma, and CK5/6, having more specificity than p63, showed significant immunoreactivity with regards to lung squamous cell carcinoma.

KEYWORDS

lung, non-small cell, immunohistochemistry.

INTRODUCTION

Lung malignancies are commonly diagnosed cancer since 1985 and are the leading cause of cancer-related mortality in industrialized countries. An estimated 1.61million new cases of lung malignancies are diagnosed worldwide, of which 1.38 million deaths occur per year, thus surpassing the other common causes of cancer deaths.[1]

In India, approximately 63,000 new lung cancer cases are being reported each year, with a five-year survival rate of (17.7 percent). The five-year survival rate for lung malignancies is 55 percent for cases detected when the disease is still localised. Only 16 percent cases are diagnosed at an early stage. More than half of people with lung malignancies die within one year of diagnosis.[2]

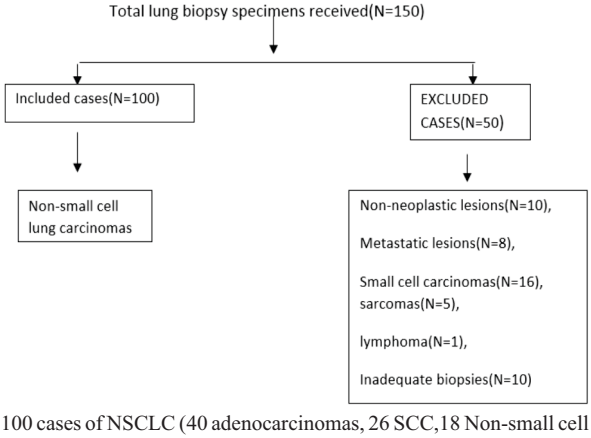
Traditionally, lung malignancies are classified as Non-Small Cell Lung Carcinoma and Small Cell Lung Carcinoma. The NSCLCs are the most common type and account for approximately 85% of cases. Currently, NSCLC is further classified into adenocarcinoma (ACA) (approximately 40%–50%), squamous cell carcinoma (SCC; 30%), and large cell carcinoma (9%).[3] Although it was previously acceptable to classify lung carcinomas as either small cell or NSCLC without further subdivision, newer treatment modalities require that ACA be distinguished from SCC. Recently, from 2015 onwards, the classification of lung cancers has been revised with the need for specific subtyping of NSCLC. New insights regarding molecular targeted therapy, in particular, have necessitated the revision of the traditional classification. Differentiating between squamous cell carcinoma and adenocarcinoma is now recommended, because of the availability of targeted therapy (tyrosine kinase inhibitors) in cases of adenocarcinoma. Most of the well to moderately differentiated NSCLC can be easily subtyped by using haematoxylin and Eosin stain (H & E) alone, but the difficulty arises in small biopsies with poorly differentiated morphology. Various Immunohistochemical (IHC)markers play a significant role in the subtyping of NSCLC.

In contrast to resection specimens where we can differentiate the morphology precisely, in small biopsy specimens it becomes challenging. With the advent of new imaging-guided biopsy techniques and the unresectable stage at presentation in most lung cancer cases, the small biopsies become the solely available tissue for the diagnosis. So it seemed necessary to assess IHC markers in addition to H and E sections for small lung biopsy for the subtyping of poorly differentiated NSCLC.

Our study deals with the subtyping of NSCLC into adenocarcinoma and squamous cell carcinoma with the help of a panel of immunohistochemical markers. We elected to focus our current study on p63, CK5/6, TTF-1, and napsinA. Our study aimed to study the efficiency of Thyroid Transcription Factor-1 (TTF-1), cytokeratin 5/6 (CK5/6), and p63 in distinguishing between ACA and SCC and to study the contribution of these markers to the diagnosis in NSCLC. The purpose of the present study is to examine the accuracy in the diagnosis of NSCLC on true-cut biopsy samples first based on morphology and then with IHC by using different relevant markers such as CK5, CK6, CK7, Napsin-A, TTF-1, P63, Synaptophysin, and Chromogranin-A and to recommend an optimal panel of IHC markers for precise diagnosis of NSCLC.

MATERIALS AND METHODS

This is a cross-sectional study done between 2019 and 2021 at the tertiary cancer centre at Jharkhand. All cases of NSCLC, diagnosed by histopathological examination of lung biopsy are included in this study. A total of 150 histopathological examinations of lung biopsies were received out of which 50 cases were excluded from the study (10 non-neoplastic cases, 8 metastatic, 5 sarcomas,1 lymphoma, 16 small cell Carcinomas, and 10 inadequate biopsies). \



100 cases of NSCLC (40 adenocarcinomas, 26 SCC,18 Non-small cell carcinoma unclassifiable, and 16 poorly differentiated carcinoma) were selected.

Table 1: Correlation between the initial diagnosis and final diagnosis.

INITIAL DIAGNOSIS [H&E]	FINAL DIAGNOSIS AFTER IHC

Diagnosis	No of cases(n)	Adenocarcinoma	Squamous cell carcinoma
Adenocarcinoma	40	40	0
Squamous cell carcinoma	26	0	26
Non-small cell carcinoma is unclassifiable	18	12	06
Poorly differentiated carcinoma	16	08	08
Total	100	60	40

IHC examinations of P63, TTF-1, Napsin A, and CK 5/6 were performed on the tissue sections obtained from formalin-fixed (neutral buffered 10% formalin) and paraffin-embedded blocks.

STATISTICALANALYSIS

Data analysis was done by SPSS20. The diagnosis value (sensitivity, specificity, positive predictive value, and negative predictive value) of immunoreactivity pertaining to each marker was calculated. Percentages were rounded to an integer and may not add up to 100.

RESULTS

A total of 150 biopsies were received out of which 50 cases were excluded from the study (10 non-neoplastic cases, 8 metastatic, 5 sarcomas,1 lymphoma, 16 small cell Carcinomas, and 10 inadequate biopsies) (Table-1). In this study, 100 Non-small cell lung carcinoma cases were included and categorized into two groups by histologic examination with different grades of differentiation: 60 cases were of adenocarcinoma and 40 cases of SCC. Each case was stained with Napsin A, TTF-1, P63, and CK5/6. The results of IHC expression in each histologic subtype of carcinoma are summarized in table 3. Adenocarcinoma was mostly immunoreactive for Napsin A (85%) and TTF-1(93%), and the reactivity of these markers was homogeneously strong and diffuse in the majority of cases; however, few cases of adenocarcinoma were positive for CK5/6 (07%) and p63 (08%), but intensity of staining was weak in these cases. On the contrary, SCC cases were immunoreactive for CK5/6 (92%) and p63 (85%) with a strong and diffuse pattern, certain cases were positive for TTF-1(25%) while no cases were positive for Napsin A. Sensitivity, specificity, PPV, NPV of IHC markers were calculated and summarized in tables 4-8.

Table 2. Frequencies of Napsin A, TTF1, CK5/6, and p63 in different subtypes of lung carcinoma (diagnosis by histologic examination)

	TTF-1	Napsin A	CK5/6	P63
Adenocarcinoma (60)	93% (56/60)	85% (52/60)	7% (04/60)	8% (5/60)
SCC (40)	25% (10/40)	0	92% (37/40)	85% (38/40)

The Sensitivity (SEN), Specificity (SPEC), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) results of IHC for napsin A, TTF-1 (Dako and SPT24), CK5/6, and p63 are summarized in Tables 3 and 4.

Table 3. Sensitivity (SEN), Specificity (SPEC), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Immuno histochemistry in Adenocarcinoma of lung.

Adenocarcinoma	TTF-1 (Dako), %	Napsin A, %
SEN	93	86
SPEC	80	100
PPV	87	100
NPV	88	83

Table 4. Sensitivity (SEN), Specificity (SPEC), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Immunohi stochemistry in Squamous Cell Carcinoma of lung.

Squamous Cell Carcinoma	CK5/6, %	p63, %
SEN	92	95
SPEC	93	91
PPV	90	88
NPV	94	96

DISCUSSION

In this study, we employed a panel comprised of p63, CK 5/6 (as markers for SCC) and TTF- 1, Napsin A (as markers for adenocarcinoma) for a reliable differential diagnosis of SCC and adenocarcinoma in small biopsy and large specimens. Several recent studies have evaluated IHC markers to diagnose non-small cell carcinoma; however, given the recent advances in targeted therapies, the subclassification of the NSCLC category into adenocarcinoma and SCC is becoming increasingly important.[3] In fact, the subclassification can be done via routine H&E examination without IHC in the majority of cases; however, in poorly differentiated cancers and specimens with few tumor cells, it is difficult and may be necessary to use IHC studies. TTF-1 is a 35-kDa homeodomain-containing DNA binding protein of the NKx-2 gene family that shows nuclear-specific staining. This marker is expressed in the thyroid and lungs. TTF 1 is used for the subtyping of lung malignancies. TTF1 is also expressed by normal pneumocytes.Therefore a clear differentiation should be made between the normal pneumocytes and malignant cells. [4-12]

Napsin is an aspartic proteinase, it is expressed as a cytoplasmic marker in the lung parenchyma. It is homologous with the polypeptide TA02 and is involved in the maturation of the biologically active surfactant protein B. It also consists of a 38-RDA protein, a protein that is expressed in type II pneumocytes, alveolar macrophages, renal tubules, and ducts of the pancreas. Napsin was used to differentiate primary lung carcinomas from metastatic. Napsin shows cytoplasmic granular positivity/ staining. Few studies suggested that Napsin is a more sensitive marker than TTF in differentiating lung primary from other metastatic adenocarcinomas and differentiate it from squamous cell carcinoma.

p63 is a member of the p53 family, located on chromosome 3q. It is a very specific and sensitive antibody for myoepithelial cells and has nuclear affinity especially for the basal layer of stratified squamous epithelium. As the cells get differentiated its reaction/positivity begins to diminish. Its expression can also be noted in basal cells of respiratory epithelium, germinal center cells, basal cells of the prostate, placental cytotrophoblasts, myoepithelial cells of the breast. Squamous cell carcinomas of lung, cervix, head, and neck and basal cell carcinoma of skin show strong nuclear positivity for P63.

CK 5/6 are intermediate-sized keratins. They are mainly expressed in keratinizing (epidermis) and non-keratinizing (mucosa) squamous epithelium. They can also be seen in the basal myoepithelial cell layers of the prostate, salivary glands, and breast. This marker is expressed in benign and malignant tumors of epidermal squamous mucosal and myoepithelial origin. They usually show diffuse cytoplasmic staining with perinuclear enhancement. These are diffusely and strongly expressed (cytoplasmic staining) in squamous cell carcinoma.

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Different studies show different positivity of markers in adeno carcinoma (Table 4). The positivity of TTF-1 ranges from 60 to 100% in different studies. In the present study, TTF-1 is positive in 93% of adenocarcinoma cases. The reason for the above variability in positivity for TTF-1 could be because TTF-1 is better expressed in differentiated adenocarcinomas. [13]

Table 5: Comparison of percentage positivity of IHC markers in pulmonary adenocarcinoma in various studies

	TTF-1	Napsin A	CK5/6	p63
Xu et al.[14]	94.6	-	4.5	9
Whithaus et al.[15]	60	83	4	14
Mukhopadhyay S et. al[16]	80	58	0	10
Brunnstrom H et al.[17]	88	87	-	-
Zhao W et.al[18])	80	64	0	0
Bhatti V et al[19]	85.4	92.3	46	40
Present study	93	85	07	08

In our study, Napsin-A positivity (85%) is lesser than TTF-1 positivity (93%). The positivity of Napsin-A ranges from 60 to 100% in different studies. CK5/6 and p63 are expressed in 7% and 8% cases respectively.

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Table 6: Comparative immunohistochemical profile of squamous cell carcinoma

	TTF-1	Napsin A	CK5/6	p63
Xu XY et al.[14]	0	-	91.9	97
Whithaus K *et al*.[15]	2	2	53	95
Mukhopadhyay S et al[16]	0	0	73.3	100
Brunnstrom H et al[17]			97	97

Zhao W et al[18]	0	0	81	100
Bhatti V et al[19]	23.1	0	100	92.3
Present study	25	0	92	95

Positivity of CK5/6 ranges from 70 to 100% in different studies. In the present study, CK5/6 is positive in 37 out of 40 SCC cases i.e. 92% cases. p63 was positive in 38 cases i.e. 95% cases in our study. 3 cases were negative for CK5/6, were positive for p63. 2 cases that were negative for p63 were positive for CK5/6. The reported positivity for p63 is 90 to 100% in different studies to date. TTF-1 is positive in 25% cases (Table5). Napsin-A was negative in all the SCC cases.

Table 7: Sensitivity and specificity of Napsin A and TTF-1 in lung adenocarcinoma

	Sensitivity of TTF-1 (%)	Sensitivity of Napsin A	specificity of TTF-1	specificity of Napsin A
Grzegorz TG et.al[20]	84.5	92	96.4	100
Whithaus K et al.[15]	60	83	98	98
WANG, J. Y.et. al[21]	79.6	56.14	93.44	95.08
Sisakht TM et. al[22]	94	91	69	97
Present study	93	86	80	100

TTF1 had 93% sensitivity and 80% specificity with PPV-87% and NPV-88%, and Napsin A had 86% sensitivity and 100% specificity with PPV-100% and NPV-83% as regards the diagnosis of adenocarcinoma. The PPV of TTF-1 was 73% and 100% respectively in Mohsenian Sisakht T et al and Whithaus study. In the present study, Napsin A was more specific (100%) but slightly less sensitive (86%) than TTF-1 as regards adenocarcinoma.

Table 8: Sensitivity and specificity of CK5/6 and P63 in lung SCC

	Sensitivity of CK5/6	Sensitivity of P63	specificity of CK5/6	specificity of P63
Grzegorz TG et.al[20]	100	91.7	77.8	78.3
Whithaus K et.al[15]	53	95	96	86
WANG, J. Y.et. al[21]	77.05	83.6	96.44	88.93
Sisakht TM et. al[22]	100	100	69	84
Present study	92	95	93	91

p63 had 95% sensitivity and 91% specificity with PPV 88% and NPV96%, and CK5/6 had 92% sensitivity and 93% specificity with PPV90% and NPV 94% in the diagnosis of squamous cell carcinoma. CK5/6 was more specific than p63 for SCC (93% versus 91%) but did not contribute overall to distinguishing ACA from SCC. Overall, p63 was more sensitive for SCC than was CK5/6 (95% versus 92%). The reported sensitivity of CK5/6 for pulmonary SCC ranges from 73% to 100%. Zhao et. al [23] and Turner et al[24] showed that CK5/6 was highly specific for the SCC of the lung. In previous reports, the sensitivity of p63 ranges from 75% to more than 95%; whereas, specificity is between 70% and 90%. [13-15]

In our study, Napsin A was more specific than TTF-1 regarding the diagnosis of lung adenocarcinoma, and CK5/6, having more specificity than p63, showed significant immunoreactivity with regards to lung SCC. Similar results were observed in other studies. [15-17]

CONCLUSION

Based on the results of our study, it is suggested that IHC studies are necessary in all cases of NSCLC, in which morphological differentiation is difficult on H&E stained slides. Our recommended IHC profile is composed of Napsin A, TTF-1, CK5/6, and P63. Moreover, the most cost-effective tissue-preserving panel for small biopsy specimens regarding the differential diagnosis of lung adenocarcinoma versus Squamous cell carcinoma is a combination of P63 and Napsin A.

CONFLICT OF INTEREST- NIL
FINANCIAL SUPPORT- NIL

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