

# Immunohistochemical Staining by TTF-1 and P63 Markers for Typing of Non Small Cell Lung Carcinomas: A Three Year Study of A Tertiary Care Health Centre

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## ABSTRACT

**Introduction:** Studies have shown that selection of therapies such as vascular endothelial growth receptor (VEGR) and epithelial growth factor receptor (EGFR) inhibitors may depend on the correct differentiation of squamous cell carcinoma (SCC) versus adenocarcinoma (ADC). This study was done to evaluate role of thyroid transcription factor-1 (TTF-1) and P63 markers in differentiating SCC and ADC in lung mass biopsies.

**Material and methods:** This study was conducted over a period of 2 years from October 2015 to December 2017. Total 52 cases of non-small cell carcinomas (NSCLC) including ADC and SCC were included. IHC staining for thyroid transcription factor-1 (TTF-1) and P63 were carried out for all the cases along with routine histopathological examination of tumour.

**Results:** Out of total 52 cases, 16 cases were diagnosed as ADC, 22 cases as (SCC), 12 cases as NSCLC and 2 cases as papillary adenocarcinoma on histopathology. All the 16 cases of ADC and 2 cases of papillary adenocarcinoma were positive for TTF-1, all the 22 cases of SCC were positive for P63 and out of 12 cases as NSCLC, 5 cases were positive for TTF-1 and 7 were positive for P63. None of the case showed simultaneous positivity for both IHC markers.

**Conclusion:** SCC constituted 55.8% while ADC constituted for 44.2% cases. All the SCCs showed P63 positivity while All the ADCs showed TTF-1 positivity. Hence it can be concluded that TTF-1 and P63 are reliable IHC markers for further sub typing of NSCLC.

**Keywords:** Vascular Endothelial Growth Receptor (VEGR), Epithelial Growth Factor Receptor (EGFR), Squamous Cell Carcinoma (SCC), Adenocarcinoma (ADC), Thyroid Transcription Factor-1 (TTF-1), P63

The observed patterns in lung cancer rates reflect the historical prevalence and variation in the trends of smoking among men and women. Incidence of the previously predominant squamous cell variety appears to be declining (although not universally), with a corresponding increase in adenocarcinoma (ADC) variety in both genders.<sup>4</sup>

The standard therapy for operable non-small cell carcinoma of the lung is complete surgical excision through thoracotomy.<sup>5</sup> Adenocarcinoma and squamous cell carcinoma are the two major subtypes of non-small cell lung carcinoma. Until recently, therapeutic approaches to non-small cell lung carcinoma were largely guided by tumour stage, and there was no difference in treatment for adenocarcinoma vs. squamous cell carcinoma. This monolithic approach to non-small cell lung carcinoma (NSCLC) has dramatically changed in the last few years as a result of three major advances in thoracic medical oncology for advanced disease. These include (1) Endothelial Growth Factor Receptor (EGFR) targeted therapies, Erlotinib (Tarceva) and Gefitinib (Iressa), which are currently recommended as the first-line treatment for non-small cell lung carcinoma with EGFR mutations, and these mutations occur primarily in adenocarcinoma,<sup>6</sup> (2) Bevacizumab (Avastin), which is contraindicated in squamous cell carcinoma due to the risk of pulmonary hemorrhage,<sup>7</sup> and (3) Pemetrexed (Alimta), which is also contraindicated in squamous cell carcinoma due to the lack of effectiveness.<sup>8</sup>

Immunohistochemistry is a highly effective ancillary tool for distinguishing adenocarcinoma and squamous cell carcinoma. Common markers used for non-small cell carcinoma sub typing include TTF-1 for adenocarcinoma vs. P63 for squamous cell carcinoma. Present study was done to evaluate role of Thyroid Transcription Factor-1 (TTF-1) and

## INTRODUCTION

Lung cancer remains a major cause of morbidity and mortality worldwide, accounting for more deaths than any other cancer. Lung cancer has been the most common cancer worldwide in the world since several decades, and in 2012, there were an estimated 1.8 million (13% of all cancers) new cases, representing 13% of all new cancers. It was also the most common cause of death from cancer, with 1.6 million deaths (19.4% of all cancer-related deaths).<sup>1</sup>

Compared to the western population<sup>2</sup>, the prevalence of lung cancer in India appears to be increasing. According to the Indian Council of Medical Research cancer registry, 57,795 new cases were reported in 2010, which is projected to rise up to 67,000 new cases annually by the year 2020.<sup>3</sup>

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P63 markers in differentiating non-small cell lung carcinoma (NSCLC).

## MATERIAL AND METHODS

This retrospective observational study was conducted in a tertiary level hospital in Central India. Total 52 cases of non-small cell carcinomas including adenocarcinoma and squamous cell carcinoma were included in present study. IHC staining for TTF-1 and P63 markers was carried out for all the cases along with routine H and E staining and histopathological typing of tumours as per WHO classification for lung tumours.<sup>9</sup> The Staining pattern of TTF-1 and P63 in terms of percentage of cells stained, pattern of staining and intensity was noted for each case.

Scoring of TTF-1 and P63 expression was done semi quantitatively:

According to the percentage of cells displaying nuclear staining (0-weak positive or negative, <10% cells -positive +, 10-50% positive; ++, >50% positive+++ along with the intensity of that staining (graded + to +++).<sup>10</sup>

Intensity of reactivity was recorded as weak (1+, less than normal cells); moderate (2+, same as normal cells); strong (3+, stronger than normal cells).<sup>11</sup>

### Exclusion criteria

Patient known and already diagnosed cases of lung cancers.

Patients already on chemotherapy for lung cancer.

Specimen which were improperly fixed or with more necrotic tissue.

Microscopically diagnosed case of small cell lung carcinoma.

In our study, data was collected which was then distributed and tabulated according to gender, age, presenting symptoms, smoking habits, site of tumour, histopathological diagnosis, TTF-1 and P63 staining pattern, final diagnosis, percent proportion was calculated for different parameters. Correlation between staining intensity of TTF-1 and P63 markers and correlation between histological grading with staining pattern of TTF-1 and P63 markers was derived. Statistical analysis

Descriptive statistics lime mean and percentages were used to interpret the data with the help Microsoft office 2007.

## RESULTS

During a period of 2 years from January 2015 to December 2016, a total of 52 patients diagnosed as having non-small cell lung carcinomas. Total 52 cases were studied, of which 39 cases (75%) were male and 13 cases (25%) were female. In age wise distribution of these cases highest number (36.5%) of cases were seen in age group 51-60 years followed by 61-70 years age group total of 16 cases (30.76%) (Table -1). In our study, weight loss (46.1%) and cough (44.2%) were the commonest presenting symptoms in study followed by dyspnea 36.53% and weakness in 33% cases. Hoarseness of voice and fever were the least common symptoms seen in 1.9% cases each. Chest pain was seen in 11% cases and hemoptysis in 25% cases (Table -2).

Overall 34 cases (65.38%) were associated with smoking and 18 cases (34.62%) were seen in non-smokers.

Age group in years	Male	Female	Total of Cases
31 to 40	2	1	3
41 to 50	8	1	9
51 to 60	13	6	19
61 to 70	12	4	16
71 to 80	4	1	5
Total	39	13	52
Percentage	75%	25%	100%

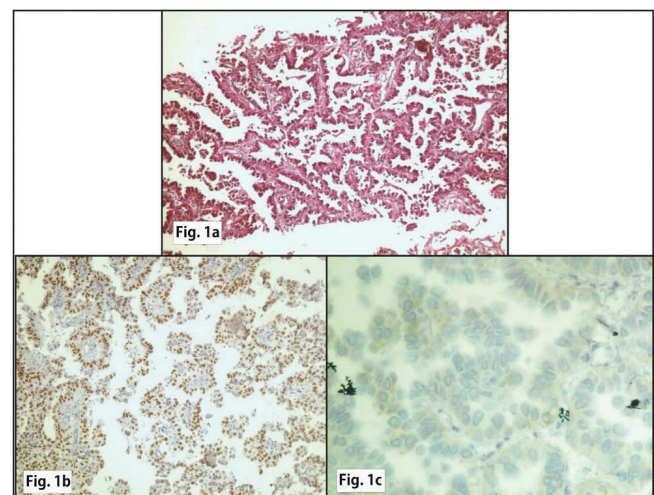
**Table-1:** Age and gender wise distribution of study cases

Symptoms	No. of cases
Weight loss	24 (46.1%)
Cough	23 (44.2%)
Dyspnea	19 (36.53%)
Weakness	17 (33%)
Chest Pain	12 (23.07%)
Hemoptysis	13 (25%)
Hoarseness	1 (1.9%)
Fever	1 (1.9%)

**Table-2:** Distribution of cases as per presenting symptoms

Histopathological diagnosis	Final Diagnosis	
	ADC	SCC
ADC	16	0
NSCLC	5	7
PADC	2	0
SCC	0	22
Total	23	29

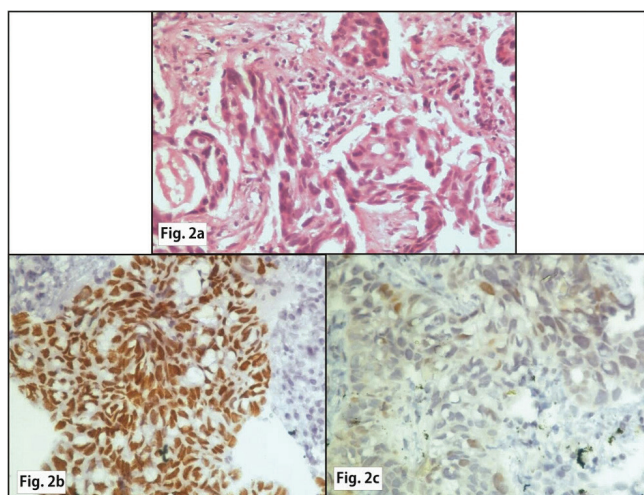
**Table-3:** Distribution of cases based on histopathological diagnosis and final diagnosis after IHC stud



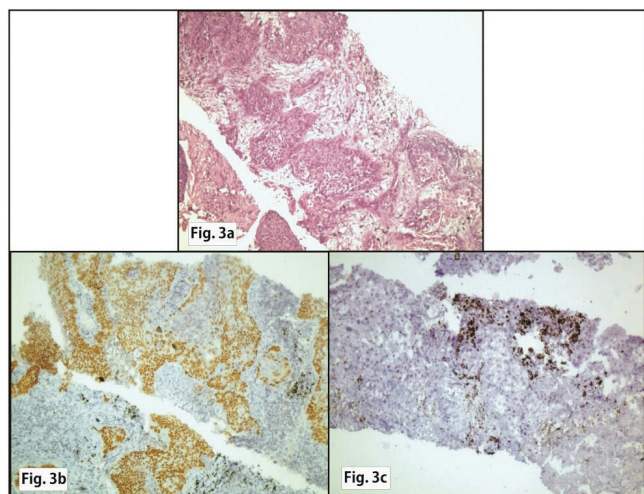
**Figure-1(a):** Photomicrograph of Papillary Adenocarcinoma showing branched papillae lined by columnar cells with fibrovascular core. (H and E, 10x10X); **Figure-1(b):** Photomicrograph of Papillary Adenocarcinoma showing TTF-1 nuclear positivity (3+) (IHC, 10x10X); **Figure-1(c):** Photomicrograph of Papillary Adenocarcinoma showing negative for P63 (10x10X);

All of 16 cases of adenocarcinoma (ADC) and 22 cases of squamous cell carcinoma (SCC) as on histopathology were found to be as ADC and SCC respectively on final diagnosis based on IHC staining as well. Out of 12 cases of non-small





**Figure-2(a):** Photomicrograph of Adenocarcinoma showing atypical cells forming acini pattern. (H and E, 40x10X); **Figure-2(b):** Photomicrograph of Adenocarcinoma showing TTF-1 nuclear positivity (3+) (IHC, 40x10X); **Figure-2(c):** Photomicrograph of Adenocarcinoma showing negative for P63 (IHC, 40x10X);



**Figure-3(a):** Photomicrograph of squamous cell carcinoma showing atypical cells forming sheets and lobules. (H and E, 10x10X); **Figure-3(b):** Photomicrograph of squamous cell carcinoma showing P63 nuclear positivity (3+) (IHC, 10x10X); **Figure-3(c):** Photomicrograph of squamous cell carcinoma showing negative for TTF-1 (IHC, 10x10X)

cell lung carcinoma on histopathology 5 cases were diagnosed as ADC and 7 cases diagnosed as SCC respectively on IHC staining pattern. Out of 2 cases of papillary adenocarcinoma on histopathology both were found to be ADC on IHC staining pattern (Table- 3).

## DISCUSSION

The lung tumor spectrum varied from male to female. In present study, there was an overall predominance of male patients 39 (75%), whereas the female patients constituted 13 (25%) cases out of total 52 cases. Thus male to female ratio was 3:1.

Our sex ratio was similar to studies of Noronha et al.<sup>12</sup>, which described a higher age-adjusted lung cancer incidence rate in males with male to female ratio of 3.5:1

Our finding did not correlate with sex ratio described by Gurda et al.<sup>13</sup>, who showed slight female predominance with M:F of 1:1.1. The overall male preponderance in lung cancer in India can be attributed to more prevalence of tobacco abuse among males and higher exposure to industrial pollutants compared to females.

We also noted that lung cancer was equally prevalent in smoker and non smoker females which was consistent with findings noted in studies done by Koo et al.<sup>14</sup>, Parkin et al.<sup>15</sup>, Toh et al.<sup>16</sup> and Noronha et al.<sup>12</sup>

The clinical symptoms among the patients studied showed that weight loss was the most common symptom being reported by 46.1% followed by cough in 44.2%, dyspnea in 36.53%, weakness in 33%, chest pain in 23.07%, Hemoptysis in 25%, Hoarseness and fever in 1.9% respectively. Noronha et al.<sup>12</sup>

In our study, Squamous cell carcinoma was the most common histological subtype of lung carcinoma which was similar to findings of studies done by Akram et al.<sup>17</sup>, Behera D et al.<sup>18</sup>, Singh N et al.<sup>19</sup> and Prasad R et al.<sup>20</sup>

The current study demonstrates that the TTF-1 staining pattern was consistently expressed in adenocarcinomas (100%). There was no correlation seen between staining intensity and histologic grading of adenocarcinomas with non-significant pearson correlation value (0.330) was obtained. Thus TTF-1 can prove a good and specific IHC marker for lung ADC irrespective of histologic grade of ADC. All the ADC cases were negative for P63 staining.

In the present study 100% cases diagnosed as ADC on histomorphology showed TTF-1 positivity which closely correlates with the study by K. Kadota et al.<sup>21</sup>, followed by Stenhouse et al.<sup>10</sup> and Gurda et al.<sup>13</sup> studies.

The current study demonstrates that the P63 staining pattern was consistently expressed in all squamous cell carcinomas (100%). There was no correlation seen between P63 staining intensity and histologic grading of SCC with not-significant pearson correlation value (0.441). None of the SCC case was positive for TTF-1 staining.

In the current study 100% cases diagnosed as SCC on histomorphology showed P63 positivity which was in league with studies by Mukhopadhyay et al.<sup>22</sup> and Gurda et al.<sup>13</sup> Pelosi G et al.<sup>23</sup> showed positivity in 92% cases. Staining percentage of nuclear staining was not significantly associated with tumor differentiation or grade, and high TTF-1 immunoreactivity was observed in 8 well differentiated (34.7%), 7 moderately differentiated (30.4%) and 3 poorly differentiated (13%), while high P63 immunoreactivity was observed in 8 well differentiated (27.5%), 1 moderately differentiated (3.44%) and 6 poorly differentiated (20.6%).

Several similar studies reported by Pelosi G et al.<sup>24</sup>, Fabbro D et al.<sup>25</sup> and Anagnostou et al.<sup>26</sup> showed no correlation between TTF-1 staining intensity and tumor growth pattern and differentiation.

Lack of significant relation between P63 staining intensity and SCC tumor grading is also noted in studies by Shimada Y et al.<sup>27</sup>, Massion PP et al.<sup>28</sup>, Au NH et al.<sup>29</sup>, Wu M et al.<sup>30</sup> and Kargi A et al.<sup>31</sup>

In present study we reported 12 cases as Non-small cell carcinoma on histomorphology. On applying TTF-1 and P63 IHC staining on these cases we found that 5 cases showed positivity for TTF-1 and 7 cases showed positivity for P63 cases. None of the case had dual IHC staining pattern. Thus we classified these finally into ADC and SCC based on their positivity for TTF-1 and SCC respectively.

Loo Ps et al.<sup>32</sup> found that p63 and TTF-1 allows specific subtyping in 73% of non-small cell lung carcinomas cases with 86% accuracy and this staining panel showed 100% concordance with specific non-small cell carcinomas morphologic subtyping in small biopsies.

Gurda et al.<sup>13</sup> study demonstrates that P63 have the sensitivity of 91.7% and specificity of 100%, while that of TTF-1 has the sensitivity and specificity of 84.5% and 96.4%. Mukhopadhyay et al.<sup>22</sup> shows TTF-1 positivity in 80% of cases while P63 positivity in 100% of cases.

Bir F et al.<sup>33</sup> showed P63 positivity in 96% squamous cell carcinoma and mild positivity was also seen in 25% cases of adenocarcinoma. Ma Y et al.<sup>34</sup> found P63 sensitivity and specificity of 87% and 81% in squamous cell carcinoma. Stenhouse G et al.<sup>10</sup> showed 75% positivity of TTF-1 in pulmonary adenocarcinoma.

As a result, immunochemistry has been increasingly used to aid in the subclassification of non-small cell carcinoma.

## CONCLUSION

Hence it can be concluded that TTF-1 and P63 are reliable markers for further sub-tying of Non-small cell carcinoma of lung. TTF-1 and P63 IHC are complimentary for differentiating Adenocarcinoma and Squamous cell carcinoma and is now important because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type. Therefore, consideration should be given to the addition of immunohistochemistry to the WHO criteria for classifying non-small cell lung carcinomas.

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