THE ROLE OF IMMUNOHISTOCHEMICAL MARKERS (P63 AND TTF-1) IN THE DIAGNOSIS AND CLASSIFICATION OF NON-SMALL CELL LUNG CARCINOMA

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ABSTRACT

BACKGROUND

Lung carcinoma is the most common cause of cancer mortality worldwide. Most non-small cell lung carcinomas are inoperable and histologic diagnosis made on small biopsy samples are prone to diagnostic inaccuracy. So, precise subtyping of poorly differentiated non-small cell lung carcinoma (NSCLC) into adenocarcinoma and squamous cell carcinoma has a direct impact on management of patient and prognosis.

The aim of this study was to investigate the role of immunohistochemistry profile using p63 and TTF-1 for differentiating non-small cell lung carcinoma into squamous cell carcinoma and adenocarcinoma.

MATERIALS AND METHODS

This descriptive study was conducted to assess p63 and TTF-1 expression in 80 cases of non-small cell lung carcinoma received in the Department of Pathology, Government Medical College, Kottayam during a period of 18 months (March 2016 - September 2017).

RESULTS

Present study includes 80 cases of histologically diagnosed NSCLC. Of the study population, 73 cases (91.25%) were males and 7 cases (8.75%) were females. Among males, squamous cell carcinoma is the predominant histological subtype and adenocarcinoma is the predominant subtype in females. In this study, peak incidence is between 60-69 years of age. The mean age is 61 years. Out of the 80 histologically diagnosed cases of NSCLC studied, after IHC confirmation 47 were squamous cell carcinoma (58.75%), 27 were adenocarcinoma (38.75%), 3 were adenosquamous (3.75%) and rest 3 were NSCLC-NOS (3.75%). Histologically diagnosed cases of squamous cell carcinoma show 100% positivity for p63 and adenocarcinoma shows 100% positivity for TTF-1.

CONCLUSION

Both p63 and TTF-1 have 100% expression in squamous cell carcinoma and adenocarcinoma respectively. Hence, these markers may be recommended for subtyping of poorly differentiated non-small cell lung carcinoma (NSCLC).

KEY WORDS

p63; TTF-1; Immunohistochemistry; Non-Small Cell Carcinoma.

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BACKGROUND

Lung carcinoma is currently the most frequently diagnosed major cancer in the world and the most common cause of cancer mortality worldwide. An increasing incidence of lung cancer has been observed in India. The case fatality is high with lung cancer. It is also the leading cause of cancer-related deaths accounting for 57.8% of all cancer deaths in males and 37% in females.

Histologically, primary lung cancer is broadly classified into small cell and non-small cell types. The distinction is clinically useful, as the treatment strategies differ significantly. Most of the non-small cell lung carcinomas are inoperable and pathologic diagnosis is made usually on small biopsy samples that are prone to diagnostic inaccuracy. In recent

years new targeted therapies are available, which necessitates the importance of specific pathologic accuracy in the subtyping of non-small cell lung carcinoma. Precise subtyping of poorly differentiated NSCLC into adenocarcinoma and squamous cell carcinoma has a direct impact on management of patient and prognosis. With this background use of immunohistochemistry is an important diagnostic tool, which could help to predict the final sub-type of non-small cell lung carcinoma diagnosed on pre-operative bronchial biopsy samples. This study is to be conducted to investigate the role of immunohistochemistry profile of p63 and TTF-1, as a useful marker panel for differentiating non-small cell lung carcinoma into squamous cell carcinoma and adenocarcinoma. This also helps to provide prognostic and predictive information, have a significant impact on patient management.

Objective

To study the expression of immunohistochemical markers (p63 and TTF-1) in different subtypes of non-small cell lung carcinoma (NSCLC).

MATERIALS AND METHODS

Type of Study

A descriptive study.

'Financial or Other Competing Interest': None.

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Period of Study

18 months (March 2016 - September 2017).

Study Setting

Department of Pathology, Government Medical College, Kottayam.

Study Population

FOB biopsy specimens and resected lung specimens of non-small cell lung carcinoma received in the Department of Pathology, Govt. Medical College, Kottayam during the study period.

Sample Size

Sample size $N = Z\alpha^2 pq / d^2$

$Z\alpha = 1.96$ at 95% CI

$p =$ prevalence/ proportion in previous study, $q = 100 - p$

$d =$ precision/ allowable error

Proportion of immunohistochemically proven primary adenocarcinoma (TTF-1 positive, p63 negative) in the study done by Shanmugapriya Shankar et al was 55%, $q = 1 - p = 45$

Taking absolute precision as 11

$$\text{Sample size, } N = \frac{Z\alpha^2 pq}{d^2} = \frac{(1.96)^2 \times 55 \times 45}{11 \times 11} = 78.578$$

Hence, sample size is taken as 80.

Sampling Method

Continuous sampling method. Study sample include first 80 cases of non-small cell lung carcinoma cases received in the Department of Pathology, Government Medical College, Kottayam during the study period.

Inclusion Criteria

Histopathologically diagnosed cases of non-small cell lung carcinoma in FOB biopsy specimens and resected lung specimens.

Exclusion Criteria

Inadequate biopsy samples, cases without proper data and cases with diagnosis of other subtypes of lung carcinoma. If we receive FOB biopsy and resected lung specimen of the same patient, the resected specimen is preferably taken for the study.

Study Procedure

72 FOB biopsies, 2 wedge resections, 3 lobectomy specimens and one pneumonectomy specimen were received in formalin. Gross examination of the resected lung specimens were done. Appropriate bits of tissues of representative areas were taken for study. All specimens were fixed in formalin and embedded in paraffin. 4 microns thick sections were stained with H and E for routine histological examination. Immunohistochemical staining were done on histopathologically diagnosed cases of NSCLC, using rabbit monoclonal anti-p63 and anti-TTF-1 29 antibody.

Immunoreactivity assessed according to the distribution and intensity of staining. P63 and TTF-1 shows diffuse nuclear positivity which is considered as positive, if present in more than 10% of malignant cells.

Data Management and Analysis

The data was entered in Microsoft Excel and further statistical analysis was done using SPSS software (Version 20). The statistical methods used were mean, frequency, proportion and non-parametric test (chi-square test). The level of significance was indicated as 'p' value < 0.001 .

RESULTS

- Lung carcinoma was more common in males.
- The mean age of onset was 61 years.
- Eighty two percentage of cases in this study were found in the age range of 50 to 79 years.
- Peak incidence was between 60 and 69 years (46.25%).
- In males, lung carcinoma was commonly found in the 60-69 age group.
- In females, lung carcinoma mostly occurred in the age group of 40 - 49 yrs.
- Major histological type was squamous cell carcinoma (58.75%).
- Squamous cell carcinoma was the predominant histological subtype in males (61.6%).
- Adenocarcinoma was the predominant histological subtype in females (57.1%).
- 95.7% of squamous cell carcinoma and 85.2% of adenocarcinoma were found in males.
- Squamous cell carcinoma showed 100% p63 positivity.
- TTF-1 expression was found in 100% of primary lung adenocarcinoma.
- Squamous cell carcinoma did not show TTF-1 expression.
- After IHC marker study 58.75% cases were squamous cell carcinoma, 33.75% were adenocarcinoma, 3.75% cases were adenosquamous and 3.75% cases were diagnosed as NSCLC-NOS.
- 20 cases were histologically diagnosed as squamous cell carcinoma, after IHC confirmation study 19 cases (95%) were diagnostically confirmed as squamous cell carcinoma.
- Among the 11 histologically diagnosed cases of adenocarcinoma, 10 cases (90.9%) were diagnosed as adenocarcinoma.
- Out of the 26 cases of poorly differentiated squamous cell carcinoma, 20 cases (76.9%) were diagnosed as squamous cell carcinoma and 4 cases (15.38%) as adenocarcinoma.
- Among the 7 cases of poorly differentiated adenocarcinoma, 6 cases (85.7%) were diagnosed as adenocarcinoma.
- 16 cases were histologically diagnosed as poorly differentiated carcinoma, of which 8 cases (50%) were diagnosed as squamous cell carcinoma, 7 cases (43.75%) as adenocarcinoma after IHC confirmation study.

Figure 1. Percentage distribution of cases based on IHC Diagnosis

Figure 2. Percentage distribution of cases based on Histological Diagnosis and IHC Diagnosis

Figure 3. Squamous Cell Carcinoma H and E Stain 40x

Figure 4. Squamous Cell Carcinoma showing p63 Nuclear Positivity 40x

Figure 5. Adenocarcinoma H and E Stain 40x

Figure 6. Adenocarcinoma showing TTF1-1 Nuclear Positivity 40x

DISCUSSION

The incidence of lung carcinoma is on the rise in India and worldwide. Non-small cell lung carcinoma accounts for 80-85% of all lung carcinomas. Adenocarcinoma is the predominant histologic type in non-smokers and females. Globally, the overall lifetime risk of lung cancer is 1 in 13 for men and 1 in 16 for women. Most of the Non-Small Cell Lung Carcinoma (NSCLC) are inoperable and so the pathologic diagnosis made on small biopsy samples is more prone to diagnostic inaccuracy. The immunohistochemical markers play an important role in the subtyping of poorly differentiated NSCLC. Primary adenocarcinoma is positive for TTF-1, Napsin A and CK7. Squamous cell carcinoma is typically positive for p63 and CK5/6. Present study indicates the role of immunohistochemical markers, p63 and TTF-1 in the diagnosis and classification of non-small cell lung carcinoma. This study includes 80 cases of primary lung carcinomas, which are morphologically diagnosed as non-small cell lung carcinomas. Among the 80 cases of NSCLC, 91.25% (73 cases) were males and 8.75% (7 cases) were females. Male-to-female (M: F) ratio is approximately 10.4: 1. This higher incidence in males can be attributed to the higher prevalence of cigarette smoking in males, which is a definite risk factor for lung carcinoma. In the study conducted by Shanmugapriya Shankar et al in 2014 has obtained M: F ratio of 1.7: 1. In 2003, Vigg et al has obtained a M: F ratio of 6: 1.

Sex Ratio	Shanmugapriya Shankar et al ^[1]	Vigg et al ^[2]	Present Study
M: F Ratio	1.7: 1	6: 1	10.4: 1

Table 1. Comparison of Sex Ratio with Similar Studies

Lung cancer is commonly occurring in elderly people. In this study, peak incidence was between 60 to 69 years of age. 46.25% of the study population (37 cases) found between 60 and 69 years and 82.5% (66 cases) between 50 and 79 years. The mean age was 61 years. These values are comparable with study done by Shanmugapriya Shankar et al,^[1] in which the peak incidence was between 61 and 70 years of age. In our study, lowest age was 42 years and highest age was 86 years. 11.25% of patients were < 50 years of age and 6.25% of patients were 80 years of age and above. In the study conducted by M Gangopadhyay et al,^[3] the peak incidence was between 60 and 69 years and the mean age was 64 years. Out of the 73 males, 36 patients (49.31%) were in 60 - 69 age group and out of the 7 females 3 cases (42.85%) were in

the age group of 40 - 49 years. As per our study, in males elderly age group (60 - 69 years) was commonly affected and in females the incidence were more in the age group of 40 - 49 years.

Age in Years	Shanmugapriya Shankar et al ^[1]	M Gangopadhyay et al ^[3]	Present Study
Peak Incidence	61-70	60-69	60-69
Mean Age	62	64	61

Table 2. Comparison of Peak Incidence and Mean Age with Similar Studies

Among the 80 cases of non-small cell lung carcinoma majority were squamous cell carcinoma 58.75% (47 cases), adenocarcinoma constituted 33.75% (27 out of 80 cases), adenosquamous carcinoma 3.75% (3 out of 80 cases) and NSCLC-NOS 3.75% (3 out of 80 cases). These results can be comparable with the study done by Shankar S et al in 2014. In their study NSCLC accounts for about 93% and among these 55% was adenocarcinoma and rest of the 45% is composed of squamous cell carcinoma, adenosquamous carcinoma and NSCLC-NOS. This study can also be comparable with the studies done by Argon A et al^[4] in 2015 and Peh Sun Loo et al^[5] in 2010.

Histological Type of Carcinoma	Shanmugapriya Shankar et al ^[1]	Argon A et al ^[4]	Peh Sun Loo et al ^[5]	Present Study
Squamous cell Ca	24%	74.20%	53.65%	58.75%
Adeno Ca	55%	22.50%	33%	33.75%
Adenosquamous Ca	4%	3.30%	0%	3.75%
NSCLC-NOS	18%	0%	13.40%	3.75%

Table 3. Comparison of percentage distribution of Cases based on IHC Diagnosis

In our study, squamous cell carcinoma is the histologically predominant type. This is because the number of males are more in the study population compared to females, in which cigarette smoking is a definite risk factor and smoking is more attributable to squamous cell carcinoma.

Among the 20 histologically diagnosed cases of squamous cell carcinoma, all the 20 cases were positive for p63. Out of the 11 histologically diagnosed cases of adenocarcinoma, only one showed p63 positivity. 26 cases were histologically diagnosed as poorly differentiated carcinoma, possibly squamous, of which 20 cases showed p63 positivity and 6 cases were negative for p63. Among the 7 cases of poorly differentiated adenocarcinoma, 1 case showed p63 positivity. 16 cases were histologically diagnosed as poorly differentiated carcinoma. Out of these 16 cases, 8 were positive for p63 and 8 were negative. Out of the 20 cases of squamous cell carcinoma, which were histologically diagnosed only one showed TTF-1 positivity which was also positive for p63 and finally diagnosed as adenosquamous carcinoma. TTF-1 was positive in all the 11 cases of histologically diagnosed adenocarcinoma. Of the 26 cases of poorly differentiated squamous cell carcinoma, 4 cases showed TTF-1 positivity which were negative for p63 and were considered as adenocarcinoma. 22 cases of poorly differentiated squamous cell carcinoma were negative for TTF-1. All the 7 cases of

poorly differentiated adenocarcinoma showed TTF-1 positivity. Among the 16 cases of poorly differentiated carcinoma, 7 cases were positive for TTF-1. Rest of the 9 cases which were TTF-1 negative, one case was also negative for p63 and was diagnosed as NSCLC-NOS. The interpretation is that, 20 cases were histologically diagnosed as squamous cell carcinoma. After IHC confirmation study, 19 are diagnostically confirmed as squamous cell carcinoma and one case diagnosed as adenosquamous carcinoma which showed both p63 and TTF-1 positivity. Among the 11 histologically diagnosed cases of adenocarcinoma, 10 cases were diagnosed as adenocarcinoma and one case as adenosquamous after IHC marker study. Out of the 26 cases of poorly differentiated squamous cell carcinoma 20 cases were diagnosed as squamous cell carcinoma, 4 cases as adenocarcinoma and for 2 cases the diagnosis was given as NSCLS-NOS since they were negative for both p63 and TTF-1. Among the 7 cases of poorly differentiated adenocarcinoma, 6 were diagnosed as adenocarcinoma and one as adenosquamous carcinoma after IHC study (showed positivity for both markers). 16 cases were histologically diagnosed as poorly differentiated carcinoma, of which 8 cases were diagnosed as squamous cell carcinoma, 7 cases as adenocarcinoma and 1 case as NSCLC-NOS (negative for both markers) after IHC confirmation study.

Chi-square test was done for both p63 and TTF-1 expression and got P value < 0.001, which was statistically significant. This study is comparable with the studies conducted by Peh Sun Loo et al^[5] in 2010 and Argon A et al^[4] in 2015.

Author	H and E Diagnosis	Total No. of Cases	IHC Diagnosis			
			Squamous cell Carcinoma	Adenocarcinoma	Adeno-Squamous	NSCLC-NOS
Peh Sun Loo et al ^[5]	Squamous cell carcinoma	25	25	-	-	-
	Adenocarcinoma	16	-	16	-	-
	NSCLC-PD	41	19	11	-	11
Argon A et al ^[4]	Squamous cell carcinoma	72	72	-	-	-
	Adenocarcinoma	19	-	15	4	-
	NSCLC-PD	29	17	12	-	-
Present Study	Squamous cell carcinoma	20	19	-	1	-
	Adenocarcinoma	11	-	10	1	-
	NSCLC-PD	49	28	17	1	3

Table 4. Comparison of cases based on Histological Diagnosis and IHC Diagnosis

Limitations of the Present Study

- Short study period and inability to follow-up the cases.
- Small sample size.

CONCLUSION

The present study was conducted to evaluate the role of immunohistochemical markers p63 and TTF-1 in subtyping of non-small cell lung carcinoma. Both p63 and TTF-1 have 100% expression in squamous cell carcinoma and adenocarcinoma respectively. Hence, these markers may be recommended for subtyping of non-small cell lung carcinoma (NSCLC).

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