



A STUDY OF THE EXPRESSION OF EGFR AND TTF1 ON PRIMARY LUNG CARCINOMA

Pathology

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ABSTRACT

Background : Since it is an era of targeted therapies, identification of molecular differences between the specific subtypes of lung carcinomas are necessary. So the pathologists are challenged with the need to offer the clinicians a more detailed classification of lung carcinomas with the help of immunohistochemistry.

Methodology: The study is a cross sectional study with immunohistochemical analysis of 41 lung biopsies of primary lung carcinomas received in our department. The expression of EGFR and TTF1 were analysed and scored by H score and diffuse nuclear positivity respectively.

Results: 70% (14 out of 20 cases) of adenocarcinoma and 25% (1 out of 4 cases) of small cell carcinoma shows TTF 1 positivity. None of the squamous cell carcinoma is TTF 1 positive. In the present study 94% (16 out of 17 cases) of squamous cell carcinoma and 85% (17 out of 20 cases) of adenocarcinoma show EGFR positivity. None of the small cell carcinoma show EGFR positivity. Majority of cases of primary lung adenocarcinomas showed both EGFR and TTF1 expression (65%), showing a good correlation between the two.

Conclusions: Epidermal growth factor receptor protein expression is more in squamous cell carcinoma. Thyroid transcription factor 1 expression is more in adenocarcinoma. Majority of cases of primary lung adenocarcinomas show both EGFR and TTF 1 expression.

KEYWORDS

INTRODUCTION

Carcinoma of lung is one of the leading causes of cancer deaths [1]. The five-year survival rate of patients with lung carcinoma is 15% [2]. Surgical resection with curative intent is the current modality of treatment for lung carcinoma. The prognosis of patients with lung carcinoma is generally poor [3].

Primary lung carcinoma is classified into two clinically relevant groups: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). Because of the differences in the response of NSCLC to new targeted therapies, the identification of molecular differences between the specific subtypes are necessary [4]. Immunohistochemistry (IHC) aids in the separation of small cell carcinoma from non-small cell carcinoma and helps in further classifying non-small cell carcinoma. The most useful application of IHC is in distinguishing primary lung tumors from metastatic lung tumors, particularly in small biopsy specimens with viable tumour tissue [5].

Epidermal growth factor receptor (EGFR) is a member of HER or ErbB family and consists of four members - EGFR (HER1/ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). They regulate many physiological processes that are involved in the modulation of cell proliferation, apoptosis, cell motility and neovascularisation. Thus these receptors play an important role in carcinogenesis [6,7].

Thyroid transcription factor 1 (TTF1) is a transcription factor that regulates the expression of many genes involved in lung development [8]. As lung is one of the most common sites of metastasis, TTF1 is a reliable marker to distinguish between primary lung carcinoma and metastases to the lung, especially when dealing with adenocarcinoma. It is also considered as a reliable marker in the differential diagnosis of pleural localization of a peripheral lung carcinoma and malignant mesothelioma [9].

Since targeted therapy is being offered on the basis of the diverse tumour characteristics and expression of the prognostic marker EGFR, pathologists are now challenged with the need to offer clinicians a more detailed typing of lung carcinomas, especially that of non-small cell lung carcinoma (NSCLC).

AIMS AND OBJECTIVES

1. To evaluate the expression of immunohistochemical marker, TTF 1 (Thyroid transcription factor 1) in primary lung carcinomas.
2. To evaluate the expression of EGFR (Epidermal growth factor receptor) protein in primary lung carcinomas by immunohistochemistry.
3. To assess the relationship between TTF 1 and EGFR protein expression in primary lung carcinomas.

MATERIALS AND METHODS

Study setting: Department of Pathology, MES Medical College.
Study population: All diagnosed cases of primary lung tumor in Respiratory Medicine Department of MES Medical College.
Sample size: 40 cases approx.

INCLUSION CRITERIA:

1. Both males and females with biopsy proven primary lung carcinoma
2. Samples with adequate tumour tissue

EXCLUSION CRITERIA:

1. Inadequate biopsy specimen
2. Proven cases of metastatic lung carcinoma

METHODS:

EGFR and TTF-1 staining was done by the standard IHC techniques and the positivity is graded accordingly

Study design: Cross sectional study.

EGFR AND TTF 1 SCORING

EGFR shows diffuse membrane positivity. EGFR scoring is done by H-Score method. Here a score of 0 - 300 is assigned for each case. This is obtained by multiplying percentage of cells stained and intensity of staining. Intensity of staining is divided into absent, mild, moderate and severe staining. Absent staining is given 0 staining, mild staining is given 1 score, moderate staining is given 2 score and strong staining is given 3 score. H-Score \leq 200 is considered as negative and $>$ 200 is taken as positive [10].

TTF 1 shows diffuse nuclear positivity which is considered as positive.

OBSERVATIONS AND RESULTS

GENDER DISTRIBUTION

Out of the 41 primary lung carcinomas, 80.5% (33cases) were in males and 19.5% (8cases) were in females.

TABLE -1 showing gender predilection

GENDER	No. of cases	Percentage of cases
MALES	33	80.5
FEMALES	8	19.5

Out of the total 41cases, 48.78% of cases (20 cases) were between 56-65 years and 83% of cases (34 cases) falls between 46-75 years. Mean age is 62.5 years. Out of the total, 33 males 51.5% (17cases) and 8 females 37.5% (3cases) falls between 56-65years.

SMOKING AND LUNG CARCINOMA

	SQCC	ADCC	SMCC	TOTAL
	No. of cases (%)			
No smoking	1 (5.88)	6 (30)	1 (25)	8 (19.51)
No significant smoking	3 (17.65)	4 (20)	0 (0)	7 (17.07)
Significant smoking	13 (76.47)	10 (50)	3 (75)	26 (63.42)
TOTAL	17	20	4	41

Table 2 - showing relation between smoking and histological type of lung carcinoma

In this study out of 41 cases, 63.41% (26cases) are significant smokers. Significant smoking is seen in 76.47% (13 out of 17 cases) of squamous cell carcinoma, 75% (3 out of 4 cases) of small cell lung carcinoma and 50% (10 out of 20 cases) of adenocarcinoma. All the histological types of lung carcinomas are more common in significant smokers.

DISTRIBUTION OF LUNG CARCINOMA

Majority of cases are non small cell lung carcinoma and it accounts for about 90.25% (37 out of 41cases) . Small cell lung carcinoma forms about 10% (4 out of 41cases).

Among all lung carcinomas, 48.78% (20 cases) are adenocarcinoma, 41.46% (17cases) are squamous cell carcinoma and 9.76% (4cases) are small cell lung carcinoma.

PERCENTAGE OF EACH HISTOLOGICAL SUBTYPE

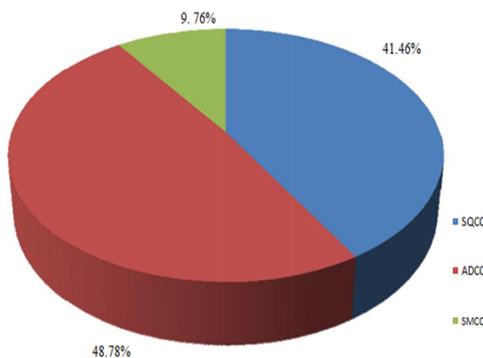


Figure 1- showing the distribution of histological types of lung carcinoma

Table 3- showing the distribution of histological types of lung carcinoma in males and females

TYPE OF CARCINOMA	MALES	FEMALES
	NO. OF CASES (%)	NO. OF CASES (%)
SQCC	16 (48.48)	1 (12.5)
ADCC	14 (42.43)	6 (75)
SMCC	3 (9.09)	1 (12.5)
TOTAL	33	8

TTF1 EXPRESSION IN LUNG CARCINOMA

Table 4 - showing the expression of TTF1 in the histological types of primary lung carcinoma

TYPE OF CARCINOMA	TTF 1 +	% +	TTF 1 -	% -	Total
SQCC	0	0%	17	100%	17
ADCC	14	70%	6	30%	20
SMCC	1	25%	3	75%	4

70% (14 out of 20cases) of adenocarcinoma and 25% (1 out of 4 cases) of small cell carcinoma shows TTF 1 positivity. None of the squamous cell carcinoma is TTF 1 positive.

EGFR EXPRESSION IN LUNG CARCINOMA

TYPE OF CARCINOMA	EGFR +	% +	EGFR -	% -	Total
SQCC	16	94%	1	6%	17
ADCC	17	85%	3	15%	20
SMCC	0	0%	4	100%	4

Table 5 - showing the expression of EGFR in the histological types of primary lung carcinomas.

In the present study 94% (16 out of 17cases) of squamous cell carcinoma and 85% (17 out of 20 cases) of adenocarcinoma show EGFR positivity. None of the small cell carcinoma show EGFR positivity.

RELATIONSHIP BETWEEN EGFR AND TTF 1 EXPRESSION IN PRIMARY ADENOCARCINOMA LUNG.

ADENO CARCINOMA	TTF 1 +	TTF 1 -	Total
EGFR +	13 (65%)	4 (20%)	17 (85%)
EGFR -	1 (5%)	2 (10%)	3 (15%)
Total	14 (70%)	6 (30%)	20

Table 6 - showing the relation between EGFR and TTF1 positivity in primary lung adenocarcinoma.

Majority of cases of EGFR positive lung carcinomas also showed TTF1 positivity(65%).

DISCUSSION

As the diagnostic and therapeutic approach to lung carcinomas mainly adenocarcinoma, have changed a lot, earlier classification of lung carcinoma into small cell carcinoma and non-small cell lung carcinoma is not sufficient for offering the appropriate treatment. There is a multidisciplinary approach towards the diagnosis of lung carcinoma that incorporates clinical, radiologic, molecular, surgical and pathologic findings.

The incidence of lung carcinoma is increasing nowadays. Present study includes 41 primary lung carcinoma cases of which 80.5% (33 cases) were males and 19.5% (8 cases) were females. Male to female (M:F) ratio is approximately 4:1

In 2007 studies done by Gaur et al has reported a M:F ratio of 3.6:1 which is comparable with our study[11]. This indicates that there is an increase in incidence of lung carcinoma in females. Our results also showed that 50% of significant smokers (13 out of 26 cases) have squamous cell carcinoma and 75% of non smokers (6 out of 8cases) have adenocarcinoma. Significant smoking is seen in 76.47% (13 out of 17 cases) of squamous cell carcinoma, 75% (3 out of 4 cases) of small cell lung carcinoma and 50% (10 out of 20 cases) of adenocarcinoma. Jedrychowski et al showed that attributable risk of smoking in squamous and small cell lung carcinoma is (90% and 88% respectively) much higher than adenocarcinoma (64%)[12]. In this study, majority of cases are non small cell lung carcinoma and it accounts for about 90.25% (37 out of 41cases) . Small cell lung carcinoma forms about 10% (4 out of 41cases). Among NSCLC , incidence of adenocarcinoma is more than squamous cell carcinoma. Adenocarcinoma forms about 54% (20 out of 37cases) and squamous cell carcinoma forms about 46% (17 out of 37cases). These results can be compared with the studies 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, NSCLC NOS and adenosquamous carcinoma[13].

In the present study 94.12% (16 out of 17 cases) of SQCC, 75% (3 out of 4 cases) of SMCC and 70% (14 out of 20 cases) of ADCC occurred in males. Squamous cell carcinoma accounts for about 48.48% (16 out of 33 cases) of lung carcinoma in males followed by adenocarcinoma which forms about 42.43% (14 cases). Predominant histological type in females is adenocarcinoma which accounts for about 75% (6 out of 8 cases). The incidences of squamous and small cell lung carcinoma are equal in females.

In our study 82.35% (14 out of 17 cases) of squamous cell carcinoma falls between 55 to 75 years where as adenocarcinoma have slightly younger age of onset than squamous cell carcinoma. In the present study 70% (14 out of 20 cases) of adenocarcinoma falls between 45 to 65 years.

Precise subcategorisation of NSCLC is possible with the immunohistochemical marker TTF 1. It is expressed more in well differentiated than poorly differentiated carcinomas. In our study, 70% (14 out of 20 cases) of adenocarcinoma and 25% (1 out of 4 cases) of small cell carcinoma shows TTF 1 positivity. None of the squamous cell carcinoma is TTF 1 positive. But the studies done by Shankar S Et al showed that TTF 1 is expressed in 55% of lung adenocarcinoma (13). Yatabe et al had studied the expression of TTF 1 in adenocarcinoma. Our results can be compared with this study who got 72% positivity for TTF 1 in adenocarcinoma[14]. Combination of TTF 1 with other immunohistochemical marker Napsin A helps to increase the sensitivity to detect primary lung carcinoma. EGFR protein expression is seen both in squamous cell carcinoma and adenocarcinoma. In the present study 94% (16 out of 17 cases) of squamous cell carcinoma and 85% (17 out of 20 cases) of adenocarcinoma show EGFR positivity. None of the small cell carcinoma show EGFR positivity. Studies done by Vaela D et al showed higher expression of EGFR in squamous cell carcinoma than adenocarcinoma (87.5% in squamous cell carcinoma and 70% in adenocarcinoma) [15].

Eightyfive percentage EGFR positivity of adenocarcinoma in our study is comparable with the results obtained by Shankar S Et al. They obtained 89% positivity in lung adenocarcinoma[13].

Studies done by Sheffield BS et al have shown that EGFR mutation is more in TTF 1 positive adenocarcinoma[16]. In our study also we got EGFR positivity in majority of cases of TTF1 positive adenocarcinoma.

Sheffield BS et al in 2014 has shown that absence of TTF 1 can exclude EGFR mutation with an accuracy of 94.2%. Thus TTF1 status helps in selecting the patients for *EGFR* mutation testing and cannot replace EGFR mutation testing[16]. Newer monoclonal antibodies are being designed to detect the specific mutated EGFR protein. EGFR expression in the present study can be used as a screening test before mutation analysis.

CONCLUSIONS

1. Epidermal growth factor receptor protein expression is more in squamous cell carcinoma than adenocarcinoma.
2. Small cell carcinomas are EGFR negative.
3. Thyroid transcription factor 1 expression is more in adenocarcinoma.
4. None of the squamous cell carcinoma show TTF 1 positivity.
5. Majority of cases of primary lung adenocarcinomas showed both EGFR and TTF1 expression (65%), showing a good correlation between the two.

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