The Extent of Expression of Thyroid Transcription Factor 1, Cytokeratin 7, and Anaplastic Lymphoma Kinase in Lung Adenocarcinoma

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Abstract

Background: New forms of genetic alteration were developed giving a new era of lung adenocarcinoma (AdC) with diverse clinical and pathological features. **Aim and Objectives:** The aim is to investigate the ALK-rearranged gene as one of these genetic alterations in AdC. The role of TTF-1 and CK7 is to exclude the metastases. **Material and Methods:** Paraffin-embedded 50 AdC specimens were cut into 4-mm thick sections and stained with the primary antibody, using an anti-TTF-1 antibody, (all at a 1:200 dilution, mouse monoclonal antibody; Dako, Denmark), anti-CK 7 antibody (DAKO, Carpentaria, CA), and the rabbit monoclonal anti- ALK antibody (D5F3) (Ventana, USA). Positive reactivity was considered as brown nuclear immunostaining for TTF-1 and cytoplasmic for CK 7and both cytoplasmic and nuclear for ALK. **Results:** The median age was 56±2, with male to female ratio 7:3. Forty-four cases revealed a mixed pattern (88%), with focal intraluminal mucin. Forty-one cases (82%) were positive for TTF-1, of these; weak (13; %), moderate (16; %) and strong (12; %). Regarding CK7; 48 cases (96%) were positive; weak (7 cases; 14.5; %), moderate (18 cases; 37.5%) and strong (23 case; 47.9 %) of the positive cases. Regarding D5F3; five cases (10%) were positive; weak (1 case; 20 %), moderate (2 cases; 40 %) and strong (2 cases; 40%) out of the positive cases. Four ALK-positive cases were male and ALK-positive patients ranged from 42-55 years with median 48. **Conclusion:** Despite the incidence of ALk-AdC is uncommon, it might be suspected in lesions of lower age group especially with mucinous foci.

Keywords: Adenocarcinoma, anaplastic lymphoma kinase, cytokeratin 7, immunohistochemistry, thyroid transcription factor 1

Introduction

Lung cancer is the first cause of mortality from malignancy worldwide. Lung adenocarcinoma (AdC) had increased dramatically especially in the last decades[1,2] According to the World Health Organization lung carcinoma classification 2004, AdC is defined as an epithelial tumor with the glandular differentiation. They show different pattens including acinar, papillary, solid, bronchioloalveolar, or mixed pattern. The International Association for the Study of Lung Cancer excluded bronchioloalveolar carcinoma and mixed morphological pattern of AdC from use and reclassified the invasive lung AdC as lepidic, acinar, papillary, micropapillary, and solid subtypes.[3]

Many immunohistochemical markers were utilized in the diagnosis of primary AdC, including thyroid transcription factor 1 (TTF-1) and cytokeratin 7 (CK7). TTF-1 is one of the Nk×2 gene families and act as a transcription factor.[4] It is expressed in the lung and thyroid.[5] The main role of TTF-1 in the lung is a regulation of surfactant protein expression. TTF-1 is expressed in follicular neoplasms, medullary carcinomas, and lung neoplasm mainly AdC.[6] Hence, it is considered one of the major diagnostic markers for lung AdC.[7]

CK7 is a type II keratin presents in the ductal epithelium, and mesothelial cells. The varied staining of CK7 expression in carcinomas is valuable in distingushng various types of some

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carcinomas.[8,9] The positivity of CK7 is the most discriminative marker in distinguishing lung AdC from colorectal AdC. On contrary, this positivity cannot help in the the differential diagnosis of ovarian, endometrial, and lung AdCs because CK7 positivity has been found over 80% of breast, lung, and endometrial AdC, and over 90% of ovarian AdCs.[10-14] Hence, CK7 alone cannot exclude lung metastasis, so its association with TTF-1 can differentiate between primary and metastatic lung AdC.

The anaplastic lymphoma kinase (ALK)-rearranged gene is a newly discovered in AdC. It occurs in chromosome two through an inversion in its short arm, resulting in fusing echinoderm microtubule-associated protein-like 4 (EML4) to the ALK, resulting in the activation of ALK tyrosine kinase. EML4-ALK rearrangement is present nearly in 4% of all dC. In addition, ALK-rearranged AdC showed several histopathological features that distinguished it from other different types: cribriform pattern, presence of cells distended with mucin in addition to psammoma bodies.[15] Kim *et al.*[15] recognized a unique feature for ALK-rearranged tumors that is the close connection with the adjacent bronchial epithelium. In some cases, tumor cells cross the bronchiolar epithelium and form small clusters of the epithelial cell into the lumen, giving a budding off appearance[16,17] Other ALK fusion partners have been identified in non-small-cell lung carcinoma, for example, KIF5B-ALK,[18] TFG-ALK,[19] multiple EML4-ALK isoforms,[10-22] and KCL1-ALK,[23] but their clinical consideration and application are still unknown.

In the previous study,[7] investigation of the TTF1 in AdC revealed a high sensitivity, and some of these diagnosed cases have occurred in relatively lower age groups than usual and hence, a new mutation may be considered in these cases. Hence, the study aims to investigate the extent of new mutation as an ALK-rearranged gene using D5F3 clone in lung AdC and find any associations between its expressions with TTF-1 and CK-7. The utility of TTF-1 and CK7 in the study is to confirm that AdC is of pulmonary origin and also to exclude metastases, especially from colorecral AdC.

MATERIALS AND METHODS

Fifty archival paraffin-embedded specimens of lung AdC were collected following instructions of the ethical guidelines and after taking the written permission. These specimens were collected from the period between 2016 and 2019. The data as age and gender were collected from reports of these archival specimens. All specimens were subjected to histopathological examination before the immunohistological study. The 4-mm thick sections were done and the staining system was automated including incubation for 45 min using an anti-TTF-1 antibody (mouse monoclonal antibody, 1:200 dilution,; by Dako, Denmark), anti-CK 7 antibody (Dako, Carpentaria, CA), and the rabbit monoclonal anti-ALK antibody using D5F3 (Ventana, D5F3, Tucson, USA), rinsed by a brief buffer, and then incubated with biotinylated anti-mouse IgG/IgM manufactured by (Abcam,

Cambridge, UK) for 30 min. The slides were consequently incubated with avidin/biotin (Abcam, Cambridge, UK) for 30 min and reacted with diaminobenzidine and hydrogen peroxide (H_2O_2). Positive reactivity was considered as nuclear brownish for TTF-1, cytoplasmic for CK-7, and both cytoplasmic and nuclear for ALK-D3F5 immunoreactivity.

The reactivity was considered according to the percentage of positive cells and calculated as follows: weak = <10%, moderate = 11%–50%, and strong = more than 50% tumor cells stained positively.[24] Statistical analysis was carried out using the Chi-square test, and the two-sided $P \leq 0.05$ was considered. Statistical Package for Social Sciences (SPSS) SPSS, version 18, IBM, NY, USA version 18 was used in this study.

RESULTS

In the present study and from the reported data, we found that the age group ranged from 42 to 69 years of age, with a median 56 ± 5, and mean 57.28% with male predominance (28 male; 12 female, and male-to-female ratio 7:3). The histological pattern revealed that most of the cases revealed a mixed pattern of 44 cases; 88%, six cases; 12% has a solid pattern with focal intraluminal and intracellular mucin which need histochemical stain [Figure 1a] [Figure 2a].

Forty-one out of 50 cases (82%) revealed positivity for TTF-1, while the remaining 9 cases; 18% showed negative results. In the positive cases, the degree of staining was as follows: weak (13%), moderate (16%), and strong (12%), of positive cases. TTF-1 immunostaining is expressed in [Figure 1b].

Regarding to CK7, 2 cases (4%) were negative, 7 cases) (14.5%) were weak, 18 cases (37.5%) were moderate, and 23 cases (47.9%) were strong [Figure 2c].

Regarding ALK-D5F3, 45 (90%) were negative and 5 cases (10%) were positive, of the positive case; 1 case (20%) was weak, 2 cases (40%) were moderate, and 2 cases (40%) were strong, all these results are represented in Table 1 and
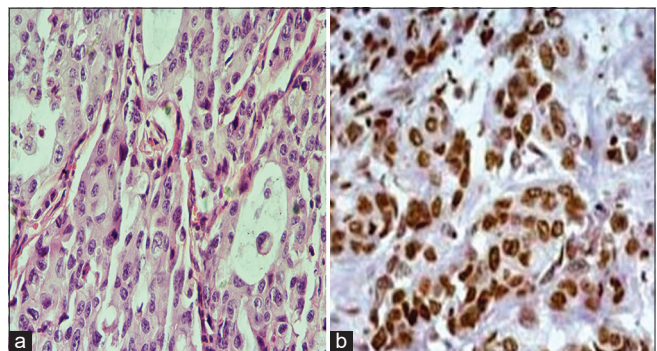
**Figure 1:** (a) Invasive adenocarcinoma of lung. This section shows acinar type despite this case exhibits mixed pattern as a solid pattern. Some of the glands contain intraluminal and intracellular wispy blue mucin. The intracellular mucin must be demonstrated with histochemical stains (H and E, ×200). (b) This tumor has intense nuclear reactivity to thyroid transcription factor 1 (anti-thyroid transcription factor-antibody, ×400)
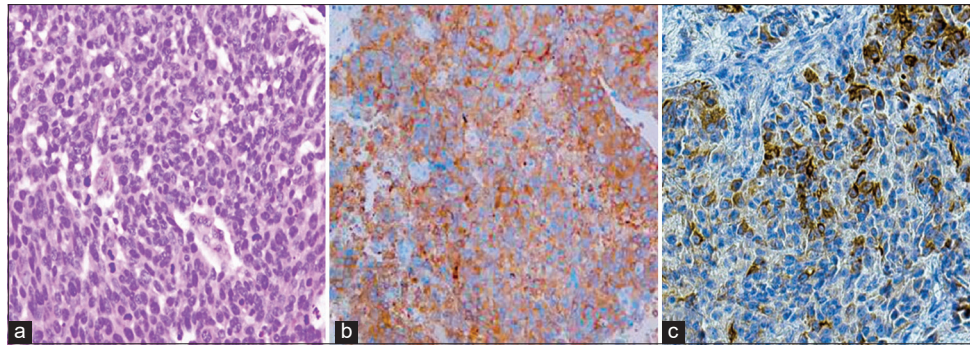
Figure 2: (a) A case of pulmonary adenocarcinoma showing mainly solid pattern with few acinar pattern (H&E stain, x200), (b) the case showing strong cytoplasmic reactivity for anaplastic lymphoma kinase-antibody using D5F3 (anti anaplastic lymphoma kinase antibody, D5F3) (×200), (c) the same case showing moderate cytoplasmic activity for cytokeratin 7 ((anti-cytokeratin 7 antibody, ×200) (DAB)
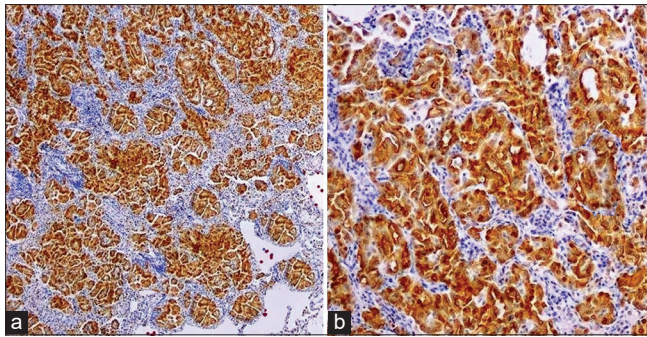
Figure 3: (a) A case of lung adenocarcinoma showing diffuse strong cytoplasmic and nuclear reactivity for anaplastic lymphoma kinase-antibody using D5F3 (anti anaplastic lymphoma kinase antibody, D5F3) (×100), (b) the same case (x,200)
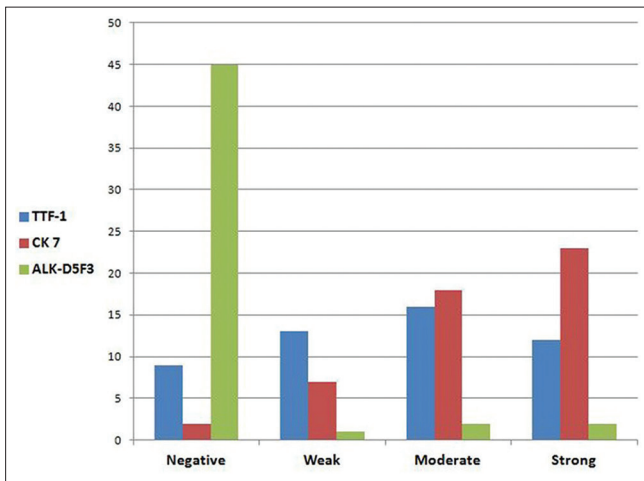
Figure 4: A graph showing the reactivity of thyroid transcription factor 1, cytokeratin 7 and anaplastic lymphoma kinase in the present study

Figure 2b, 3 and illustrated in 4. In figure 2a the reaction is cytoplasmic while in figure 3 the reaction is strong cytoplasmic and nuclear. In five ALK-D5F3-positive cases, four cases were male and the age group of the ALK-D5F3-positive patients was ranged from 42 to 49 years with median 48 and mean 46.8. All these results are summarized in Table 2. The panel reactivity to TTF-1, CK 7 and ALK-D5F3 is summarized in Table 3.

Using Chi-square test, significant P values were obtained between reactivity of TTF-1 and CK7 ($P = 0.02$), between TTF-1 and ALK-D5F3 ($P = 0.00001$), CK7 and ALK ($P = 0.0001$), and TTF-1, CK 7, and ALK-D5F3 ($P = 0.0001$).

DISCUSSION

In the present work, we found that the age group ranged from 42 to 69 years of age, with median 56 ± 5, and mean 57.28% with male predominance (28 male; 12 female, and male-to-female ratio 7:3), the age presentation at the time of diagnosis and male predominance is matched with many studies.[25-29] Also, the majority of examined cases revealed a mixed pattern and this finding is closely met with other studies.[29-33] Regarding TTF-1, the shown results revealed that 41 out of 50 cases of AdC were positive, which is in concurrence with many findings that revealed positivity in AdCs ranged from 81 to 89%,[4,24-28] while our results are distant from the result of 50% that revealed in the study of Kostovski and Petrushevska.[29]

In the current research, we found 48 cases (96%) showed positivity for CK7; this result is parallel to many studies. On the other hand, we found one case revealed negativity for both CK7 and TTF-1; this case may be metastasized from other sites especially from colorectal and so, CK20 would be valuable in this case.[30] On the other hand, we found that one case revealed negativity for both TTF-1 and ALK-D5F3 with positivity for CK 7, this case may be metastasized from other organs as CK positivity has been found over 80% of breast, and endometrial AdC, and over 90% of ovarian AdCs.[11,12]

In the present study, we found five cases (10%) showing positivity for D5F3, this is slightly higher than to results obtained in some studies that revealed that ALK-rearranged lung AdC, is found in 4%–7% of lung AdC, also, our results revealed that all positive cases showed positivity for TTF-1, this is in agreement with the several studies that confirmed that ALK-rearranged lung AdC is characterized by a TTF-1 cell lineage.[7,31-34] The percentage of positive cases may be due to the application of D5F3 in the study in contrast to previous studies that used ALK1 antibody. D5F3 is more sensitive in detecting the ALK-rearranged lung AdC which is proved in the study

Table 1: Results of thyroid transcription factor 1/cytokeratin 7/D5F3 staining in the adenocarcinoma cases

Stain	Degree of reactivity				*P*-value using Chi-square test
	Negative	Weak	Moderate	Strong	
TTF-1	9	13	16	12	0.02
CK_7	2	7	18	23	
ALK-D5F3	45	1	2	2	
P			0.00001		

TTF-1: Thyroid transcription factor 1, CK 7: Cytokeratin 7

Table 2: The five positive ALK-D5F3 cases in relation to age and gender

Serial number	Age	Gender	TTF-1	CK-7	ALK
1	42	Male	3+	2+	3+
2	48	Male	3+	2+	1+
3	48	Female	2+	3+	3+
4	49	Male	-	3+	2+
5	47	Male	3+	-	2+

TTF-1: Thyroid transcription factor 1, CK 7: Cytokeratin 7

Table 3: Panel reactivity in the present study

Serial number	Panel reactivity	Number of AdC cases	Remarks
1	TTF-1+/CK+/D5F3+	3	
2	TTF-1+/CK+/D5F3−	37	
3	TTF-1−/CK+/D5F3+	1	
4	TTF-1+/CK-/D5F3+	1	
5	TTF-1−/CK+/D5F3−	7	? metastasis
6	TTF-1−/CK−/D5F3−	1	? metastasis
7	TTF-1−/CK−/D5F3+	0	
8	TTF-1+/CK−/D5F3−	0	

AdC: Adenocarcinomam, TTF-1: Thyroid transcription factor 1, CK: Cytokeratin

of Taheri *et al*.[35] which revealed that a new anti-ALK-D5F3 has been demonstrated to be of superior sensitivity over the ALK1 antibody which is currently used in most studies. Also, many studies supported the superiority of D5F3 in the finding of Alk-positive Adc cases.[36-38] Four out of five positive AdC cases were of mixed pattern with mucinous foci, this is matched with previous studies[31-33] which stated that the majority of ALK-positive AdC occurs in tumors with at least mucinous foci. In addition, we found the reaction is mainly cytoplasmic but in one case [Figure 3] the reaction is diffuse cytoplasmic and nuclear this is attributed to predominant patten that in this current case it is glandular or acinar type or may be attributed to presence of more than different Alk-fusion patterns as more than 19 fusion patterns are recently discovered.[18,20,23,39]

In the present study, the age for cases of ALK-D5F3 positive cases was ranged from 42 to 49 with a median of 48 and a mean 46.5 [Figures 2b,3,4]. This is in agreement with previous studies as Soda *et al*., Inamura *et al*., Shaw *et al*., and Inamura *et al*.[17,31-33] The detailed clinical history as presence or absence of smoking is

deficient and this considers as limitation of this study. Also, our results are in agreement with Uruga and Mino-Kenudson[40] who found that ALK positive AdC cases are characteristically found in younger age group than conventional AdC and most cases exhibit a solid growth pattern with either signet cells and or presence of focal mucinous pattern.[18,34,41]

This study is retrospective depends mainly on the patient report which includes the age and gender of the patient, so the deficiency of other clinical data is considered as one of the limitations in addition to a low study sample of the study.

CONCLUSION

Although the incidence of ALK rearrangement AdC is uncommon, it might be suspected in lesions especially that of a lower age group than conventional lung AdC. Its association with TTF-1 and CK 7 supports the diagnosis of the primary lung AdC and excludes the lung metastases.

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Conflicts of interest
There are no conflicts of interest.

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