Cytomorphological Features of Lung Adenocarcinoma with Anaplastic Lymphoma Kinase Gene Rearrangement

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Abstract

Background: The anaplastic lymphoma kinase (ALK) gene rearrangement is a predictive and prognostic marker in pulmonary adenocarcinoma. A series of clinical and pathological features have been documented in patients who harbor this translocation. Aims: The objectives of this study were to analyze the specific cytomorphological features of lung adenocarcinoma that harbored the ALK gene rearrangement and to compare the morphological features with the mutation-negative cases. Materials and Methods: The study sample of 40 cases included 15 ALK-positive cases and 25 ALK-negative cases. After the diagnosis was established, testing for ALK rearrangement was on the histopathology sample. The corresponding fine-needle aspiration cytology slides were retrieved from the records and cytomorphological features were analyzed. Results: A comparison of pattern between the ALK-positive and ALK-negative group revealed that the sheet-like pattern and singly scattered cells were more common in the ALK-positive group. Moderate-to-severe nuclear pleomorphism was identified in 80% of ALK-positive cases and 72% of ALK-negative cases. The presence of mucinous features and/or signet ring morphology was the most striking feature of ALK-positive cases with \( P \) value of 0.002. The presence of necrosis in the background was identified in 80% of ALK-positive cases and was statistically significant (\( P = 0.001 \)). Conclusion: This is among the few studies from India, where a detailed analysis of the cytomorphological features of cases with ALK phenotype versus the mutation negative cases has been performed.

Keywords: Anaplastic lymphoma kinase rearrangement, cytomorphological features, pulmonary adenocarcinoma

INTRODUCTION

The anaplastic lymphoma kinase (ALK) gene rearrangement is a predictive and prognostic marker in pulmonary adenocarcinoma.[1] It is identified in 3–5% of non-small cell lung carcinoma (NSCLC) with adenocarcinoma phenotype. The presence of this gene rearrangement leads to unrestricted cell growth that forms the major pathway of oncogenesis.[2] The novel ALK fusion-echinoderm microtubule-associated protein-like 4 (EML4-ALK) was first discovered as a somatic gene rearrangement by Hiroyuki Mano et al. An inversion event on the short arm of chromosome 2, resulting in the fusion of ALK gene with the EML4 gene locus, is the most common aberration of the ALK gene in lung cancer. This rearrangement leads to the production of a chimeric protein, which has constitutive ALK kinase activity resulting in inhibition of apoptosis and promotion of cell proliferation in tumor cells.[3,4]

A series of clinical and pathological features have been documented in patients who harbor this translocation. It is most frequently detected in nonsmokers and is associated with hepatic, brain metastasis and pleural and pericardial effusions. No apparent differences in the ethnicity and the sex have been identified.[5,6]

Crizotinib is an oral selective inhibitor of ALK. Based on the response rates reported in the phase 1 and 2 clinical trials, crizotinib received accelerated approval by the Food and Drug Administration in August 2011 for treatment of locally advanced or metastatic NSCLC that is ALK positive.[7,8]

The objectives of this study were to analyze the specific cytomorphological features of lung adenocarcinoma that harbored the ALK gene rearrangement and to compare the morphological features with the mutation-negative cases.

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**Materials and Methods**

This was a retrospective case series conducted in the Department of Pathology of a tertiary care hospital. The study included 40 cases of NSCLC–adenocarcinoma subtype, which were diagnosed on histology with or without the aid of immunohistochemistry (IHC). IHC was performed on formalin-fixed paraffin-embedded (FFPE) tissue sections using the standard protocol for napsin, p40, and thyroid transcription factor 1. After the diagnosis was established, testing for ALK rearrangement was done using IHC with the Ventana anti-ALK (D5F3) clone. The FFPE tissue blocks were sectioned at 3–4 µm, using a microtome (Leica, Germany), mounted on tissue bond-coated slides (Biocare, USA). The immunostaining using the D5F3 antibody immunostaining was performed on the benchmark XT system using the Optiview DAB IHC detection kit and Optiview Amplification kit. The prediluted Ventana anti-ALK (D5F3) rabbit monoclonal primary antibody was used along with the rabbit monoclonal negative antibody in every case. A positive control (appendix) was run with all the batches. The staining was performed as per the manufacturer’s instructions. Cytoplasmic and/or membranous staining was assessed. A sample was positive when there was presence of strong granular cytoplasmic staining in any percentage of tumor cells and negative when cytoplasmic staining was absent.

The study sample of 40 cases included 15 ALK-positive cases and 25 ALK-negative cases. The corresponding fine-needle aspiration cytology slides of all the 40 cases were retrieved from the records. The cytology smears retrieved were both air dried and wet-fixed smears that had been stained with May–Grünwald Giemsa, hematoxylin and eosin, and Papanicolaou stains per the standard staining protocol. The cytomorphological features of all the 40 cases were analyzed using the following parameters: (1) cellular arrangement papillary, acinar, sheets, clusters, or singly scattered; (2) nuclear features – nuclear pleomorphism and presence/absence of nucleoli; (3) presence of mucinous features and signet ring morphology was assessed; and (4) necrotic background. A two-dimensional arrangement was “sheet.” “Cluster” was used to indicate a three-dimensional group of cells.

Statistical analysis was performed using the IBM-Statistical Package for Social Sciences (SPSS, International Business Machines Corporation, New York, USA) analysis software, version 16. Chi-square test was used for the categorical variables. All *P* values were calculated with two-sided tests and *P* ≤ 0.05 was considered significant and highly significant when *P* ≤ 0.01.

**Results**

This was a retrospective case series that included a total of 40 cases (15 ALK positive and 25 ALK negative) of NSCLC-adenocarcinoma phenotype where testing for ALK protein expression was performed on the corresponding histology sample.

The cytomorphological features of the ALK-positive versus ALK-negative cases were analyzed [Table 1]. Majority of the cases had a mixed pattern of cellular arrangement [Figures 1 and 2].

**Cellular pattern**

The most common pattern of cellular arrangement in the ALK-positive group was the sheet-like arrangement of cells

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**Table 1: Cytomorphological features of ALK-positive versus ALK-negative cases**

| Cytomorphological characteristics | ALK negative (n=15) | ALK negative (n=25) | *P* |
|-----------------------------------|---------------------|---------------------|-----|
| **Cellular arrangement**          |                     |                     |     |
| Papillary                         | Present             | 2 (13.33%)          | 10 (40%) | 0.074 |
|                                  | Absent              | 13 (86.67%)         | 15 (60%) |
| Acinar                            | Present             | 7 (46.67%)          | 11 (44%) | 0.866 |
|                                  | Absent              | 8 (53.33%)          | 14 (56%) |
| Sheet                             | Present             | 14 (93.33%)         | 16 (64%) | 0.038* |
|                                  | Absent              | 1 (6.67%)           | 9 (36%)  |
| Cluster                           | Present             | 13 (86.67%)         | 18 (72%) | 0.282 |
|                                  | Absent              | 2 (13.33%)          | 7 (28%)  |
| Singly scattered                  | Present             | 13 (86.67%)         | 14 (56%) | 0.044* |
|                                  | Absent              | 2 (13.33%)          | 11 (44%) |
| **Nuclear features**              |                     |                     |     |
| Nuclear pleomorphism              | Mild                | 3 (20%)             | 7 (28%)  | 0.571 |
|                                  | Moderate or severe  | 12 (80%)            | 18 (72%) |
| Nucleoli                          | Present             | 12 (80%)            | 15 (60%) |
|                                  | Absent              | 3 (20%)             | 10 (40%) |
| Mucinous features/Signet ring morphology | Present | 11 (73.33%) | 6 (24%) | 0.002* |
|                                  | Absent              | 4 (26.67%)          | 19 (76%) |
| Necrotic background               | Present             | 12 (80%)            | 7 (28%)  | 0.001* |
|                                  | Absent              | 3 (20%)             | 18 (72%) |

*P* value <0.05 is significant
present in 93.33% cases followed by clusters (86.67%) and singly scattered cells (86.67%). Papillary pattern of cellular arrangement was the most uncommon pattern and was present in only 13.33% cases. Amongst the ALK-negative group, the most common pattern of arrangement was clusters in 72% cases followed by sheet-like arrangement in 64% cases. Similar to the ALK-positive group, papillary pattern was the least common pattern in the ALK-negative group and was identified in 40% cases. A comparison of pattern between the ALK-positive and ALK-negative group revealed that the sheet-like pattern and singly scattered cells were more common in the ALK-positive group and was statistically significant with \( P \) value of 0.038 and 0.044, respectively.

Nuclear features
Moderate-to-severe nuclear pleomorphism was identified in 80% of ALK-positive cases and 72% of ALK-negative cases. Nucleolar prominence was identified in 80% of ALK-positive cases and 60% of ALK-negative cases. However, none of these criteria was statistically significant.

Mucinous features/Signet ring morphology
This finding was present in 73.33% of ALK-positive cases, whereas only 24% of ALK-negative cases demonstrated either presence of mucinous features and/or signet ring morphology.

This feature was statistically significant and the most striking feature of ALK-positive cases with \( P \) value of 0.002.

Necrotic background
The presence of necrosis in the background was identified in 80% of ALK-positive cases, whereas only 28% of ALK-negative cases demonstrated necrosis. This finding was statistically significant with \( P \) value of 0.001.

DISCUSSION
In this current era of personalized medicine, the identification of genetic alterations is essential for targeted therapy. Predictive and prognostic marker testing is now mandatory in NSCLC-adenocarcinoma phenotype.[4] The ALK gene rearrangement was first identified in the year 2007. The cases that harbor ALK gene rearrangement have distinct clinical and histomorphological features. ALK gene rearrangement is more commonly found in females and nonsmokers and at younger age. The characteristic histomorphological features include acinar, solid pattern with mucinous features, or signet ring morphology.[9-11] However, there are very few studies published in literature, wherein the cytomorphologic features of ALK-rearranged lung adenocarcinoma have been evaluated.

Cytological specimens represent a significant number of samples on which diagnoses of lung adenocarcinoma are rendered. Additionally, now cytology samples are being widely used for molecular marker testing, especially in cases where the disease presentation is at an advanced stage and obtaining a biopsy is difficult.[12]

In the present study, the most common pattern of cellular arrangement in the ALK-positive group is sheet-like pattern. This finding was statistically significant when compared with the ALK-negative group. In the study conducted by Li et al., the most common pattern observed in the ALK-positive group was the acinar or glandular pattern.[13] In the current study, acinar pattern was present in 46.67% of ALK-positive cases. This finding is in concordance with the observations of Nishino et al.; the authors stated that the acinar predominant morphology is significantly reduced.
in the ALK-positive group. In the study conducted by Ha et al., sheets were present in 93.8% and clusters were observed in 56.3% of ALK-positive cases. In the current study, clusters were observed as the most common pattern of arrangement in the ALK-negative group. In the study conducted by Pareja et al., the most common architectural pattern was papillary or micropapillary pattern. This finding is contradictory to the current study where papillary pattern was the least common and was present in only 13.33% of ALK-positive cases.

In the current study, the ALK-positive cases displayed moderate-to-severe nuclear pleomorphism along with nucleolar prominence in 80% cases. This finding was not statistically significant when compared with the ALK-negative group. This finding is in concordance with the conclusions derived from the study conducted by Ha et al. Pareja et al. in their study documented the presence of mucinous features associated with signet ring morphology in 67% cases. Nishino et al. stated that the presence of mucinous features associated with signet ring morphology was the single most significant cytomorphological feature in primary pulmonary adenocarcinomas. This characteristic can serve as the most vital criteria for the identification of cases that have the likely probability to harbor the ALK rearrangement.

The detailed examination of the background revealed the presence of necrosis in 80% of ALK-positive cases. This finding is similar to the results of the study conducted by Li et al., where a necrosis in the background was present in 67% of ALK-positive cases. EGFR-positive cases were described to harbor a clean background. Hence, necrosis may also serve as the most vital criteria for the ALK rearrangement. Consequently, an attempt to morphological features supportive of ALK rearrangement may aid in more judicious use of the biopsy tissue.

**Conclusion**

The current study is among the few studies from India, where a detailed analysis of the cytomorphological features of cases with ALK phenotype versus the mutation-negative cases has been performed. In cases that harbor the ALK rearrangement, the common pattern of arrangement is sheet-like associated with singly dispersed cells. These tumors are generally high grade with moderate-to-severe nuclear pleomorphism and presence of necrosis in the background. The identification of these cytomorphological features and patterns may aid in triaging cases that have the probability to harbor ALK phenotype, which further has predictive and prognostic implications.

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**Conflicts of interest**

There are no conflicts of interest.

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