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Original Article

ALK Gene Rearrangemnet in Lung Adenocarcinoma

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ABSTRACT

Objective: To assess the frequency of ALK gene rearrangement among patients presenting with lung adenocarcinoma at tertiary care hospital of Karachi, Pakistan.

Study Design: Prospective longitudinal study

Place and Duration of Study: Department of Medical Oncology, Jinnah Postgraduate Medical College Karachi, Pakistan from May 2019 Mar 2020.

Methodology: Total 185 patients with confirmed diagnosis of stage IV lung adenocarcinoma who were unresectable and metastatic of age 20-75 years of either gender were included in the study using non-probability consecutive sampling technique. Patients were subjected for ALK mutation analysis using fluorescent in situ hybridization technique.

Results: Out of 185 patients, the ALK rearrangements were found positive in 27 tumors whereas it was negative in 158 tumors. Among ALK positive cases, majority of the patients were of aged less than 50 years (n=18, 66.7%).The statistically significant difference was found between age and ALK gene rearrangement (p<0.05). Whereas no statistically significant relationship was found between ALK rearrangements and gender, ethnicity and smoking status (p>0.05).

Conclusion: The results showed positive rearrangement of ALK gene in 15% of the patients which is in contrast to 7% patients found in western studies. ALK gene testing has become standard of care in patients of adenocarcinoma who are EGFR negative so that treatment like ALK gene inhibitors such as alectinib, brigatinib, ceritinib, crizotinib, and lorlatinib can be tailored accordingly.

Keywords: Adenocarcinoma, ALK status, Carcinoma, Lung, Tumors.

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INTRODUCTION

Globally, lung cancer is regarded as the prime etiological factor for mortality.[1] Adenocarcinoma, a subtype of Non-small cell lung cancer (NSCLC) originates in glandular cells and produces mucus that accumulate in small airways such as alveoli. It is located at the outer sided of lungs and has been known to grow gradually in comparison with other types of lung cancers.[2] Advanced stage of non-small cell lung cancer accounts for more than eighty five percent of lung cancer having only fifteen percent survival rates in five years.[3] Translocation among chromosomes are albeit the most well-known change in genetics in the field of oncology and are also explicated in different types of leukemias and now also in lung cancers.[4]

At the molecular level, heterogeneity of non-small cell lung malignancy has been acknowledged which has led to identify the molecular subgroups. In adenocarcinoma of lungs, anaplastic lymphoma kinase or ALK is one of the molecular abnormality like EGFR, Ros1, Kras. The reorganization in the ALK gene have been found out which is sensitive to therapy with ALK tyrosine kinase inhibitors. Generally, ALK tyrosine receptor are enzymes that are coded by ALK gene. ALK belongs to the insulin family of receptor known as tyrosine kinases and is expressed in lower levels in central nervous system.[5] ALK gene is found on chromosome number 2p23. The physiology of ALK gene in humans is still debatable. It is said that ALK gene is responsible for various adhesions at cellular level. It further plays a vital role in development of neuronal tissues and synapses.[6] In lung cancer, genomic alteration involving echinoderm microtubule-associated protein like 4(EML4)-ALK gene fusion (EML4–ALK) was found. The pathology of ALK gene can be comprehended as either union with other gene or mutation of its own gene or may be adding up another gene.[7]

In lung cancer, genomic alteration involving EML4 – ALK fusion was found.[8] This expression is present in up to six percent non-small cell lung malignancy (NSCLC) cases. Even though there is variety of other genetic expression that adds up to ALK rearrangement, however, ALK gene is somehow preserved intracellularly but remains oncogenic.[9] In lung adenocarcinoma, ALK gene expression rearrange-

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ment is used as diagnostic modality. Fluorescent in situ hybridization and immunohistochemical technique (IHC) are the two methods to analyze ALK gene. The EML4-ALK fusion gene is found to be the sole reason of developing lung carcinoma along with histological changes. In Asian population, fusion of EML4-ALK gene has been identified in five percent lung adenocarcinoma cases.[10] Hence, the current study will be the second study in Sindh Pakistan which will report the occurrence of ALK rearrangement in cases of lung adenocarcinoma. Thus, it supports the clinician to tailor appropriate treatment.

METHODOLOGY

The prospective longitudinal study conducted at the Department of Medical oncology of Jinnah Postgraduate Medical Center Karachi, Pakistan from May 2019-Dec 2019. Sample size was estimated as 185 by using statistics of frequency of ALK as 14%[11], margin of error as 5% and 95% confidence level.

Inclusion Criteria: All the patients with confirmed diagnosis of stage IV lung adenocarcinoma who were unresectable and metastatic of age 20-75 years of either gender were included in the study.

Exclsuion Criteria: Patients who received previous chemo-therapy or radiotherapy were excluded from the study.

Ethical review committee approval (ERC NO.2-81/2019-GENL/20231/JPMC) was sought before conduct of study. Data regarding socio-demographics and smoking habits was noted in predesigned proforma. All the patients were subjected for ALK mutation analysis using fluorescent in situ hybridization technique.

SPSS version 23 was used to analyze data. Frequency and percentage was reported for qualitative variables and outcome. Chi-square test was applied to see the difference in the frequency of ALK with respect to effect modifiers. p-value less than and equal to 0.05 was taken as statistically significant.

RESULTS

Total 185 patients with lung adenocarcinoma were included in the study. Majority of the patients were of age more than and equal to 50 years (n=115, 62.2%). About 114 patients were males (61.6%) and 71 were females (38.4%). Most of the patients were Sindhi (n=88, 47.6%) followed by Urdu speaking (n=42, 22.7%). One fifty eight patients were Muslim (85.4%) and 27 were non- Muslim (14.6%). About 62 patients with adenocarcinoma were smokers (33.5%) (Table-I).

Table-I: Descriptive Statistics (n=185)

Age Groups	n(%)
<50 years	70(37.8)
=>50 years	115(62.2)
Gender	
Male	114(61.6)
Female	71(38.4)
Ethnicity	
Sindhi	88(47.6)
Punjabi	26(14.1)
Pathan	17(9.2)
Baloch	12(6.5)
Urdu Speaking	42(22.7)
Religion	
Muslim	158(85.4)
Non-Muslim	27(14.6)
Smoking	
Yes	62(33.5)
No	123(66.5)

The ALK rearrangements were found positive in 27 tumors whereas it was negative in 158 tumors (Fig 1) Among ALK positive cases, majority of the patients were of aged less than 50 years (n=18, 66.7%). In the patients of age less than 50 years, 9 were males and 9 were smokers (Table-II).

Table-II: Association of Age with Gender and Smoking Status of ALK Positive Cases (n=27)

	Age Group	
	<50 years	=>50 years
Gender		
Male	9(69.2%)	4(30.8%)
Female	9(64.3%)	5(35.7%)
Smoking		
Yes	9(81.8%)	2(18.2%)
No	9(56.2%)	7(43.8%)

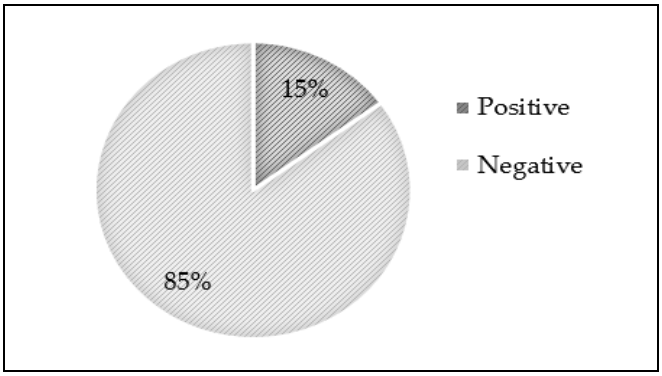

Figure: Frequency of ALK Gene Rearrangement (n=185)

Among younger patients (age group less 50 years) majority of the patients had negative ALK rearrangements and the relationship was statistically significant between age and ALK gene rearrangement (p<0.05).

Whereas no statistically significant relationship was found between ALK rearrangements and gender, ethnicity, religion and smoking status (p>0.05) (Table-III).

Table-III: Association of ALK Gene Rearrangement with Different Risk Factors (n=185)

Variables	ALK		*p*-value
	Positive	Negative	
Age Group			
< 50 years	18(25.7%)	52(74.3%)	
≥50 years	9(7.8%)	106(92.2%)	0.001
Gender			
Male	13(11.4%)	101(88.6%)	
Female	14(19.7%)	57(80.3%)	0.119
Ethnicity			
Sindhi	11(12.5%)	77(87.5%)	
Punjabi	6(23.1%)	20(76.9%)	
Pathan	0	17(100%)	
Balochi	3(25%)	9(75%)	
Urdu speaking	7(16.7%)	35(83.3%)	0.207
Religion			
Muslim	21(13.3%)	137(86.7%)	
Non-Muslim	6(22.2%)	21(77.8%)	0.47
Smoking			
Yes	11(17.7%)	51(82.3%)	
No	16(13%)	107(87%)	0.627

DISCUSSION

The present study focuses on determining occurrence of ALK gene rearrangement in patients having lung adenocarcinoma stage IV. Several studies have been carried out in this context in western population along with its treatment.[12-15] However, Asian population still needs statistics for ALK gene rearrangement. Therefore, according to the current study, the ALK rearrangements were found positive in 27 tumors whereas it was negative in 158 tumors. A study conducted in Pakistan which claims to be the foremost study recording frequency of ALK rearrangement in lung adenocarcinoma. The study also compare the two main diagnostic test for ALK modification namely Fluorescent in situ hybridization and the immunohistochemistry as suggested in other study. The results showed that out of sixty four samples, fourteen percent were positive for ALK rearrangement by FISH.[11] Selinger *et al*. reported the frequency and clinicopathological characteristics of lung carcinoma having translocation of ALK gene in five hundred ninety four resected non-small cell lung carcinoma among which four hundred seventy adenocarcinomas, eighty three squamous carcinomas, twenty six large cell carcinomas and fifteen other histological subtypes were present using a tissue microarray method. [16]

The clinical and histopathological characteristics of lung carcinoma with the fusion of EML4-ALK gene and the association with ALK expression haven't been established in western population. However, the incidence of ALK gene rearranged in tumors that were surgically resected is 0.45 percent which is less than that reported for Asian groups.[17,18] Rodig *et al*. conducted a study with the purpose of evaluating incidence and characteristics of ALK gene rearrangement among patients having lung adenocarcinoma among western population in comparison to Asian population and evaluated the frequency of ALK gene rearrangement in a cluster of patients belonging to three different institutions and found out that a total of twenty ALK-rearranged genes were identified in lung adenocarcinomas.[19]

Paik *et al*.[20] in his study, consisting of sample size of 735 patient having lung carcinoma, 3.8% patients had ALK rearrangement analyzed by FISH analysis. The patients who had adenocarcinoma had statistically significant *ALK gene* rearrangement with age and gender. Non-Smokers were also found strongly associated with ALK gene rearrangement. Nevertheless, the present study also showed statistically significant relationship between age and ALK gene rearrangement (p<0.05).

Bal *et al*. also found out seven percent cases to be ALK gene modified that were analyzed by immunohistochemistry. He found almost eight percent frequency of ALK gene rearrangement among patients having lung adenocarcinoma.[21] The ALK-rearrangement in lung adenocarcinomas are much more prevalent in Asian population compared to western population. Also the limited number of studies with limited sample size and variation in diagnostic method requires large number of studies to be conducted.

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CONCLUSION

The results showed positive rearrangement of ALK gene in 15% of the patients which is in contrast to 7% patients found in western studies. ALK gene has become standard of care in patients of adenocarcinoma which EGFR negative so that treatment options like ALK gene inhibitors such as alectinib, brigatinib, ceritinib, crizotinib, and lorlatinib can be tailored accordingly.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

BR & GH: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

SS & VRR: Concept, data analysis, data interpretation, critical review, approval of the final version to be published.

FR & MA: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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