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Original Research Paper

Pathology

MOLECULAR PROFILE OF NON-SMALL CELL CARCINOMA LUNG: DIAGNOSTIC UTILITY IN CANCER THERAPEUTICS.

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ABSTRACT

Background: Lung cancer is one of the most common malignancies in the world. About 80–85% of lung cancers are non-small cell lung cancer (NSCLC) patients. The development of therapeutic agents targeting products of epidermal growth factor receptor (EGFR) gene mutation and anaplastic lymphoma kinase (ALK) rearrangements has significantly improved survival in patients with non-small cell lung cancer (NSCLC). Thus, the patients eligible for the treatment should be selected through appropriate molecular tests. **Aims and Objective:** To ascertain the prevalence of lung carcinoma cases, their histological types and the mutations present , in a tertiary care hospital , over a period of 1 year. **Inclusion criteria:** All cases diagnosed as Non small cell Lung carcinoma on histopathology. **Exclusion criteria:** All cases diagnosed as non-malignant on histopathology. **Methodology:** A prospective study was carried out among 228 patients presenting to pulmonology OPD, in a tertiary care hospital. Radiology, Histopathology, Immunohistochemistry and molecular study by PCR technique were used in this study. **Result:** Out of 138 cases of NSCLC on histopathology, 78 cases(56.7%) were reported as Squamous cell carcinoma,46 cases (33.3%)as Adenocarcinoma and 14 cases (10.1%)as Non small cell lung carcinoma, NOS. IHC was used for categorization of NSCLC,NOS cases which resulted in 4 as Adenocarcinoma and 10 as squamous cell carcinoma. EGFR deletion mutation 19 was the predominant mutation in Adenocarcinoma. ROS1 mutation was found in approximately 2.2% NSCLC patients. **Conclusion:** Molecular study for genetic analysis has improved the scope for targeted therapy in Non small cell carcinoma patients, thereby reducing mortality and morbidity in cases of lung carcinoma.

KEYWORDS : Non-small cell lung cancer, Adenocarcinoma, EGFR mutation, ALK mutation, ROS1, smoking.

INTRODUCTION:

Lung cancer is the most common cause of cancer death, with approximately 1.4 million deaths worldwide annually.[1] Owing to the absence of clinical symptoms and effective screening programs, most of the patients have advanced stages or metastatic disease at the time of presentation.[1] Deaths in the USA attributable to lung cancer are more than the next three most common cancers combined.[2]WHO classifies Non-small-cell lung cancer (NSCLC), as adeno carcinoma, squamous cell carcinoma and large-cell carcinoma subtypes –adeno squamous, sarcomatoid carcinoma.[2,3]In the past we have seen increased rate of lung carcinoma among the male population predominantly the smokers.[3]It is well known that smoke exposure can lead to well-characterized series of morphological changes of the bronchial epithelium progressing from basal cell hyperplasia to metaplasia, severe dysplasia to carcinoma in situ and, finally, frank carcinoma.[3] This series of changes is primarily associated with the squamous sub-type of NSCLC. At present Non-small-cell lung cancer (NSCLC) accounts for more than 85% of lung cancers in western countries, with 20–30% of NSCLC occurring in never smokers.[4]

This finding has coincided with the discovery that a proportion of lung cancers occurring in never or light smokers, are driven by oncogenic mutations which, when selectively inhibited, can lead to dramatic tumor regression and prolonged survival.[5] Hence targeted therapies for genes such as EGFR, ALK and KRAS have become the hotspot of attention and study in lung cancer treatment for the last 10 years. In the past these two molecular alterations have been viewed as mutually exclusive, but recent cases of lung cancer with concurrent EGFR mutation and ALK rearrangement have been identified.[5,6] Clinical guidelines have now incorporated molecular testing and the use of drugs targeting those genes.[6] In this paper our aim was to study the distribution of the various histological subtypes of Non small cell Lung carcinoma and spectrum of mutations in non small cell lung carcinoma cases and its use for targeted therapy and better prognosis.

MATERIAL AND METHODS:

Place of study: IPGMER & SSKM Hospital, Kolkata, West Bengal, India.

Period of study: 1 year (From February 2022 to February 2023).

Type of study: Prospective study.

Inclusion criteria: All cases diagnosed as Non small cell Lung carcinoma on histopathology.

Exclusion criteria: All cases diagnosed as non malignant on histopathology.

Method of study: We have done a correlation study based on the observations from radiological, histopathological and molecular study findings of patients reported as Lung carcinoma on biopsy

No statistical analytical methods were used in the study

Procedures opted: Chest X-ray and Contrast enhanced computed tomography (CECT) of lung followed by CT guided

Trucut biopsy of lung lesions. Prior to the biopsy patients' complete hematological profile including coagulation profile (PT/INR) was obtained. Immunohistochemistry (IHC) was done for those cases where categorization of the carcinoma was not possible on histopathological examination alone.

Molecular study by ARMS PCR (AMPLIFICATION RESTRICTED POLYMERASE CHAIN REACTION) technique using the paraffin blocks and fresh tissue was carried out in all the cases of lung carcinoma (detected on histopathology), to detect EGFR mutations.

ALK and ROS1 mutation study was done using Immunohistochemistry.

RESULTS:

Among 228 cases of lung lesions studied,156 cases were reported as Lung carcinoma of which Non small cell was 138 cases(88.5%) and small cell carcinoma were 18 cases(13.05%) (Table 1).

Predominant clinical presentation among the patients was cough, chest pain and breathlessness.

Among 228 patients, male patients were predominant (82.5%) with average age ranging from 46 to 74 yrs. Average age among females ranged from 52 to 67 years.

On histopathological examination alone 78 cases (56.7%) were reported as Squamous cell carcinoma, 46 cases (33.3%) as Adenocarcinoma and 14 cases (10.1%) as NSCLC-NOS. IHC was used for categorization of NSCLC-NOS cases which resulted in 4 as Adenocarcinoma and 10 as squamous cell carcinoma (Table 2).

Male predominance was seen in all cases of NSCLC (3:1 in squamous cell carcinoma and 2.6:1 in Adenocarcinoma cases) (Table 3).

Average age ranged from 63-65 yrs in males and 47-55 yrs in females. Middle aged females with no smoking habit showed increased incidence of Adenocarcinoma.

140 patients out of 156 had a history of smoking for minimum of 11 yrs. (table 4)

Molecular study using PCR technique was carried out in 134 cases of NSCLC, for detection of EGFR mutation. Out of 50 cases diagnosed as Adenocarcinoma, 12 cases (24.0%) showed EGFR deletion mutation of exon 19 and 4 cases (8.0%) showed EGFR deletion mutation of exon 21. 4 cases of morphologically diagnosed squamous cell carcinoma showed EGFR deletion mutation of exon 19 .(table 5)

On Histopathological examination Squamous cell carcinoma showed large islands, broad sheets and infiltrating nests of pleomorphic squamoid cells having large round to oval hyper chromatic nucleus and moderate amount of cytoplasm(Fig1)

Histology showing poorly differentiated malignant cells in large aggregates and sheets was diagnosed as Non small cell lung carcinoma, NOS (Fig2)

Cases diagnosed as Adenocarcinoma shows histological features of malignant epithelial cells arranged in glands, tubules and infiltrating cords (Fig 3)

ALK was positive in 4 cases of adenocarcinoma and 7 cases of squamous cell carcinoma.ROS1 mutation was positive in 2 cases of Adenocarcinoma and 1 case of Squamous cell carcinoma.(Fig4) Immuhistochemistry for TTF1 and Napsin A was positive in Adenocarcinoma cases (Fig 5)

P63 and P40 IHC markers were positive in cases of Squamous cell carcinoma (Fig 6)

On follow up the patients were started on targeted therapy with favorable prognosis.

Table 1: Distribution Of Lung Lesions.

Histology (n=228)	Cases
Small cell carcinoma	18
Squamous cell carcinoma	78
Adenocarcinoma	46
Non small cell Ca(non differentiated)	14
Others(SFT, TB, NHL, METS, Pneumocyte hyperplasia, germ cell tumor)	60
Inconclusive	12

Table 2: Distribution of NSCLC.

NON SMALL CELL CARCINOMA (n=138)	HISTOPATHOLOGY	IHC(TTF1, NAPSIN,P40,P63)
Adenocarcinoma (n=50)	46	
Squamous cell carcinoma (n=88)	78	
NSCLC-NOS	14	Adenocarcinoma- 04 Squamous cell carcinoma- 10

Table 3: Sex distribution of NSCLC

SEX DISTRIBUTION IN NSCLC(n=138)	Adenocarcinoma	Squamous Cell CA
Male	36	66
Female	14	22

Table-4: Smoker vs Non smoker in Lung carcinoma (n=156)

Distribution of smokers	Males	Females
Smoker	100	40
Non smoker	02	14

Table 5: Molecular analysis of NSCLC.

HISTOLOGY	EGFR exon 19	EGFR exon 21	ALK	ROS1
Adenocarcinoma (n=50)	12	04	3	2
Squamous cell carcinoma (n=88)	04	03	4	1

DISCUSSION:

The molecular basis of lung cancer is complex and heterogeneous. The molecular changes (genetic, epigenetic, protein expression) and the improvements in the understanding of the functional expressiveness of these changes have potential effects on the diagnosis, prognosis and treatment of lung cancer.[7]EGFR mutations in NSCLC are seen in the intra cellular tyrosine kinase domain. Deletion of exon 19 is the most common followed by missense mutations, exon 21, a single nucleotide point mutation leading to a single amino acid change from leucine to arginine at codon 858.[5,7] Ture et al in their study of 132 patients of NSCLC found 30.3% (19) cases of lung carcinoma had EGFR exon 19 mutation. Eighteen of these cases were Histologically diagnosed as Adenocarinoma.[6] Marino et al found EGFR mutation in 32.7% cases (336/977) of lung carcinoma in a Chinese population.[3] In our present study we found EGFR mutation in 32% cases (16/50) out of which exon 19 mutation was 24%(12/50) and exon 21 mutation was 8.0% (4/50). Sweis et al in their retrospective study of 20 cases of non-small cell lung carcinoma found the disease control rates in patients treated with EGFR inhibitors was 46%(6/13).[4] Wang et al in their study of 300 cases of NSCLC found ALK rearrangement mutation in 4.33% cases of Adenocarcinoma (13/300).[8] In our study ALK

mutation was found to be positive in 5.2% (7/134) cases.

Won et el in their study screened 1458 patients, out of which 91 patients were selected for molecular study by direct sequencing FISH method. EGFR and ALK mutations were found to be 42.4% and 6.3% respectively. Concomitant mutations were found in 4.4%(4 cases)[9]. Lee T et al in their study found that 12 out of 6637 patients had concomitant mutations.[10] In our study none of the cases showed a concomitant mutation.

Concomitant genetic alteration of NSCLC is unusual because EGFR, KRAS, and ALK mutations are widely known as mutually exclusive. Most are associated with acquired mutation after targeted therapy and are related to drug resistance.[10]

In a meta-analysis of 146 scientific articles ROS1 mutation was found in 1-2% of patients with NSCLC with 11 different fusion partners were isolated[11].Our study demonstrated incidence of 2.2% of ROS1 mutation positive NSCLC. We of course we did not go for fusion partner's study.

Wang et al in their study found the distribution to be squamous cell carcinoma 239 cases (79.67%), adenocarcinoma 36 cases (12%) and adeno-squamous carcinoma 10 cases(3.33%).[8] In our study the distribution of non-small cell carcinoma was found to be Adenocarcinoma 36.23% (50/138) and squamous cell carcinoma 63.7% (88/100). Ture et al and Wang et al in their study found male population predominant in lung carcinoma, 74.2% (98/132) and 59.33% (178/300) respectively.[6,8]In our study we also found 72.7% patients of lung carcinoma were males with an average age of 63.2 yrs.

CONCLUSION:

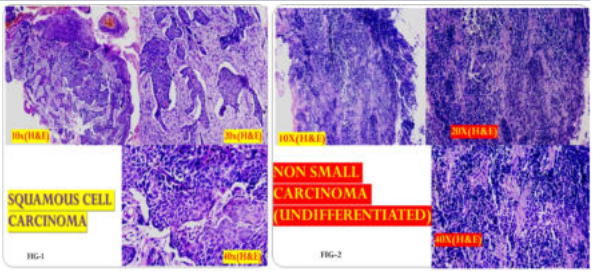
Histomorphological examination and Immunohistochemistry are essential for definite diagnosis of non-small cell lung carcinoma. EGFR mutation, single and concomitant with ALK mutation is the most common genetic alteration seen in patients diagnosed as Adenocarcinoma among Non-small cell carcinoma cases. Males with smoking history with more than ten years are at risk for developing lung carcinoma. The detection of EGFR, ALK and ROS1 mutation has widespread therapeutic implications.

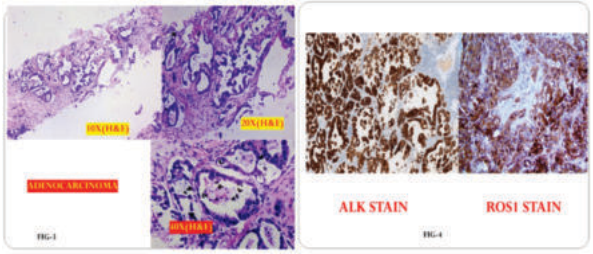
Patients with EGFR mutation positive NSCLC were subjected to EXON18, 19, 20 and 21 mutations. Those who were positive for EXON20 mutations were subjected to platinum doublet therapy. If these subset of patients despite therapy show evidence of clinical or radiological progression. They were added Docetaxel to their therapeutic regimen. In spite of that if they show clinical or radiological progression, they were considered for PDL1 testing. Positive patients were stared on PDL1 inhibitors (Nivolumab, Pembrolizumab or Atezolizumab) or they are subjected to clinical trials.

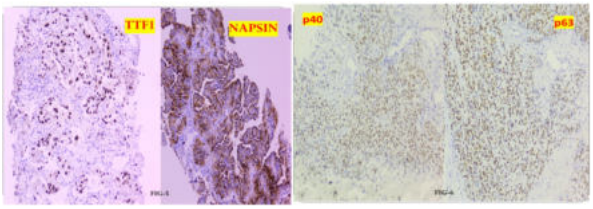
Those who were PDL1 negative were initially planned for allocation to clinical trials or may be given a test therapy of PDL1 inhibitors. The subset of patients who were positive for EXON 18, 19 and 21 mutations are subjected to first generation (Geftinib/Erlotinib) or second generation (Afatinib) EGFR tyrosine kinase inhibitors.

If they clinical or radiological progression were subjected to T790M testing. Those who are T790M positive were subjected to third generation EGFR TKI (Osimertinib). Negative patients were again allocated to platinum doublet therapy group.

Even after starting third generation TKI if there is clinical or radiological progression they are given a trial of platinum doublet therapy. This protocol is followed in our Institute and patients are routinely followed up.







REFERENCES:

1. Casadio C, Guarize J, Dongi S, Tonno CD, Fumagalli C, Vacirca D et al.Molecular Testing for Targeted Therapy in Advanced Non–Small Cell Lung Cancer. Suitability of Endobronchial Ultrasound Tran bronchial Needle Aspiration. *Am J Clin Pathol* 2015; 144:629-34.
2. Lindeman N, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al.Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors.*Arch Pathol Lab Med*2013; 137:828–860.
3. Marino FZ, Ronchi A, Accardo M, Franco R.Concomitant ALK/KRAS and ALK/EGFR mutations in non small cell lung cancer: different profile of response to target therapies.*Transl Cancer Res* 2017; 6(Suppl 3):S457-S460.
4. Sweis RF, Thomas S, Bank B, Fishkin P, Mooney C, Salgia R. Concurrent EGFR Mutation and ALKTranslocation in Non-Small Cell Lung Cancer. *Cureus*2016; 8(2): e513.
5. Sculier JP. Nonsmall cell lung cancer. *Eur Respir Rev* 2013; 22: 33–36.
6. Sag SO, Gorukmez O, Ture M, Gorukmez O, Deligonul A, Sahinturk S et al. Spectrum of EGFR gene mutations and ALK rearrangements in lung cancer patients in Turkey. *SpringerPlus* 2016; 5:482.
7. Forde PM, Ettinger DS.Targeted therapy for non-small-cell lung cancer: past, present and future. *Expert Rev Anticancer Ther*2013; 13(6): 745–758.
8. Wang S, Mao N, Zuo C, Pan H, Xie T, Huang Y et al.A study of EGFR wild-type non-small cell lung cancer ALK genetic mutation. *Transl Cancer Res* 2017;6(5):976-980.
9. Won JK, Keam B, Koh J, et al. Concomitant ALK translocation and EGFR mutation in lung cancer: a comparison of direct sequencing and sensitive assays and the impact on responsiveness to tyrosine kinase inhibitor. *Ann Oncol* 2015; 26:348-54.
10. Lee T, Lee B, Choi YL, et al. Non-small Cell Lung Cancer with concomitant EGFR, KRAS, and ALK Mutation: clinicopathologic features of 12 Cases. *J Pathol Transl Med* 2016; 50:197-203.
11. Rica Zinsky: Metaanalysis of ROS1-positive lung cancer cases: European Respiratory Journal 2016 48:64.
12. Thunnissen E, Noguchi M, Aisner S, et al. Reproducibility of histopathological diagnosis in poorly differentiated NSCLC: an international multiobserver study.J Thorac Oncol 2014;9(9):1354–62.
13. Hasanovic A, Ang D, Moreira AL, et al. Use of mutation specific antibodies to detect EGFR status in small biopsy and cytology specimens of lung adenocarcinoma.Lung Cancer 2012;77(2):299–305.
14. Seo AN, Park TI, Jin Y, et al. Novel EGFR mutation-specific antibodies for lung adenocarcinoma: highly specific but not sensitive detection of an E746_A750 deletion in exon 19 and an L858R mutation in exon 21 by immuno histochemistry.Lung Cancer 2014;83(3):316–23.
15. Mino-Kenudson M, Chirieac LR, Law K, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res 2010;16(5):1561–71.
16. Li Y, Pan Y, Wang R, et al. ALK-rearranged lung cancer in Chinese: a comprehensive assessment of clinicopathology, IHC, FISH and RT-PCR. PLoS One 2013;8(7):e69016.
17. Sholl LM, Sun H, Butaney M, et al. ROS1 immunohistochemistry for detection of ROS1-rearranged lung adenocarcinomas. Am J Surg Pathol 2013; 37(9):1441–9.
18. Thunnissen E, van der Oord K, den Bakker M. Prognostic and predictive biomarkers in lung cancer. A review. Virchows Arch 2014;464(3):347–58.
19. Linardou H, Dahabreh IJ, Bafaloukos D, et al. Somatic EGFR mutations and efficacy of tyrosine kinase inhibitors in NSCLC. Nat Rev Clin Oncol

2009;6(6):352–66.
20. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for nonsmall-cell lung cancer with mutated EGFR. N Engl J Med 2010; 362(25): 2380–8.
21. Li CM, Chu WY, Wong DL, et al. Current and future molecular diagnostics in nonsmall-cell lung cancer. Expert Rev Mol Diagn 2015;15(8):1061–74.
22. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350(21):2129–39.