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Original Article

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Epidermal Growth Factor Receptor Mutation Frequency in Squamous Cell Carcinoma and Its Diagnostic Performance in Cytological Samples: A Molecular and Immunohistochemical Study

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Abstract

Background: Epidermal growth factor receptor (EGFR) mutation is the most frequent mutation tested in lung cancer for targeted therapy in the era of personalized medicine. Knowledge about EGFR mutation is constantly expanding regarding its frequency, clinicopathological association, advancements in testing methodology and sample requirement. We investigated EGFR mutation frequency in non-small cell lung cancer (NSCLC) in North Indian patients and evaluated its diagnostic performance in cytological samples.

Methods: Molecular EGFR testing was done in 250 cases of NSCLC by both real-time polymerase chain reaction (PCR) (Therascreen) and mutation-specific EGFR immunohistochemistry (IHC). Thirty cases had both cytology samples and biopsy including 20 pleural effusions and 10 fine-needle aspirates. EGFR mutation concordance between pleural effusion and biopsy was studied.

Results: EGFR mutation was overall 31.6% in NSCLC with 36.5% in adenocarcinoma and 15% in squamous cell carcinoma. L858R mutation accounted for 50.7% and DEL19 for 39.3% of total EGFR mutations. Complex mutations were seen in 2% of cases. Sensitivity of mutation-specific EGFR IHC was 48.3% and specificity was 92.3%. L858R showed higher sensitivity (55% vs. 33.3%) but similar specificity (93.2% vs. 91.3%) compared to DEL19. EGFR mutation was successful in 95% of pleural effusion and showed 83.3% concordance with tissue biopsy.

Conclusions: EGFR mutation frequency in North Indian patients was comparable to that of Asia-Pacific region and showed a similar pattern of histological distribution. EGFR mutation in squamous cell carcinomas is increasingly recognized which was 15% in our study. Mutation-specific EGFR IHC shows variable but generally low sensitivity and considering its significant pre- and post-analytical variables, it should be highly discouraged in patient management. Cytological samples may not only serve as suitable alternative but may be complementary to tissue biopsies.

Keywords: EGFR; Pleural effusion; Mutation-specific IHC; Squamous cell carcinoma; NSCLC

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Introduction

Lung cancer is the most common cancer as well as the leading cause of cancer-related death. Over the years, understanding of lung cancer biology has evolved and the histological classification has been supplemented with molecular classification. Targetable driver mutations with development of oral tyrosine kinase inhibitors (TKIs) have changed the management of non-small cell lung cancer (NSCLC) dramatically. Of all the targetable mutations, epidermal growth factor receptor (EGFR) mutation is the most frequent and important marker in terms of management particularly in adenocarcinoma where TKIs have resulted in improved overall and progression-free survival with better tolerance compared to systemic chemotherapy.

The EGFR mutations have a wide prevalence all over the world being more prevalent in Asians [1-3]. Though EGFR mutations have been reported largely in lung adenocarcinoma, in the recent years, it is being increasingly reported in squamous cell carcinoma (SCC) as well in Asians, particularly in Chinese. Studies from Caucasians have reported very low rate of EGFR mutation in SCC (0-3%) [4, 5], while it is reported in the range of 0-19.2% in Chinese [3, 6, 7].

The early studies for EGFR mutation used mutation-specific EGFR immunohistochemistry (IHC) against two mutations (L858R, and DEL19) to detect EGFR mutations in NSCLC. These two mutations account for 80-90% of to-

tal EGFR mutations. However, IHC results have shown lower sensitivity with both false-positive and false-negative results [8-10]. Intra-tumoral heterogeneity in IHC staining as well as pre-analytical and analytical variability is the major problems with EGFR mutation-specific antibodies. According to the current recommendations molecular methods such as quantitative real-time polymerase chain reaction (qPCR) is the method of choice for detecting EGFR mutations; however mutation-specific IHC is advised only for small biopsies having scant tumor cells where molecular testing is not feasible. In resource poor centers, mutation-specific EGFR IHC is still a viable option for guiding patient management [11].

One of the other issues relevant to EGFR mutation is that of sample type. Since large number of cases are detected in late stages particularly with effusion or poor patient performance scores, accessing tissue biopsy or even fine-needle aspirate cytology (FNAC) for EGFR testing becomes difficult. Studies are being done on malignant pleural effusion and peripheral blood samples to detect targetable mutations in NSCLC. Comparison studies between malignant pleural effusion and biopsy samples have shown good concordance between the two suggesting effusion samples to be an alternative of tissue biopsy in advanced stage NSCLC patients [12-15].

We studied the concordance of EGFR mutation by qPCR and mutation-specific EGFR IHC, as well as its feasibility in malignant effusion samples.

Materials and Methods

All patients with NSCLC after histological/cytological diagnosis, from November 2015 to April 2019, were tested for EGFR. Histological or cytological diagnosis was reviewed and IHC for TTF1, P63 and CK5/6 was used wherever necessary.

Paraffin blocks of endobronchial biopsies, cell blocks of fine-needle aspirates (FNAs) or effusion fluids were used to extract DNA using QIAamp extraction kit (Qiagen, Hilden, Germany). EGFR testing was done by qPCR using Qiagen Therascreen kiton QuantStudio 6 Flex real-time thermal cycler (Life Technologies, Carlsbad, CA, USA) according to the recommended protocol. Briefly, in the first step, samples were tested with internal control provided with the kit. Once the amplification was successful, then in second step primer, probes for 29 different mutations were tested in eight wells for each patient. ΔCt method was used to detect EGFR mutation according to the recommended cut-offs provided.

Mutation-specific IHC was used for exon 19 deletion (E746-A750) and L858R in exon 21 from Cell Signalling Technologies (Danvers, MA, USA) (dilution, 1:50; clone, 6B6 and 43B2 respectively). Membranous staining of 2+ (strong incomplete membranous) or 3+ (strong complete membranous) was considered as positive staining.

The EGFR mutation concordance between biopsy and effusion samples and that between qPCR and mutation-specific IHC were evaluated. Categorical variables were analyzed by Chi-square test and survival was analyzed by Kaplan Meier log rank test using SPSS version 20.

The study was approved from the Institute's Ethics Committee vide letter no. IEC-2016-01-IMP-89. It was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration and informed consent was obtained from the patients.

Results

Five hundred thirty patients were diagnosed as NSCLC (adenocarcinoma, SCC, NSCLC-not otherwise specified (NOS)), of whom 250 patients were tested for EGFR mutation. EGFR testing was done based on tissue adequacy, patient's willingness for molecular testing and receiving anti-EGFR therapy. There were 175 men and 75 women with an age range of 26 - 87 years (mean: 59.3 years, median: 60 years). Pleural effusion was present in 99 patients of whom 45 (45.5%) had malignant effusion. Biopsies alone were available in 205 patients, FNAC alone in eight patients and malignant effusion alone was available in 16 patients. In another 21 patients, both biopsy and malignant effusion (paired samples) were available. Mutation-specific IHC was done in 112 patients.

Of the 226 biopsies including 21 paired samples, adenocarcinoma comprised of 169 cases (74.8%) followed by 43 cases of SCC (19.5%), 10 cases of NSCLC-NOS (4.4%) and three cases of adenosquamous (1.3%). Thirty-six of the above cases were NSCLC-NOS of which 22 cases turned out to be adenocarcinoma, and four as SCC. Rest remained as NSCLC. Additionally only cytological material was present in 24 cases of which 16 were pleural effusion and eight were transbronchial FNAs. This included 20 (83.3%) adenocarcinoma, three (12.5%) SCC and one (4.2%) NSCLC-NOS. So finally including both histology and cytology samples, there were 189 cases of adenocarcinoma, 46 cases of SCC and 11 cases of NSCLC.

EGFR mutation

EGFR mutation was found in 31.6% (79/250) of all patients with NSCLC. The two most common mutations, L858R (40 cases, 50.7%) and DEL19 (31 cases, 39.3%) together accounted for 90% of all mutations. Other mutations comprised of G719X (four cases, 5%), T790M (two cases, 2.5%) and INS (two cases, 2.5%). Five cases harbored double EGFR mutations. Of these four were adenocarcinoma which showed L8585R + INS in two cases, L858R + DEL19 in one case and DEL19 + G719X in one case. The fifth case was that of SCC harboring L858R + T790M mutation.

EGFR mutation was higher in women (40.5%) than in men (28.3%) (P value = 0.05) and in young patients with age ≤ 40 years (47% vs. 30.7%, P value = 0.1). Patients with pleural effusion (38.8% vs. 27.5%, P value = 0.06) or malignant pleural effusion (43.2% vs. 18.2%, P value = 0.1) also had higher EGFR mutation rate. Smoking was present in 115 patients of whom 36.8% were mutant whereas 63.2% of non-smokers showed EGFR mutation (P value = 0.05) (Table 1).

Adenocarcinoma showed 36.5% (69/189) EGFR mutation followed by 15% (7/46) in SCC, 27.3% (3/11) in NSCLC and none (0/3) of the three cases of adenosquamous carcinoma (P

Table 1. EGFR Genotype With Clinicopathological Parameters

Features	EGFR mutant (n = 79)	EGFR wild (n = 171)	P value
Age			
≤ 40 years (n = 17)	8 (47.0%)	9 (53%)	0.1
> 40 years (n = 233)	71 (30.5%)	162 (59.5%)	0.1
Gender			
Men (n = 175)	49 (28%)	126 (72%)	0.06
Women (n = 75)	30 (40%)	45 (60%)	0.06
Smoking			
Present (n = 115)	29 (36.8%)	86 (50.3%)	0.05
Absent (n = 135)	50 (63.2%)	85 (49.7%)	0.05
Pleural effusion			
Present (n = 99)	38 (38.4%)	61 (61.6%)	0.06
Absent (n = 151)	41 (27.2%)	110 (72.8%)	0.06
Malignant effusion			
Present (n = 45)	19 (42.2%)	26 (57.8%)	0.1
Absent (n = 12)	2 (16.7%)	10 (83.3%)	0.1
Histology			
Adenocarcinoma	69 (36.5%)	120 (63.5%)	0.02
Squamous cell carcinoma	7 (15%)	39 (85%)	0.02
NSCLC-NOS	3 (27.3%)	8 (72.7%)	0.02
Adenosquamous carcinoma	0	4 (100%)	0.02

value = 0.02). Similar to adenocarcinoma, L858R was the frequent mutation in SCC followed by DEL19, G719X and INS. Thus, EGFR mutation pattern in both the histological types was largely similar.

Mutation-specific EGFR IHC

Mutation-specific EGFR IHC was done in 112 cases for L858R and DEL19 of which 29 cases were mutant for L858R (20 cases) and DEL19 (nine cases) on qPCR whereas mutant-specific IHC was positive for L858R in 19 cases and DEL19 in 10 cases. Mutation-specific IHC correctly picked up 11/20 (55%) cases of L858R and 3/9 (33.3%) cases of DEL19 (Table 2). The sensitivity and specificity of mutation-specific EGFR antibodies were calculated considering the molecular testing as gold standard. Both the antibodies together showed a sensitivity of 48.3% and specificity of 92.3% with L858R IHC having greater sensitivity (55% vs. 33.3%) but nearly similar specificity (93.2% vs. 91.3%) than DEL19 IHC (Table 3).

EGFR testing in cytological specimens

EGFR was tested in 30 cases having paired biopsy and cytology samples (pleural effusion: 20 cases, FNA: 10 cases). Of the

Table 2. Comparison Between EGFR Mutation by Real-Time PCR and Immunohistochemistry

Real-time PCR (n = 112)	Mutation-specific antibody immunohistochemistry		
	L858R (n = 19) (43B2)	DEL19 (n = 10) (6B6)	Wild (n = 87)
L858R (n = 20)	11[a]	02[a]	09
DEL19 (n = 9)	01[b]	03[b]	06
G719X (n = 3)	00	01	02
T790M (n = 2)	01	00	01
INS (n = 1)	00	00	01
Wild (n = 76)	06[c]	04[c]	68

[a]Two cases of L858R were positive for both mutation-specific antibodies; [b]One case of DEL19 was positive for both mutation-specific antibodies; [c]One case of wild type was positive for both mutation-specific antibodies.

Table 3. Sensitivity and Specificity of Mutation-Specific EGFR Antibodies

Mutation-specific antibodies	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)
L858R (n = 112)	11	84	08	09	55	91.3
DEL (n = 112)	03	96	07	06	33.3	93.2
Total	14	180	15	15	48.3	92.3

10 cases having FNA and biopsy, EGFR mutation was detected in 2/10 cases in biopsy (L858R: one case, DEL19: one case) and 4/10 cases in FNA with two additional cases (L858R: two cases, DEL19: two cases). In 20 cases with pleural effusion and biopsy, one case had very scant malignant cells and did not amplify; however it was successfully amplified in biopsy sample. Another case that did not show amplification on biopsy was successful on effusion sample. Thus, mutation testing in effusion samples was successful in 95% (19/20) cases. The concordance rate of EGFR mutation between biopsy and effusion (18 paired cases) was 83.3% (15/18) as effusion samples showed mutation in three extra cases (16.7%) (3/18) (L858R: one, G719A: two), where biopsy was wild, giving a higher mutation detection rate in effusion sample (64.7%) as compared to biopsy (47%) (Table 4). Thus considering all the cytological samples together, EGFR mutation was detected in 10/30 (33.3%) biopsy samples and 15/30 (50%) of cytological samples.

Follow-up was available in 228 cases ranging from 2 - 53 months with a mean follow-up duration of 7.6 months and median of 4 months. Fifteen deaths were recorded. In EGFR wild patients mean and median survival was 6.8 and 4 months whereas in EGFR mutant it was marginally higher with 9.4 and 5.5 months (P value = 0.5) respectively.

Discussion

Lung cancer bears the load of cancer worldwide with approximately 11.6% incidence and 18.4% cancer-related mortality. In India, nearly 67,800 new cases and 48,700 deaths related to lung cancer were reported in 2018 according to the GLOBOCAN 2018 India factsheet [16]. The role of EGFR TKIs in patients with NSCLC harboring EGFR mutations has emerged as a key oncogenic outcome and EGFR status has become a major prognostic factor in lung cancer. A study by Midha et al as well as those by others [2, 8, 17-23] have shown EGFR mutation frequency to be highest in Asia-Pacific region averaging 47% but with a very wide range of 20-76% in different parts of this region. They also showed that the wider variability of EGFR mutation status was prevalent within each country of the specific regions of world.

India is a culturally and ethnically versatile country. In the present study from northern part of India which caters patients largely from the state of Uttar Pradesh and its neighboring states such as Bihar, Orissa and country like Nepal showed an overall EGFR mutation frequency of 31.6% in NSCLC, irrespective of histology, which is similar to the previously published EGFR mutation frequency reported from Asia-Pacific region. Other studies from India which have been mostly reported from south and central parts of India had shown EGFR mutation ranging between 16-43% [24-26] with two studies from North India itself reported by Maturu et al [25] and Kasana et al [26] showed EGFR mutation frequency of 16.6% and 35.1% respectively. This shows that the genetic changes are influenced by various environmental factors, geographical variations, ethnicity and lifestyle habits. From the Asia-Pacific region, Taiwan shows highest EGFR mutation of 57% (range 36-76%), while Singapore shows the lowest frequency of 40% (range 39-43%). However, in the extreme south-east Australia lowest EGFR mutation frequency ranging between 7-36% [27, 28] was reported. The widest range of EGFR mutation in NSCLC has been reported from South America ranging between 9-67% with an overall frequency of 36% [2, 29, 30]. Reports from Europe also show great variability with mutation frequency ranging between 7.3-40.8% (Table 5) [4, 8, 17-27, 29-40].

The combined mutation frequency of DEL19 and L858R was 89.8% similar to the published literature; however we found L858R to be higher than DEL19 with 50.6% and 39.2% respectively. Similar frequency of higher L858R mutation than DEL19 has been reported by Kim et al (53.3%, 40.3%) and Yotsukura et al (56.3%, 40%) [8, 17]. Complex mutations with more than one type of EGFR mutations were found in 5/250

Table 4. Comparison of EGFR Mutation Between Biopsy and Malignant Effusion Specimen

Biopsy specimen (n = 20)	Malignant effusion specimen (n = 20)						
	L858R	DEL19	T790M	G719A	INS	Wild	Unsuccessful
L858R	5	-	-	-	-	-	1
DEL19	-	3	-	-	-	-	-
T790M	-	-	-	-	-	-	-
G719A	-	-	-	-	-	-	-
INS	-	-	-	-	-	-	-
Wild	1		-	2	-	7	-
Unsuccessful	-	-	-	-	-	1	-

Table 5. Frequency Distribution of EGFR Mutation in Non-Small Cell Lung Cancer

Area	Authors	Country	Year	EGFR mutation (%)
Europe	Leduc et al [32]	France	2017	37
	Isaksson et al [27]	Sweeden	2013	11
	Sarosi et al [33]	Hungary	2016	9.8
	De Greve et al [34]	Belgium	2016	27
	Gervas et al [31]	Serbia	2015	19
	Chatziandreou et al [4]	Greece	2015	10.6
	Schmid-Bindert et al [35]	Germany	2013	40.8
	Schmid et al [36]	Austria	2009	7.3
	Arrieta et al [37]	Mexico	2015	27.0
	Martin et al [38]	Canada	2016	29.7
	Lopez-Chavez et al [39]	USA	2016	21.9
South America	de Castro et al [29]	Brazil	2017	17
	Arrieta et al [30]	Argentina	2011	18
Asia-Pacific	Zhou et al [19]	China	2017	34.9
	Yotsukura et al [17]	Japan	2017	46.9
	Rahman et al [18]	Japan	2014	22.9
	Kim et al [8]	Korea	2015	40.3
	Toh et al [20]	Singapore	2010	39
	Chang et al [21]	Taiwan	2007	76.5
	Shi et al [22]	Vietnam	2014	64
Australia	Cooper et al [23]	Australia	2013	14.7
	Sriram et al [40]	Australia	2011	7.1
India	Kasana et al [26]	India	2016	35.1
	Maturu et al [25]	India	2016	16.6
	Doval et al [24]	India	2013	25.9

(2%) cases. Complex EGFR mutations have also been reported in the similar range (2-3.4%) in other studies [28, 41-43].

EGFR mutation frequency in NSCLC irrespective of any region has shown a consistent association with female gender. In the present study, 40% women and 28% men showed EGFR mutation which is similar to other studies like 28% versus 19% in North America, 22% versus 9% in Europe, 60% versus 37% in Asia-Pacific region and 48% versus 8% in Africa in women and men respectively [2]. Liu et al from China [13] (54.95% men, 71.6% women) and Hsu et al from British Columbia (19% men, 24.5% women) also reported similar female predilection for EGFR mutation [43]. Other studies from India have also shown similar female predominance in EGFR mutation [1, 17]. A study from Bangladesh reported an exception to this pattern where Rahman et al reported higher EGFR mutation rate in men than women (25.5% men, 14.3% women) [18].

Histology versus mutation

EGFR mutation has been largely associated with adenocarcinoma; however, in the last few years EGFR mutation is consistently being reported in SCC, both in endobronchial biopsies where it may be considered as component of adenosquamous carcinoma and in pure resected specimens with squamous histology proven by IHC. In the present study EGFR mutation was 36.5% in adenocarcinoma and 15% in SCC. L858R was also more frequent (6.4%) in SCC followed by DEL19 (4.6%), G719X (2%) and INS (2%). Studies from China and Korea have reported EGFR mutation in SCC ranging from 2-19% [6, 7] whereas reports from American and European cohorts have shown lower frequency of SCC ranging from 0-2% [5, 44, 45]. Han et al reported 9.9% and 3.7% EGFR mutation in SCC and 49.3% and 18% mutation in adenocarcinoma from Asia-Pacific region and Russia respectively [3]. EGFR mutation in SCC varies between 0-25% in different regions of the world [6, 29, 31].

EGFR mutation by mutation-specific antibodies

EGFR mutation detection by using mutation-specific IHC for exon 19 deletion (E746-750) and exon 21 (L858R) point mutation are recommended for very small biopsies which have insufficient tumor cells for molecular testing. However, it is still

Table 6. Sensitivity and Specificity of Mutation-Specific EGFR IHC With Molecular Testing

Author	Country	Year	Sensitivity (DEL19 + L858R)	Specificity (DEL19 + L858R)
Brevet et al [9]	USA	2010	84	98.9
Kitamura et al [47]	Japan	2010	47	96
Seo et al [10]	Korea	2014	76.6	89
Kim et al [8]	Korea	2015	75.6	94.5
Zhang et al [46]	China	2016	83.7	98.6

practiced in low resource centers due to unavailability of infrastructure and expertise. The sensitivity and specificity of EGFR mutation vary widely between different studies (sensitivity 47-84%, specificity 89-99%), which may be due to the inherent technical problems of IHC [8-10, 40, 46-47]. In the present study EGFR mutation-specific IHC was available in 112 cases, of which 19 cases showed L858R mutation with a sensitivity and specificity of 55% and 91.2% respectively. DEL19 was detected in 10 cases by IHC with a sensitivity and specificity of 33.3% and 93.1% respectively. The overall specificities for the two mutation-specific antibodies have been reported to be high (77-100%); however the sensitivity has been quite low (47-87.7%). Sensitivity for E746-A750 deletion on IHC is 40-100% and for L858R point mutation is 36-100% [8-10]. The E746-A750 specific antibody detects the common 15 base pair deletion, which accounts for only 66-81% of E746-A750 deletion missing out approximately 20-34% of the exon 19 deletion [8, 9, 40, 47]. Other reasons for low expression may be the different cut-offs of IHC pattern and intensity of expression considered used in different studies. Most studies have included incomplete (2+) or complete (3+) membranous staining as positive whereas a few others have included all grades of staining (1+, 2+ and 3+ pattern) as positive. The interobserver variability in interpretation of IHC is also one of the important reasons of variable sensitivity and specificity. Ragazzi et al showed the sensitivity and specificity for DEL19 (E746-750) IHC as 56% and 100%, respectively and sensitivity and specificity for exon 21 (L858R point mutation) as 70.4% and 89%, respectively [45]. Zhang et al from China included all grades of staining as positive (1+ to 3+ staining) and showed a sensitivity and specificity of 99.6% and 99.3%, respectively for L858R whereas low sensitivity (86.0% and 82.7% respectively) for DEL19 (Table 6) [8-10, 46, 47]. The overall sensitivity and specificity of mutation-specific EGFR IHC were 48.3% and 92.2% in the present study. Our IHC results of DEL19 (E746-750) gave more inconsistent results than for L858R. DEL19 IHC showed more commonly cytoplasmic staining most of the time with either weak or moderate intensity and was not crisp giving a blurred transition from positive to negative stained field whereas in L858R, the staining was crisper and more intense with well delineated difference between positive- and negative-stained area.

EGFR mutation in cytology samples

Mutation detection in effusions has several potential advantages over analysis of tissue biopsies. First, tumor samples from patients with lung cancer are often limited, particularly when obtained from bronchial washings or fine-needle aspiration. These samples are used mainly for pathological diagnosis, frequently leaving insufficient numbers of cancer cells to be tested for an increasing number of targetable genomic abnormalities in addition to EGFR mutations, like ALK and ROS1. Because of the risks of complications from procedures used to obtain a tissue diagnosis and the frequent requirement for prompt initiation of therapy in patients with metastatic lung disease, some patients with targetable mutations may not be exposed to the effective drugs. The current standard for detection of EGFR mutations is tissue biopsy; however, obtaining tissue is one of the challenges in management of lung cancer. Cytological specimen particularly pleural effusion or a metastatic neck lymph node is valuable minimally invasive sample for EGFR mutation testing in advanced stage NSCLC patient. Though there is smaller chance of DNA degradation in cytology samples, the poor cellularity remains a major concern for false-negative tests particularly in effusion samples.

EGFR mutation was detected in 10/30 (34.5%) biopsy samples and 15/30 (51.7%) of cytological samples in the present study. The EGFR mutation was successfully detected in 95% of pleural effusion. In both paired biopsy and FNA samples, mutation detection rate was 20% (2/10) and 40% (4/10) respectively, while in the paired biopsy and pleural effusion it was 61.1% (11/18) and 44.4% (8/18) respectively. Three cases (16.7%) showed wild EGFR genotype on biopsy but a mutant genotype effusion with L858R in one and G719A mutations in other two cases. This discrepancy could be either because of heterogeneity of tumor cells or presence of very scant mutant cells in the biopsy (< 5%) that were not picked up by qPCR. Liu et al showed a sensitivity of 81.8% and specificity of 80% for EGFR mutation detection in effusion sample with tissue biopsy sample [12]. Davies et al had successful EGFR mutation analysis in nine of 10 (90%) cases with malignant pleural effusion [14]. Liu et al studied 192 paired malignant effusion and biopsy and found a higher mutation rate in biopsy of 62% compared to effusion of 58.9%. They found concordance between biopsy and effusion in 87% cases similar to the present study [13]. Guan et al also showed higher mutation rate on biopsies of 34% compared to 30% on effusions with a concordance of 88.2% [15]. Although the mutation rate in different studies was somewhat higher in biopsies than in effusions, the present study showed opposite results; however no statistical difference was noted in any of the studies. Malignant effusion samples are easier to obtain in patients with advanced stage of disease as minimally invasive technique. The DNA quality also remains

superior to biopsy which has the disadvantage of formalin fixation. Thus malignant effusions may be considered complementary to biopsies in management of NSCLC patients.

Conclusions

The present study shows EGFR mutation from a tertiary care referral academic institution in northern part of India with 36.5% in adenocarcinoma and 31.6% in overall NSCLC similar to that reported from Asia-Pacific region. Female gender predilection for EGFR mutation is also similar to the worldwide data. Our observations were different from the reported studies regarding frequencies of DEL19 and L858Rmutations. We found L858R mutation higher than DEL19 which has been reported in few studies from Japan, China and Korea. EGFR mutation in SCC has been largely reported from Asia-Pacific region. The present study also showed 15% EGFR mutation in SCC on the similar trend. Although role of anti-EGFR therapy in SCC is still in its naive phase, there are fair evidences for trials in this histological group. The current international guidelines do not recommend EGFR mutation-specific IHC except for cases where tumor tissue is insufficient for molecular studies, but still it is practiced in centers with limited resources. Considering the variable but low sensitivity of mutation-specific IHC with a lot of analytical variables involved, it is not suitable for routine use. Cytological samples in any form such as effusion fluids or FNAs are good alternatives to tissue biopsies in cases where biopsies are not accessible. In the present study, cytological samples yielded a better mutation yield than biopsy which may be due to better preservation of DNA as well as absence of contaminating non-tumorous cells and supporting tissues which could be there in biopsies. Cytological samples should be used as complementary to tissue biopsy rather than as alternative where both are available because there is always a chance of tumor heterogeneity in these tumors.

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Conflict of Interest

None.

Informed Consent

Informed consent was obtained from the patients.

Author Contributions

N Kumari, N Krishnani, SS, AN and ZN contributed to conception and design of the study; N Kumari, DH, SKM, SS, AN and ZN contributed to acquisition of data; N Kumari, DH, and SKM were involved in analysis and interpretation of data; N Kumari, DH, and N Krishnani contributed to drafting and revising the article; N Kumari, N Krishnani gave final approval of the version to be submitted.

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