CHARACTERISTICS OF THE PATHOLOGICAL, IMMUNOHISTOCHEMICAL FINDINGS AND *EGFR* GENE MUTATION ON BIOPSIES IN 193 LUNG CANCER PATIENTS

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SUMMARY

Objectives: Pathological classification of lung cancer is based on the expression of immunohistochemical markers, and evaluating the characteristics of EGFR gene mutations of lung cancers. Subjects and methods: The cross-sectional, descriptive, retrospective and prospective study of 193 patients, 150 males and 43 females, who were diagnosed with lung cancers on lung tumor surgical specimens, bronchoscopy biopsies, transthoracic biopsies, and the cell blocks obtained by pleural fluid at 103 Military Hospital, from April 2014 to June 2018. The new WHO pathological classification of lung cancer (2015) was applied, as well as the EGFR gene mutation was analyzed. Results: Patients with mean age of 61.48 ± 10.88, male/female ratio: 3.49/1. 4 pathological types were determined: adenocarcinoma (62.7%), squamous cell carcinoma (21.8%), small cell carcinoma (8.3%) and carcinoid tumor (3.1%). The panel of TTF-1, p63, CK5/6 was valuable in the differential diagnosis of adenocarcinoma and squamous cell carcinoma with sensitivity over 70%. The panel of synaptophysin, chromogranin, NSE was valuable in the diagnosis of small cell carcinoma, carcinoid tumor with high sensitivity. 54.55% of these cases had an EGFR mutation which mainly appeared on adenocarcinoma (83.33%), and 19th deletions (56.68%). Conclusion: Immunohistochemistry is valuable to identify the pathological classification of lung cancers on biopsies. EGFR gene mutations are mainly found on adenocarcinoma, and on 19th exon.

** Keywords: Lung cancer; EGFR mutations; Pathological diagnosis; Immunohistochemical markers.*

INTRODUCTION

Lung cancers are the common malignant tumor and have a high mortality rate in both sex. According to Globocan (2012), there are 1.8 million new cases of lung cancer in the world, and 1.59 million deaths each year. In addition to surgical specimens, small biopsies specimens are important in cases of old age, failure, or no indication of surgery. However, pathological diagnosis is difficult to determine the type of lung cancers based on hematoxylin - eosin (H.E) of bronchoscopy biopsy specimens, transthoracic biopsies, or the cell blocks. Thus, further investigation of immunohistochemical characteristics is needed to determine the nature of the tumor. The treatment of cancer in general and lung cancer, in particular, are more and more interested in targeted treatments.

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Result of histopathological diagnosis and gene mutation characteristics in lung cancers are essential for clinicians to have more accurate indications for treatment and prognosis. Therefore, we conducted this study with the two following aims:

- *Pathological classification of lung cancer is based on the expression of immunohistochemical markers on small biopsy specimens and on lung tumor surgical specimens.*

- To evaluate the characteristics of EGFR gene mutations of lung cancer.

SUBJECTS AND METHODS

1. Subjects.

193 patients were diagnosed with lung cancer based on lung tumor surgical specimens, bronchial endoscopic biopsies, transthoracic biopsies, and the cell blocks obtained by pleural fluid at the Department of Pathology, 103 Military Hospital from April 2014 to June 2018.

2. Methods.

- The cross-sectional, descriptive, retrospective and prospective study.

- The new WHO pathological clasification of lung cancer (2015) was applied. Immunohistochemistry was used throughout the classification process. 193 patients diagnosed with lung cancer that have not yet identified the type of pathology on H.E staining specimens directed to two large groups of non-small cell and small cell carcinoma.

+ 177 cases of non-small cell carcinoma were stained with immunohistochemical markers CK7, CKAE1/AE3, TTF-1, p63, CK5/6, Napsin A.

+ 16 cases of small cell carcinoma were stained with immunohistochemical markers such as CK7, CKAE1/AE3, TTF-1 and other neuroendocrine markers such as NSE, chromogranin, synaptophysin.

- There were 55 cases with 48 cases of adenocarcinoma and 7 cases of other types that had the potential to test *EGFR* gene mutations.

** Data analysis:* Statistical analysis was performed by using SPSS 22.0 for Window. Results were expressed by variables average values, percentage (%) and showed in figures and tables. The statistically test is valid when $p < 0.05$.

RESULTS AND DISCUSSION

Table 1: Distribution of patients by age, sex (n = 193).

Gender / Age	Male	Female	Total
30 - 39	3	2	5
40 - 49	13	9	22
50 - 59	44	12	56
≥ 60	90	20	110
Total	150	43	193
The mean age	62.29 ± 10.32	58.67 ± 12.38	61.48 ± 10.88
p	0.055		

The average age was 61.48 ± 10.88, the lowest was 31, the highest was 92. Our results were higher than those of previous authors such as Pham Nguyen Cuong (2014) with mean age was 57.6 ± 8.6 years.

An incidence of lung cancer increased with the age of the patients, and over the age of 50 accounted for 86% of the patients. The morbidity rate increased with the age of the patients, and the highest rate was age group > 60 with 110/193 cases accounting for 56.99%, the remaining 43.01% patients aged ≤ 60 years. This result was consistent with Pham Van Luan's (2017) study of 320 non-small cell lung cancer patients with 62.2% (> 60), and 37.8% (≤ 60), respectively [3]. In terms of sex, male was predominantly with a male/female ratio of 3.49/1, lower than the study results of authors in recent years such as Pham Van Luan (2017): over 320 lung cancer patients had a 4/1 ratio [3].

Table 2: Histopathology of lung cancer and the expression of immunohistochemical markers.

Type / Marker	AC		SCC		SmC		Carcinoid tumors		ACC		LCC		ASC		NDC	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
TTF-1	99	5	3	34	11	3	4	0	1	1	0	1	1	0	0	1
CK7	97	1	15	19	3	9	0	1	1	1	0	1	1	0	1	0
CKAE1/AE3	20	0	18	0	9	1	4	0	1	0						
p63	10	75	19	15	1	6	1	1	2	1	0	1	1	0	0	1
CK5/6	2	65	18	6			0	1	1	1	0	1	1	0	0	1
Napsin A	48	8	2	10	0	4			1	0	1	0	1	0		
Synap	2	0			1	1	1	2	1	0						
Chomogranin	0	3	0	2	11	4	2	2								
NSE	0	5	0	3	8	4	4	0								
Total	121 (62.7%)		42 (21.8%)		16 (8.3%)		6 (3.1%)		3 (1.6%)		1 (0.5%)		1 (0.5%)		3 (1.6%)	

(AC: Adenocarcinoma; SCC: Squamous cell carcinoma; SmC: Small cell; NDC: Non-differentiated carcinoma; ACC: Adenoid cystic carcinoma; LCC: Large cell carcinoma).

Adenocarcinoma accounted for 62.7%, squamous cell carcinoma (21.8%), small cell carcinoma (8.3%), carcinoid tumors (3.1%), non-differentiated carcinoma (1.6%), adenoid cystic carcinoma (1.6%), and large cell carcinoma (0.5%).

The expression of immunohistochemical markers of 121 cases of adenocarcinoma

with TTF-1, CK7, CKAE1/AE3, Napsin A, p63, CK5/6 were: 95.19%, 98.98%, 100%, 85.71%, 11.76%, 2.99%, respectively.

The expression of immunohistochemical markers of 42 cases of squamous cell carcinoma with TTF-1, CK7, CKAE1/AE3, p63, CK5/6 were: 8.11%, 44.12%, 100%, 55.88%, 75%, respectively.

The expression of immunohistochemical markers of 16 cases of small cell carcinoma with TTF-1, CK7, CKAE1/AE3, synaptophysin, chromogranin, NSE were respectively: 78.57%, 25%, 90%, 50%, 73.33%, 66.67%.

The expression of immunohistochemical markers of 3 cases of adenoid cystic carcinoma with TTF1, CK7, CK5/6, p63 were all 50%.

3 cases of non-differentiated carcinoma were positive with CK7, negative with TTF-1, CK5/6, p63 markers.

1 case of large cell carcinoma was negative with all for TTF-1, CK7, p63, CK5 / 6, only positive for Napsin A.

1 case of adenosquamous carcinoma was positive with all for TTF-1, CK7, p63, CK5/6, and Napsin A. According to a study by Pham Nguyen Cuong (2014), the number of adenocarcinomas was high (67.1%), while squamous cell carcinoma ranked second (11.4%), non-differentiated carcinoma ranked third (6.4%), others accounted for a low ratio [1]. According to a study by Montezuma et al (2013), 325 cases which were diagnosed primary lung carcinomas, 198 cases of adenocarcinoma (44.7%), 127 cases (28.7%) were squamous cell carcinoma and 40 cases (9%) were non-small cell carcinoma with no further classification; 10 cases (2.3%) were classified as unknown original adenocarcinoma, 9 cases (2%) were squamous cell carcinoma [5].

Thus, although the results of different types of lung cancers of authors were different, the current adenocarcinoma is more dominant than the squamous cell carcinoma, the other types are very rare.

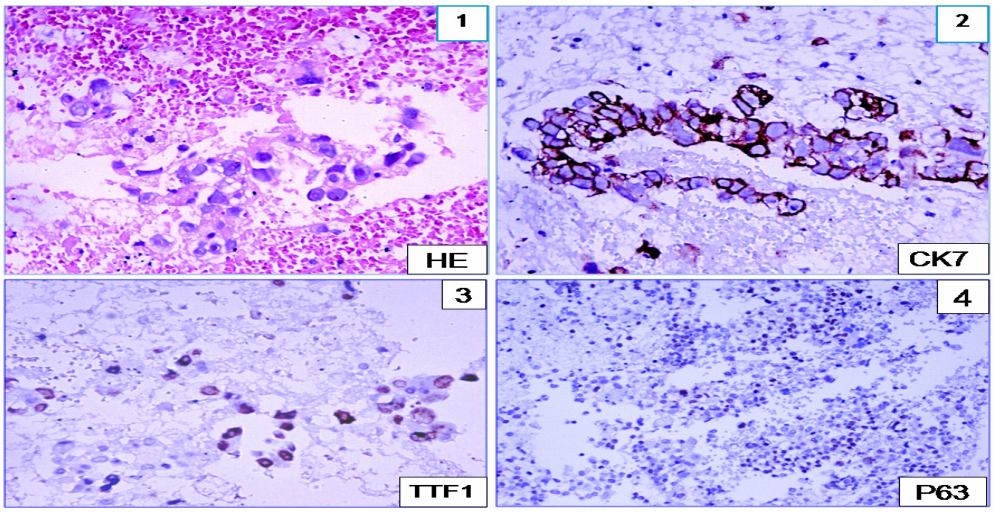

Figure 1: Adenocarcinoma (number of specimens: R1810).

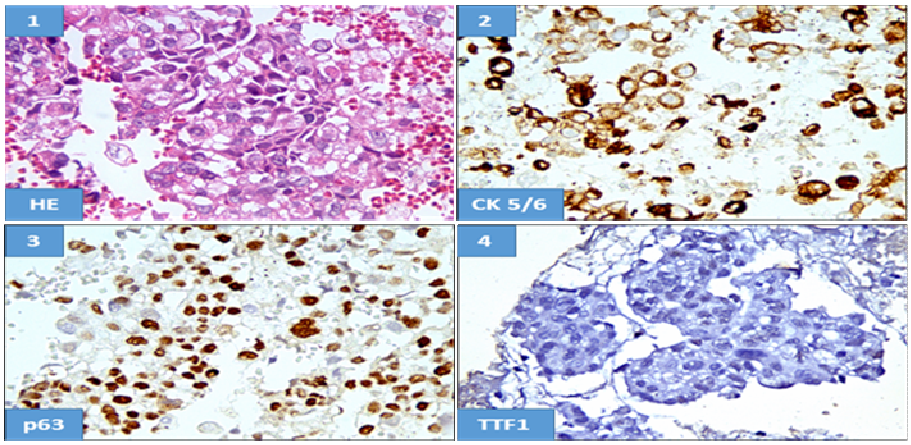

Figure 2: Squamous cell carcinoma (number of specimens: R1815).

Table 3: Distribution of immunohistochemical markers according to histopathology.

Marker \ Type	AC	SCC	SmC	Carcinoid tumors	ACC	LCC	ASC	NDC
TTF-1	95.19	8.11	78.57	100	50	0	100	0
CK7	98.98	44.12	25	0	50	0	100	100
CKAE1/AE3	100	100	90	100	100			
Napsin A	85.71	0	0		100	100	100	
p63	11.76	55.88	14.29			0		
CK5/6	2.99	75			50	0	100	0
p63 (+), CK5/6 (+)	0	16.67			50			
TTF-1 (+), p63 (-), CK5/6 (-)	85	0						
TTF-1 (-), p63 (+), CK5/6 (+)	0	52.38						
Synapto Physin			50	33.33				
Chromo Granin			73.33	50				
NSE			66.67	100				
Chromo (+), NSE (+)			45.45	100				

TTF-1 had a high sensitivity in adenocarcinoma that was 95.19%. Sensitivity was lower in small cell carcinoma with 78.57%. In squamous cell carcinoma, the sensitivity of TTF-1 was very low (8.11%). The result was suitable with Pham Nguyen Cuong's study (2014) [1]: lung adenocarcinoma had a high

positive rate with TTF-1 (71.8%), followed by endocrine carcinoma (60%), very low in squamous cell carcinoma.

CK7 (+) had a high sensitivity of 98.98% in adenocarcinoma that was similar to Pham Nguyen Cuong (2014) [1]; however, there was a difference in the incidence of squamous cell carcinoma between 44.12% of us and 81% of this author.

CKAE1/AE3 (+) was very sensitive to all types of lung cancers from 90.00% in small cell carcinoma, up to 100% in adenocarcinoma, squamous cell carcinoma, carcinoid tumors.

When combined p63 (+) with CK5/6 (+) in squamous cell carcinoma, the sensitivity was 16.67%. None of the cases were positive for all in adenocarcinoma. According to Kagi et al, Pham Nguyen Cuong (2014) found that p63, CK5/6 were high in squamous cell carcinoma, and very low in other types of carcinoma which is significant in distinguishing between squamous cell carcinoma and other types [1]. According to Argon (2015), TTF-1, CK5/6, p63 have been shown to be useful in the differential diagnosis of adenocarcinoma and squamous cell carcinoma with high sensitivity from 87% to 100% [6].

Synaptophysin, NSE, and chromogranin have gradually increased sensitivity tor small cell carcinoma (50%, 66.67% to 73.33%, respectively). These results were lower than Le Trung Tho's (2007) study, which conducted on 50 small cell carcinoma cases on a large biopsy specimen, the positive rate were 60%; 100% and 88%, respectively [4]. Similarity, in Tarvinder K Taneja's study (2004), the hight expression rate of synaptophysin, chromogranin, NSE on small cell carcinoma showed the neuroendocrine source of this tumor [7]. Synaptophysin, chromogranin, and NSE had gradually increased in carcinoid tumor that were 33.33%, 50%, 100%, respectively.

Table 4: EGFR gene mutation of lung cancers by pathological type (n = 55).

***EGFR* gene mutations** / **Type**	**Number of tests**	**Number of cases**	**Range (%)**	**p**
Adenocarcinoma	48	25	52.1	< 0.05
Squamous cell carcinoma	5	3	60.0	
Non-differentiated carcinoma	2	2	100	
Total	55	30	54.55	

There were 55 cases of lung cancer tested for EGFR mutation, 54.55% of which had mutations and mainly in type adenocarcinoma (52.1%), squamous cell carcinoma (60%), in particular, 2 cases of non-differentiated carcinoma had *EGFR* gene mutations. This finding was higher than the study by Mai Trong Khoa (2016): 40.1% of patients with the non-small cell lung cancer had mutations [2], and Pham Van Luan (2017): 320 cancer patients with non-small cell lung had mutations with rate of 39.4% [3].

In the research, of which 54.55% had mutations and mainly in type adenocarcinoma (52.1%), squamous cell carcinoma (60%),

in particular, 2 cases of non-differentiated carcinoma had *EGFR* gene mutations, with p > 0.05. Pham Van Luan's research (2017) also showed similar results with 42.6% of adenocarcinoma cases had *EGFR* gene mutations, while the others were only 28.2% [3]. Mai Trong Khoa's research (2016) had a lower rate (41.2% of patients had this mutation in adenocarcinoma, while the others were positive with 25.7%) [2]. These results of us may be due to the smaller sample size of EGFR mutation.

Table 5: Mutative position on *EGFR* gene (n = 30).

Location	Number	Range (%)	p
19th exon	17	56.68	< 0.05
21st exon	10	33.33	
19th exon + 20th exon	1	3.33	
21st exon + 20th exon	2	6.66	
Total	30	100	

Mutations on the 19th exon in 17 cases accounted for the majority (56.68%). On the 21st, there were 10 mutative cases, accounted for 33.33%, that similar to the results of Pham Van Luan (2017): there were 60.3% of mutations occurring in 19th exon and the less common mutations were 18th, 20th, 21st exon [3]. Especially, 1 case had both mutation on the 19th and 20th exon (3.33%) and 2 cases of both 20th exon and 21st exon (6.66%) (p < 0.05). Previously, in the study by Mai Trong Khoa (2016), the rates of mutations in 19th and 21st exons were 56.4% and 37.4%. There were 4/211 cases in the 20th exon. The T790M accounted for 1.9% [2].

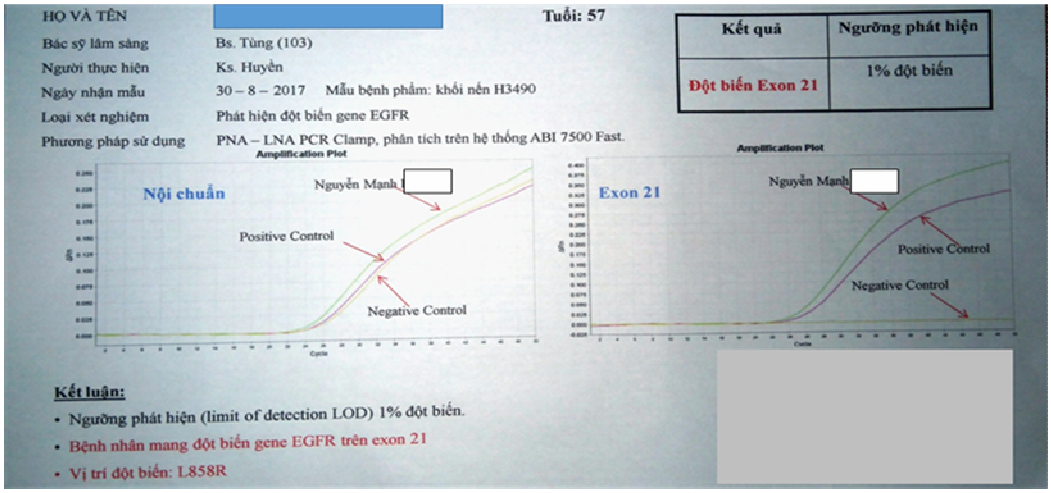

Figure 3: Mutation test results of the *EGFR* gene of patient (Nguyen M.H.) showed a mutation in 21st exon.

CONCLUSION

Adenocarcinoma was the highest with 62.7%, followed by squamous cell carcinoma accounting for 21.8%; small cell carcinoma (8.3%), carcinoid tumors (3.1%), non-differentiated carcinoma (1.6%), adenoid cystic carcinoma (1.6%), large cell carcinoma (0.5%), and the last is adenosquamous carcinoma (0.5%).

- Using TTF-1, CK5/6, p63 markers to distinguish adenocarcinomas or squamous cell carcinoma has high sensitivity. The same results when using the panel of synaptophysin, chromogranin, NSE to identify the group of neuroendocrine tumors.

- The rate of *EGFR* gene mutation was 54.55%, type of adenocarcinoma was higher than other types (44.28%). 19th exon accounted for the highest (56.68%).

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