Archives of Razi Institute, Vol. 77, No. 1 (2022) 95-100

Copyright © 2022 by
Razi Vaccine & Serum Research Institute
DOI: 10.22092/ARI.2021.356552.1870

Original Article

Immunohistochemical and Electron Microscopy Evolution in the Diagnosis of Lung Cancer in Iraq

Saleem Abd Alkreem Alsaqi, S[1] *

1. Department of Anatomy, College of Medicine, University of Babylon, Babylon, Iraq

Received 15 October 2021; Accepted 10 November 2021
Corresponding Author: med.sabreen.saleem@uobabylon.edu.iq

Abstract

Lung cancer is considered the most commonly diagnosed cancer that kills over 1.76 million people yearly around the world. This study aimed to investigate how electron microscopy (EM) and immunohistochemistry (IHC) worked together. In the current study, a total of 50 tissue blocks were used which were embedded in paraffin wax from 20 healthy controls and 30 lung cancer patients (19 males and 11 females) to conduct a histopathological examination, which included hematoxylin and eosin stain, IHC detection regarding the protein expression in P53, and EM for lung cancer diagnosis. The findings indicated that the mean age of the patients was 51.84±15.70 years, and there was no correlation between the age of patients and the disease stage. P53 expression was measured using an immunohistochemical technique, while the positive rate showed a highly significant difference between healthy controls and patients ($P \leq 0.05$). The association of P53 expression with age and gender was not significant, although it showed a significant correlation with stage and grade. Furthermore, a correlation was found between P53 IHC and EM ($P \leq 0.05$). In conclusion, the detection of an immunohistochemical method of lung cancer patients has been (gold standard) useful in detecting the subsets of patients in addition to a good method of EM in the diagnosis of lung cancer patients.

Keywords: Electron microscopy, Immunohistochemical, Lung adenocarcinoma, Prognostic factors

1. Introduction

Despite the breakthroughs in the treatment and diagnosis, lung cancer is considered the leading cause of cancer mortalities globally, which is responsible for nearly 1.6 million fatalities yearly; its incidence rate is constantly growing in developed nations (1). Lung cancer is classified into two types of non-small cell lung cancer (NSCLC), affecting the majority of the lung cancer patients (about 80%), and small cell lung cancer (SCLC), affecting approximately 15% of the lung cancer patients. The most prevalent kind is NSCLC (2). Now, women have a low rate of lung cancer, compared to men (3). Historically, men smoked tobacco at a higher rate, compared to women, with high rates of mortality and incidence (4). Because of the biological factors, such as DNA damage over time and shorter telomeres linked to cancer development in older people, the median age of the lung cancer diagnosis is 70 years for both women and men (5).

Tobacco cigarettes are the single biggest risk factor for lung cancer, accounting for up to 90% of lung cancer cases (1). Immunohistochemistry (IHC) is important for classifying tumors into sub-types and evaluating biomarkers for making accurate and prompt therapy decisions (6). As a result, molecular-specific IHC techniques offer a lot of potential as screening methods for detecting drug-able genetic changes and evaluating biomarkers for molecular-targeted therapy (7). Despite the latest breakthroughs in immuno-cytochemistry, electron microscopy (EM) remains the

"gold standard" for determining the differential diagnosis of the epithelial cell (8). This sub-type, which is the most frequent, is also the most complicated to distinguish from metastatic adenocarcinoma or peripheral lung at the light microscopic level. The unique morphology of its microvilli is the primary distinguishing feature regarding the neoplastic epithelial cells in epithelial and mixed forms of mesotheliomas at ultra-structural level (9). This study aimed to investigate how EM and IHC worked together in lung cancer diagnosis.

2. Materials and Methods

The presented study was conducted in the laboratories of Hila Teaching Hospital, Babylon, Iraq, from November 2020 to April 2021. Out of a total of 50 samples, 30 tissue blocks embedded in paraffin wax have been acquired from 30 patients (19 males and 11 females) experiencing lung adenocarcinoma; moreover, pathologic and clinical information was also acquired, including the operation type, gender, age, and smoking history. On the other hand, 20 samples were obtained from healthy volunteers as the controls.

2.1. Histopathological Examination Study

Sections were obtained with 5-µm thick from paraffin-embedded tissues. These sections have been stained with the use of hematoxylin and eosin through a staining technique. In addition, the tissue was fixed in buffered formalin of 10%, frequently processed, and embedded in paraffin. Furthermore, the sections have been stained with eosin and hematoxylin (10).

2.2. Immunohistochemical Staining

For IHC staining, 5-µm-thick sections have been de-paraffinized and placed on +ve charged slides, and p53 Monoclonal Mouse antibody (anti-human primary antibody) Abcam (Cambridge, UK) was used for the detection of p53 proteins. The antibody datasheet was code 3214, and the recommended dilutions were 1/500. The atomic protein articulation of p53 was scored by (6).

1 – Score 0: >10% +ve recoloring of malignant cells considered –ve and

2 – +1 score: >25% Score, +2: >50% positive (moderate), and +3 score: >50% positive (strong)

2.3. Electron Macroscopically Study

The samples were arranged for EM evaluation in the Faculty of Science, University of AL-Kufa, Kufa, Iraq. According to the preparation procedure for EM, the following protocol was utilized. Representative sections of paraffin blocks were taken for ultra-structural evaluation and put in xylene for a total of 30 min, with numerous changes for removing paraffin at room temperature. After that, the sections were put for 20 min in graded alcohol (absolute, 90%, 70%, and 50%) to bring them to water, and subsequently, they were placed in the Sorensen's buffer for another 20 min. The sections have been then embedded in epoxy resin after being post-fixed in 1% osmium tetroxide. Furthermore, the ultra-thin sections have been put on copper grids, which were stained by lead citrate and uranyl acetate.

2.4. Statistical Analysis

The statistical analysis was performed in SPSS software (version 23), and the results were expressed as mean±SD through the Chi-square test. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Clinicopathologic Characteristic in Patients and Controls

This study was conducted on 19 male and 11 female patients, while the controls included 11 males and 9 females. The mean age of the patients was estimated at 51.84±15.7 years, and the median age of them was obtained at 54 years (age range: 17-70 years). On the other hand, the mean age of the controls was 39.266±21.43, and the median age of them was estimated at 38.50 years (age range: 20-70) years. The male/female ratio of the patients has been 2:1, while that of the controls was estimated at 1.75:1. There was no statistical difference between

the patients and controls regarding the male/female ratio (P>0.05). Regarding the site of the tumor, 7 patients had a right-sided lung tumor, accounting for 56.7%, while 13 patients had a left-sided lung tumor, accounting for 43.3%. Considering the grade of tumor, 16 (53.3%), 12 (40%), and 2 (6.7%) patients showed grades I, II, and III, respectively. Furthermore, 14 (46.7%), 13 (43.3%), and 3 (10%) patients were in stages I, II, and III of lung carcinoma, respectively (Table 1 and Figure 1). According to the results of Kendall's tau-b Test, there was no correlation between stage and grade of cancer (r=0.474, P=0.116).

Table 1. Clinicopathologic characteristics in patients

Variables	n (%)
Age	**(Mean±SD) (years)**
Patients	51.84±15.70
Controls	39.26±21.43
Gender	
Male	19 (63.30)
Female	11 (36.70)
Site of tumor	
Right-sided	16 (56.7)
Left-sided	14 (43.3)
Grade of tumor	
Well differentiated	16 (53.3)
Moderately differentiated	12 (40)
Poorly differentiated	2 (6.7)
Stage of tumor	
Stage I	14 (46.7)
Stage II	13 (43.3)
Stage III	3 (10)

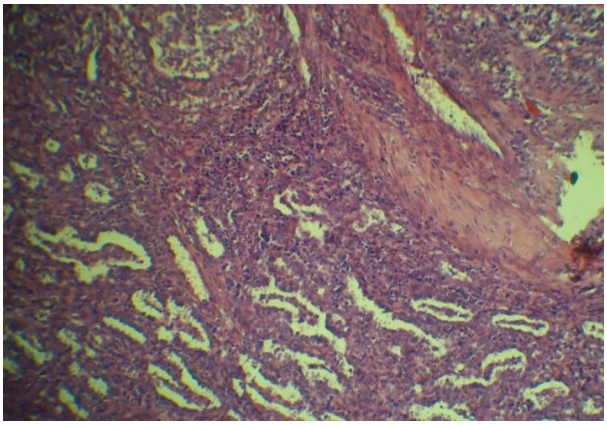

Figure 1. Histological section of the lung showing grade II differentiated adenocarcinoma with almost micropapillary; H & E (400×)

3.2. Immunohistochemical Study of the Expression of p53

The results of the IHC expression of p53 showed significant differences between patients and healthy controls in terms of p53 positive rate (P<0.001). A positive p53 rate was obtained at 44% in patients with lung carcinoma, whereas it was estimated at 0% in the controls. There was no correlation between gender and p53 carcinoma (P> 0.05). The patients with right-sided cancer showed the highest rate of p53 expression (70.6%); however, the results of this study showed a significant association between the grade of tumor and p53 expression (P>0.05). On the other hand, no correlation was found between the stage of disease and p53 expression (P>0.05) (Figures 2 and 3, Table 2).

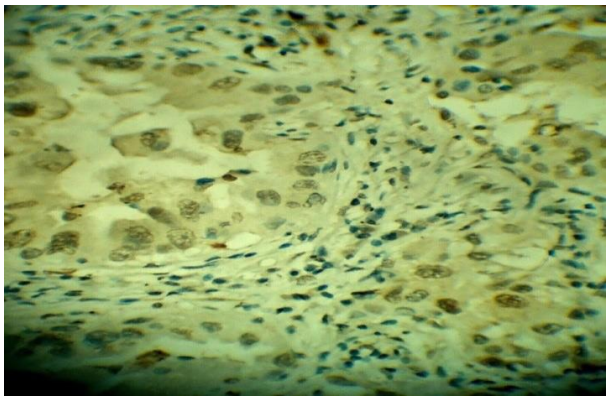

Figure 2. Immunohistochemical section of the p53 expression in the form of brown nuclear stain score 2 (100×)

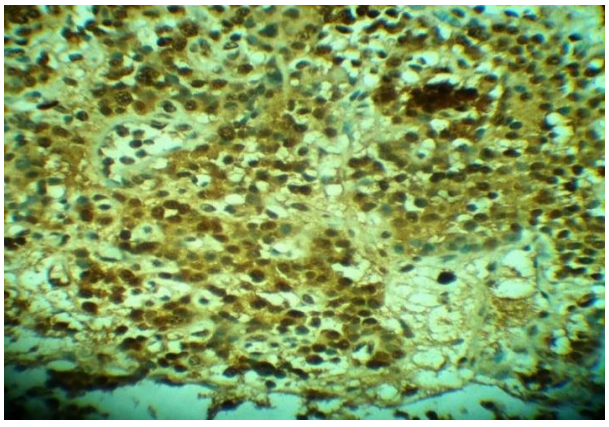

Figure 3. Immunohistochemical section of the p53 expression in the form of brown nuclear stain score 3 that is > 50% of the cell (100×)

Table 2. The associated p53 of Immunohistochemical with pathological features

Immunohistochemical of P53					
		Negative	**Positive**	**Total**	**P-value**
Gender	Male	7 (36.8%)	12 (63.2%)	19	0.979
	Female	4 (36.4%)	7(63.6%.)	11	
Site	Left	6(46.2.1%)	7 (53.8 %)	13	0.346
	Right	5(29.4 %)	9 (70.6 %)	17	
Stage	I	9(64.3%)	5 (35.7%)	14	0.012
	II	2 (15.4%)	11 (84.6%)	13	
	III	0 (0%)	3 (100%)	3	
Grade	I	11 (68.8%)	5 (31.3%)	16	0.001*
	II	0 (0%)	12 (100%)	12	
	III	0 (0%)	2 (100%)	2	

3.3. Electron Microscope Results

EM of the lung tissue showed a normal structure of the tissue appears with a simple squamous layering of the cell grow along the alveolar wall, while the cell surfaces were peculiarly smooth without cellular projections (Figure 4). Moreover, this figure illustrates the micrograph cell carcinoma of the lung view of the disordered pattern of the growth that is the characteristic of this tumor. Figure 5 shows the central area of the cluster cell containing cellular and blood cells in grade III. According to the results of Kendall's tau-b Test, a correlation was found between IHC and EM (P=0.04, r=0.816).

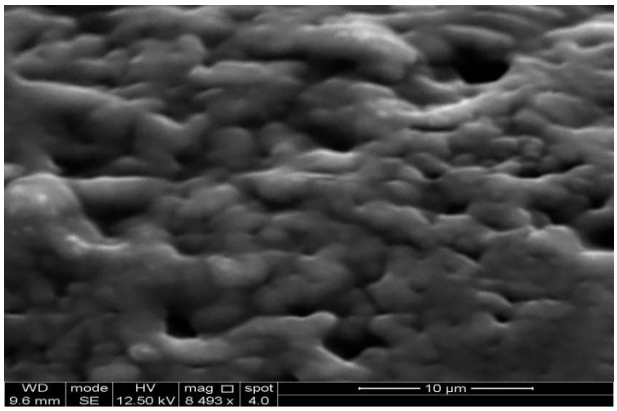

Figure 4. Scanning electron micrograph showing individual cells with ruffled cell surface in grade II

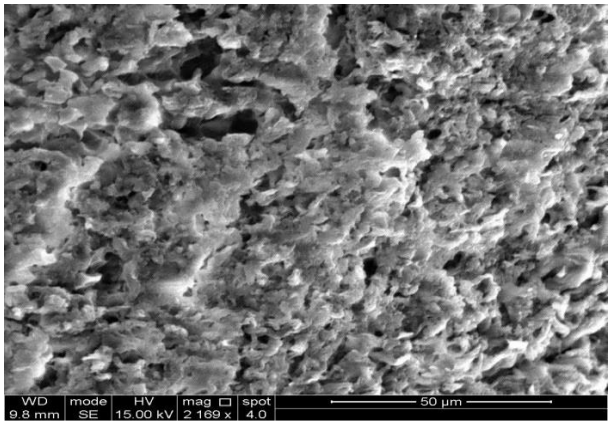

Figure 5. Scanning electron micrograph showing the central area of the cluster cell containing cellular and blood cells in grade III

4. Discussion

According to the results, the mean age of the patients with carcinoma was 51.8 4+15.70 years, and the median was obtained at 52 years (age range: 14-79 years). The best possible explanation for the discrepancy of age at diagnosis is some differences in racial/ethnic characteristics which may be attributed to a complicated interaction between genetic and lifestyle factors (5, 11). Another reason can be related to a higher mortality rate due to cardiovascular events among young smokers. On the other hand, most patients with lung carcinoma were more than 50 years of age.

Mousawi (12) expresses that smoking is the main source of lung disease, and the explanations behind the predominance of smoking in Iraq are people and social conditions that advance smoking, accounting for 85%-90% of the lung malignant growth patients. The male to female ratio has been 2.4:1 in the present study. One of the hypotheses is that smoking, DNA capacity repair, and alcohol consumption, as well as hormonal factors, such as estrogens, (another hypothesis), have been shown to play a role in an increase in the male to female ratio (13, 14). Habib, Al-Asadi (15) showed that the *ER* gene polymorphisms were related to lung adenocarcinoma in never smokers. The present study showed that p53 immunohistochemical expression was not correlated with age, grade, and stage of tumor in patients with lung carcinoma. To explain these results, three groups of the hotspot mutations (group1), the nonsense mutations (group2), and strongly positive missense mutations (group3) were investigated. Accordingly, group 2 obtained the absence of immune labeling due to the non-appearance of the gene product at an early age (16).

In previous studies, Dubinski, Leighl (17) showed the IHC expression of p53 that was typically interpreted to likely indicate a p53 gene mutation. An IHC analysis has the ability to rapidly detect an altered protein that has been produced by the mutant gene, even though it is neither 100% sensitive nor 100% specific. In a study performed by Gibbons, Byers (18), it was shown that the relationship between smoking and TP53 mutation in lung adenocarcinoma was high, compared to other tumors. IHC, along with EM, plays a significant role in the pulmonary adenocarcinoma classification. They have shown the benefits of ultra-structural analysis in terms of resolution in the pulmonary adenocarcinoma classification. In tumors that are unclassifiable after ultra-structural examination, the sample size for EM is still a limiting factor (19, 20). This study presents the gross microscopic findings and detailed ultra-structural features of 30 cases of lung adenocarcinoma and compares the diagnosis based on IHC and EM features based on the ultra-structural findings. Moreover, the ultra-structural findings in a given case were compared with that of EM both in the well-differentiated and poorly-differentiated areas to document the presence of regional differences**.**

In conclusion, the significance of EM in lung adenocarcinomas classification is highlighted in this study. Immunohistochemical and light microscopic observations suggest the direction of differentiation, which is validated via ultra-structural data. In the case when IHC is ambiguous, EM can offer crucial information in sub-typing lung adenocarcinoma. In the case of lung adenocarcinoma, EM could be used as a supplement to clinical decision-making.

Authors' Contribution

Study concept and design: S. S. A. A. A.
Acquisition of data: S. S. A. A. A.
Analysis and interpretation of data: S. S. A. A. A.
Drafting of the manuscript: S. S. A. A. A.
Critical revision of the manuscript for important intellectual content: S. S. A. A. A.
Statistical analysis: S. S. A. A. A.
Administrative, technical, and material support: S. S. A. A. A.

Ethics

All the procedures are approved by the Department of Anatomy, College of Medicine, University of Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. Cancer J Clin. 2016;66(1):7-30.
2. Le Péchoux C. Role of postoperative radiotherapy in resected non-small cell lung cancer: a reassessment based on new data. Oncologist. 2011;16(5):672.

3. Shamseddine A, Saleh A, Charafeddine M, Seoud M, Mukherji D, Temraz S, et al. Cancer trends in Lebanon: a review of incidence rates for the period of 2003–2008 and projections until 2018. Popul Health Metr. 2014;12(1):1-8.
4. Miller KD, Goding Sauer A, Ortiz AP, Fedewa SA, Pinheiro PS, Tortolero-Luna G, et al. Cancer statistics for hispanics/latinos. Cancer J Clin. 2018;68(6):425-45.
5. Torre LA, Siegel RL, Jemal A. Lung Cancer Statistics. In: Ahmad A, Gadgeel S, editors. Lung Cancer and Personalized Medicine: Current Knowledge and Therapies. Cham: Springer International Publishing; 2016. p. 1-19.
6. Thunnissen E, Allen TC, Adam J, Aisner DL, Beasley MB, Borczuk AC, et al. Immunohistochemistry of pulmonary biomarkers: a perspective from members of the Pulmonary Pathology Society. Arch Path Lab. 2018;142(3):408-19.
7. Mino-Kenudson M. Immunohistochemistry for predictive biomarkers in non-small cell lung cancer. Transl Lung Cancer Res. 2017;6(5):570.
8. Hammar SP, Bockus DE, Remington FL, Rohrbach KA. Mucin-positive epithelial mesotheliomas: a histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing pulmonary adenocarcinomas. Ultrastruct Pathol. 1996;20(4):293-325.
9. Henderson D. Transmission electron microscopy of malignant mesothelioma. MSA Bull. 1993;23:288-97.
10. Falkeholm L, Grant CA, Magnusson A, Möller E. Xylene-free method for histological preparation: a multicentre evaluation. Lab Invest. 2001;81(9):1213-21.
11. Ausubel F, Brent R, Kingston R, Moore D, Seidman J, Smith J, et al. Current Protocols in Molecular Biology. New York: John Willey & Sons. Inc; 1994.
12. Mousawi AA. The prevalence of smoking among Karbala/Iraq university students in Iraq in 2005. Tob Use Insights. 2014;7:12238.
13. Babacan NA, Yucel B, Kilickap S, Seker MM, Kacan T, Olcas IK, et al. Lung cancer in women: a single institution experience with 50 patients. Asian Pac J Cancer Prev. 2014;15(1):151-4.
14. Chen K-Y, Hsiao C-F, Chang G-C, Tsai Y-H, Su W-C, Chen Y-M, et al. Estrogen receptor gene polymorphisms and lung adenocarcinoma risk in never-smoking women. J Thorac Oncol. 2015;10(10):1413-20.
15. Habib OS, Al-Asadi JN, Hussien OG. Lung cancer in Basrah, Iraq during 2005-2012: Incidence and time trend. Saudi Med J. 2016;37(11):1214.
16. Lee SH, Kim H, Kim WY, Han HS, Lim SD, Kim WS, et al. Genetic alteration and immunohistochemical staining patterns of ovarian high-grade serous adenocarcinoma with special emphasis on p53 immnnostaining pattern. Pathol Int. 2013;63(5):252-9.
17. Dubinski W, Leighl NB, Tsao M-S, Hwang DM. Ancillary testing in lung cancer diagnosis. Pulm Med. 2012;2012.
18. Gibbons DL, Byers LA, Kurie JM. Smoking, p53 mutation, and lung cancer. Mol Cancer Res. 2014;12(1):3-13.
19. Clavé S, Gimeno J, Muñoz-Mármol AM, Vidal J, Reguart N, Carcereny E, et al. ROS1 copy number alterations are frequent in non-small cell lung cancer. Oncotarget. 2016;7(7):8019.
20. Righi L, Graziano P, Fornari A, Rossi G, Barbareschi M, Cavazza A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. Cancer. 2011;117(15):3416-23.