Detection of Relationships of Immunohistochemical, Molecular and Electron Microscopy Study of EGFR of Mutation in NSCLC in Patients

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Abstract

Background: Mutations in the gene EGFR can lead to expression of mutant EGFR proteins that accumulate in tumor tissues and is responsible for approximately 1.76 million deaths each year worldwide. The aim of this study by found relationships of EGFR mutation status NSCLC between mutation-specific immunohistochemistry (IHC), molecular biology and electron microscopy. Methods: 30 tissue blocks embedded in paraffin wax of NSCLC patients were evaluated for (11 females and 19 males) while controls were 20 samples from healthy, detection of protein expression in by EGFR IHC and used Molecular study, electron microscopy. Results: Our study results have find the mean age of patients group was 51.84±17.70 years was don study shows no correlation between age of patients with the stage,site and grade of tumor, while recorded in immunohistochemical method highly significant difference between EGFR expression with age, gender, grade and stage tumor, we found not correlation between the immunohistochemical of EGFR protein and genetic study in NSCLC but its correlation with electron microscopy in ($P < 0.05$). Conclusion: Our results indicate the presence of relationships clear between Clinicopathologic characteristic in patients of immunohistochemistry EGFR and Electron Macroscopically but were found not clear role with Molecular study of NSCLC patients.

Keywords: NSCLC, Immunohistochemically, gantic study, electron microscopy, Prognostic factors.

1. Introduction

The most common reason for cancer-related fatalities worldwide is NSCLC. The cure rate for advanced non-small-cell lung cancer (NSCLC) is still poor despite recent improvements in management., Non-small cell lung carcinoma are composed of Adenocarcinoma, Squamous Anaplastic and Large cell carcinoma, the most common of(NSCLC), the adenocarcinoma (ADC) more than 80% of lung cancer cases [1, 2].

This is attributable to a variety of factors, including longer life expectancies, earlier pregnancy ages, poor nutrition, sedentary lifestyles, and cigarette smoking [3]. Tobacco cigarettes are the single largest risk factor for lung cancer, accounting for up to 90% of lung cancer cases [4].

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor that plays important roles in physiologic and neoplastic situations, as well as in the genesis and progression of NSCLC [5, 6]. The EGFR gene is found on the short (p) arm of chromosome 7 at location 12exons 18/19/20/21 are crucial for oncologists dealing with lung cancer patients7. A deletion in exon 19 and a point mutation known as L858R8 are the mutations that are most prevalent.

About 30% of all epithelial malignancies have EGFR or family member mutations, amplifications, or miss regulations. The diagnosis is lung epithelial cancers. Because to the vast majority of patients afflicted with lung cancer (about 85 percent of all pathologically defined lung cancer cases). [7]. imunohistochemistry has an advantage in examining such small biopsy specimens. In this study, immunohistochemical techniques use chemical and immunological processes to identify antigens in tissue sections [8].

2. Materials and Methods

The presented work was done in the laboratories, in Hila Teaching Hospital in Babylon of Iraq, from November2021 to April 2022. 50 samples, 30 tissue blocks embedded in paraffin wax have been acquired from 30 patients (19 males and 11 females) experiencing NSCLC, pathologic and clinical information was also acquired, including the operation type, sex, age, smoking history, while controls were 20 samples from healthy volunteers, our study included the following.

A-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 staining technique. In addition, the tissue was but in buffered formalin of 10%, frequently processed, and but in paraffin. Furthermore, the tissues have been stained with eosin and hematoxylin Falkeholm et al. [9].

B. EGFR protein expression: For IHC staining, 5 µm thick sections have been de-paraffinized and placed on +ve charged slides, EDG Monoclonal Mouse antibody (anti-human primary antibody) Abcam (Cambridge,) for the detection of EGFR proteins. Immunoreactions were revealed by a strepta vidinbiotin enhanced immunoperoxidase technique Diaminobenzidine was used as the chromogenic substrate. Thunnissen et al. [10].

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

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

C. Genetic study: DNA Extraction was prepared from Paraffin embedded Lung cancer specimens with sections thick 5-10 µm. Isolation of DNA genomic protocol according to Genomic DNA Mini Kit (Geneaid biotech. Ltd., Feline. (No GS100), Lot no. FE16205-N) 13. Taq Polymerase and 100 ng DNA are both included in a 25 L PCR reaction mixture. The following was done for amplification: DNA was expanded Initial Denaturation: 5 minutes in 94 oC for 35 cycles of 94 oC for 30 seconds, 55 oC for 30 seconds, and 72 oC for 30 seconds, followed by an extended period of 5 minutes at 72 oC. In addition, Performing PCR of samples. RFLP-PCR technique the 1 st PCR products done using MseI 10 IU at 37oC overnight.

D. Electron Macroscopically study

The samples were done in Electron microscopic of the Faculty of Science/ University of AL-Kufa. According to the preparation procedure for Electron Microscope, as follows protocol.

1- Put paraffin block in xylene for 30 minutes,

2-put theis for 20 minutes each in alcohol - absolute, 90%, 70%, and 50%.

3-wching of the water and put in Sorensen's buffer for 20 minutes.

4- Put in 1% osmium tetroxide and put in epoxy resin and mounted on copper grids, stained with uranyl acetate and lead citrate, examined with electron micrmoscope.

3. Statistical Analysis

Statistical Package for the Social Sciences Version 23 was expressed in the mean and standard deviation form (mean ± standard deviation), and Chi-square test, it P < 0.05.

4. Results

1-Clinicopathologic Characteristic in Patients and Control

Our results have, the patients male 19 and female 11 while control male 12 and female 8confirmed that the mean age of patients group was 51.8 4 ± 17.7 0 years and the median age of patients was 53 years (range23– 70 years) while the mean age of control was 39.26 ± 21.43 and median age of control was 38.500 years (range20–70) years, The male/female ratio of patients was 2:1 while the control was 1.75:1. The male/female ratio here of both patients and control was no statistical difference (P > 0.05). The distribution of patients according to site of tumor was as the following: 17 patients had a right-sided lung tumor, accounting for 56.7%, while 13 patients had a left-sided lung tumor, accounting for 43.3%. The distribution of patients according to site of tumor was as the following: 16 patients had a right-sided lung tumor, accounting for 53.3 %, while 14 patients had a left-sided lung tumor, accounting for 46.7 %. Distribution of patients according to the grade of tumor was as follows: 15patients (50.0 %) showed Grade I, 11patients (36.7%) had Grade II pattern, and 4 patients (13.3 %) had Grade III; distribution of patients according to the stage of lung carcinoma was as follows: There were 10 patients (33.3. %) in Stage I disease, there were 15patients (50.0 %) in Stage II disease, and there were 5patients (16.7%) in Stage III disease [Table 1 and Figures 1 By using chi-square Test, a was no correlated between stage and grade of cancer (P=0.001

Table 1: Correlation between Clinicopathologic characteristic in patients

Variables	*n* (%)
Mean age± standard deviation (years)	
Patient	51.84±15.70
Control	39.26±21.43
Gender	
Male	17 (56.7)
Female	13 (43.3)
Site of tumor	
Right sided	16(53.3)
Left sided	14(46.7)
Grade of tumor	
Well	15 (50.0)
differentiated	
Moderately differentiated	11 (36.7)
Poorly differentiated	4(13.3)
Stage of tumor	
Stage I	10 (33.3)
Stage II	15 (50.0)
Stage III	5 (16.7)

2- EGFR tumour expression and clinicopathologic parameters:

In our study showed that highly correlated difference in EGFR Positive between patients and control subjects, Positive EGFR was limited to patients with NSLC (25%) whereas none of control a positive EGFR expression (0%). No correlated were found both gender and EGFR expression patients with NSCLC (P> 0.05), No statistically significant difference was observed for between Grade, Stag and Sit of NSCLC with EGFR

expression (P> 0.05) show the (Table2 & figure2).So in our study showed that EGFR expression patients were correlated with Electron microscopic study but a not clear

With genetic study of mutation EGFR (figure3& figure4).

Table2: The Relationship between EGFR tumor staining with pathological feature

Variables		Negative	Positive	Total	p-value
Gender	Male	11(57.1 %)	6(54.5 %)	17	0.858
	Female	8(42.1%)	5 (45.5 %.)	13	
Site	Left	9 (47.4.1%)	10(52.6 %)	19	0.9 19
	Right	5(45.5 %)	6(54.5 %)	11	
Stage	I	7(36.8 %)	3 (27.3 %)	10	0.866
	II	8(42.1%)	6(54.5 %)	15	
	III	3 (15.8%)	2(18.2%)	5	
Grade	I	10 (52.6 %)	5 (45.5 %)	15	0.221
	II	8(42.1%)	3(27.3%)	11	
	III	1(5.3%)	3(27.3%)	4	

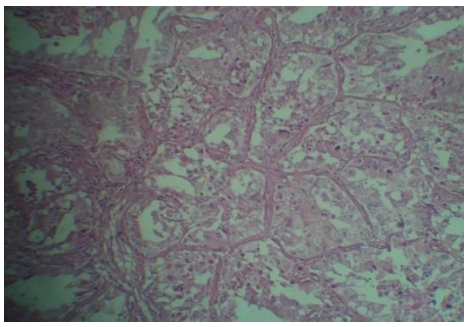

Figure1: Histological section of lung showing moderately differentiated adenocarcinoma with almost micropapillary;H & E (10x).

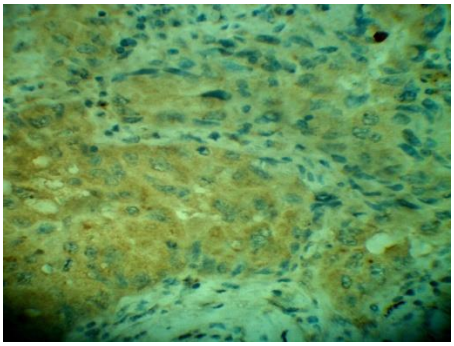

Figure 2: Immunohistochemical section EGFR expression inthe form of brown cytoplasm stain of the cytoplasm are stained 10X)

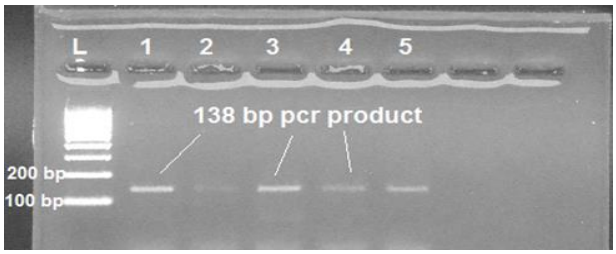

Figure 3: Amplified gene EGFR exon19by (PCR).

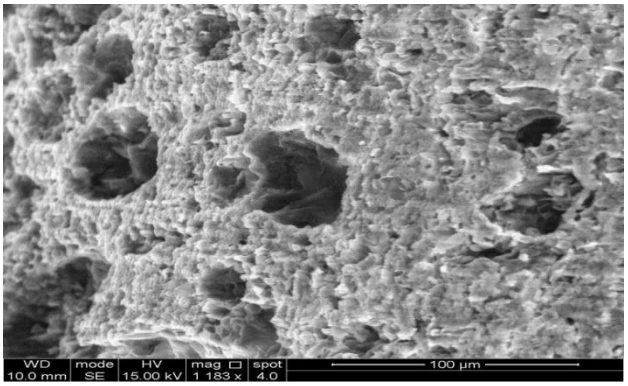

Figure 4: Scanning electron micrograph shows lunge issues in patient's disorderly growth pattern in grade I

5. Discussion

In the present study the mean age of patients with carcinoma was51.84 +/- 15.70 years and the lung median was 54 years while the age range was from23 years through 70 years, expressed that smoking is the main source of lung disease and the explanations behind the predominance of smoking in Iraq are people conduct and social condition that advance smoking and it was accounted for that 85-90% of lung malignant growth patients expressed that older age was related with disease advancement because of biologic components that incorporate DNA damage over time and shortening telomeres, In a recent study done in
Iran, on 1353 patients with Lung carcinoma, the mean age was 60 years and the age range was16-94years
these findings are substantially higher than that of the present study. It's shown that changes
in prevalence and types of tobacco smoking, physical activity, air pollution in indoor, radon environments can affect the pattern of lung cancer in terms of gender and The best possible explanation which may be attributed to a complex interaction between genetic and lifestyle factors [11].
Male to female ratio was2:1 in the present study. One of the hypotheses is that the results further suggest that smoking, DNA capacity repair, alcohol consumption and obesity anther hypotheses exogenous and endogenous estrogens in particular, hormonal factors, have been shown to play a role in the development of adenocarcinoma [12, 13] The length of the menstrual cycle, age at menarche, age at menopause, exogenous hormone replacement treatment, and reproductive factors have also been researched and may have an impact on the development of lung cancer in females [14].
The results of the present study show that EGFR immune - histochemical expression was not correlated with age,sex, grade,stage of tumor in patients that agree with the results of the tow study [15-17].
To explain The IHC staining was graded according to the proportion of tumor cells' cytoplasm and/or membrane that was stained, with the negative control being the absence of a primary antibody. demonstrated that there was no association between the tumor and the patients' lung cancer.
The results of the present study showed of deletion of exon 19 mutation was not detected, so its not correlated with EGFR immune histochemical,The best possible explanation which may be. In a found that RFLP-PCR frequently misses mutations in tumor samples with a low percentage of EGFR-mutant cancer cells, but immunohistochemistry using antibodies specific to the mutation can pick them up [18]. This is significant because immunohistochemistry is frequently used to assess tiny biopsy samples, which frequently do not give enough high quality RFLP-PCR method [19].
addition, the spatial distribution of mutant cells (such as those that are limited to the tumor's developing edge or connected to in situ lesions), localization with cancer stem cell markers, etc.),done that was agree with present study that most of sample take by small biopsy.
Finally, in our study we a not clear role for EGFR mutations and EGFR overexpression, but Electron Macroscopically study find significant correlation with immunohistochemical EGFR [20].

6. Conclusions

In our investigation no significant association between, EGFR protein expression and gender, grade, site, stage tumors so were found not clear role for between Molecular study and Immunohistochemical study of EGFR gene, but the presence of relationships clear between Clinicopathologic characteristic in patients of immunohistochemistry EGFR and Electron

Macroscopically of NSCLC patients.

References

1. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH. Comparison of four chemotherapy regimens for advanced non–small-cell lung cancer. New England Journal of Medicine. 2002;346(2):92-8. https://doi.org/10.1056/NEJMoa011954
2. Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, Nishiwaki Y, Saijo N, Ariyoshi Y, Fukuoka M. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Annals of oncology. 2007;18(2):317-23. https://doi.org/10.1093/annonc/mdl377
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015;65(2):87-108. https://doi.org/0.3322/caac.21262
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34. https://doi.org/10.3322/caac.21551
5. Kuan C, Wikstrand C, Bigner D. EGF mutant receptor vIII as a molecular target in cancer therapy. Endocrine-related cancer. 2001;8(2):83-96.
6. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. The lancet oncology. 2012;13(3):239-46. 6.
7 Rosell R, Carcereny E , Gervais R ,(2012).Erlotinib versusstandard chemotherapy as first-line treatment for European patients with advanced EGFR mutation positive non small-cell lung cancer (EURTAC): amulticentre, open-label, randomised phase 3trial,Lancet On col;13239-46.
8 Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Translational lung cancer research. 2015;4(1):36. https://doi.org/10.3978%2Fj.issn.2218-6751.2014.05.01
9. Sun J-M, Ahn M-J, Choi Y-L, Ahn JS, Park K. Clinical implications of T790M mutation in patients with acquired resistance to EGFR tyrosine kinase inhibitors. Lung cancer. 2013;82(2):294-8. https://doi.org/10.1016/j.lungcan.2013.08.023
10. Falkeholm L, Grant CA, Magnusson A, Möller E. Xylene-free method for histological preparation: a multicentre evaluation. Laboratory investigation. 2001;81(9):1213-21.
11. Thunnissen E, Allen TC, Adam J, Aisner DL, Beasley MB, Borczuk AC, Cagle PT, Capelozzi VL, Cooper W, Hariri LP. Immunohistochemistry of pulmonary biomarkers: a perspective from members of the pulmonary pathology society. Archives of pathology & laboratory medicine. 2018;142(3):408-19. https://doi.org/10.5858/arpa.2017-0106-SA
12. Hamid MS, Shameem R, Gafoor K, George J, Mina B, Sullivan K. Non-Small-Cell Lung Cancer Clinicopathologic Features and Survival Outcomes in Asian Pacific Islanders Residing in the United States: A SEER Analysis. Journal of Cancer Epidemiology. 2015;2015:269-304. https://doi.org/10.1155/2015/269304
13. North CM, Christiani DC, editors. Women and lung cancer: what is new? Seminars in thoracic and cardiovascular surgery; 2013: Elsevier. https://doi.org/10.1053/j.semtcvs.2013.05.002.
14. Babacan N, YÜCEL B, Kilickap S, Seker M, Kacan T, Olcas I, Eren A, Odabas H. Lung cancer in women: a single institution experience with 50 patients. Asian Pacific Journal of Cancer Prevention. 2014;15(1). http://doi.org/10.7314/apjcp.2014.15.1.151
15. Tan HS, Tan M-H, Chow KY, Chay WY, Lim W-Y. Reproductive factors and lung cancer risk among women in the Singapore Breast Cancer Screening Project. Lung Cancer. 2015;90(3):499-508. https://doi.org/10.1016/j.lungcan.2015.10.003
16. Sugio K, Uramoto H, Ono K, Oyama T, Hanagiri T, Sugaya M, Ichiki Y, So T, Nakata S, Morita M. Mutations within the tyrosine kinase domain of EGFR gene specifically occur in lung adenocarcinoma patients with a low exposure of tobacco smoking. British journal of cancer. 2006;94(6):896-903.
17. Tomonaga N, Nakamura Y, Yamaguchi H, Ikeda T, Mizoguchi K, Motoshima K, Doi S, Nakatomi K, Iida T, Hayashi T. Analysis of intratumor heterogeneity of EGFR mutations in mixed type lung adenocarcinoma. Clinical lung cancer. 2013;14(5):521-6.
18. Tang X, Varella-Garcia M, Xavier AC, Massarelli E, Ozburn N, Moran C, Wistuba II. Epidermal growth factor receptor abnormalities in the pathogenesis and progression of lung adenocarcinomas. Cancer Prevention Research. 2008;1(3):192-200. https://doi.org/10.1158/1940-6207.CAPR-08-0032
19. Chen Z, Liu H-b, Yu C-h, Wang Y, Wang L, Song Y. Diagnostic value of mutation-specific antibodies for immunohistochemical detection of epidermal growth factor receptor mutations in non-small cell lung cancer: a meta-analysis. PLoS One. 2014;9(9):e105-940. https://doi.org/10.1371/journal.pone.0105940
20. Zhou J, Ben S. Comparison of therapeutic effects of EGFR-tyrosine kinase inhibitors on 19Del and L858R mutations in advanced lung adenocarcinoma and effect on cellular immune function. Thoracic cancer. 2018;9(2):228-33. https://doi.org/10.1111/1759-7714.12568
21. Bondgaard A-L, Høgdall E, Mellemgaard A, Skov BG. High specificity but low sensitivity of mutation-specific antibodies against EGFR mutations in non-small-cell lung cancer. Modern Pathology. 2014;27(12):1590-8. https://doi.org/10.1038/modpathol.2014.67