Immunohistochemical Correlates of Epidermal Growth Factor Receptor Mutations in Lung Adenocarcinoma in Iraq Patient

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Abstract

We aimed to investigate the Detection of epidermal growth factor receptor (EGFR)mutations in lung adenocarcinoma by Immunohistochemical and correlation with Clinicopathological features. We used Fifty tissue blocks embedded in paraffin wax were obtained from 50 patient (16 female and 34males) suffering from lung adenocarcacinoma, while controls were 30 samples from healthy, Histopathological examination Study by (H and E stain)and detection of Mutation in EGFR(exon19) by RFLP-PCR, detection of protein expression EGFR by immunohistochemical method Our results have, confirmed the mean age of patients group was 51.84 +/-15.70 years and the median was 54, performed study the non correlation between stage of disease and age of patients. site of tumor was right sided of lung tumor, accounting for 58 % .Distribution of patients according to grade of tumor was, The results of this study showed non-significant correlation between age and grade, results of this study showed significant difference in EGFR Positive between patients and control healthy in Imunohistochemical, had found as not significant with age ,gender, sit of tumor, grade, stage, in Immunohistochemical method of EGFR Expression. while Gene EGFR deletion of exon 19were analyzed using mutant-enriched polymerase chain reaction (PCR) was not detected in all patients and control

Keywords: *Epidermal growth factor receptor (EGFR); Immunohistochemical; lung adenocarcacinoma; prognostic factors.*

Introduction

Lung cancer is currently the most frequent cause of major cancer incidence and mortality worldwide. [1] Two main histological categories are included: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC comprises about 80% of all diagnosed lung cancer, and lung adenocarcinoma is the most common subtype of NSCLC.[2]This fact is due to several reasons, including increases in life expectancy, higher pregnancy age, poor nutrition ,sedentary lifestyle and cigarette smoking.[3] The use of tobacco cigarettes is the single greatest risk factor in the development of lung cancer, with up to 90% of lung cancers attributed to smoking.[4]

The EGFR (also referred to as: ERBB, ERBB1, HER1) gene is located on the short (p) arm of chromosome 7 at position 12 (cytogenetic chr band 7p12.1).[5]Among its main exons (n=28), exons 18/19/20/21are critical for oncologists handling lung carcinoma patients, The most common mutations are a deletion in exon 19 and L858R point mutation.[6,7,8]. clustered around the amino-acid residues 747–750(delE746-A750,delL747-T751insS, and delL747- P753insS) and a specific exon21 point mutation L858R(leucine to arginine [L858R] and leucine to glutamine [L861Q]) have been reported to comprise up to 90% of all activating EGFR utations.[9,10] Mutations ,amplifications or miss regulations of *EGFR* or family members are implicated in about 30% of all epithelial cancers lung epithelial malignancies constitute the diagnosis attributed to the majority of patients suffering from lung cancer (about 85%of all pathologically defined lung cancer cases).[11] Immunohistochemical techniques detect antigens in tissue sections by means of immunological and chemical reactions. [12] while PCR is a method for the in-vitro enzymatic synthesis of specific DNA sequences using two synthetic oligonucleotides primers that each hybridizes to opposite strands and flanks the region of interest in the target DNA, permitting the amplification of small amounts of genetic material[13].

The aims of our study were to fined correlation between the IHC expression and presence of EGFR mutations with study of clinicopathological (age, sex, stag, grade) in the Patients.

Patients and Methods

This study was done in the department of Biology Lab, Faculty of Science, Kufa Universityduring the period from April 2018 to May2019A total at 80 samples, Fifty tissue blocks embedded in paraffin wax were obtained from 50 patient (16 female and 34males) suffering from lung denocarcacinoma ,while controls were 30 samples from healthy volunteers, our study included the following

A-Histopathological Eamination Study:

Sections were obtainedwith 5 µm-thichfrom paraffin embeded tissues, these sections were stained by using Haematoxylin and Eosin staining method (H and E stain). [14]

B-**Immunohistocemical staining.**

For IHC staining, 5-micrometer-thicksections were deparaffinized and placed on +ve charged slides,EGFR Monoclonal Mouse antibody (Anti-Human Primary antibody) Abcam, (Cambridge, UK for detection EGFR proteins not specific. Three scoring scales were connected at 40X goal .[15] as pursues Zero score with no stain.,+1score,faint staining of cytoplasmic considered (weak). +2score, moderate staining of smooth cytoplasmic considered (moderate). +3score,intense staining of granular cytoplasmic tumor cells considered.

C**. Molecular study** :DNAExtraction was prepared from Paraffin embedded Lungcancer 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). and The extracted DNA concentration (ng/µL)assessedby using Nanodrop Spectro- photometer according to.[16] Amplification of the gene by the reaction prepared it used 8 µl of PreMix and 2µl of DNAsamples, , 1µl of primers (0.5µl forward 5′-ATCCCAGAAGGTGAG AAAGATAAAATTC-3 ′and 0.5 µl reverse from5′-CCTGAGGTTCAG AGCCATGGA -3′)and added8.5 µl distilled water and 0.5MgCl . mixed with- vortex ,DNA was amplifi ed Initial Denaturation5 min in94oC for 35 cycles at 94°C for 30seconds, 55°C for 30 seconds,and 72°C for 30 seconds, extension at 72°C for5minute, and Performing PCR of samples. The 1 st PCR productsdone using MseI 10 IU at 37oC over.night PCR aliquotswere used as a template for the 2nd round of PCR amplification as the first round PCR conditions but for 40 cycles. The PCR products of the 2nd amplification was analyzed on 12% PageVia Sliver staining.

Statistical analysis: Statistical Package for the Social Sciences (SPSS) Version 23, were expressed in the of mean&standard deviation form(mean±SD), Pearson t-test and Chi-square test , corrolation with($P < 0.05$) .

Results

I- Clinicopathologic Characteristic in Patients and Control: Our results have ,the patients Male33,Female17 while control Male19 ,Fmale11, confirmed the mean age of patients group was 51.84 +/-15.70 years and the median age of patients was 54 years (range 13-76 years) while the mean age of control 39.266+/-21.43 and median age of control was 38.500 years(range 10-79) years, The Male/female ratio of patients were 2:1 while the control were 1.75:1. Male/ female ratio there both of patients and control was no statistical difference ($P > 0.05$),, The distribution of patients according to site of tumor were as the following (29) patients had a right sided lung tumor, accounting for 58 %, while (21) patients had a left sided lung tumor, accounting for 42 %. .show in (Table 1and Figure 1), our study by using Kendall's tau-b yielded not correlated between stage and grade .

Table 1: Cinicopathologic Characteristic in Patients and control

Variable	No%	p-value
Mean age(years)		
patients		
control	was51.84	
Gender	+/-15.70	
Male	39.266	
Female	+/-21.43 to	0.809
site of tumor	33(66%)	
right sided	17(43%)	0.507
left sided	29(58%)	0.261
grade of tumor	21(42%)	
well differentiated	25(50%)	0.1
Moderately differentiated	16(32%)	
Poorly differentiated	9(18%)	0.4
stage of tumor	16(32%)	
stage I	26(52%)	
stage II	8(16%)	
stage III		

II-Immunohistochemical Expression of EGFR:

Immunohistochemica of EGFR					
Variables			**Negative**	**Positive**	**Total P-vule**
Gender	Mal	25 (75.8%)	8 (24.2%)	36(72%)	
0.410					
	Female	11 (64.7%)	6 (35.3%)	14(28%)	
Sit	Left	19(90.5%)	2 (9.5%)	21	
	0.01				
	Right	17(58.6%)	12(41.4%)	29	
Stage	I	9(56.25%)	7(43.75%)	16	
II	13(50%)	13(50%)	26	0.54	
	III	6(75%)	2(25%)	8	
Grade	I	5 (83.3%)	1 (16.7%)	6	
	II	11(61.1%)	7(38.9%)	18	0.416
	III	20 (76.9%)	6 (23.1%)	26	

In our study showed that highly correlated difference in EGFR Positive between patients and control subjects,. Positive EGFR was limited to patients with lung carcinoma (28%) whereas non of control a positive EGFR expression (0%). EGFR of IHC expression was detected as brown cytoplasm, The score of EGFR was as follows: score 1 accounted for 3 (21.42%) of cases, score 2 was seen in 9 (64.28%) of cases and score 3 was present in 2 (14.28%) of

Table 2 Immunohistochemica of EGFR in Patients

cases show in figure(5,6,7,8) , No correlated were found both gender and EGFR expression patients with Lung carcinoma (P> 0.05), No statistically significant difference was observed for between Grade and Stag with EGFR expression but significant with Sit of Tumor (P> 0.05) show the(Table2 & figure2).

III- Molecular: The associated of gene *EGFR* exon19:

Gene *EGFR* deletion of exon19were analyzed using mutant-enriched polymerase chain reaction (PCR) was not detected in all patients and control as shown in figure(3).

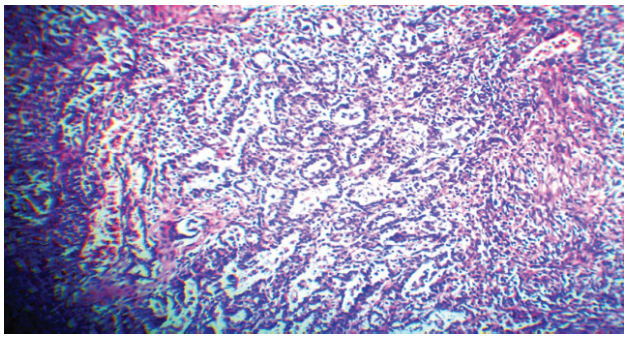

Figure1: Histological section of lung showing moderately differentiated adenocarcinoma with almost micropapillary;: H & E (10x).

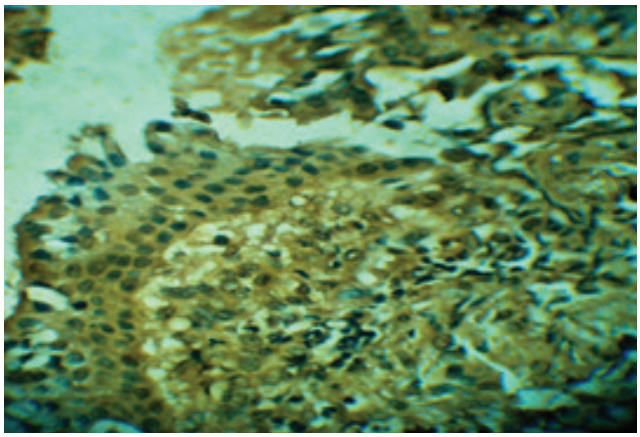

Figure 2: Immunohistochemical section EGFR expression in the form of brown cytoplasm stain 50 % of the cytoplasm are stained 10X).).

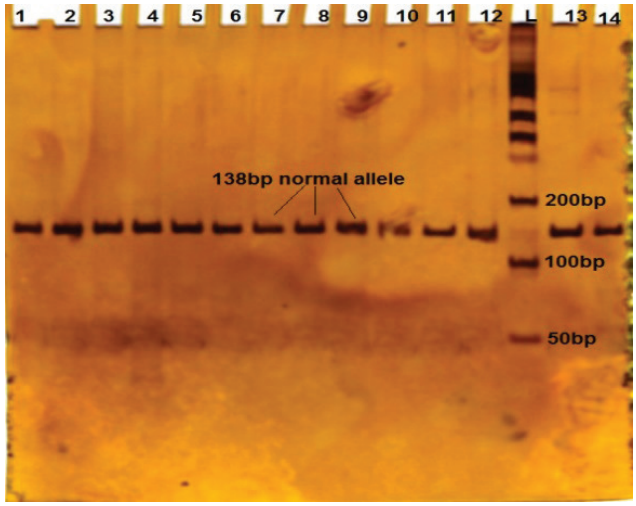

Figure 3 : resulting mutant-enriched products (PCR) of gene EGFR exon19normal allele.

Discussion

In the present study the mean age of patients with carcinoma was 51.8 4+/- 15.70 years and the lung median was 52 years while the age range was from 14 years through 79 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 the social and social condition that advance smoking and it was accounted for that 85-90% of lung malignant growth patients Torre *et al*[17]expressed that older age was related with disease advancement because of biologic components that incorporate DNA damage over time and shortening telomeres, Al khuzaie *et al*[18] stated a study the first most common site in males on newly diagnosed Iraqi cancer patients. performed during a 3 years period (2005-2007),Al-Hashimi and Wang[19] the reported among 2000 and 2010 demonstrated the rate is most noteworthy (19%) in the age gathering 60–69 years in Iraq/Mousl, Jaloudi *et al*[20], An aggregate of 25 NSCLC patients were enrolled in the examination at standard (7/25 Bahrain; 11/25 UAE; 7/25Qatar) illness, The mean age of the was 56.9 which are slightly higher than that of the present study 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 Khosravi *et al*[22]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, While the men age range was (56 years) in India[22] . In an Saudi Arabia, Kuwait and UAE the men age range were (66 years) [23],the multiple studies which are higher than

of the present study. Male to female ratio was 2.4:1 in the present study one of the hypotheses is that results further suggest that smoking, DNA capacity repair, alcohol consumption and obesity anther hypotheses hormonal factors such as estrogens which is not present in normal lung tissue Temraz *et al* [24] reported a male to female ratio of Lebanon 2.5 :1, which is again, in accordance with the present study while Jaloudi *et al*[20]a total of 25 NSCLC patients (Bahrain; UAE; Qatar) disease were 2.8:1 which is again, in accordance with the present The present study showed that 54% of tumor masses were located in the Right region and that46 % of cases were located in the Left region These findings differences in patients characteristics, According to Jaloudi *et al* [20] the tumor masses of lung is the most common in the upper right lobe approximately (57.1%) of the cases and followed by the upper left lobe , The present study showed that the majority carcinoma (50%) had a well differentiated grade I histological pattern, its findings differences in patients characteristics ,other factors that showed a prognostic effect independent of disease grad include age and gender several genetic bio markers ,while ,other factors like obesity and smoking history associated with lung cancer. [(25,26)] Well to Moderately differentiated morphology was the major histological grade reported by some authors.([27,21,20)] The present study showed that majority of patients enrolled in the present study (52%) had stage II disease The finding of the present study that majority of patients had stage II disease is in agreement with many authors [28] .

Conclusion

our results indicate the non correlation between stage &gread of disease withe age of patients and Expression of EGFR of Immunohistochemical method highly significant difference between patients and control healthy, had found as not significant with age ,gender, grade, stage, but significant with sit while *EGFR* significant difference between patients and healthy controls but not significant with, gender, sit of tumor ,grade and stage the end non correlation between Expression of EGFR Immunohistochemical and mutation.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology, Faculty of Science, University of Kufa/Iraq and all experiments were carried out in accordance with approved guidelines.

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