RESEARCHARTICLE

Frequency of Epidermal Growth Factor Receptor Mutations among Iraqi Non-small Cell Lung Cancer Patients

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ABSTRACT

Background: Epidermal Growth Factor Receptor (EGFR) mutations are actually currently utilized as genetic biomarkers for focused lung carcinoma treatment. Unfortunately, there is minimal data on Iraq's proportion of mutations in EGFR gene. The current study's objective is to find the mutations prevalence of EGFR gene mutations and the dominant EGFR mutations in patients with lung carcinoma type non-small cell (NSCLC) from Iraq.

Methods: On tissues taken from paraffin-embedded blocks of NSCLC patients, RT-PCR was performed to investigate the mutations in EGFR gene.

Results: This study involved 234 paraffin tissue blocks of NSCLC patients in addition to17 paraffin tissue blocks with invalid results, although it was tested more than three times. This study was used Polymerase chain reaction (PCR) technique to determine mutations in EGFR gene. Forty-seven out of 234 patients had an EGFR mutation (20%). The most prevalent anomaly found in exon19 Del (70.3%) followed by L 858 R (15%). High mutations were found among two age groups (60–69) and 70–79 which suggest increased mutation among old ages when compared with young people. However, no significant difference between EGFR mutation status and sex or age status was found.

Conclusion: The prevalence of EGFR mutation among Iraqi patients is 20% which is compatible with a percentage of cases documented in Asian populations and the most prevalent EGFR mutant in Iraq cases is the EX19del.

Keywords: EGFR mutation, Lung cancer, NSCLS, Real-Time PCR.

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INTRODUCTION

One of the major factors contributing to cancer-related death in both men and women is lung cancer, and the quantity of effective medications for an advanced stage of this disease remains limited. Approximately 75% of lung cancer cases are non-small cell lung cancers (NSCLC), therapy has been difficult due to the poorly understood pathological pathways. In contrast to lung cancer-type squamous cell (SCLCs), NSCLCs are often less responsive to chemotherapy and radiotherapy than SCLCs. NSCLC is the primary malignant in which activating mutations in the Epidermal Growth Factor Receptor (EGFR) gene occur. These mutations cause the kinase activity of the EGFR protein to be constitutively activated, which leads to the carcinogenic pathway. The EGFR is found on the cell membrane and is triggered by attaching its specialized ligands. The EGFR gene undergoes somatic mutations that result in the receptor's continual stimulation, which results in uncontrollable cell replication.[1] Gefitinib (Iressa) and erlotinib (Tarceva) are two oral anti-cancer medications that suppress EGFR and have been recommended for treatment of aggressive NSCLC, EGFR mutations have been recognized in correlation with certain lung malignancies.[2] This study will investigate the incidence and frequency of EGFR mutations, furthermore the genotyping of EGFR mutations in NSCLC of Iraqi patients which may provide a pathway to their medication.

MATERIALS AND METHODS

Patients

This study included 251 paraffin blocks tissues with NSCLC collected from NSCLC patients in oncology centers all over the country. The duration of the study was one year; it was started on (23/7/2018) and ended on (24/7/2019). These samples were

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Table 1: Prevalence of EGFR mutations

Items	*No.*	*%*
Mutation detection	47	20
No mutation detection	187	80
Total	234	100

Table 2: Number of EGFR mutations per sex

Sex	*No. of mutated*	*No. of non-mutated*	*Total (%)*
Male	24	110	134 (18)
Female	23	77	100 (23)
Total	47	187	234 (20)
Statistical analysis between Male and female (Z test)	Z test = 0.42074 p > 0.05, No significant difference		

Table 3: Distribution of EGFR mutations by age groups

Age (years)	*No. of mutated*	*No. of non-mutated*	*Total*	*%*
20–39	3	21	24	12,5
40–49	5	34	39	12.8
50–59	9	32	41	22
60–69	15 48 63			23.8
70–79	10	33	43	23.2
80–89	5	19	24	20
Total	47	187	234	20
Statistical analysis for age group (Chi Square Test)		p value = 0.961 p > 0.05, No significant difference		

Table 4: Frequency of Genotypes of EGFR Mutations

Mutations types	*No.*	*(%)*
EX19Del	33	70.3
L858R	7	15
L861Q	2	4.2
G719X	2	4.2
EX19Del+T790M	2	4.2
EX20Ins	1	2.1
Total	47	100

tested for EGFR mutations using a fully automated system (Real-Time PCR) at Baghdad's central public health laboratory.

Methods

The paraffin tissue cases of NSCLC patients are examined by utilizing the cobas® DNA sample preparation kit for DNA extraction and the cobas z 480 analyzer for auto amplifying and diagnosing EGFR gene mutations. EGFR gene has 42 mutations in exons 18, 19, 20, and 21, including the T790M resistant mutation as described in the following:

- Exon 18: G719X (G719A, G719C, and G719S)

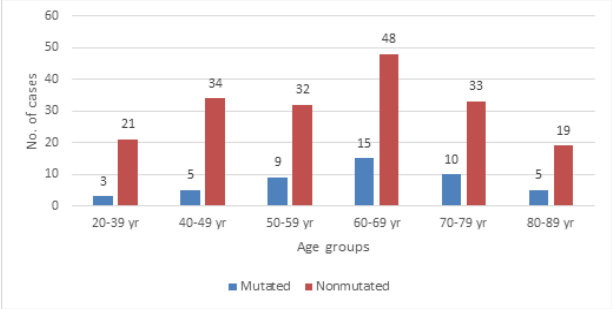

Figure 1: Distribution of EGFR mutations by age group

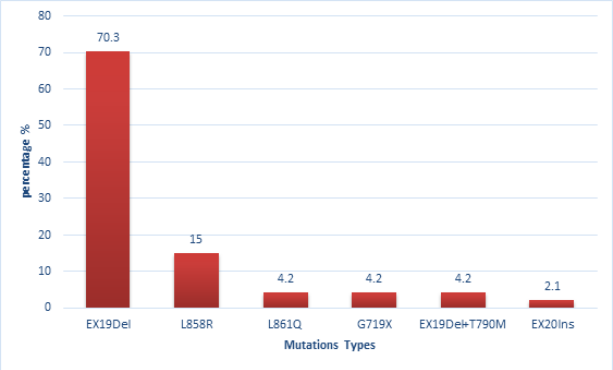

Figure 2: Frequency of Genotypes of EGFR Mutations among NSCLC patient

- Exon 19: deletions and complex mutations
- Exon 20: S768I, T790M, and insertions
- Exon 21: L858R and L861Q

Using the cobas z 480 analyzer and PCR analysis to determine mutations. To ensure accuracy, each run comprises a mutant control and negative control.

Statistical Analysis

Chi Square and Z tests were applied, and a *p* value of 0.05 or less than was considered significant.

RESULTS

In these results, the diagnosis of EGFR mutations was performed on 251 paraffin block tissues from Iraqi patients with lung carcinoma type NSCLC, 17 out of 251 showed invalid results although it was tested more than three times. From 234 of malignant cases, 47 (20%) had mutations in EGFR gene (Table 1).

This study found no significant difference of EGFR mutations between male (18%) and female (23%) (Table 2).

Although older patients were more likely to have EGFR mutations than younger patienes, there was no correlation between EGFR mutations and age groups (Table 3 and Figure 1).

Also this data showed the EX19Del EGFR mutation is the predominant type (70.3%) followed by the L858R mutation (15%), while the percentage was (4.2%) in L861Q, G719X and

for combined (EX19Del and T790M). The lowest mutation type (2.1%) is found in EX20Ins (Table 4 and Figure 2).

DISCUSSION

Asians are most likely to have EGFR mutations (27–60%), followed by Indians (20–25%), white americans (16%), europeans (8–13%) and africans (12%).[3] Several research studies were conducted in the middle east region, the prevalence of EGFR mutations in most of these studies was (21.2%), in Turkey, the frequency was (44%), whereas in Saudi Arabia, it was (2.1%).[4,5] The present research detects that 20% of NSCLC patients had EGFR mutations, which was in line with the incidence of other research studies in Iraq and the Middle East. In the current data, mutations of EGFR gene were found in 20% of the NSCLC patients. This result was compatible with the findings of other studies in Iraq and the Middle East region.[6,7]

Invalid results for EGFR mutation test in this study were (6.7%) despite that the samples were re-tested more than 3 times. The extraction of the DNA from FFPET is complicated and may cause defragmentation of target DNA which may lead to invalid results. The invalidity results of these samples may be due to pre-examination factors such as formalin-fixation time and a paraffin-embedding procedure which always results in significant fragmentation of nucleic acids.

In several Middle Eastern investigations, male patients with EGFR mutations predominated; the average male to female ratio was 2.15.[7] Gender had no appreciable impact on the prevalence of EGFR mutations in our sample, which included 23% female and 18% male patients. The findings of this study were in agreement with those of another study carried out in Iraq.[6] The difference in the EGFR mutations between males and females may be due to sample size or to the specific situation of each country. With regard to the relationship of age with mutations, we note that the elderly patients showed the highest percentage of mutations in EGFR. Despite this, there was no significant difference when comparing EGFR mutations among age groups.

The current results showed that the EX19Del is a predominant type of EGFR abnormality (70.3%) followed by L858R (15%) of all mutations. These results are consistent with those from studies in Europe (Exon 19 deletion, 62.2%; and Exon 21, 37.8%) and Asia (Exon 19, 60%; and Exon 21, 40%), studies from the Middle East discovered that the exon 19 mutation, which affects EGFR as a whole, occurs at a frequency of 40% on average.[8,9] Contrarily, Iraqi study revealed that the dominant mutations are point mutation (35.5%) in exon 18 (G719X) and the next (8%) found in exon 21 (L858R),[6] the difference may be due to sample size.

The most common EGFR mutations in Iraq were EX19Del and L858R, both of which account for up to about (85–90%) of identified mutations that activate EGFR in individuals with NSCLC, while G719X and T790M gene mutations accounted for (5% each).[10-13]

According to clinical research, exon 19 deletion mutations or the L858R substitution mutation in exon 21 were discovered in advanced NSCLC patients treated with tarceva. For the diagnosis of tarceva, the EGFR test is utilized. The tarceva is a therapy that reversibly restricts EGFR kinase function, stopping autophosphorylation of tyrosine residues related to the ligand that blocks downstream activation of stimulating cell cycle progression. Erlotinib is more sensitive to binding to EGFR exon 21 L858R mutations or exon 19 deletions than the wild-type receptor. Compared to patients receiving chemotherapy, they seem more likely to benefit clinically when used as first-line treatment.[14,15]

The EGFR exam on paraffin tissue samples is utilized as a diagnostic method for tagrisso (osimertinib), an irreversibly blocker of both EGFR TKI-sensitizing and T790M resistant mutants in advanced NSCLC. A series of intracellular downstream signaling pathways that promote cell survival, proliferation, and angiogenesis are blocked by Tagrisso's action on the EGFR kinase enzyme. Based on clinical findings, patients with advanced non-squamous NSCLC and an EGFR TKI-sensitizing mutation have progressed after receiving treatment with a first-generation EGFR TKI and those who received tagrisso (osimertinib) and have a T790M resistant mutation in exon 20 are more likely to benefit from the medication.[16]

Our results show that most Iraqi NSCLC patients have mutations (EX19Del and L858R) (85%) that can be treated with the TARCEVA drug, therefore we recommend conducting another study with a larger sample size for the purpose of confirming the types of mutations that dominate in Iraq to find out whether these drugs are useful or not for those patients.

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