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Original article

Correlation of Epidermal Growth Factor Receptor (EGFR), Mutation Status and Plasma Level

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ABSTRACT

Lung cancer remains a leading cause of cancer-related mortality, with non-small-cell lung cancer (NSCLC) being the most prevalent form. Across populations, there are differences in the frequency of EGFR mutations, which are a crucial component of NSCLC. This study aimed to investigate the potential use of soluble EGFR (sEGFR) as a biomarker for diagnosis NSCLC and its association with EGFR gene mutations. A total of 76 NSCLC tissue samples and corresponding plasma samples, along with 12 control samples, were analyzed. PCR was used to test for EGFR gene mutations, and enzyme-linked immunosorbent assay (ELISA) was used to determine the plasma's sEGFR levels. The overall mutation rate in EGFR gene was 44.7%, with exon 19 deletions being the majority. Notably, there was not a significant difference in sEGFR levels between patients with NSCLC and healthy controls, or between different EGFR mutation statuses. These findings suggest the complexity of sEGFR's role as a diagnostic biomarker, influenced by NSCLC heterogeneity and biological factors. Additional study is required to understand the clinical importance and possible applications of sEGFR in NSCLC and EGFR mutation analysis.

1. Introduction

Globally, lung cancer is the one of the most prevalent cause of death. This hazardous disease kills many people every year and is responsible for the high mortality rate majority of mortality from cancer worldwide. The major public health issue is a lung cancer that requires continued study, early identification, and innovative therapies [5]. This is largely because it is typically discovered at advanced stages. Among the various subtypes of lung cancer, there are two primary categories: non-small-cell lung cancer (NSCLC), which manifests in roughly 83% of cases, and small-cell carcinoma (SCLC), which manifests in roughly 13% of instances. [1, 6] Lung cancer that is not small cell (NSCLC) is the most prevalent kind. Research indicates that between 12.5% and 50.2% of people with advanced lung cancer have epidermal growth factor receptor (EGFR) genetic mutations. [13, 15] NSCLC patients frequently have mutations in the EGFR, with a reported prevalence of 17% in Caucasian populations and roughly 40% in Asian cultures [11]. The EGFR mutation prevalence is higher in nonsmokers (60.7%) than in smokers, and women (61.1%) have high greater risk than males to have mutation in EGFR [4, 21]. The incidence of mutations in EGFR in African and Middle Eastern nations remains unknown, however a meta-analysis study indicates that the frequency of EGFR mutations among the examined NSCLC patients in African and Middle Eastern nations was 17.2% [24].

EGFR, a tyrosine kinase transmembrane receptor, is essential for cellular growth, differentiation, and survival. EGFR mutations are identified as important cancer-causing factors in NSCLC, particularly in histology of adenocarcinoma [7]. As a result, the accurate and timely detection of EGFR mutations has become essential for guiding personalized treatment strategies and improving patient outcomes [20]. Tyrosine kinase inhibitor TKIs that block the EGFR pathway, like gefitinib and erlotinib, are more effective when applied to patients with EGFR mutations. A greater response to EGFR TKIs, like gefitinib and erlotinib, is linked to EGFR mutations [14].

Therefore, EGFR mutation status is an essential biomarker for determining whether patients may benefit for EGFR TKIs. The soluble epidermal growth factor receptor (sEGFR) is a 110 kDa peptide that is produced when an alternatively spliced EGFR isoform's cell surface precursor is broken down by proteases [22]. The discovery of a variant in this circulating sEGFR prompted an investigation into its potential utility as a circulating biomarker. sEGFR is made up of a variety of extracellular domain fragments of the receptor, mostly through alternative mRNA splicing or proteolytic cleavage [2, 17]. The level of sEGFR in circulatory fluids may be regulated and influenced by

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both healthy and cancerous tissues [16]. Some publications reflect the intriguing finding that the levels of circulating sEGFR in healthy individuals were greater and in patients with malignant tumors tended to decrease; nevertheless, the data from these preliminary research are still unclear [3, 12, 18]. However, the association between EGFR gene mutation status and sEGFR levels in body fluid (plasma) in patients with lung cancer is not well established. Here, we looked at whether sEGFR might be a possible biomarker for diagnosing NSCLC patients and if there is a relationship between sEGFR levels and NSCLC. We further investigated the association between sEGFR levels in lung cancer patients and mutation status of EGFR gene.

2. Materials and methods

2.1. *Sample Collection*

A total of 76 tissue samples of lung cancer patients diagnosed as NSCLC were obtained from pathology department. Additionally, plasma samples of those patients were retrieved from the biobank of Shefaa Al-Orman Oncology Hospital (SOH), after approval of the Scientific Advisory Committee (SAC) and the institutional Review Board (IRB)of SOH. The control group comprised 12 healthy volunteers who did not have any acute or chronic inflammatory conditions. All individuals, including healthy volunteers and patients, provided their informed consent.

2.2. *DNA Extraction:*

The QIAamp® DNA FFPE Tissue Kit (Qiagen, Cat. No.: 56404), was used to extract the DNA according to the manufacturer's instructions. The purified DNA samples were divided into aliquots and stored at low temperature -80°C until further analysis.

2.3. *Polymerase Chain Reaction (PCR) Amplification:*

To detect EGFR gene mutations, the PCR Kit Therascreen® EGFR RGQ (cat: 870111) was used to perform PCR. This kit is specifically designed for EGFR mutations detection and has been validated for accurate and sensitive mutation analysis, which enables the detection of DNA alterations vs wild-type. The kit can detect the following mutations: 3 insertions in exon 20,T790M,19 deletions in exon 19, L858R, L861Q, S768I and G719X .

2.4. *Soluble EGFR Measurement:*

The levels of soluble EGFR were quantified in plasma samples. Human EGFR ELISA Kit Cat: ELK2110. A total of 76 NSCLC patient samples and 12 samples from healthy controls were analyzed. The plasma samples were obtained from the Shefaa Al-Orman Oncology Hospital biobank, which had received ethical approval for sample collection and storage.

2.5. *Statistical Analysis:*

Statistical approaches were used to assess the correlation between EGFR mutations and soluble EGFR levels. We examined the levels of plasma EGFR in the healthy group (control) and the group of cancer patients, as well as in the cancer patients' group categorized by their gene mutation status. Examine age disparities across several mutant categories. An analysis of the dataset was conducted through the utilization of the SPSS, Statistical Package for the Social Sciences version 28. Mann-Whitney U test, a statistical method, was applied to ascertain the significance of the observed variances.

3. Results and Discussion

3.1 Results

The demographic characteristics of the participants in this study were defined as follows: The median age for cancer patients was determined to be 61 years, the range of age spanning from 22 to 86 years. Healthy controls were 47.5 years old on average, ranging from 27 to 66 years. The gender distribution in the patient exhibited a predominance of males, comprising 68.4% of the total population.

An important finding of this study was the wild type percentage 55.3%.The overall EGFR mutation rate, which came out to be 44.7% (34 out of 76 patients) table 1. Exon 19 deletion mutations are 61.8% of all the mutations in this study, making them the majority (Table 2). The frequency and distribution of several EGFR mutation types were then determined by analysis; exon 19 deletion mutations had the highest percentage (61.8%), followed by exon 21's L858R mutation (26.5%), and other less common variants. Table 2 presents the information of many EGFR mutations.

Table 1 : The Mutant and wild type percentage

	Frequency	Valid Percent	Cumulative Percent
Mutant	34	44.7	44.7
Wild type	42	55.3	100.0
Total	76	100.0	

Table 2: The rate of EGFR mutation types

Mutation type	Frequency	Valid Percent	Cumulative Percent
Deletion mutation in exon 19	21	61.8	61.8
G712X Mutation in exon18	1	2.9	64.7
L858R mutation in exon 21	9	26.5	91.2
T790 M mutation in exon 20 & 5 L858R mutation in exon 21	2	5.9	97.1
T790M mutation in exon 20	1	2.9	100.0
Total	34	100.0	

Concurrent representation, either by means with standard deviation or median with minimum and maximum, was required for a comprehensive understanding. Because of the divergence from normality assumptions, The Mann-Whitney U test was employed to find differences between groups. P-values < 0.05 were used to determine significance in all analyses.

Table 3: Plasma EGFR in the Healthy group (control) and the cancer patients' group

	Healthy group	Cancer patients' group	p
Mean ±SD	6.5±1	7.2±2.5	0.8
Median (min-max)	6.4(4.7-8.3)	6.4(4-21.2)	

P <0.05 significant, the p-value was determined using the Mann-Whitney U test.,

The median plasma EGFR level in the healthy group was 6.4, ranging from a minimum of 4.7 to a maximum of 8.3. In contrast, the cancer patients' group exhibited a median plasma EGFR level of 6.4, with a range of 4 to 21.2. A Mann-Whitney U test was employed to evaluate the disparity in plasma EGFR between the healthy group and NSCLC patients. The test results indicated that the plasma EGFR levels in the two groups do not differ statistically (p-value = 0.8) as present in Table 3.

Table 4: Plasma EGFR in the cancer patients' group stratified by the gene mutation status.

	Mutant	Wild type	P
Mean ±SD	7.3±3.3	7.1±1.8	0.2
Median (min-max)	5.9(4-21.1)	6.9(4-12.2)	

P <0.05 significant, the p-value was determined using the Mann-Whitney U test.

The median plasma EGFR level in the mutant type group was 5.9, ranging from a minimum of 4 to a maximum of 21.1. In contrast, the wild type group exhibited a median plasma EGFR level of 6.9, with a range of 4 to 12.2. A Mann-Whitney U test was employed to evaluate the disparity in plasma EGFR between the healthy group and NSCLC patients. The test results indicated that there is no statistically significant difference in plasma EGFR between the two groups, with a p-value of 0.2 as present in Table 4.

In examining age differences among different mutation types, using an Independent-Samples Mann-Whitney U Test, the age is equivalent across mutation and wild types the test yielded a p-value of 0.855 Fig (1). Consequently, there is insufficient evidence to suggest a significant difference in age distribution among individuals with different mutation types. This result is based on asymptotic significances, with a significance level set at 0.05.

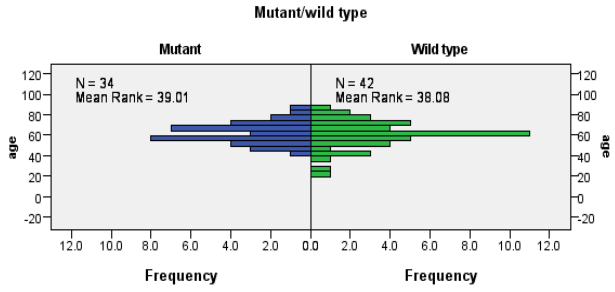

Fig (1): Distribution of age across mutation and wild types.

3.2. Discussion

Biological fluids like serum or plasma can be used to measure the soluble isoform of EGFR, or sEGFR. This protein may be a promising biomarker although its overall role in cancer is still unclear.

Numerous studies have been conducted on the detection of sEGFR in plasma as possible biomarkers for NSCLC. These biomarkers hold promise for aiding in diagnosis, prognosis, and treatment selection. However, the link between EGFR mutation status and sEGFR levels in plasma remains a topic of ongoing investigation, and the results have been inconsistent.

In the current study, the measurement of sEGFR in plasma has been investigated as a possible biomarker for NSCLC and the possible presence of EGFR gene mutations. A single study evaluated the usefulness of plasma sEGFR as an NSCLC biomarker, while only a small number of studies looked at serum sEGFR as an NSCLC prognostic biomarker. [8, 19, 23]. However, the current investigation did not find any difference in sEGFR levels between NSCLC patients and healthy controls. This finding is not compatible with some earlier research studies that showed that patients with non-small cell lung cancer (NSCLC) had significantly lower plasma sEGFR levels than controls. When contrasting healthy controls with NSCLC [9], this study found a significant difference when using plasma samples to compare the level of sEGFR in patients with NSCLC and healthy controls. In earlier studies, when serum was employed as the sample matrix, no statistical significance was found between NSCLC patients and healthy controls, which could be attributed to the production of proteases during coagulation and the release of platelet contents and other blood cells [10]. As a result, they determined that EDTA plasma was the best matrix for their research on the function of EGFR as a biomarker in NSCLC. Following these recommendation, we used plasma samples in our study .However, patients with wild-type EGFR and those with EGFR mutations did not show difference in their sEGFR levels, which is consistent with prior research that found similar results [19, 23].

This discrepancy in findings regarding sEGFR levels between lung cancer patients with NSCLC and healthy individuals could be attributed to several factors. NSCLC is a complex illness with different molecular profiles and clinical features. The presence of various genetic alterations and different stages of the disease within the NSCLC patient population might contribute to differences in sEGFR levels. It is possible that sEGFR levels are influenced by specific subtypes of NSCLC or other concurrent molecular alterations that were not fully explored in our study or in some of the previous studies. Furthermore, the statistical power of a study is influenced by the sample size. Larger, well-powered studies might be needed to accurately capture variations in sEGFR across different populations. Moreover, the biology of EGFR signaling is intricate, involving numerous ligands, receptors, and downstream pathways. The release of sEGFR could be influenced by various biological factors, including the presence of other receptors and ligands [12].

4. Conclusion

In conclusion, the lack of significant differences in sEGFR levels between healthy and lung cancer with

NSCLC individuals, as well as between patients with different EGFR mutation statuses, suggests that the role of sEGFR as an NSCLC diagnostic biomarker is intricate and multifaceted. The evolving understanding of EGFR biology, along with advancements in technology and methodologies, will continue to shape our understanding of the potential clinical utility of sEGFR in the context of lung cancer diagnosis and management. Non-significant results indicatez that sEGFR may not be a reliable biomarker for distinguishing these groups. However, further research is necessary to address the limitations and discrepancies in previous studies and to assess the true clinical value and possible applications of sEGFR in NSCLC and EGFR mutation analysis.

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