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Original paper

Identification of EGFR Gene Exon 21 and 20 Mutations Based on the Method of PCR Sequencing in Paraffin Biopsy of Lung Cancer

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Abstract

Background and aim: One of the causes of cancerous tumours is mutations that occur in essential genes, such as genes that control cell growth and division. EGFR is an intermembrane protein that plays a role in cell growth, proliferation and differentiation with its tyrosine kinase activity.

Considering the increasing prevalence of lung cancer and the identification of EGFR gene mutations as one of its causes, designing a non-invasive and quantitative method with early detection power to investigate and identify biomarkers related to lung cancer can play a significant role in determining this cancer. We designed a more accurate and cost-effective molecular system to detect exon 20 and 21 mutations to identify responses to targeted drug therapy and monitor cancer treatment, considering the predictive value of this gene and screening in patients.

Materials and methods: In this research, 20 positive paraffin biopsy samples of lung cancer were used. Since the samples used are embedded in paraffin, we must first deparaffinize the samples and then perform the steps. We used Hexadecane to deparaffinize the samples. After deparaffinization of the tissue for extraction, it was necessary to digest the tissue, which was performed with Proteinase k.

In the next step we used PZP kit for DNA extraction. As a last step we performed quantitative and qualitative analysis on them to confirm the presence of DNA, and PCR-Sequencing of exon 20 and 21 was performed.

Results: During the research conducted on lung cancer tissue, in general, out of 20 samples examined from exon 20 and 21, 9 of our samples mutations with rs 1151171 were observed, all of which were in exon 20, where Nucleobase G was changed to A, and in exon 21 No mutations were observed in our samples. The sequencing PCR method identified the mutation in exons 20 and 21.

Conclusion: For people with lung cancer, their treatment methods include chemotherapy, which has various drugs available for chemotherapy, and their effect is on the growth of cells. Before chemotherapy, the patient's tissue must be examined for the presence or absence of mutations. Examine the EGFR gene so that they can prescribe a suitable drug for chemotherapy. According

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to the results, a point mutation in exon 20 of the EGFR gene is related to cancer progression and the response to drug therapy.
Keywords: *Lung cancer, EGFR Gene, PCR- Sequencing, Exon 21 and 20*

Introduction

Lung cancer is one of the most common and deadliest cancers globally. This type of cancer is the leading cause of cancer death in men and the second leading cause of cancer death in women worldwide. About 1.6 million cases of lung cancer are diagnosed worldwide each year [1]. There are two main types of lung cancer, 1- Non-small cell lung cancer (NSCLC)-2-Small cell cancer (SCLC) [2], Non-small cell lung cancer (NSCLC) originating from bronchial epithelial cells, it is divided into three types: Squamous cell carcinoma, adenocarcinoma and large cell lung cancer, originating from central nervous cell precursors. Small cell lung cancer (SCLC) is a malignant epithelial tumour composed of small cells with diffuse cytoplasm, indistinct cell borders, granular nuclear chromatin, absent or indistinguishable, round, oval, and spindle- shaped nuclei [3].

The EGFRs family has three parts with different functions, which include an external domain that binds to the ligand, a hydrophobic hydrophobic domain, and a cytoplasmic domain with tyrosine kinase capability [4]. Ligand binding to EGFR induces and brings together the receptors, and finally, It causes the formation of homo or heterodimeric structures of receptors [5]. The formation of dimeric structures leads to the activation of the tyrosine kinase property of the receptors and the phosphorylation of the intracellular structures of the receptors, which leads to the activation of the intracellular signaling pathways [6].

Epidermal growth factor receptor (EGFR) is a glycoprotein with a weight of 171 kDa and a length of 1186 amino acids, which is located on the short arm of chromosome number 7 (11.2p7), and the EGFR gene has 28 exons with an approximate length of 211 nucleobases. The EGFR gene also has repetitive elements throughout its genome, including SINEs and LINEs, as well as GC-rich repetitive regions in intron 15 and CA repetitive sequences in intron27 [7].

EGFR is a membrane receptor tyrosine kinase type 1 (RTKs) that belongs to the ErbB family [8]. EFGR (epidermal growth factor receptor) is vital in regulating normal cell proliferation, apoptosis and cell functions. Approximately 11% of NSCLC patients in the United States and 35% in East Asia are associated with this mutation. The structure of EGFR is tyrosine and plays a role in the activation of various AKT/K3PI (phosphoinositol trikinase) and RAF/RAS as well as MAPK pathways [9]. The most common mutation known in this gene is in exons 19 and 21, which includes 89% of mutations [10]. Four receptor tyrosine kinases are involved in the cancer pathway after binding to the activation of receptor tyrosine kinase, protein kinase MAPK and phosphor 3-inositol kinase (PI3K) [11]. The common mutation that was seen in the other 45% of EGFR mutation cases is the CTG to CGG point mutation at nucleotide 2573 of exon 21, which leads to The amino acid leucine being replaced by arginine in codon 858 [12].

To identify EGFR as an oncogene led to the development of anticancer drugs against EGFR (called EGFR inhibitors)—small molecules that inhibit EGFR tyrosine kinase, which is on the cytoplasmic side of the receptor. EGFR cannot activate without kinase activity itself a prerequisite for binding stable adapter proteins. It is reduced by stopping the signalling cascade in cells that rely on this pathway for tumour growth, proliferation, and migration. Gefitinib, Erlotinib, Brigatinib and lapatinib are examples of small kinase inhibitors [13], [14]. This study identifies EGFR gene exon 21 and 21 mutations based on PCR-sequencing method.

Material and Methods

In this study, 20 positive lung cancer paraffin biopsies were used. Since the samples used are embedded in paraffin, the samples must first be deparaffinized before performing the steps. We used Hexadecane to de-paraffin the samples. After deparaffinizing the tissue for extraction, it is necessary to digest the tissue with proteinase k. In the next step, we use the PZP kit to extract DNA. In the final step, we performed quantitative and qualitative analyses to confirm the presence of DNA and performed PCR sequencing of exons 20 and 21.

Table 1. Information of the primer used for exon 20

EGFe20-SF: CAC AGC CCT GCG TAA ACG TCC, 21-mer, Tm: 65.9ºC, hp: 42
EGFe20-SR: GCA CGC ACA CAC ATA TCC CCA, 21-mer, Tm: 65.5 ºC, hp: 0

Table 2. Information of the primer used for exon 21

EGFe21-SF: CGT TCG CCA GCC ATA AGT CCT C, 22-mer, Tm: 65.3ºC, hp: 0
EGFe21-SR: CCC CTG CAT GTG TTA AAC AAT ACA GC, 26-mer, Tm: 64.9ºC, hp: 38

Results

In this research, 20 paraffin tissue samples of lung cancer were randomly used, 10 samples for exon 21 and 10 samples for exon 20. The band appeared in the region of bp474 and the sample with exon 21 primer, the band appeared in the region of bp375.

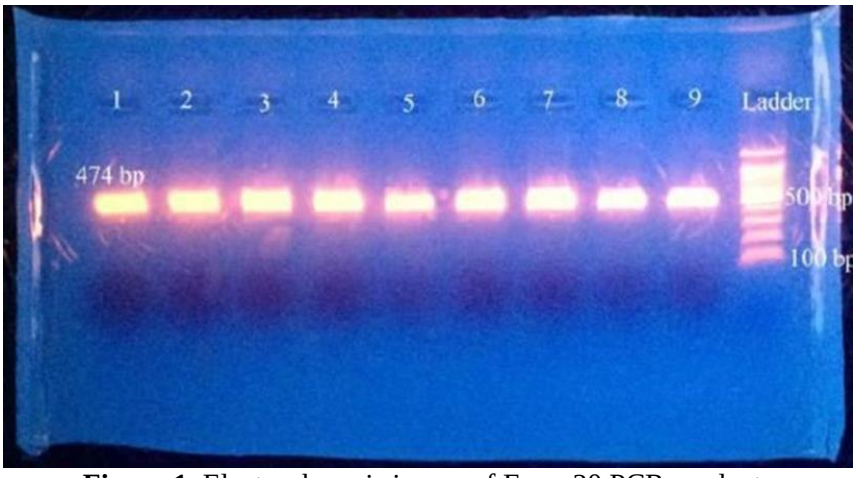

Figure 1. Electrophoresis image of Exon 20 PCR products

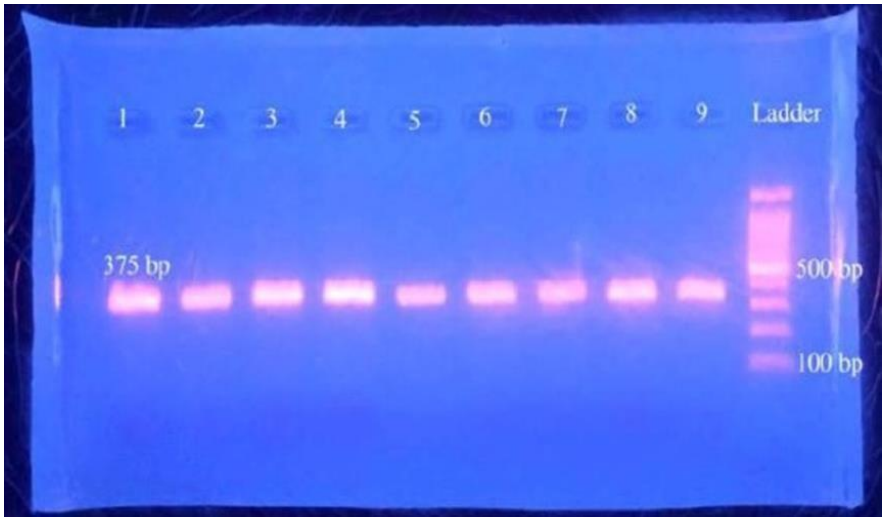

Figure 2. Electrophoresis image of Exon 21 PCR products

The results of sequence alignment of exon 21 and 20 with DNAMAN 2.1 VERSION software, during the research conducted on lung cancer tissue, in general, out of 20 samples examined from exon 21 and 20, 9 point mutations with rs 1151171 were observed, all of them in They were exon 20, where the G base was changed to A, and no mutation was observed in exon 21 in our samples. Sequencing PCR method was able to identify and detect mutations in exons 20 and 21.

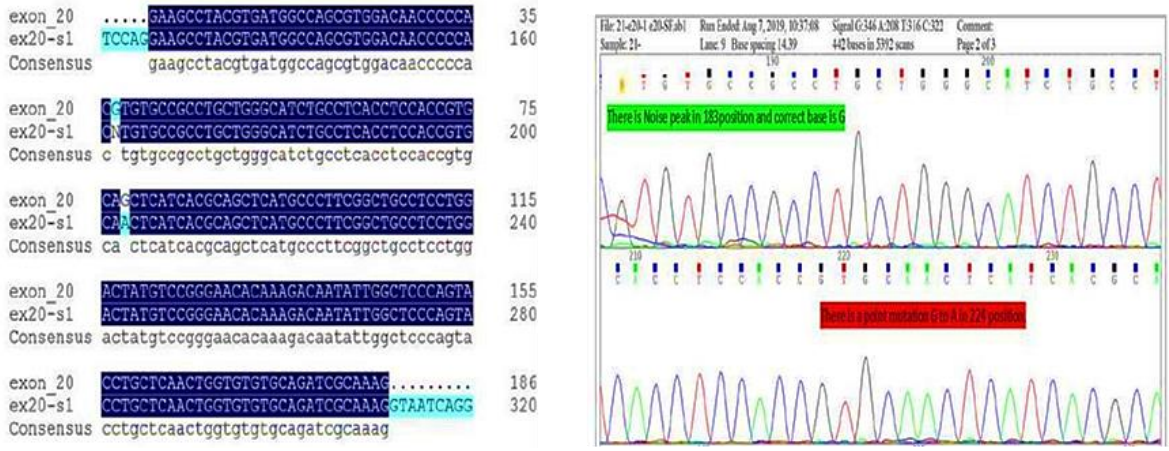

Figure 3. Results of exon 20 sequencing of e20-1 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

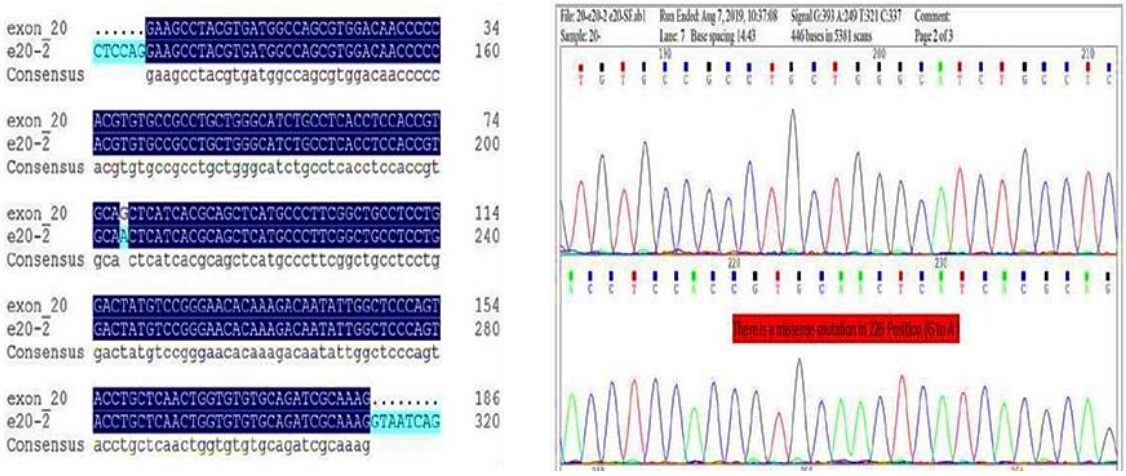

Figure 4. Results of exon 20 sequencing of e20-2 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

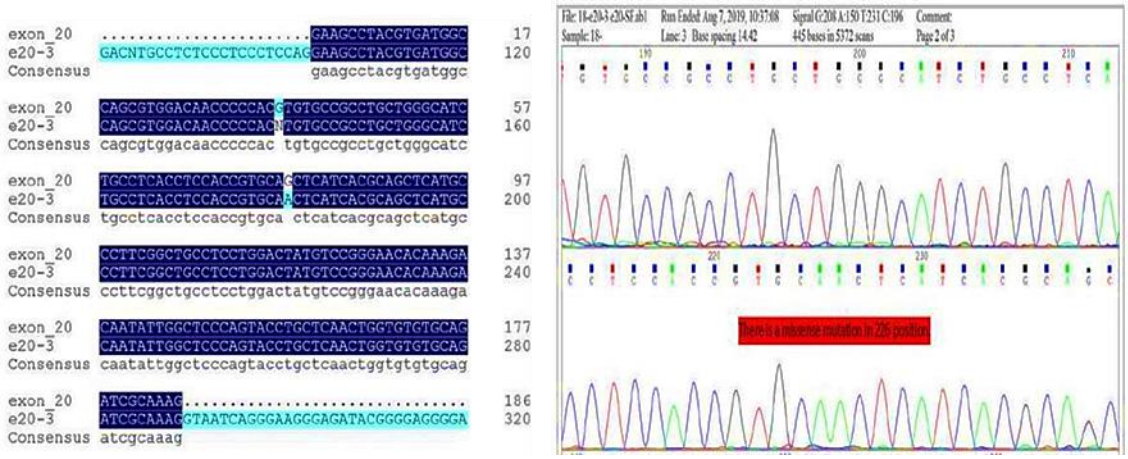

Figure 5. Results of exon 20 sequencing of e20-3 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

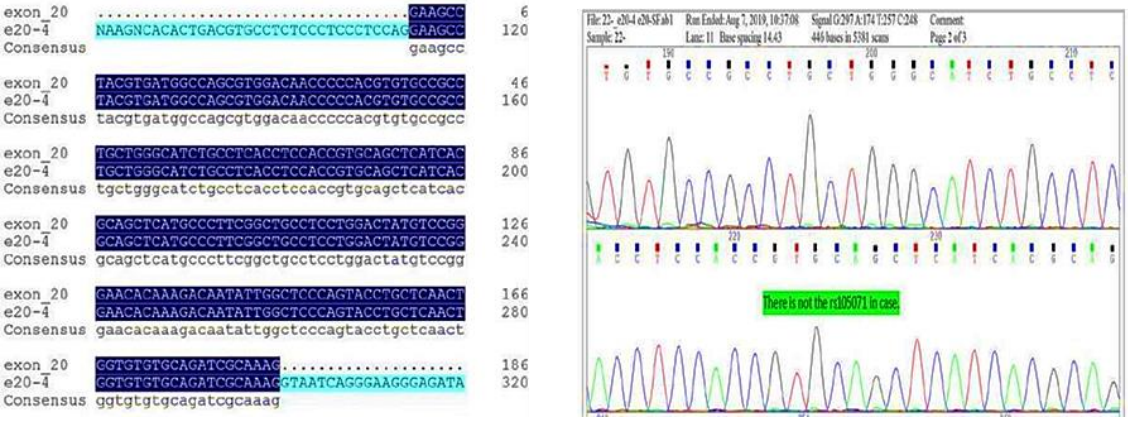

Figure 6. Results of exon 20 sequencing of e20-4 sample with DNAMAN VERSION 2.1 software (No mutation)

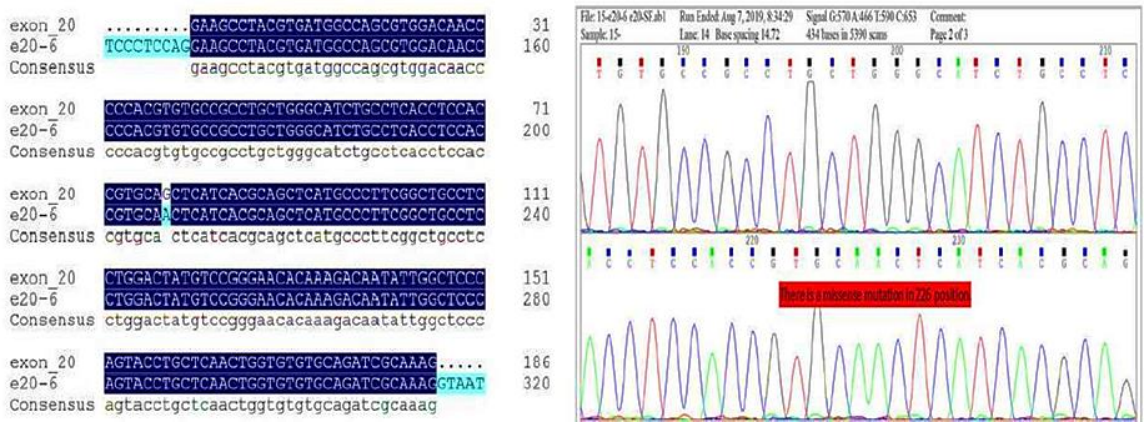

Figure 7. Results of exon 20 sequencing of e20-6 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

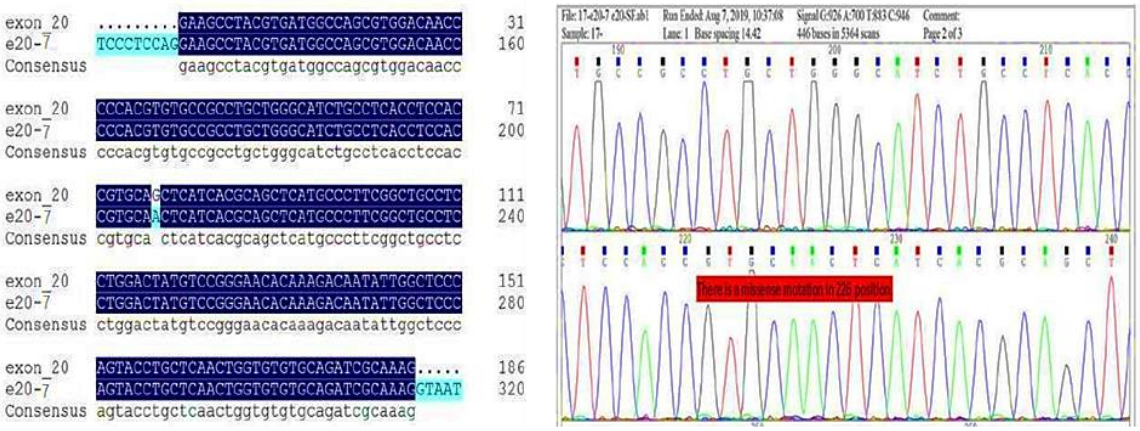

Figure 8. Results of exon 20 sequencing of e20-7 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

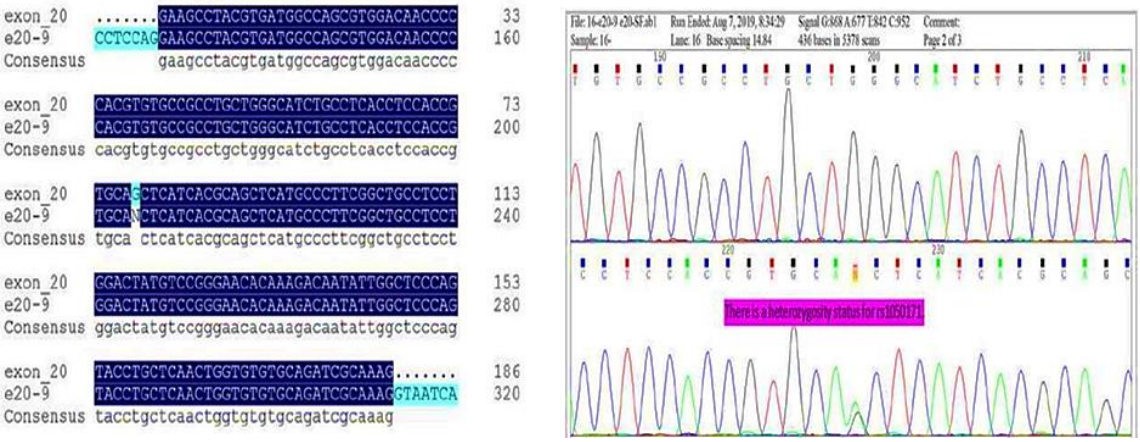

Figure 9. Results of exon 20 sequencing of e20-9 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

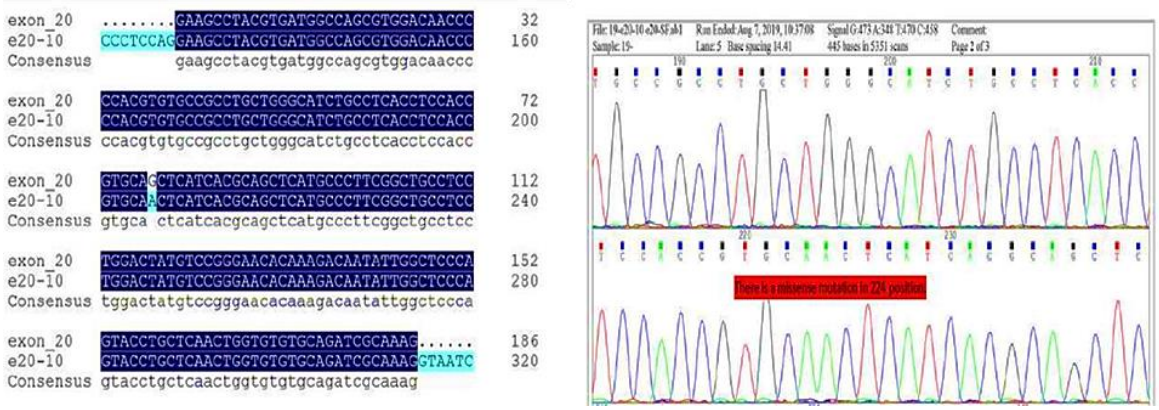

Figure 10. Results of exon 20 sequencing of e20-10 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

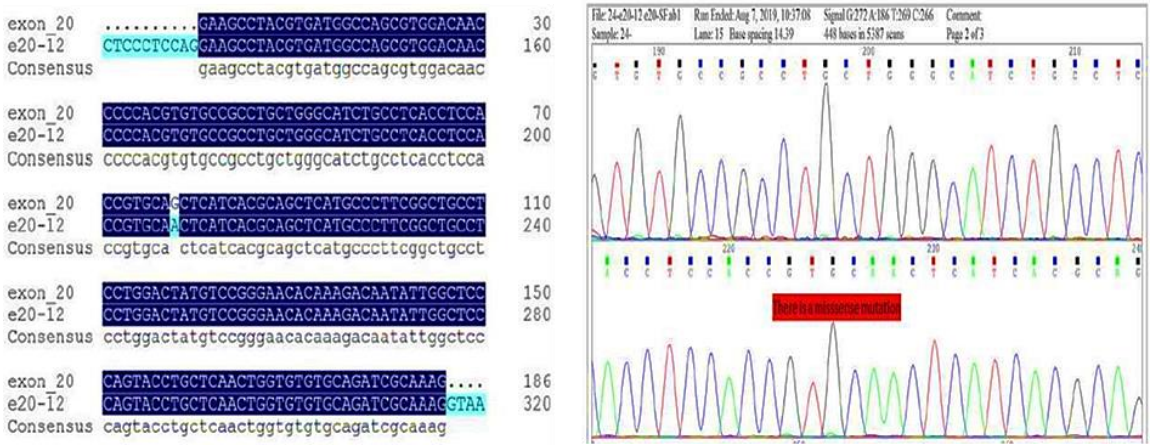

Figure 11. Results of exon 20 sequencing of e20-12 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

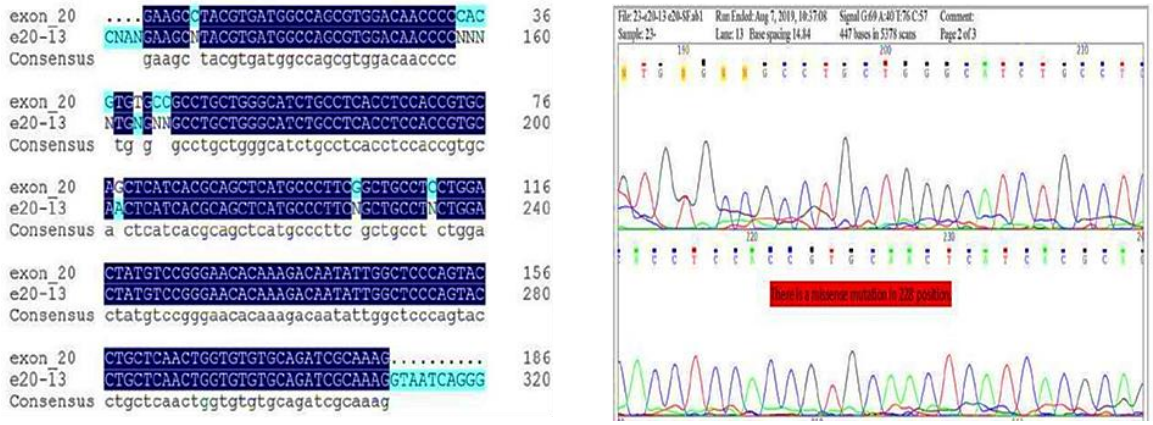

Figure 12. Results of exon 20 sequencing of e20-13 sample with DNAMAN VERSION 2.1 software (contains missense mutation with rs1050171)

rs1050171 [*Homosapiens*]
- *Variant Type:*
SNV
- *Alleles:*
G>A,C
- *Chromosome:*
7:55181370
- *Gene:*
EGFR (Varview), EGFR-AS1 (Varview)
- *Functional Consequence:*
non_coding_transcript_variant, genic_downstream_transcript_variant,coding_seque
nce_variant,missense_variant,synonymous_variant
- *Clinical Significance:*
benign,likely-benign
- *Validated:*
by frequency,by cluster
- *HGVS:*
NC_000007.14:g.55181370G>A, NC_000007.14:g.55181370G>C, NC_000007.1
3:g.55249063G>A, NC_000007.13:g.55249063G>C, NG_007726.3:g.167339G>A
, NG_007726.3:g.167339G>C, NM_005228.5:c.2361G>A,
The mutations we obtained are the conversion of G to A, and the mutation of the conversion of G to C was not observed, and all were of the G to A type.

Discussion

According to the results obtained in this project on lung cancer tissue, in general, out of 20 samples examined from exon 21 and 20, 9 point mutations were observed with rs 1050171 and the PCR Sequencing method is able to identify and diagnose. 40 % of EGFR gene mutations have been identified in patients with lung cancer in Asia [15].The main cause of lung cancer is mutation in proto-oncogene KRAS and mutation in Epidermal growth factor (EGFR) is common in non-small cell lung cancer, especially adenocarcinoma [16]. The EGFR gene has 4423 single nucleotide polymorphisms, of which 16 polymorphisms in the EGFR gene have been confirmed to be associated with lung cancer [17]. In a study in 2011, researchers investigated EGFR exon 19 mutations. They used the direct PCR sequencing method in this research. Their results showed that 172 lung adenocarcinoma patients had mutations in their exon 19 and the mutation in this exon had caused this disease [18]. Also, in another study, the researchers investigated EGFR gene mutation and its expression in esophageal squamous cell carcinoma. In this study, direct sequencing was used. The samples included 152 people who had ESCC disease. Exons 18-21 were analyzed. 14 samples showed EGFR changes, among which 4 people had lung cancer during the research [19].

In a real clinic world, there are factors associated to the study of EGFR mutations in lung cancer patients. Detection of EGFR mutation may be one test among several tests [20]. Thus, there is no consensus on how to prioritize the different assays that are often performed in different laboratories [21]. After the importance of determining the mutational status of EGFR in lung cancer was first demonstrated, there is no standard approach for performing this mutation analysis. There are wide variety of methods, some of them developed in vitro assays, have been used to analyze EGFR

mutations. However, they are not suitable for routine clinical use for various reasons such as cost, complexity, long time, low specificity or unavailability [22], [23].
In an aggressive tumor, choosing the right treatment option according to the recognition of the mutation in the invasive gene of lung cancer, we need to use a very fast and reliable identification method [24].

Conclusion

During the study performed on general lung cancer tissues, out of 20 samples tested from exons 21 and 20, 9 of our samples had a point mutation with rs 1050171, all in exon 20, where the G base is changed to A, and there is no mutation. It was observed in our samples in exon 21, and the PCR sequencing method identified and detected mutations in exons 20 and 21Their treatment for people with lung cancer includes chemotherapy, which provides various drugs to stimulate and influence cell growth. Before chemotherapy, the patient's tissues need to be tested for the presence or absence of mutations. Test for the EGFR gene so that appropriate medication can be prescribed for chemotherapy.
According to the results obtained, point mutations in exon 20 of the EGFR gene are associated with cancer progression and response to drug treatment.

Acknowledgment

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Conflict of interests

The authors declare that there are no competing interests.

Reference

[1]. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, Bray F. International variation in prostate cancer incidence and mortality rates. European urology. 2012;61(6):1079-92.
[2]. Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type: male: female differences diminishing and adenocarcinoma rates rising. International journal of cancer. 2005;117(2):294-9.
[3]. Zakowski MF. Pathology of small cell carcinoma of the lung. InSeminars in oncology 2003 Feb 1 (Vol. 30, No. 1, pp. 3-8). WB Saunders.
[4]. Yarden Y. The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. European journal of cancer. 2001;37:3-8.
[5]. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature reviews Molecular cell biology. 2001;2(2):127-37.
[6]. Qu CK. Role of the SHP-2 tyrosine phosphatase in cytokine-induced signaling and cellular response. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2002;1592(3):297-301.
[7]. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R. 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.
[8]. Boeckx C, Weyn C, Vanden Bempt I, Deschoolmeester V, Wouters A, Specenier P, Van Laer C, Van den Weyngaert D, Kockx M, Vermorken JB, Peeters M. Mutation analysis of genes in the EGFR pathway in Head and Neck cancer patients: implications for anti-EGFR treatment response. BMC research notes. 2014;7:1-8.
[9]. Greulich H, Pollock PM. Targeting mutant fibroblast growth factor receptors in cancer. Trends in molecular medicine. 2011;17(5):283-92.
[10]. Xu W, Li Y, Wang X, Chen B, Wang Y, Liu S, Xu J, Zhao W, Wu J. FGFR4 transmembrane domain polymorphism and cancer risk: a meta-analysis including 8555 subjects. European journal of cancer. 2010;46(18):3332-8.
[11]. Dacic S, Flanagan M, Cieply K, Ramalingam S, Luketich J, Belani C, Yousem SA. Significance of EGFR protein expression and gene amplification in non–small cell lung carcinoma. American Journal of Clinical Pathology. 2006;125(6):860-5.
[12]. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proceedings of the National Academy of Sciences. 2004;101(36):13306-11.
[13]. Yan L, Beckman RA. Pharmacogenetics and pharmacogenomics in oncology therapeutic antibody development. Biotechniques. 2005;39(4S):S565-8.
[14]. Roskoski Jr R. The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacological research. 2014;79:34-74.
[15]. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proceedings of the National Academy of Sciences. 2004;101(36):13306-11.
[16]. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science. 2004;305(5687):1163-7.
[17]. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS medicine. 2005;2(3):e73.
[18]. Araki T, Shimizu K, Nakamura T, Baba M, Kawai Y, Nakamura K, Mitani Y, Obayashi K, Aomori T, Fujita Y, Miyamae Y. Clinical screening assay for EGFR exon 19 mutations using PNA-clamp smart amplification process version 2 in lung adenocarcinoma. Oncology reports. 2011;26(5):1213-9.
[19]. Hainaut P, Malekzadeh R, Saidi F, Mounawar M, Villar S, Martel-Planche G, Lone MM, Zargar SA, Mir MM, Dar NA, Abedi-Ardekani B. Epidermal growth factor receptor (EGFR) mutations and expression in squamous cell carcinoma of the esophagus in central Asia. BMC cancer. 2012;12:1-7.
[20]. Hirsch FR, Wynes MW, Gandara DR, Bunn Jr PA. The tissue is the issue: personalized medicine for non-small cell lung cancer. Clinical Cancer Research. 2010;16(20):4909-11.
[21]. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. The lancet oncology. 2011;12(2):175-80.
[22]. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. Clinical Cancer Research. 2007;13(17):4954-5.
[23]. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G,

Isla D, Provencio M, Insa A. Screening for epidermal growth factor receptor mutations in lung cancer. New England Journal of Medicine. 2009;361(10):958-67.
[24]. Angulo B, Conde E, Suarez-Gauthier A, Plaza C, Martínez R, Redondo P, Izquierdo E, Rubio-Viqueira B, Paz-Ares L, Hidalgo M, López-Ríos F. A comparison of EGFR mutation testing methods in lung carcinoma: direct sequencing, real-time PCR and immunohistochemistry. PLoS ONE. 2012;7(8): e43842.