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YEDITEPE UNIVERSITY
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THE ROLE OF KLK10 GENE (RS7259451) POLYMORPHISMS IN SUSCEPTIBILITY TO NON-SMALL CELL LUNG CANCER

MASTER THESIS

Büşra BIÇAK

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SUPERVISOR

ASSOC. PROF. DR. G. SEDA GÜLEÇ YILMAZ

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	Title, Name-Surname (Institution)
Chair of the Jury:	Prof. Dr. Turgay İsbir
Supervisor:	Assoc. Prof. Dr. Seda Güleç Yımaz
Member/Examiner:	Prof. Dr. Bedia Çakmakoğlu

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 20.06.2022 and numbered .....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for an award of any other degree except where due acknowledgment has been made in the text.

BÜŞRA BIÇAK

DEDICATION

I dedicate my thesis to my lovely family,

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	Microliter
ALK	Anaplastic lymphoma kinase
CI	Confidence interval
CNS	Central nerve system
COVID-19	Coronavirus disease
CT	Computed tomography
CXR	Chest radiography
DEAE	Diethylaminoethyl
DNA	Deoxyribose nucleic acid
DNase	Deoxyribonuclease
EBUS	Endobronchial ultrasound
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor gene
EUS	Endoscopic ultrasound
FDG	Fluoro-Deoxy-Glucose
FISH	Fluorescent in situ hybridization
hMSH2	Human mismatch repair genes
HPV	Human papillomavirus
IASLC	International association for the study of lung cancer
KLK	Kallikrein

KLK10	Kallikrein 10
LCLC / LCC	Large-cell lung cancer/carcinoma
MAF	Minor allele frequency
MRI	Magnetic resonance imaging
NaCl	Sodium chloride
Nm	Nanometer
NSCLC	Non-small-cell lung cancer/carcinoma
OD	Optical density
OR	Odd ratio
PD-L1	Programmed death ligand 1
PET-CT	Positron emission tomography-computed tomography
pH	Potential of hydrogen
PI3K	Phosphoinositide 3-kinase
Rb gene	Retinoblastoma gene
RNase	Reoxyribonuclease
ROS1	Proto-oncogene 1
RT-PCR	Real-time polymerase chain reaction
SBRT	Stereotactic radiation therapy
SCC	Squamous-cell lung cancer/carcinoma
SCLC	Small-cell lung cancer/carcinoma
SNP	Single nucleotide polymorphism

SPSS	Statistical package for social sciences
TBNA	Transbronchial fine needle aspiration
TNM	Tumor-lymph nodes-metastasis
Tp53 gene	Tumor protein p53
Tris-Cl	Trisaminomethane chloride
UV	Ultraviolet light
VATS	Video-assisted thoracic surgery
WHO	World health organization
X-ray	X-radiation
χ^2	Chi square

ABSTRACT

Bicak, B. (2022). The Role of KLK10 Gene (rs7259451) Polymorphisms in Susceptibility to Non-small Cell Lung Cancer. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. MSc thesis, Istanbul.

Nowadays, with the COVID-19 disease, the significance of lung cancer specially NSCLC has increased. Moreover, it is also important because it is the second most deadly cancer among other cancers. Kallikrein 10 (KLK10) is one of 15 kallikrein family members. It is a serine endopeptidase and also it has a potential of tumor suppressor protein in prostate and prostate malignant cells. The aim of this study, observation of the impact of KLK10 gene (rs729451) in NSCLC. Blood sample were collected from 48 NSCLC patient and 46 healthy control groups. All DNA isolated from blood samples by using DNA Isolation Robot. Then DNA samples were prepared to make Real-Time PCR (RT-PCR) by using KLK10 detection kit. After RT-PCR, allelic discrimination was obtained from both patient and control groups. Then statistical analyzes were done in SPSS 26.0 by using Fisher's Exact Tests and Chi-square tests and also student's t-test was utilized to get numerical values. P values less than or equals to 0.05 are considered statistically significant. According to analysis, GG wild type homozygote 52.1% (n=25), GT heterozygote 45.8% (n=22) and TT 2.1% (n=1) has been recorded in patient. In addition to this, GG wild type homozygote 60.9% (n=28), GT heterozygote 32.6% (n=15) and TT 6.5% (n=3) has been obtained in control group. Moreover, G allele 80.2% (n=72) and T allele 19.8% (n=24) in patient, and G allele 77.2% (n=71) and T allele 22.8% (n=21) in control group. In the control group, the TT genotype was three times greater (p=356). These findings suggest that the TT genotype is a carrier genotype. Nonetheless, the number of TT genotypes (both control and patient) was extremely low, requiring more research. As a consequence, there is no statistically significant difference in KLK10 polymorphism between the control and patient groups (rs7259451). It's possible that the TT genotype is a carrier gene. More research, on the other hand, is already required. Perhaps this study might be improved by include a broader or diverse population. Otherwise, alternative SNPs in this gene can be investigated.

Keywords: Non-small cell lung cancer, NSCLC, polymorphism, KLK10, rs7259451

ÖZET

Bıçak, B. (2022). Küçük Hücreli Olmayan Akciğer Kanserine Duyarlılıkta KLK10 Geni (rs7259451) Polimorfizminin Rolünün Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Yüksek lisans tezi, İstanbul.

Günümüzde COVID-19 hastalığı ile birlikte akciğer kanserinin özellikle KHOAK'nin önemi artmıştır. Ayrıca diğer kanserler arasında en ölümcül ikinci kanser olması nedeniyle de önemlidir. Kallikrein 10 (KLK10), kallikrein ailesinin 15 üyesinden biridir. Bir serin proteazdır ve ayrıca prostat ve prostat malign hücrelerinde tümör baskılayıcı protein potansiyeline sahiptir. Bu çalışmanın amacı, KLK10 geninin (rs729451) KHOAK'deki etkisinin gözlemlenmesidir. 48 KHOAK hastası ve 46 sağlıklı kontrol grubundan kan örneği alındı. DNA İzolasyon Robotu kullanılarak kan örneklerinden tüm DNA izole edildi. Daha sonra KLK10 tespit kiti kullanılarak Real-Time PCR (RT-PCR) yapmak için DNA örnekleri hazırlandı. RT-PCR sonrası hem hasta hem de kontrol gruplarından allelik değerler elde edildi. Daha sonra Fisher's Exact Testleri ve Ki-kare testleri kullanılarak SPSS 26.0'da istatistiksel analizler yapıldı ve sayısal değerler elde etmek için Student t-testi kullanılmıştır. 0,05'ten küçük veya 0,05'e eşit olan P değerleri istatistiksel olarak anlamlı kabul edilir. Analize göre hastalarda GG doğal tip homozigot %52,1 (n=25), GT heterozigot %45,8 (n=22) ve TT %2,1 (n=1) kaydedildi. Ayrıca kontrol grubunda GG doğal tip homozigot %60,9 (n=28), GT heterozigot %32,6 (n=15) ve TT %6,5 (n=3) elde edilmiştir. Ayrıca hastalarda G alleli %80,2 (n=72) ve T alleli %19,8 (n=24), kontrol grubunda G alleli %77,2 (n=71) ve T alleli %22,8 (n=21) idi. Kontrol grubunda TT genotipi üç kat daha fazlaydı (p=356). Bu bulgular, TT genotipinin bir taşıyıcı genotip olduğunu düşündürmektedir. Bununla birlikte, TT genotiplerinin sayısı (hem kontrol hem de hasta) oldukça düşük olduğundan daha fazla araştırma gerektirir. Sonuç olarak, kontrol ve hasta grupları arasında KLK10 polimorfizminde (rs7259451) istatistiksel olarak anlamlı bir fark yoktur. TT genotipinin bir koruyucu gen olması mümkündür. Öte yandan, daha fazla araştırma zaten gereklidir. Belki de bu çalışma daha geniş veya çeşitli bir popülasyon dahil edilerek geliştirilebilir. Diğer taraftan, bu gendeki alternatif SNP'ler araştırılabilir.

Anahtar Kelimeler: Küçük hücreli olmayan akciğer kanseri, KHOAK, polimorfizm, KLK10, rs7259451

1. INTRODUCTION AND PURPOSE

Lung cancer has been increasingly prevalent in recent years as a result of the COVID-19 disease. The rationale for this is that COVID-19 illness has been related to an increased risk of lung cancer in patients (1).

Any kind of epithelial lung cancer other than small-cell lung cancer (SCLC) is referred to as NSCLC. NSCLC is responsible for around 85% of all lung malignancies. In comparison to small-cell cancer, NSCLCs are relatively insensitive to chemotherapy as a group. They are mainly treated with surgical resection with curative purpose, when possible, while chemotherapy is highly being utilized either preoperatively (neoadjuvant chemotherapy) or postoperatively (adjuvant chemotherapy) (2, 3).

KLK-related proteases are a subclass of serine endopeptidases with a wide range of physiological roles. A growing body of research shows that several kallikreins are involved in tumorigenesis, and that some of them might be used as new cancer and disease biomarkers. This gene includes 15 kallikrein subfamily member found on chromosome 19 in a region. Its encoded protein in same name with gene and may can be involved in carcinogenesis suppression in breast and prostate malignancies. Multiple transcript variants encoding the same protein emerge from alternative splicing of this gene (4).

Kallikrein10 (KLK10) is a protein that affects hormone-receptor complexes and so plays a role in steroid hormone activation. The steroid hormone-regulated genes encode the human tissue KLK, which is found on chromosome 19q13.4. Multiple disorders, including cancer, have been linked to deregulation of KLK expression (5). KLK10 expression has been linked to tumorigenic development, indicating that KLK10 proteolytic cleavage products are likely to be involved in epithelial cell negative growth control. As a result, KLK10 might be a tumor suppressor gene. (6).

The goal of this study is to see if the rs7259451 polymorphism in the KLK10 gene is linked to NSCLC. The COVID-19 illness, which created a pandemic, has raised the importance of lung disorders in recent years. Lung cancer is also one of the most often diagnosed cancers, and it is the leading cause of cancer-related mortality globally. The KLK10 gene polymorphism might be impact in lung cancer, much as it does in breast and prostate malignancies. As a result, steroid hormone control may be crucial for healthy lung cells.

2. LITERATURE REVIEW

2.1. The Lung

Lung is a fundamental organ of the human's respiratory system as shown in Figure 1. Also, mammals, most other vertebrates, some snails and a few fish have two lungs which are located on both side of the heart near the backbone. The main function of lung is to pH balance in the body and it does this by taking in oxygen and giving out CO_2 in a process of gas exchange (7).

Moreover, the lung tissues may be affected by several respiratory diseases, such as lung cancer and pneumonia. Chronic obstructive pulmonary disease contains emphysema & chronic bronchitis, and it can be associated with either smoking or exposure to harmful substances or both (8).

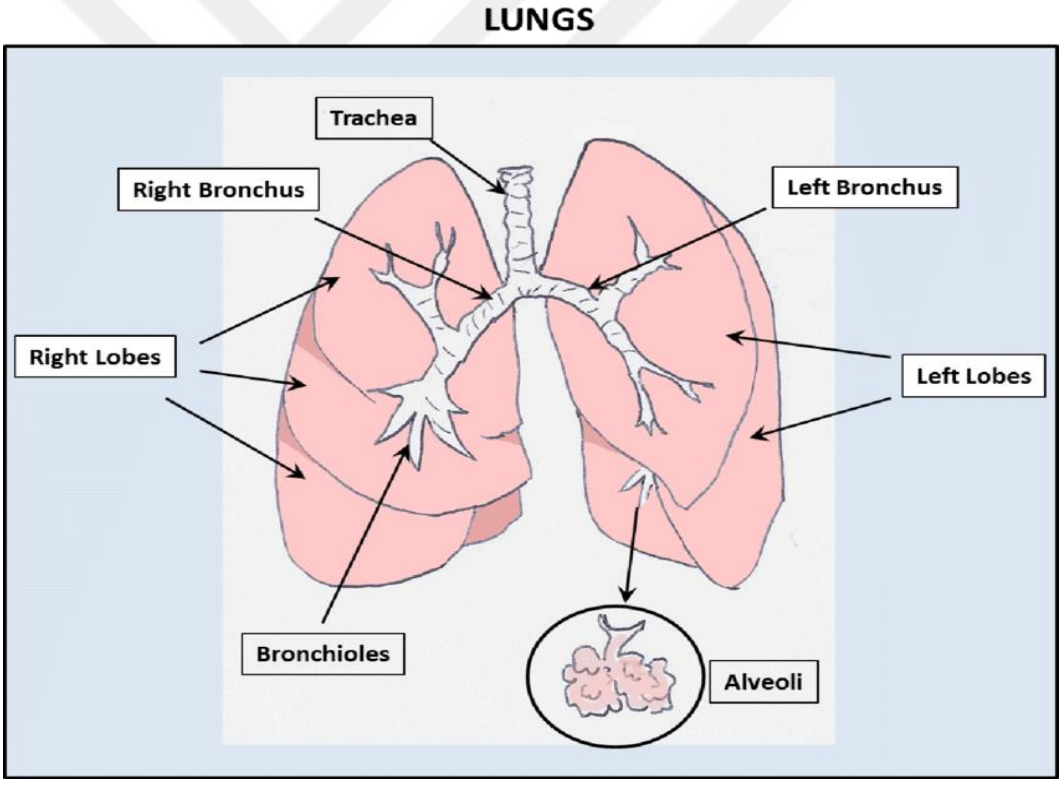

Figure 1: Schematic representation of the lung. The lung is divided into five lobes, three on the right and two on the left. Two bronchi connect these lobes, branching into smaller bronchioles that culminate in little sacs known as alveolar sacs (9).

2.2. Lung Cancer

Cancer is a situation that cells in a partially specific area of the body grow and multiply uncontrollable. The cancerous cells can be offending, invade and damage around

the tissues and organs. Cancer sometimes starts in one area and spreads to another area. The process is called as metastasis. There are over 200 different cancer types. Each of them has their own diagnose and treatment (10,11). Skin cancer, lung cancer, prostate cancer, breast cancer and colorectal cancer are the 5 most common cancers in the world (12). Nowadays, lung cancer become more important because of the COVID-19 disease. The reason for this, COVID-19 infection has been linked to an elevated risk of lung cancer in patients (13).

Lung cancer is a malignant tumor that develops uncontrollably in the tissues of the lungs (14). Because carcinomas account for 98–99 percent of all lung cancers, it is also known as lung carcinoma. Lung carcinomas develop from epithelial cells that have been transformed into malignant cells or from epithelial-based tissues. The malignant transformation of mesenchymal cells' connective tissues such as nerve, muscle, fat, and bone cause other lung malignancies, such as the uncommon sarcomas of the lung. Lymphomas and melanomas, which are lymphoid and melanocyte lineage cells, can also cause lung cancer (15).

Over time, this uncontrolled development might spread to nearby tissues or further away sections of the body, either directly into the lymphatic circulation or hematogenously, through blood the process called metastasis (16).

Moreover, there are 2 main types of lung cancer. First of them is small-cell lung carcinoma known as SCLC and the other one is non-small-cell lung carcinoma known as NSCLC (17). The wide symptoms of lung cancer are coughing, chest pain, difficulty in breathing and weight loss. Sometimes, blood comes up with coughing (18).

2.2.1. Non-small-cell lung cancer incidence and etiology

2.2.1.1. Non-small-cell lung cancer incidence

Lung cancer has been the main cause of cancer-related mortality in the world for decades, accounting for nearly 1 in every 5 fatalities (19). Moreover, NSCLC is the most often seen subtype cancer in the World. It causes high rate of death. NSCLC comprises the pathologically different adenocarcinoma, squamous-cell lung carcinoma, and large-cell lung carcinoma sub-types and accounts for roughly eighty-five percent of all lung malignancies. If found early, when surgery is most successful (20), NSCLC has a good prognosis; nonetheless, despite advances in chemotherapy, 5-year survival rates for

advanced disease remain low (21). This emphasizes the importance of developing new therapeutic options for people with unresectable or metastatic tumors.

The main problem is that cancer leads to death all over the world, to clarify, approximately 10 million patient dead in 2020. 2.26 million patients (breast cancer), 2.21 million patients (lung cancer), 1.93 million patients (colon and rectum cancer), 1.41 million patients (prostate cancer), 1.20 million patients (non-melanoma skin cancer), and 1.09 million patients (stomach cancer) were death leads to most fatal cancer types in 2020. Moreover, lung cancer (1.8 million mortality), colon & rectum cancer (916 thousand mortality), liver cancer (830 thousand mortality), stomach cancer (769 thousand mortality) and breast cancer (685 thousand mortality) are leading the most frequent of cancer death in 2020. Addition to these, around 400 thousand child develops the cancer (22).

In order to indicate the severity of the death rate in lung cancer, as seen in the Figure 2, the sum of the death numbers of these 4 cancers, including pancreatic, prostate, breast and colon cancers, and the number of fatalities from lung cancer are almost equal in United States (23).

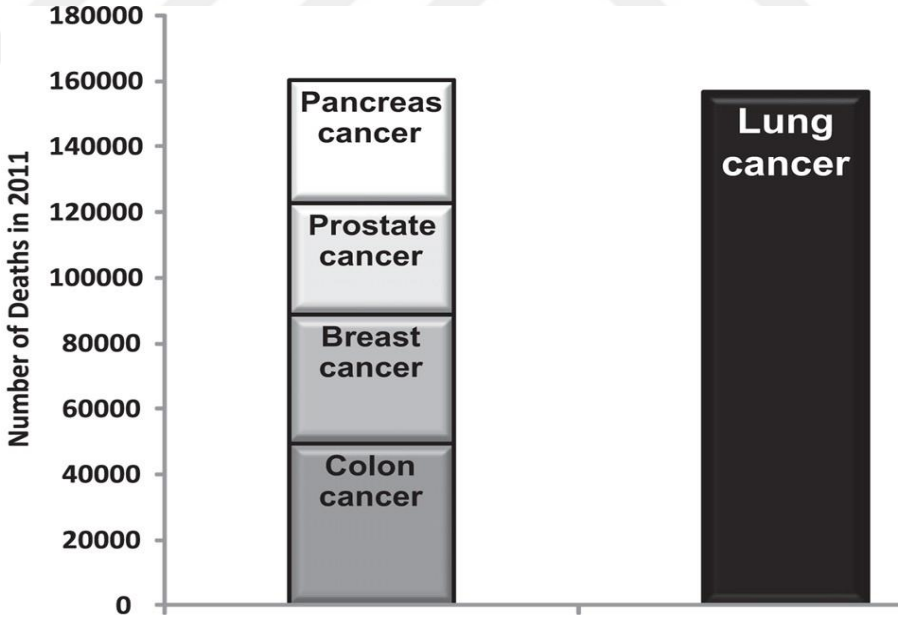

Figure 2: Number of deaths in United States from lung cancer compares to combination of death number pancreas, prostate, breast and colon cancer (23).

2.2.1.2. Non-small-cell lung cancer etiology

The World Health Organization (WHO) predicts that lung cancer mortality in worldwide will be rising, hugely as a consequence of an increasing in universal tobacco

using, specially in Asian countries. Tobacco use is the fundamental reason of lung cancer, and the consequences of cigarette smoking are responsible for a high percentage of all pulmonary carcinomas (24).

Tobacco was traditionally chewed or smoked in pipes, but following the advent of cigarette wrapping machines in the mid-1800s, it became readily available in cigarette form (25). After fifty years, tobacco consumption increased to over 3,5 thousand cigarettes per person per year, peaking at 4,4 thousand cigarettes per person per year in the mid-1960s (26). It has been shown that with increasing cigarette consumption, later smoking can adversely affect health (27).

The length of smoking and the amount of cigarettes smoked each day increased the risk of lung cancer. According to the paper, the average male smoker has a nine- to ten-fold increased risk of lung cancer, while heavy smokers have a twenty-fold increased risk (28). In spite of the forces to quit tobacco, there are around 1.1 billion global smokers, and number will rise to 1.9 billion by 2025 assuming the current pattern continues (29).

Never smokers, often known as non-smokers, are also susceptible to lung cancer. Never smokers are people who have never smoked more than hundred cigarettes in their lives, including lifetime nonsmokers (30). Although there is no one cause of lung cancer in non-smokers, risk factors for non-smokers include secondhand smoking, exposure of radon, arsenic, indoor air pollution and asbestos a history of lung illness, and genetic factors (31).

Moreover, exposure to solvents, paints, or thinners; welding equipment; and smoke, soot, or exhaust were all potential causes of elevated risk in the environment (32). However, other research suggests that the epidermal growth factor receptor gene (EGFR), the human repair gene (hMSH2), and several cytochrome P450 and glutathione-S-transferase enzymes may play a role (33, 34).

Recent research has revealed that the human papillomavirus (HPV), which is common to induce cancer in another tissues, may have a role (35). HPV DNA has been found in squamous cell carcinoma lung cancer tissues. However, the reported frequency of HPV infection in patients with lung cancer in different nations with ethnic and geographical variances is inconsistent (36).

2.2.2. Staging of non-small-cell lung cancer (NSCLC)

The operation of determining the size of the primary tumor and the amount of carcinoma dissemination inside the body is known as lung cancer staging. The TNM staging method helps doctors manage patients, offers information on prognosis and clinical trial eligibility, and enables for worldwide comparisons. TNM staging is determined by the original tumor's features (T), the extent of lymph node involvement (N), and the existence or lack of metastasis (M) (37,38). The T, N, and M characteristics are then used to provide an overall stage (I-IV) to the tumor, with the goal of classifying individuals into stages with comparable prognoses. Cure options differ depending on the stage. For NSCLC, there are two main forms of staging. One of them is clinical stages and the other one is pathological stage (39).

2.2.2.1. Clinical staging

Before prescribing primary therapy, clinical staging is based on a thorough history and physical examination, as well as laboratory, radiographic, and bronchoscopic results (40).

Radiological investigation consists of chest radiography (CXR), computer tomography (CT), integrated positron emission tomography-computed tomography (PET-CT), radionuclide bone scintigraphy and magnetic resonance imaging (MRI) (40).

All patients receive a plain chest X-ray for lung cancer evaluation to ensure a basis for future comparison. Also, it might also reveal characteristics such as pulmonary nodules, pleural effusion, direct or metastatic rib damage, and locally advanced lung disease that indicate cancer stage (41).

CT scans can be used to determine the size of the tumor, the extent of mediastinal and vascular invasion, and the presence of lymph nodes. Moreover, it determines the presence of distant metastases and the tumor's proximal extent within the airways. When a patient is being evaluated for a surgical resection, a CT scan can give valuable anatomical information (42, 43).

Disease of unknown etiology can be detected using integrated PET-CT using the tracer Fluoro-Deoxy-Glucose (FDG). In comparison to surrounding normal cells,

glycolysis, hexokinase activity, and glucose transporter-1 receptor expression are all raised in lung cancer cells with a higher metabolic rate. This causes cancer cells to absorb more FDG. Additionally, PET-CT is utilized to validate staging and detect metabolically active intrathoracic lymph nodes, especially small ones, as well as any undetected distant metastatic illness, thus reducing the need for surgery and avoiding unnecessary thoracotomy (44).

NSCLC patients are staged using bone scintigraphy to detect bone metastases. However, PET scanning provided a greater sensitivity and specificity for the identification of bone metastases than bone scintigraphy, as well as a higher overall accuracy, according to the study (45).

MRI scanning can be performed in combination with CT scanning to identify tumor invasion of the chest wall or mediastinum, as well as transdiaphragmatic spreading. The susceptibility to artefact generated by breathing is one of the limitations in evaluating lung lesions. Meanwhile, research reported similar rates for measuring T descriptor, N descriptor, and M descriptor when comparing the usefulness of PET-CT and whole-body MRI in the classification of NSCLC (46).

2.2.2.2. Pathological staging

The definition of pathological staging is a point of contention. Some view that the pathological stage of a tumor may be determined after a thorough surgical investigation of the hemithorax and mediastinum. Other ones believe that pathological classification is determined by histological findings, which determine the most advanced stage of illness. Tissue sample offers histopathologic information, allowing the diagnosis to be confirmed. Some tissue collection methods also enable for evaluation of tumor extent throughout the airways and mediastinum (40).

Tissue collection can be done in a variety of ways. The accuracy of the invasive test, the location and size of the primary cancer and suspicious lymph nodes, the degree of distant disease, test affordability, and the patient's comorbid illness all factor into the decision (47).

Nowadays, currently used tissue collection ways are: flexible bronchoscopy, blind transbronchial fine needle aspiration (TBNA), endobronchial ultrasound (EBUS) –

guided biopsy, CT/US-guided percutaneous needle biopsy, mediastinoscopy, video-assisted thoracic surgery (VATS) or thoracoscopy, thoracocentesis in pleural effusions, anterior mediastinotomy (Chamberlain procedure), esophageal endoscopic ultrasound with needle aspiration (EUS) and resection with systematic nodal dissection (48).

2.2.2.3. TNM staging (8th edition)

Researchers and the staging committee of the International Association for the Study of Lung Cancer (IASLC) examined a large database of patients with primary lung carcinoma who were cured using all treatment modalities, from surgical resection to supportive treatment alone, at forty-six institutions in nineteen countries (49). According to database, unidentified histology, not being a new diagnosis at the registered time, or insufficient data on the patient's stage, treatment methods, or follow-up were all reasons for exclusion. Patients were tracked for 5 years, and lifetime was calculated from the time they were diagnosed. TNM lung cancer classification was formed after a study of surviving info (50). Revisions to the stage groups were also advised as a result of the TNM descriptor changes, based on comparative survival rates for each TNM subgroup. It is shown that Figure 3 (40).

Stage	cTNM Subset	Five-year Survival
0	Carcinoma in situ	
IA	T1a/T1b, N0M0	50–80%
IB	T2aN0M0	47%
IIA	T1a/T1b, N1M0	36%
	T2aN1M0	
	T2bN0M0	
IIB	T2bN1M0	26%
	T3N0M0	
IIIA	T1/T2, N2M0	19%
	T3, N1/N2, M0	
	T4, N0/N1, M0	
IIIB	T4N2M0	7%
	Any T, N3, M0	
IV	Any T, Any N, M1a/M1b	2%

Figure 3: Stage grouping – cTNM subsets (50).

About the T descriptor, dividing the T stage into T1a/T1b and T2a/T2b based on the main tumor's size. T3 tumors have separate tumor nodules and their sizes are mostly

between 5 and 7 cm in diameter. T4 tumors are those that are bigger than 7 cm in diameter (51).

Preparing a generally agreed lymph node map for N descriptor would make it simpler to compare survival statistics among institutions and to organize and analyze future treatment studies. Six nodal zones are divided into fourteen regional lymph node sites (52). These are upper-nodal stations 1–4, aortopulmonary-nodal stations 5 and 6, subcarinal-nodal station 7, lower-nodal stations 8 and 9, hilar-nodal stations 10 and 11, and, peripheral-nodal stations 12–14 (53).

In M descriptor, the existence of metastasis inside the thorax, extrathoracic metastasis, and malignant pericardial or pleural effusions are the key predictors of prognosis in patients with metastatic illness (54). There are two types of M classification. One of them is M0 and the other one is M1. If there is no distant metastasis, it is called M0 and, if distant metastasis is present, it is called M1. Also, M1 has subtypes which is known as M1a, M1b and M1c. M1a: tumor with pleural or pericardial nodule(s) or malignant pleural or pericardial effusion; tumor with pleural or pericardial nodule(s) in a contralateral lobe. M1b: single extrathoracic metastasis. M1c: extrathoracic metastases to one or more organs (55).

2.2.3. Classification of non-small-cell lung cancer (NSCLC)

The non-small-cell lung cancer is any type of lung carcinoma except the small-cell lung carcinoma. Also, this type accounts around 80-90% of all lung cancers (56). Non-small-cell lung carcinoma has differences from small-cell lung carcinoma as molecular and genetic alterations. The feature that distinguishes NSCLC from SCLC are that Rb gene alterations and also lack of protein in cells of SCLC are found. Another difference is that Tp53 is not activated in around half of NSCLC while mutation of Tp53 with behavioral inhibition is quite common in among 70-100% of SCLC (57,58). The other differences are activation of PI3K catalytic gene is detected in almost half of squamous cell carcinoma patients and mutations of EGFR gene play an essential role in adenocarcinoma carcinogenesis (57).

Moreover, NSCLC are spreading to other organs slower than SCLC type (59) and SCLC are formed of smaller cells as microscopically (60). Sometimes both of lung cancer

types are diagnosed with same tests and treating with same methods such as chemotherapy, surgery and radiation (61, 62).

Addition to these, non-small-cell lung carcinoma has different subtypes. Adenocarcinoma, SCLC and LCC mostly occur in NSCLC. Pleomorphic, carcinoid tumor, salivary gland carcinoma and not classified carcinoma occur less frequently (63). They characterized by distinct cellular and molecular features (64).

2.2.3.1. Lung adenocarcinoma

The most typical kind of lung carcinoma is adenocarcinoma and it is also most commonly seen in all lung cancer types (65). Even lung cancer patients who are not smokers in their whole life, this subtype of cancer is currently the most typical kind of lung cancer in patients (66). It is more often seen peripherally than centrally located (67).

Lung adenocarcinoma is generally developing slowly from mucosal glands and adenocarcinoma forms approximately forty percent of all lung carcinomas. It generally happens in the lung periphery, can be found in scars or chronic inflammation zone (68). This type of lung carcinoma, whose pathophysiology is complicated, often progresses histologically from cells seen in healthy lungs to markedly dysmorphic or irregular cells. There are various molecular and genetic pathways which have contributed to this progress. As a lot of lung cancer, adenocarcinoma is usually progresses during diagnosis time. To diagnose, biopsy is mostly needed to confirm and a tumor or lesion is determined with several imaging methods, such as X-ray or computed tomography (CT) (69).

The treatment of cancer varies according to the cancer subtype and the extent of tumor spread. Methods such as, chemotherapy, radiotherapy, surgical resection, targeted therapy and immunotherapy is a type of treatment that is able to kill malignant cells (70).

2.2.3.2. Squamous-cell lung carcinoma

SCC which is also known squamous-cell lung cancer is a histological subtype of non-small cell lung carcinoma (NSCLC). It is the second commonly type of lung cancer after lung adenocarcinoma, and it is bronchial in origin. Its tumor cells have a squamous look similar to that of epidermal cells. Squamous-cell lung cancer has a stronger link to cigarette use than any other kind of NSCLC (71).

Squamous-cell lung carcinoma is strongly linked in men than in women and the subtype tumor is highly related with tobacco smoking (72).

2.2.3.3. Large-cell lung carcinoma

LCLC or LCC (large-cell lung carcinoma) is a broad term for a group of undifferentiated malignant tumors that arise from lung epithelial cells that have been altered. LCLCs have historically accounted for approximately 10% of all NSCLCs, although better diagnostic techniques appear to be decreasing the incidence of normal LCLC diagnosis in favor of more poorly differentiated squamous-cell lung carcinomas and adenocarcinomas (73).

LCLC is effectively a diagnosis of exclusion since the tumor cells lack light microscopic markers that would define the neoplasm as a small-cell carcinoma, SCLC, adenocarcinoma, or another more specific histologic kind of lung cancer (74). Although squamous or glandular characteristics may be evident by ultrastructural investigation, Undifferentiated NSCLC with no light microscopy or immunohistochemical evidence of squamous or glandular differentiation is known as large cell lung. LCC can only be diagnosed after a comprehensive examination of the cancer; it cannot be diagnosed using biopsy or cytology samples (75, 76).

2.2.4. Non-small cell lung cancer diagnosis

There is currently no effective biological marker or screening approach available to detect if a lesion is or will be getting malignity in a clinically limited time period in an individual patient. The use of volume doubling time or radiomics to detect if a tumor is malignant is currently invalidated; in patients with indeterminate nodules, pathological diagnosis based upon tissue biopsy keep it up the cornerstone. For patients and doctors, deciding among radiologic surveillance for high-risk image processing characteristics and invasive diagnostic methods for histologic or cytologic confirmation of the etiology of a tumor continues to be a trouble (77).

Some biomarkers can also be used to diagnose NSCLC or NSCLC subtypes. For example, a tumor that is positive for the proteins p63, cytokeratin 5 or cytokeratin 6 marker is known as squamous-cell carcinoma (78).

To make proper diagnosis, patients have central lung carcinoma which is not apparent on standard bronchoscopy may benefit from endosonography if the cancerogenic part is close to the greater airways (endobronchial ultrasonography) or the esophagus (esophageal ultrasonography). In patients with suspected or confirmed NSCLC with suspicious mediastinal or hilar nodes on CT or PET-CT, endosonography rather than surgical staging is indicated as the initial technique for mediastinal nodal staging. Endobronchial ultrasonography combined with real-time guided transbronchial needle aspiration and endoscopic esophageal ultrasonography is better to either test performed alone (Figure 4 and 5). After a negative outcome with the needle approach, surgical staging is advised if the clinical suspicion of mediastinal node involvement remains high (79).

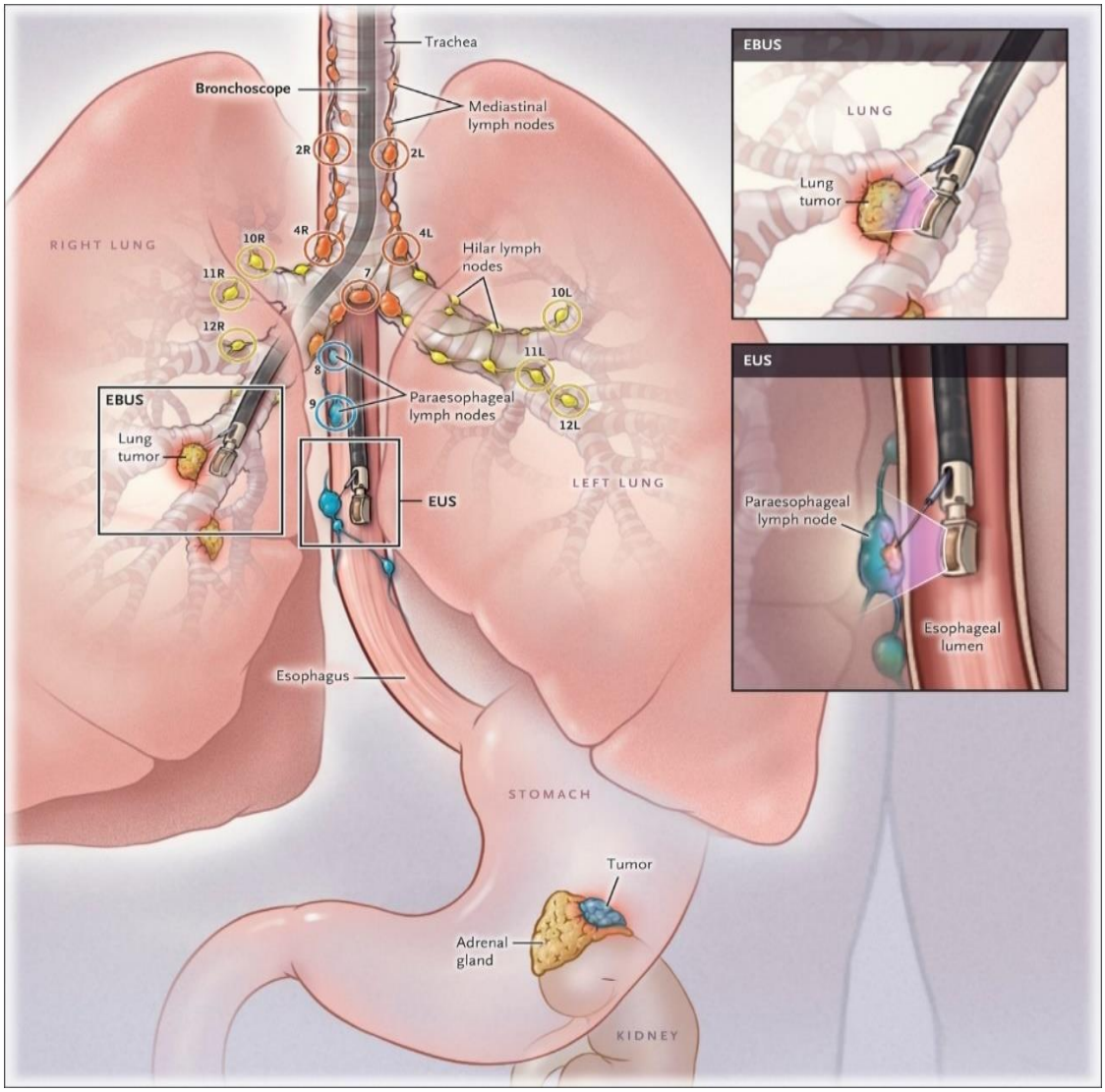

Figure 4: Endoscopic diagnosis of lung cancer. Endoscopic bronchial ultrasonography (EBUS) and endoscopic esophageal ultrasonography (EUS) are endoscopic techniques for detecting lung cancer, lymph node metastases, and adrenal metastases (79).

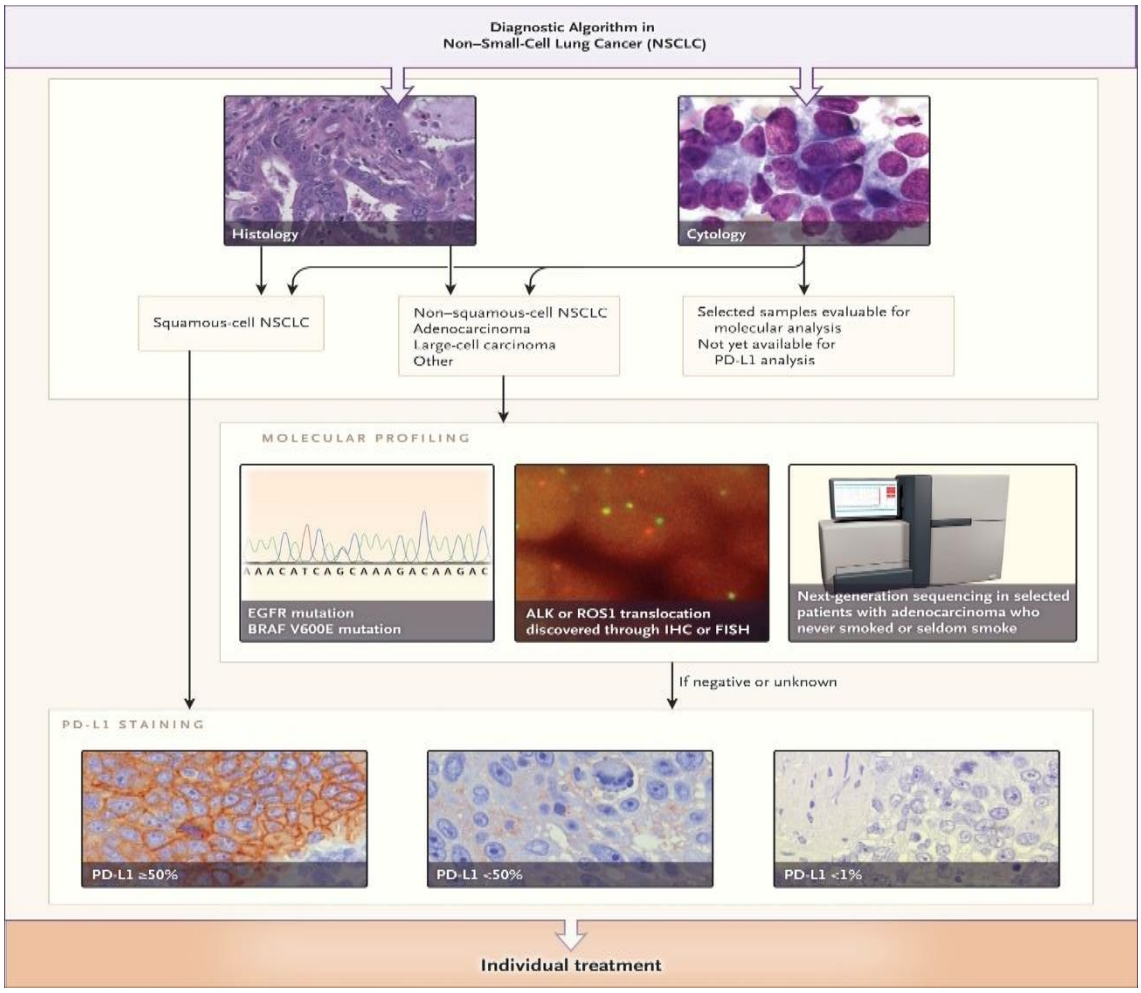

Figure 5: Methodology of diagnosis for Non–Small-Cell Lung Cancer (NSCLC) (79).

2.2.4.1. Symptoms used in the diagnosis of non-small cell lung cancer

Common symptom (exhaustion, poor appetite) and emotional stress (worry, anxiety) were among the 10 most commonly mentioned pre - treatment symptoms upon that Rotterdam Symptom Checklist, that was done by over six hundred patients enrolled in 2 MRC Lung Cancer Working Party multicenter randomized trials (shortness of breath & cough). Despite the fact that the frequency and intensity of symptoms increased as performance status declined, the most frequently symptoms were identified to be nearly identical in patients with SCLC and NSCLC, as well as in patients of various performance status levels. Women patients in NSCLC had more mental problems than men, however this distinction was not as pronounced in SCLC diseased. As a result, in order to fully assess the value of palliative therapy in patients with lung carcinoma, all symptoms present at the time of diagnosis, in addition to the conventionally recognized chest symptoms, must be included (80).

2.2.4.2. Imaging systems used in the diagnosis of non-small cell lung cancer

To make proper diagnosis, patients have central lung carcinoma which is not apparent on standard bronchoscopy may benefit from endosonography if the cancerogenic part is close to the greater airways (endobronchial ultrasonography) or the esophagus (esophageal ultrasonography). In patients with be doubtful or confirmed NSCLC with suspicious mediastinal or hilar nodes on CT or PET-CT, endosonography rather than surgical classification is indicated as the main technique for mediastinal nodal staging. Endobronchial ultrasonography combined with real-time guided transbronchial needle aspiration and endoscopic esophageal ultrasonography is better to either test performed alone. After a negative outcome with the needle approach, if the clinical suspicion of mediastinal node involvement remains strong, surgical classification is advised (79).

2.2.4.3. Molecular tests used in the diagnosis of non-small cell lung cancer

Some biomarkers can also be used to diagnose NSCLC or NSCLC subtypes. For example, Squamous-cell carcinoma is defined as a metastasis which is positive for p63, cytokeratin 5, or cytokeratin 6 markers (78).

Following the identification of curable oncogenic changes, it was suggested that molecular testing be included in the current method to further categorize NSCLC. The molecular test is making for malfunctioning in the EGFR gene, as well as BRAF V600E, ALK, ROS1, and PD-L1 genes. At present, the majority of these genomic analyses can be done on tiny biopsy and cytologic samples (81-83).

2.2.5. Non-small cell lung cancer treatment

In the palliative cure, local therapeutic techniques, particularly radiation, play a crucial role in pain and symptom control. When compared to traditional whole-tissue radiation therapy, stereotactic radiation therapy (SBRT) of tissue tumors has been proven to have equivalent effectiveness and lower toxicity (84). Additionally, certain surgical procedures for example video-assisted thoracoscopy could be beneficial in the treatment of pulmonary infection or local problems (85).

In patients with NSCLC both early-stage and advanced stage, pemetrexed maintenance therapy is frequently used after first-line treatment with platinum-based chemotherapy. With the development of new immunological and antiangiogenic medicines, standard guidelines for second and ensuing lines of therapy have shifted significantly (86).

2.3. Kallikreins

The kallikreins (kininogenases) are a set of serine endopeptidases enzymes found in many glandular organs, and also blood, lymph, and urine, in active and/or inactive form which can be activated. Prekallikreins are inactive kallikreins that can be activated by pH changes, chemical solvents, and enzymes like pepsin and trypsin (87, 88). In situ, kallikreins have strong effects on blood arteries and smooth muscles which are nearly totally indirect and are caused by the fast enzymatic cleavage of a particular substrate, an as-globulin found in plasma and lymph, which leads to the release of a peptide that causes the effects (89).

Following further inquiry, the above-mentioned viewpoints were modified. As a result, I) the sensitivity of kallikreins from distinct sources to different protease inhibitors varies (90); 2) removal of the pancreas, or chronic duct ligation with subsequent acinar cell destruction, has no effect on the concentration of prekallikrein in blood or the amount of kallikrein excreted in urine (91,92); 3) various kallikreins have unique electrophoretic mobilities, adsorbancy on diethylaminoethyl (DEAE) cellulose columns (90), expected molecular mass (93), and immune specificities (94); 4) the amino acid and carbohydrate compositions of the purest hog's pancreatic, urine, and submaxillary kallikrein arrangements exhibit significant variance in relative amounts of these items (93).

Kallikrein in plasma (EC 3.4.21.34) is an enzyme which is known as serum kallikrein, and urinary kallikrein. The following chemical reaction is catalyzed by kallikrein. Separation of several Arg- and Lys- bonds in (human) kininogen involve Lys-Arg and Arg-Ser to produce bradykinin. Factor XIIa converts plasma prokallikrein (Fletcher factor) to this enzyme (95-99).

Tissue kallikreins (KLKs) are found all over the body and have a variety of physiological activities. Because certain kallikreins can catalyze the activation of other kallikreins, many homeostatic function-regulating cascades including these proteases

have been proposed. The Kallikrein gene family in human has been extensively described, with fifteen members localized on chromosome 19q13.4 in tandem which is seen in Figure 6 (100). The salivary gland, endocrine organs, the testis, prostate, breast, and endometrial, as well as the CNS, all express kallikreins. The majority, if not all, genes are controlled by steroid hormones (101).

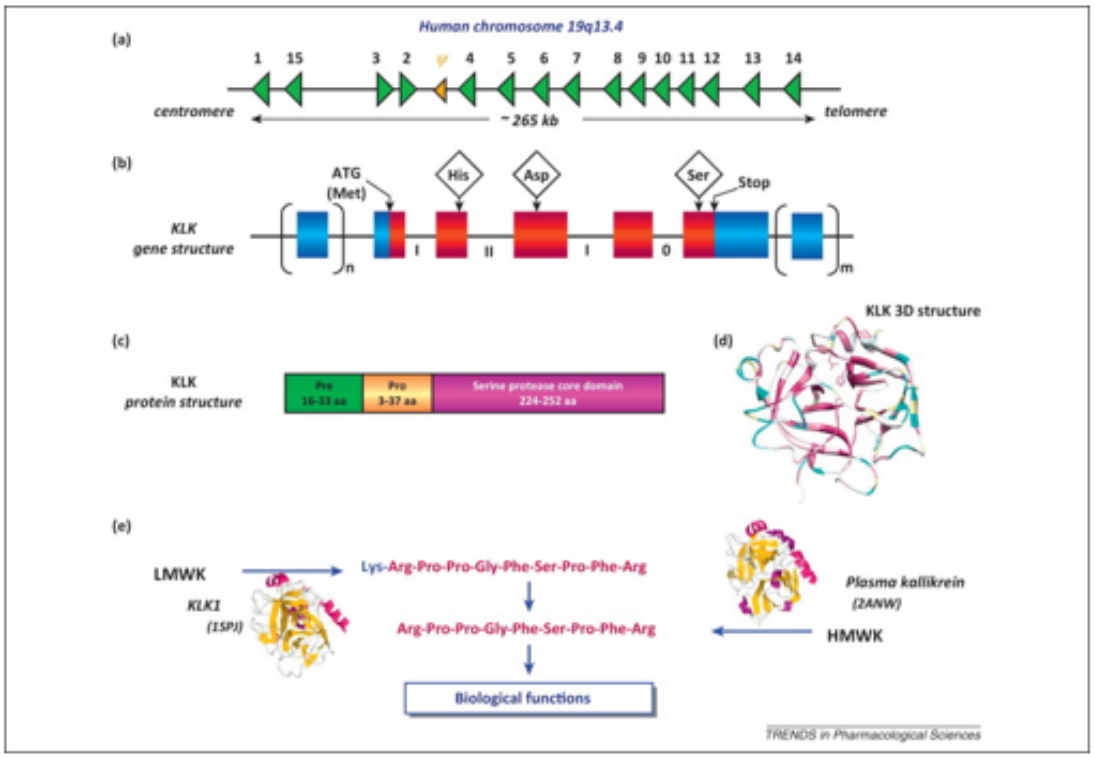

Figure 6: The kallikrein (KLK) protease family. Schematic depiction of (a) the KLK gene cluster at human chromosome locus 19q13.4 as well as the matching (b) gene and (c) protein structures. The direction of gene transcription is shown by the arrows. c is a newly identified kallikrein pseudogene; n and m represent the number of untranslated exons (n, m0). (d) Structural model of KLKs created with ConSurf (http://consurf.tau.ac.il/) and visualized with Chimera (http://www.cgl.ucsf.edu/chimera). Dark red indicates conserved sequences, whereas blue indicates non-conserved sequences. f) Functional interactions between plasma kallikrein and KLK1. Bradykinin peptide, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg; Lys-bradykinin peptide, Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (100).

KLK1, KLK2, and KLK12, 3 tissue kallikreins, also have a role in blood pressure control by activating bradykinin (102). KLKs (type 2, 3, 4, 5, and 14) are proteins found in the prostate that are considered to regulate semen liquefaction by hydrolyzing semenogelin (103,104). KLKs (type 5, 7, and 14), which are expressed in the epidermis's outer layer and break cellular adhesion proteins, are thought to be involved in skin desquamation (105). KLK6 and KLK8 have also been linked to neuroplasticity in the CNS (central nervous system) (106).

2.3.1. Kallikrein 10

The KLK10 gene in people codes for the protein kallikrein-10 (107). The gene is one of 15 kallikrein subfamily members found on chromosome 19 in a cluster. Encoded protein of KLK10 is secreted and may play a role in the prevention of carcinogenesis in breast and prostate malignancies. Multiple transcript variants encoding the same protein emerge from alternative splicing of the gene (108).

The KLK10 gene spans 5.7 kb of genomic DNA and has 5 introns and 6 exons. Alternative splicing is common in the KLK10 gene locus, as it is in the rest of the KLK gene family, since numerous KLK10 mRNA transcript variants have been identified, each with a different length of the first exon. However, the first exon is untranslated, all splice variants encode the identical 276-amino-acid prepropeptide (109). KLK10 is released as pro-KLK10, with the signal peptide consisting of the first 33 amino acids from the N-terminus (110). The structure of KLK10 is showed in Figure 7 (111).

Addition to these, KLK10 is a protein that affects hormone-receptor complexes and is involved in steroid hormone activation (112).

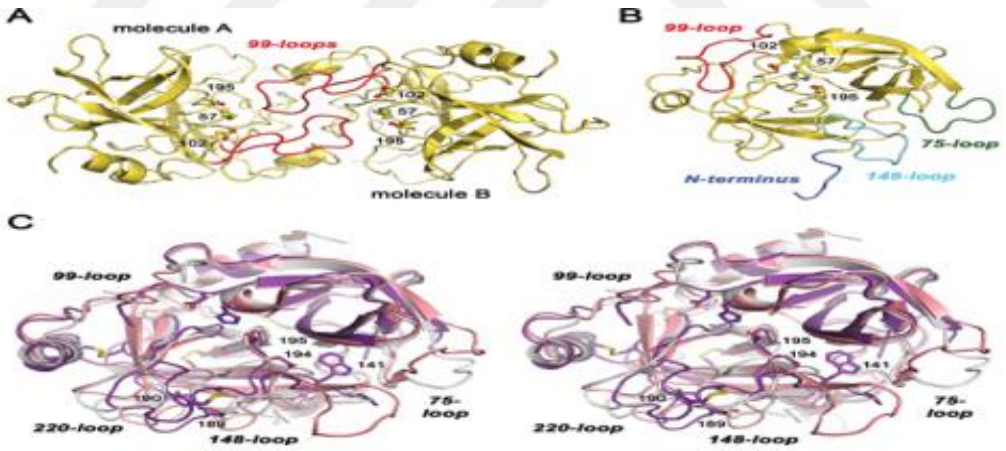

Figure 7: Structure of KLK10. (A) KLK10 (yellow) and KLK-Zn (not shown) have an asymmetric dimer with facing active sites. Molecules A and B's red 99-loops intercalate. From His95 to Arg96, the insertion from Gln95A to Pro95H is poorly defined in electron density. Ser195, His57, and Asp102 are illustrated as ball-and-stick models and labeled. (B) KLK10 monomer with ball-and-stick models of the catalytic triad. Other flexible KLK10 areas are mentioned besides the 99-loop. The disordered N-terminus (blue) lacks a salt bridge to Asp194. Both the 75-loop (green) and the 148-loop (aquamarine) lack well-defined electron density for at least six residues. (C) Superposed KLK10-Zn, pro-KLK6, and active KLK2 (light gray). Parts of KLK10 or KLK2's chaotic 99-loop have been deleted. Asp189, Pro190, and Asp194, which may form a salt bridge to the N-terminus, are shown as stick models with sequence numbers. As with other trypsin-like zymogens, Trp141's backbone amide hydrogen bonds to Asp194. Regardless of disease, relevant loops are designated (111).

2.4. Kallikreins and Cancer

KLK has been found as a possible biomarker for the diagnosis and prognosis of a number of malignancies in humans, such as ovarian, breast, and prostate cancers (112). Downregulation of KLK expression is related with late-stage disease in ovarian cancer. Also decreasing of expression means there is no disease and overall patient survival (113). In breast cancer tissue and cell lines, KLK is downregulated (114, 115). Also, in testicular tissue, KLK is downregulated in cancer compared to normal tissue (116). Downregulation of KLK gene expression is directly related with prostate cancer (117).

Serum from NSCLC patients exhibited lower levels of KLKs (type 5, 7, 8, 10, and 12) compared to normal participants, but greater levels of KLKs (11, 13, and 14). The expression of KLK11 and KLK12 was connected to stage. Except the KLK5, kallikrein expression cannot be affected by smoking or sex. KLKs (type 11, 12, 13, and 14) were linked to an increased risk of NSCLC in univariate analysis and confirmed in multivariate analysis. The aggregate area under the curve of KLKs (type 4, 8, 10, 11, 12, 13, and 14) receiver operating characteristic curves was 0.90 (95 percent confidence interval, 0.87-0.97) (118).

Addition to these, squamous cell carcinoma has much higher KLK5 expression than comparable nonmalignant lung tissue, but adenocarcinoma has lower KLK7 expression (119). Moreover, KLK10 polymorphism (rs7259451) can be marked as GG genotype can be inhibit the prostate cancer and TT genotype can lead to prostate cancer (112).

3. MATERIAL AND METHOD

3.1. Sample Selection and Definition

This project contains both patients diagnosed with NSCLC (n=48) and healthy individuals as a control group (n=46). The patient group composed of patients who get diagnosed by pulmonary medicine department and also a control group consist of healthy persons. Also, ethical approval was obtained from the Yeditepe University Ethics Committee for project (Ethics committee decision no:1532).

3.1.1. Patient group

In this study, all patients diagnosed with non-small-cell lung cancer from Yeditepe University Hospital and their ages are between 42 and 83.

3.1.2. Control group

Control group are composed with not diagnosed with non-small-cell lung cancer patient. Also control group is between 45 and 86 years old.

3.2. Materials and Devices Used in Experiment

3.2.1. Material used in DNA isolation

Isolation of DNA was performed from peripheric venous blood samples. The blood samples were stored in EDTA containing tubes at -80°C until used. EDTA is used because of prevent of coagulation. Also, to make the DNA isolation, DNA Isolation Robot (iPrep pure link, Invitrogen and the Thermo Fischer Scientific Inc) system was used. Moreover, 1.12mg/ml Proteinase K, 8 M Guanidine hydrochloride, 10.5 nM EDTA, 10.5 nM NaCl and 10.5 nM Tris-Cl of pH 8.8 were used in the DNA isolation mixture.

3.2.2. The equipment used in experiment

In the experiment, DNA Isolation Robot (iPrep Purelink, Invitrogen, Thermo Fisher Scientific Inc), Nanodrop 2000 (Thermo Fischer Scientific Inc), Real Time PCR (Fast Real Time 7500, Applied Biosystems), 7500 Fast Real-Time PCR Instrument, Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R-Beckman Coulter), +4°C Refrigerator

(Haier), -20°C Refrigerator (Haier), Ultra Pure Water (Pure Lab Option Q,EICT), Vortex (V.I Plus Biosan) and a Pipette Kit (Thermo Fischer Scientific Inc) were used.

3.3. Methods

3.3.1. Genomic DNA isolation from blood

All blood samples of patient and control group were collected in the EDTA-containing tubes volumed of 5 mL. Until the DNA isolation was started, all blood samples were stocked in refrigerator at +4°C. A robot of iPrep DNA extraction (Invitrogen) and from the genomic DNA isolation Kit with iPrep was used for DNA isolation. By using this system, DNA can be isolated from 350 µL of peripheral blood, therefore this system allows to run 13 blood samples in one time. One cartridge is used for each of the samples, and these cartridges are shaken for a while to ensure that the magnetic beads bind effectively with the DNA before placing the samples in their cartridge.

iPrep robot operates according to ChargeSwitch® (CST®) technology, acting as a calibrated extraction procedure. This procedure provides an advantage to isolate high amount of genomic DNA from the samples. In this procedure, elevated amounts of pure genomic DNA may be obtained from samples by utilizing paramagnetic particles. A DNA-binding surface encloses those particles. ChargeSwitch® (CST®) extraction procedure is unique indeed compared with other extraction methods such as silica-based DNA extraction procedure. The pH of enclosing buffer of particles alters the charge of beads. Under low pH conditions, the DNA backbone is negatively charged, then binds to positively charged beads. By the utilization of low salt buffer which has higher pH for DNA elution, those charged beams become neutralized. After the nucleic acids molecules pass into the washing buffer, the DNA samples become ready for the experiment. As a final stage of the experiment, aqueous DNA samples were acquired and stored in the refrigerator at +4°C.

3.3.2. DNA purity measurement

NanoDrop which is known as a type of UV spectrophotometer used for UV (ultraviolet) light absorbance of nucleic acids at 260 nm. OD260/OD280 ratio and OD260/OD230 ratio are measured to obtain DNA purity. Concentration of DNA are also measured by NanoDrop. Nanodrop does not include any cuvette or capillaries so samples

are measured directly away from external influences and thus more realistic results are obtained compared to other UV spectrometers.

In this experiment, to DNA purity measurement, NanoDrop 2000 (Thermo Fisher Scientific Inc.) was utilized. 0.5-1 µl of DNA sample of each individual, which is known both NSCLC patients and control group, were used. Distilled water also was used as a blank sample. After every 15-20 measurements, distilled water was used to clean sample putting stage. Then, at the end of the measurement of all samples, the NanoDrop 2000 was cleaned with distilled water.

3.3.3. Genotyping with real-time PCR

In this study, genotyping analysis were performed with 7500 Fast-Real-Time Polymerase Chain Reaction (Applied Bioystems) known as it is a Real-Time PCR device.

Detection of single nucleotide polymorphisms (SNPs) were detecting with using fluorescent dyes of probes. Detection of fluorescent signals is clearly visible. In the Real-Time PCR method, fluorescent dye probes are used and they have two different wavelengths specific to the alleles called mutant and wild type. There were two different TaqMan probes used to detect. One of them was FAM and the other one was VIC. FAM can detect wild type variant of allele. VIC can also detect mutant variant of allele. The fluorescent dye-linked probes of each allele attach with the amplified region. Taq polymerase enzyme is performed to hydrolyze the probes.

The primer was designed depending on the sequence of KLK10 gene in human. Determination of KLK10 gene (rs7259451) polymorphism was performed in Applied Biosystems 7500 Fast Real- Time PCR instrument (Applied Biosystems, Foster City, CA, USA) by using TaqMan Genotyping Assay and TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA). Based on this procedure, the polymorphism-contained region was amplified by using 5’ - TAAGGCAAGACTCAGGATAAAACAG[G>T]GTGGTGTGGCCGGGAGCGGTGG CTC 3’. Due to the Minor allele frequency (MAF) analysis, allele frequencies considered as G wild type and T mutant form.

3.3.3.1. Real-time PCR protocol

5 µl of Master Mix, 0.25 µl of Taqman genotyping assay, 3.75 µl of DNase, RNase free water, and 1 µl of template DNA were used. The total volume of reagents used in Real-Time PCR and reaction mixtures are also shown in Table 3.1. First, mixture was prepared with Master Mix, DNase, RNase free water and TaqMan genotyping assay and then mixture separates to each well of 96-well. After that, each template DNA samples were added onto the mixture one by one. Later, 96-well plate were covered carefully. Centrifuge was performed 2 min at 3000 rpm to make the solution more homogeneous and to precipitate all liquids to the bottom. Then the plate is carefully placed in the PCR device and Real-Time PCR is started.

Table 1: The mixtures of Real-Time PCR reaction

The Agent	Quantity of Agent
Master Mix	5 µL
DNAse, RNAse Free Water	3,75 µL
TaqMan Genotyping Assay	0,25 µL
Template DNA	1 µL

Real-Time PCR stages were designed. First there were 10 minutes waiting time at 95 °C, then the denaturation process was continued for 15 seconds per cycle at 92 °C and

last stage were elongation to 1 minute for each cycle at 60 °C. As it is shown in Table 2, denaturation and annealing/extension processes were applied as 40 cycles.

Table 2: Real-time PCR protocol.

	Stages	Temperature °C	Duration / Period of Time
	Hold	95 °C	10 minutes
40 cycles	Denaturation	92 °C	15 seconds
	Annealing/ Extension	60 °C	1 minute

3.3.4. Statistical analysis

In this study, student's t-test was used to get numerical values. When PCR experiments were done and allelic discrimination data were obtained, all data were entered in the SPSS 26.0 program. Moreover, the other clinical parameters were already collected and entered in the SPSS document. Clinical parameters are shown in Table 3 and 4. Then, the informations were analyzed in SPSS 26.0 by using Fisher's Exact Tests and Chi-square tests. Chi-square and Fisher's Exact Tests were applied to statistically analyze the genotype distribution and alleles in the groups. P values less than or equals to 0.05 are considered statistically significant, so possible risk factors for NSCLC can be identified and assessed by the analysis.

4. RESULTS

4.1. Demographic Characteristics

Demographic data of NSCLC patients (n=46) and healthy controls (n=48), which constitute the groups of our thesis study, are given in Table 3 comparatively.

Table 3: Demographic characteristics of the study population.

Parameter	NSCLC (n=48)	Control (n=46)	p-Value
Age (years), mean±SD	62.80±11.25	60.12±9.28	0.234
Gender (Female/Male)	6/42	18/28	0.003*
Age			
<60	n=23 (47.9%)	n=22 (48%)	0.999
≥60	n=25 (52.1%)	n=24 (52%)	
Smoking Status			
Active Smokers	n=8 (16.7%)	n=22 (47.8%)	<0.0001*
Non-smokers	n=40 (83.3%)	n=24 (52.2%)	

NSCLC: Non-small-cell lung cancer; n: number of individuals; SD: standard deviation; *p-values less than 0.05 denoted statistical significance.

As shown in Table 3, demographic data in the patient and healthy control groups were analyzed statistically. As a result of the analysis, as can be seen in the table, there was no statistically significant difference in the mean age, the number of people above and below 60 years of age, and the smoking status in the cancer patient group compared to the control group. However, a statistically significant difference in smoking status was observed in the cancer patient group compared to the control group.

4.2. Statistical Assessment of Real-Time PCR Results

Table 4: The distribution of Kallikrein 10 genotype and allele frequencies in patient and control groups

KLK10 Genotypes	NSCLC (n=48)	Control (n=46)	*P-Value*	Odd Ratio (OR)	95% Confidence Interval
			χ^2=2.453; p=0.293		
GG Genotype	52.1% (n=25)	60.9% (n=28)	0.413	0.699	0.308-1.585
GT Genotype	45.8% (n=22)	32.6% (n=15)	0.300	1.587	0.691-3.642
TT Genotype	2.1% (n=1)	6.5% (n=3)	0.356	0.305	0.031-3.044
Allelic Distributions					
G Allele	80.2% (n=72)	77.2% (n=71)	0.356	3.279	0.329-32.727
T Allele	19.8% (n=24)	22.8% (n=21)	0.413	1.431	0.631-3.247

N: number of individuals; OR: odd ratio; Cl: confidence interval; χ^2: Chi square used for comparison of patients with NSCLC and control group; *p<0.05 denoted statistically significant differences

As a result, after the experiment of KLK10 rs7259451 polymorphism, about 48 sample of NSCLC patients and healthy control groups samples. Also in this study, there is no found statistically significant differences among the two groups. The statistical analysis was done with using Chi square (χ^2) and Fisher's Exact test as shown in Table 4.

Moreover, allele types of each individual were examined in both patient and control groups by using the 7500 Fast-Real Time PCR. The allelic discrimination plot obtained from this device is seen below and the allelic discriminations were obtained with the software of this tool automatically.

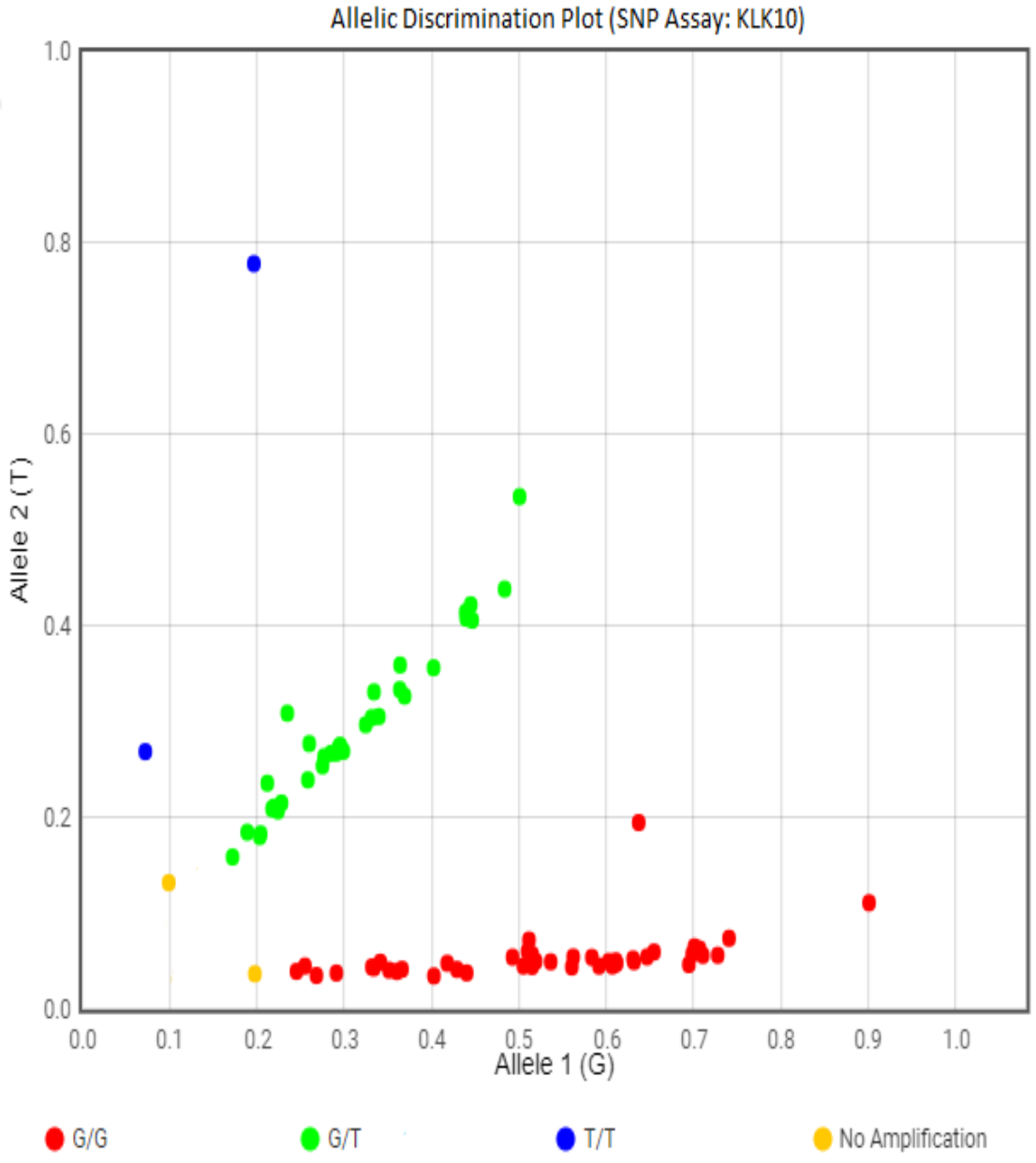

Figure 8: Allelic discrimination of KLK10 (rs7259451). GG means homozygote wild type, GT means heterozygote, and TT means homozygote mutant type.

Expression and readings of fluorescent radiation with the contribution of dyes in the probes. However, some samples could not be distinguished and interpreted. In this study, two different dyes corresponding to the FAM color blue and the VIC color green were used. ROX corresponds to a reference color for comparing FAM and VIC dyes. Allelic discrimination was evaluated by examining and interpreting radiance curves.

According to Figure 8, there is allelic discrimination shown. GG which is seen as red dots is homozygote wild type, GT which is seen as green dots is heterozygote and TT which is seen as blue dots is heterozygote mutant type.

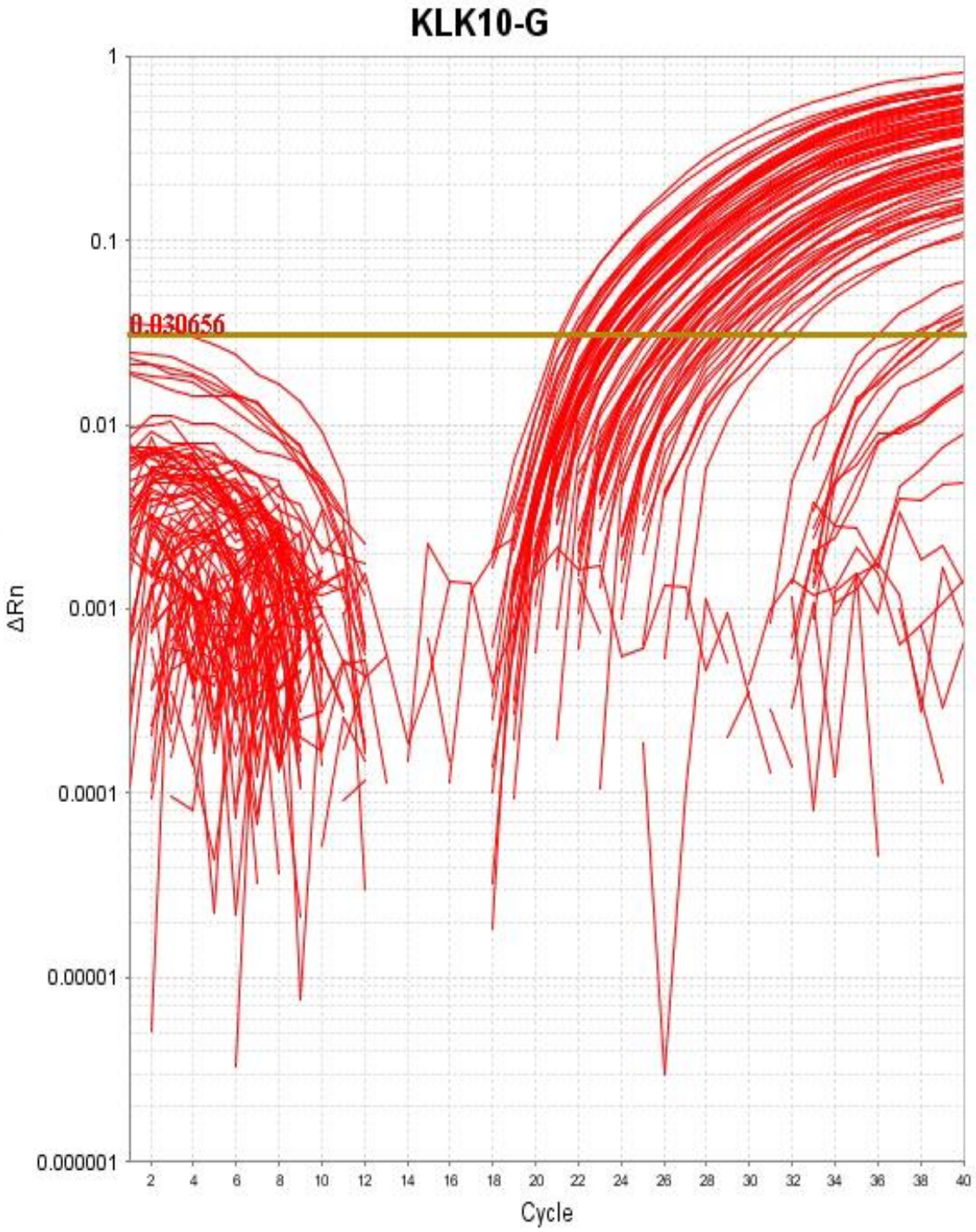

Figure 9: Allele G amplification plot.

The plot (Figure 9) represents that amplification plots of Allele G. Yellow line also shows to threshold value (0.03066).

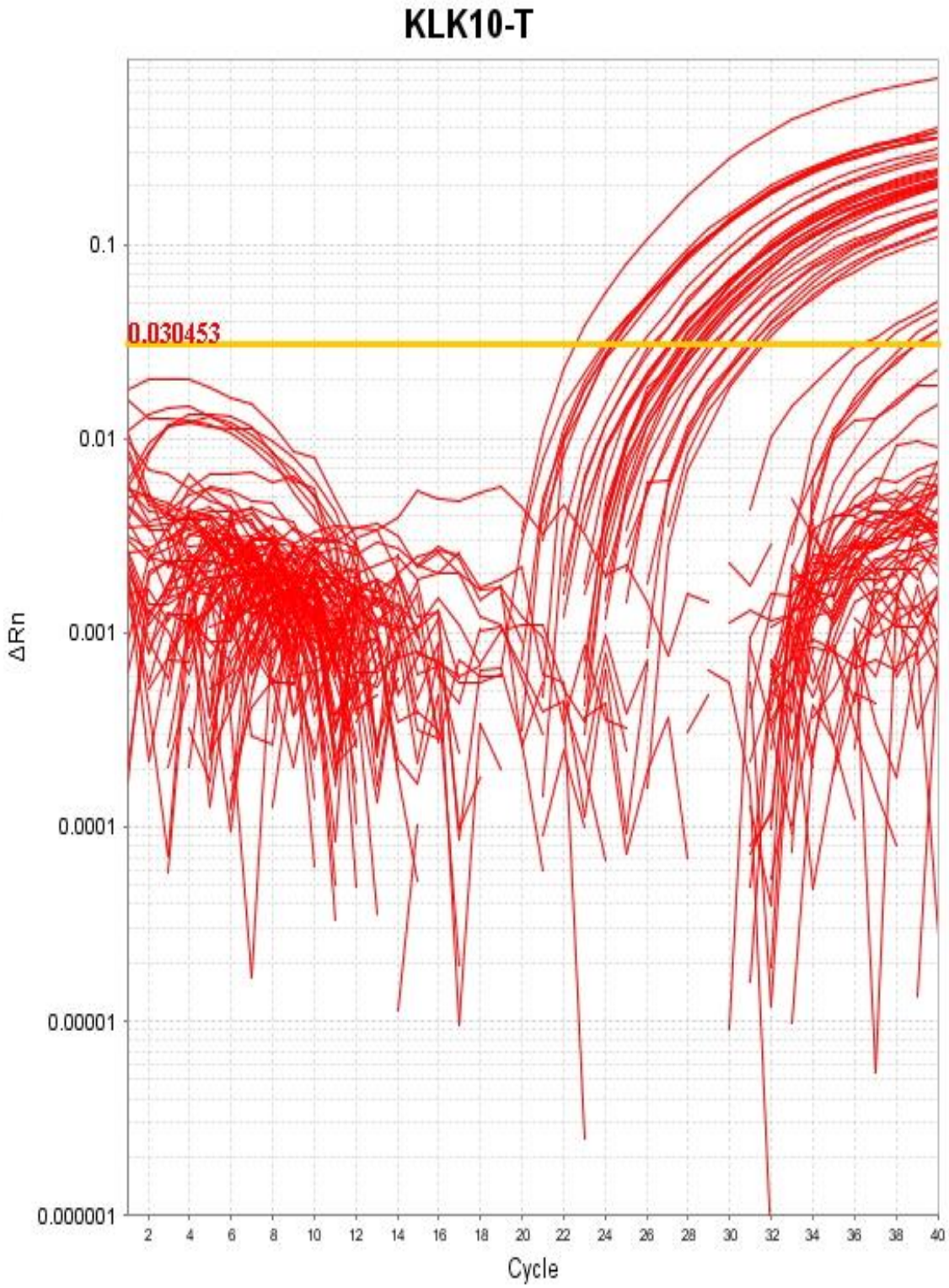

Figure 10: Allele T amplification plot.

It is indicated amplification plot of Allele T in Figure 10 and also the yellow line represents the threshold value (0.03045).

4.3. Genotype and Allele Analysis Among Patient and Control Groups

In this study, the polymorphism (rs7259451) in a specific region of KLK10 was analyzed in patients with NSCLC and healthy individuals known as the control group. Significant results were obtained from this study among the patient and healthy control groups (p=0.293). The relationship between these two groups is also shown in the table above. The calculated value of the homozygous mutant genotype corresponds to 0.356, the heterozygous genotype is 0.300, and the homozygous wild-type genotype is 0.413. According to the results of this experiment, homozygous wild type (GG), heterozygous type (GT) and homozygous mutant type (TT) ratios in the control group were determined as 60.9%, 32.6% and 6.5%, respectively. In NSCLC patient group, homozygous wild type (GG), heterozygous type (GT) and homozygous mutant type (TT) were calculated as 52.1%, 45.8%, and 2.1%, respectively. When the control and patient groups were compared for the G allele, the control group (71) was similar to the patient group (72). In addition, the T allele was higher in the patient group (24) than in the control group (21).

According to the evaluation of the characteristics of these two groups, the homozygous wild type genotype (GG) corresponds to a frequency of 60.9% in the control group and 52.1% in the patient group. In addition, the frequency of heterozygous genotype (GT) was 32.6% in the control group and 45.8% in the NSCLC patient group. Homozygous mutant type (TT) genotype was seen with a frequency of 6.5% in the control group and 2.1% in the patient group. When statistically obtained values were evaluated, each homozygous GG wild type (p = 0.413), heterozygous GT type (p = 0.300) and homozygous TT mutant type (p = 0.356) different between two groups as in Table 4.

In the NSCLC group, the G alleles were counted as 72 and the T alleles as 24. The p value of the G allele corresponds to 0.356. In addition, the G allele was noted as 71 and the T allele as 21 in the control group. The p value for the T allele was determined as 0.413. In conclusion, when KLK10 gene polymorphism was compared according to genotype and allele frequencies, no significant difference was found between the control and NSCLC patient groups in Table 4.2 (p=0.293).

To NSCLC, wild-type homozygote (GG) genotype is not much effective but can lead to protective effect (OR=0.699) than compared with other genotypes. Also, it is seen

that the mutant homozygote TT genotype has 3 times more protective effect (OR=0.305). Besides, having G allele does not show any advantage in patient with NSCLC. In addition, according to statistical analysis, heterozygous type (GT) (OR=1.587) carrying has a risk of NSCLC as seen in the Table 4. Also, carrying the G allele is not important (OR=3.279). Even possessing the T allele in heterozygous form (GT) has not a risk or protective effect, homozygous form of T (TT) constitutes a protective effect.

5. DISCUSSION AND CONCLUSION

In last decades, cancer is one of the most of the important and dangerous disease in all diseases to human life. Also, cancer leads uncontrollably cell growth and abnormally expanding of the cells which is recognized as metastasis. So that cancer can be very dangerous and fatal. If the control mechanism of these cells is not controlled, cells become divide continuously and cancer can become visible (21).

Formation of cancer, which is known as carcinogenesis, is very complicated biological mechanism. There are three stages of cancer formation; initiation, promotion and progression, respectively. Also, smoking tobacco, excess alcohol consumption and ionizing radiation can lead to generation of cancer. Moreover, some of hormones, tumor promoters and some biological factors can affect to become cancer (8, 120-122).

Based on types of cells, cancer is divided two types as malignant and benign cancers. Metastasis is the process through which malignant tumors spread across the body. Benign tumors can't infiltrate and spread like malignant ones (123). Malignant cancers include lung, prostate, liver, and cervix. Cancer cells' symptoms vary on tumor location, growth, and size. Cancer diagnostics and treatments vary. Malignancies are often diagnosed using biomarkers, imaging, MRI, and PET. Early-stage cancer patients have a greater chance of surviving (121).

Cleaning the cancerous area with surgery, radiotherapy and chemotherapy are the most common treatment methods. In addition, it can be supported by cancer-specific drug therapy and immunotherapy (84-86).

NSCLC is a subtype of lung cancer that includes adenocarcinoma, SCLC, and LCLC. It is responsible for about 85% of all lung cancers. If cancer or carcinoma discovered early, surgery is more likely to be effective (19).

While NSCLC death rates vary by country and gender, it is known as one of the deadliest cancers (22). Moreover, smoking of cigarette is the big significant thing to get lung cancer especially NSCLC. The average male cigarette consumers have a nine- to ten-fold increased risk of lung cancer, while heavy cigarette consumers have a twenty-fold increased risk (28).

Some researches show that kallikreins can be potential biological markers the diagnose and prognose of ovarian, breast, and prostate cancers (112). Many glandular organs, as well as blood, lymph, and urine, contain active or inactive kallikrein enzymes that can be triggered (87, 88). It is possible that KLK10 is involved in the inhibition of carcinogenesis in breast and prostate cancers (108). A protein called KLK10 is also involved in the activation of the steroid hormone hormone-receptor complexes (112).

On the basis of this study, the rs7259451 polymorphism in Turkish population was not found out significantly important data. However, GG homozygote wildtype genotype and GT heterozygote genotype were little higher both in control and patient groups, yet there is no statistically significant between control and patient group. Also, TT genotype was three-times higher in control group (p=356). According to these TT genotype might be carrier genotype. Nevertheless, the number of TT genotype (both control and patient) was very low it needs more study about that. Moreover, number of G allele is extremely higher than T allele in both control and patient group, but there is no statistically significant between control and patient group.

The KLK10 polymorphism (rs7259451) can be used to determine if the GG genotype inhibits prostate cancer and whether the TT genotype causes it (112).

As a result, there is no any statistically significant between control and patient group in KLK10 polymorphism (rs7259451). Maybe it is said that TT genotype can be carrier gene. However, more researches are already needed. Maybe this study can be enhanced with in larger population or different population. Otherwise, this gene's another SNPs can be study.

As a consequence,

The polymorphism of KLK10 gene (rs7259451) in Turkish patient with NSCLC has not been studied before. Also, on behalf of this thesis, it was stated that this polymorphism could not have an effect on NSCLC progression. However, according to the evaluated results, the TT homozygous mutant type can be considered as an advantage for the patients. But the T allele itself is not protective. having TT in patients may be an advantage and this type might contribute to the reduction of the risk of NSCLC.

In this study, we didn't find any statistically significant differences in KLK10 gene polymorphism (rs7259451) genotype between patients and controls based on allelic discrimination. As the KLK10 gene polymorphism effects may differ from person to person and as KLK10 gene heterogeneity is widespread, this research can be expanded by high number of individuals the sample size of the study groups in order to obtain a more accurate understanding of NSCLC.

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7. APPENDICES

Crosstabs

Notes

Output Created		02-APR-2022 12:36:26
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	Active Dataset	DataSet1
	Filter	<none>
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	N of Rows in Working Data File	94
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Grup BY Cinsiyet /TABLES=Grup BY Yaş YAŞARALIĞI /TABLES=Sigara_Hiç_İçmemiş /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL. T-TEST GROUPS=Grup(0 1) /MISSING=ANALYSIS /VARIABLES=Genotype_KLK10_rs7259451 rs7259451_GG rs7259451_GT rs7259451_TT rs7259451_G rs7259451_T /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,02
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Grup * Cinsiyet	94	100,0%	0	0,0%	94	100,0%

Grup * Cinsiyet Crosstabulation

			Cinsiyet		
			Kadın	Erkek	Total
Grup	Kontrol	Count	18	28	46
		% within Grup	39,1%	60,9%	100,0%
		% within Cinsiyet	75,0%	40,0%	48,9%
		% of Total	19,1%	29,8%	48,9%
	Hasta	Count	6	42	48
		% within Grup	12,5%	87,5%	100,0%
		% within Cinsiyet	25,0%	60,0%	51,1%
		% of Total	6,4%	44,7%	51,1%
Total		Count	24	70	94
		% within Grup	25,5%	74,5%	100,0%
		% within Cinsiyet	100,0%	100,0%	100,0%
		% of Total	25,5%	74,5%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8,761[a]	1	,003		
Continuity Correction[b]	7,417	1	,006		
Likelihood Ratio	9,055	1	,003		
Fisher's Exact Test				,004	,003
Linear-by-Linear Association	8,668	1	,003		
N of Valid Cases	94				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 11,74.

b. Computed only for a 2x2 table

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	4,500	1,590	12,736
For cohort Cinsiyet = Kadın	3,130	1,364	7,184
For cohort Cinsiyet = Erkek	,696	,539	,898
N of Valid Cases	94		

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Grup * Yaş	71	100,0%	0	0,0%	71	100,0%
Grup * YAŞARALIĞI	71	100,0%	0	0,0%	71	100,0%

Grup * Yaş

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	30,818[a]	32	,526
Likelihood Ratio	39,845	32	,161
Linear-by-Linear Association	,910	1	,340
N of Valid Cases	71		

a. 66 cells (100,0%) have expected count less than 5. The minimum expected count is ,35.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / Hasta)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Grup * YAŞARALIĞI

Crosstab

			YAŞARALIĞI		
			<60	>EŞİT60	Total
Grup	Kontrol	Count	12	13	25
		% within Grup	48,0%	52,0%	100,0%
		% within YAŞARALIĞI	35,3%	35,1%	35,2%
		% of Total	16,9%	18,3%	35,2%
	Hasta	Count	22	24	46
		% within Grup	47,8%	52,2%	100,0%
		% within YAŞARALIĞI	64,7%	64,9%	64,8%
		% of Total	31,0%	33,8%	64,8%
Total		Count	34	37	71
		% within Grup	47,9%	52,1%	100,0%
		% within YAŞARALIĞI	100,0%	100,0%	100,0%
		% of Total	47,9%	52,1%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,000[a]	1	,989		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,000	1	,989		
Fisher's Exact Test				1,000	,592
Linear-by-Linear Association	,000	1	,989		
N of Valid Cases	71				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 11,97.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	1,007	,380	2,669
For cohort YAŞARALIĞI = <60	1,004	,604	1,667
For cohort YAŞARALIĞI = >EŞİT60	,997	,625	1,590
N of Valid Cases	71		

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Grup * Sigara_Hiç_İçmemiş	46	64,8%	25	35,2%	71	100,0%
Grup * Aktif_Smoker	71	100,0%	0	0,0%	71	100,0%
Grup * Pre_Op_Evre	41	57,7%	30	42,3%	71	100,0%
Grup * Post_Op_Evre	27	38,0%	44	62,0%	71	100,0%
Grup * Evre_Son	46	64,8%	25	35,2%	71	100,0%

Grup * Sigara_Hiç_İçmemiş

Crosstab

			Sigara_Hiç_İçmemiş	
			Yok	Total
Grup	Hasta	Count	46	46
		% within Grup	100,0%	100,0%
		% within Sigara_Hiç_İçmemiş	100,0%	100,0%
		% of Total	100,0%	100,0%
Total		Count	46	46
		% within Grup	100,0%	100,0%
		% within Sigara_Hiç_İçmemiş	100,0%	100,0%
		% of Total	100,0%	100,0%

Chi-Square Tests

	Value
Pearson Chi-Square	.[a]
N of Valid Cases	46

a. No statistics are computed because Grup and Sigara_Hiç_İçmemiş are constants.

Grup * Aktif_Smoker

Crosstab

			Aktif_Smoker		
			Yok	Var	Total
Grup	Kontrol	Count	10	15	25
		% within Grup	40,0%	60,0%	100,0%
		% within Aktif_Smoker	20,0%	71,4%	35,2%
		% of Total	14,1%	21,1%	35,2%
	Hasta	Count	40	6	46
		% within Grup	87,0%	13,0%	100,0%
		% within Aktif_Smoker	80,0%	28,6%	64,8%
		% of Total	56,3%	8,5%	64,8%
Total		Count	50	21	71
		% within Grup	70,4%	29,6%	100,0%
		% within Aktif_Smoker	100,0%	100,0%	100,0%
		% of Total	70,4%	29,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	17,146[a]	1	,000		
Continuity Correction[b]	14,966	1	,000		
Likelihood Ratio	16,954	1	,000		
Fisher's Exact Test				,000	,000
Linear-by-Linear Association	16,904	1	,000		
N of Valid Cases	71				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 7,39.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval Lower	95% Confidence Interval Upper
Odds Ratio for Grup (Kontrol / Hasta)	,100	,031	,323
For cohort Aktif_Smoker = Yok	,460	,281	,753
For cohort Aktif_Smoker = Var	4,600	2,042	10,360
N of Valid Cases	71		

T-Test

Case Processing Summary

	Cases Valid N	Valid Percent	Missing N	Missing Percent	Total N	Total Percent
Grup * Genotype_KLK10_rs7259451	94	100,0%	0	0,0%	94	100,0%
Grup * rs7259451_GG	94	100,0%	0	0,0%	94	100,0%
Grup * rs7259451_GT	94	100,0%	0	0,0%	94	100,0%
Grup * rs7259451_TT	94	100,0%	0	0,0%	94	100,0%
Grup * rs7259451_G	94	100,0%	0	0,0%	94	100,0%
Grup * rs7259451_T	94	100,0%	0	0,0%	94	100,0%

Grup * Genotype_KLK10_rs7259451

Crosstab

			Genotype_KLK10_rs7259451			
			GG	GT	TT	Total
Grup	Kontrol	Count	28	15	3	46
		% within Grup	60,9%	32,6%	6,5%	100,0%
		% within Genotype_KLK10_rs7259451	52,8%	40,5%	75,0%	48,9%
		% of Total	29,8%	16,0%	3,2%	48,9%
	Hasta	Count	25	22	1	48
		% within Grup	52,1%	45,8%	2,1%	100,0%
		% within Genotype_KLK10_rs7259451	47,2%	59,5%	25,0%	51,1%
		% of Total	26,6%	23,4%	1,1%	51,1%
Total		Count	53	37	4	94
		% within Grup	56,4%	39,4%	4,3%	100,0%
		% within Genotype_KLK10_rs7259451	100,0%	100,0%	100,0%	100,0%
		% of Total	56,4%	39,4%	4,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2,453[a]	2	,293
Likelihood Ratio	2,506	2	,286
Linear-by-Linear Association	,131	1	,717
N of Valid Cases	94		

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 1,96.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / Hasta)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Grup * rs7259451_GG

Crosstab

			rs7259451_GG		
			GT+TT	GG	Total
Grup	Kontrol	Count	18	28	46
		% within Grup	39,1%	60,9%	100,0%
		% within rs7259451_GG	43,9%	52,8%	48,9%
		% of Total	19,1%	29,8%	48,9%
	Hasta	Count	23	25	48
		% within Grup	47,9%	52,1%	100,0%
		% within rs7259451_GG	56,1%	47,2%	51,1%
		% of Total	24,5%	26,6%	51,1%
Total		Count	41	53	94
		% within Grup	43,6%	56,4%	100,0%
		% within rs7259451_GG	100,0%	100,0%	100,0%
		% of Total	43,6%	56,4%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,737[a]	1	,391		
Continuity Correction[b]	,423	1	,515		
Likelihood Ratio	,739	1	,390		
Fisher's Exact Test				,413	,258
Linear-by-Linear Association	,730	1	,393		
N of Valid Cases	94				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 20,06.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,699	,308	1,585
For cohort rs7259451_GG = GT+TT	,817	,513	1,301
For cohort rs7259451_GG = GG	1,169	,818	1,670
N of Valid Cases	94		

Grup * rs7259451_GT

Crosstab

			rs7259451_GT		
			GG+TT	GT	Total
Grup	Kontrol	Count	30	16	46
		% within Grup	65,2%	34,8%	100,0%
		% within rs7259451_GT	53,6%	42,1%	48,9%
		% of Total	31,9%	17,0%	48,9%
	Hasta	Count	26	22	48
		% within Grup	54,2%	45,8%	100,0%
		% within rs7259451_GT	46,4%	57,9%	51,1%
		% of Total	27,7%	23,4%	51,1%
Total		Count	56	38	94
		% within Grup	59,6%	40,4%	100,0%
		% within rs7259451_GT	100,0%	100,0%	100,0%
		% of Total	59,6%	40,4%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,191[a]	1	,275		
Continuity Correction[b]	,776	1	,378		
Likelihood Ratio	1,195	1	,274		
Fisher's Exact Test				,300	,189
Linear-by-Linear Association	1,178	1	,278		
N of Valid Cases	94				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 18,60.

b. Computed only for a 2x2 table

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	1,587	,691	3,642
For cohort rs7259451_GT = GG+TT	1,204	,861	1,683
For cohort rs7259451_GT = GT	,759	,460	1,253
N of Valid Cases	94		

Grup * rs7259451_TT

Crosstab

			rs7259451_TT		
			GG+GT	TT	Total
Grup	Kontrol	Count	43	3	46
		% within Grup	93,5%	6,5%	100,0%
		% within rs7259451_TT	47,8%	75,0%	48,9%
		% of Total	45,7%	3,2%	48,9%
	Hasta	Count	47	1	48
		% within Grup	97,9%	2,1%	100,0%
		% within rs7259451_TT	52,2%	25,0%	51,1%
		% of Total	50,0%	1,1%	51,1%
Total		Count	90	4	94
		% within Grup	95,7%	4,3%	100,0%
		% within rs7259451_TT	100,0%	100,0%	100,0%
		% of Total	95,7%	4,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,136[a]	1	,287		
Continuity Correction[b]	,308	1	,579		
Likelihood Ratio	1,182	1	,277		
Fisher's Exact Test				,356	,292
Linear-by-Linear Association	1,124	1	,289		
N of Valid Cases	94				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 1,96.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,305	,031	3,044
For cohort rs7259451_TT = GG+GT	,955	,875	1,041
For cohort rs7259451_TT = TT	3,130	,338	29,018
N of Valid Cases	94		

Grup * rs7259451_G

Crosstab

			rs7259451_G		
			T	G	Total
Grup	Kontrol	Count	3	43	46
		% within Grup	6,5%	93,5%	100,0%
		% within rs7259451_G	75,0%	47,8%	48,9%
		% of Total	3,2%	45,7%	48,9%
	Hasta	Count	1	47	48
		% within Grup	2,1%	97,9%	100,0%
		% within rs7259451_G	25,0%	52,2%	51,1%
		% of Total	1,1%	50,0%	51,1%
Total		Count	4	90	94
		% within Grup	4,3%	95,7%	100,0%
		% within rs7259451_G	100,0%	100,0%	100,0%
		% of Total	4,3%	95,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,136[a]	1	,287		
Continuity Correction[b]	,308	1	,579		
Likelihood Ratio	1,182	1	,277		
Fisher's Exact Test				,356	,292
Linear-by-Linear Association	1,124	1	,289		
N of Valid Cases	94				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 1,96.

b. Computed only for a 2x2 table

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	3,279	,329	32,727
For cohort rs7259451_G = T	3,130	,338	29,018
For cohort rs7259451_G = G	,955	,875	1,041
N of Valid Cases	94		

Grup * rs7259451_T

Crosstab

			rs7259451_T		
			G	T	Total
Grup	Kontrol	Count	28	18	46
		% within Grup	60,9%	39,1%	100,0%
		% within rs7259451_T	52,8%	43,9%	48,9%
		% of Total	29,8%	19,1%	48,9%
	Hasta	Count	25	23	48
		% within Grup	52,1%	47,9%	100,0%
		% within rs7259451_T	47,2%	56,1%	51,1%
		% of Total	26,6%	24,5%	51,1%
Total		Count	53	41	94
		% within Grup	56,4%	43,6%	100,0%
		% within rs7259451_T	100,0%	100,0%	100,0%
		% of Total	56,4%	43,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,737[a]	1	,391		
Continuity Correction[b]	,423	1	,515		
Likelihood Ratio	,739	1	,390		
Fisher's Exact Test				,413	,258
Linear-by-Linear Association	,730	1	,393		
N of Valid Cases	94				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 20,06.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	1,431	,631	3,247
For cohort rs7259451_T = G	1,169	,818	1,670
For cohort rs7259451_T = T	,817	,513	1,301
N of Valid Cases	94		

	1	2	3	4	5	6	7	8	9	10	11	12
A	H11 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H12 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H13 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H14 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H15 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H16 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H17 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H18 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H19 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H20 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H21 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H22 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
B	H23 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H24 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H25 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H26 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H27 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H28 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H29 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H30 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H31 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H32 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H33 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H34 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
C	H35 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H36 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H37 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H38 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H39 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H40 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H41 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H42 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H43 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H44 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H45 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H46 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
D	H47 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H48 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H49 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H50 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H51 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H52 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H53 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H54 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H55 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H56 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H57 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H58 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
E	H59 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	H60 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K1 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K2 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K3 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K4 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K5 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K6 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K7 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K8 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K9 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K10 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
F	K11 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K12 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K13 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K14 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K15 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K16 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K17 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K18 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K19 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K20 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K21 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K22 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
G	K23 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K24 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K25 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K26 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K27 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K28 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K29 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K30 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K31 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K32 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K33 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K34 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB
H	K35 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K36 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K37 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K38 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K39 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K40 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K41 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K42 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K43 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K44 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	K45 U KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB	NEG N KLK10 RS7259451 G VIC-NFQ-MGB T FAM-NFQ-MGB

7.1. Ethical Committee Aproval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2252
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

25/11/2021

İlgili Makama (Büşra Bıçak)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, **"Küçük Hücreli Olmayan Akciğer Kanserine Duyarlılıkta KLK10 Geni (rs7259451) Polimorfizminin Rolünün Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2312)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **24.11.2021** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir **(KAEK Karar No: 1532)**

Prof. Dr. Turgay ÇELİK

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

Yeditepe Üniversitesi 26 Ağustos Yerleşimi, İnönü Mahallesi Kayışdağı Caddesi 34755 Ataşehir / İstanbul
T. 0216 578 00 00 www.yeditepe.edu.tr F. 0216 578 02 99

7.2. Curriculum Vitae

Personal Informations

Name	Büşra	**Surname**	BIÇAK

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate			
Master			
University	Molecular Biology and Genetics	**Istanbul Technical University**	2020
High school	Science	Nenehatun Anatolian High school	2012

[#] All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs),

Languages	Grades ([#])
English	76.25
Korean	Topik 1

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
		-
		-

Computer Skills

Program	Level
MS Programs	Excellent
SPSS, Matlab, R&R Studios	Average

***Excellent, good, average or basic**

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Articles published in other journals

Proceedings presented in international scientific meetings and published in proceedings book.

Journals in the proceedings book of the refereed conference / symposium

Others (Projects / Certificates / Rewards)
