T.C. YEDİTEPE UNIVERSITY
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THE ROLE OF IL-1 B GENE POLYMORPHISM (RS1143627) IN NON-SMALL CELL LUNG CANCER

MASTER'S THESIS

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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 ..... 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 the award of any other degree except where due acknowledgment has been made in the text.

28.06.2022

Luay Shehab

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TABLE of CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DNA= deoxyribonucleic acid,

IL1-β= Interleukin 1-β

EDTA= Ethylenediaminetetraacetic acid

NSCLC= Non-small cell lung cancer

SCLC= Small cell lung cancer

PAMP= pathogen-associated molecular pattern

DAMP= Damage-associated molecular pattern

COPD= Chronic obstructive pulmonary disease

TNF= Tumor necrosis factor

Myd88= myeloid differentiation primary response 88

interleukin-1 receptor-associated kinase 1/4 = (IRAK1/4)

TRAF6= TNFR-associated factor 6

RIP1= Receptor interacting protein 1

TRADD = TNFR1-associated death domain

LPS= lipopolysaccharide

NLRP3= NOD-like" receptor (NLR) proteins 3

PKA= Protein kinase A

ATP= Adenosine triphosphate

mRNA= messenger RNA

PET-CT= Positron Emission Tomography and Computed Tomography

PCR= polymerase chain reaction

RNA= ribonucleic acid

SNPs= single nucleotide polymorphisms

SPSS= Statistical Package for the Social Sciences

ABSTRACT

Shehab L., (2022). The role of IL 1-Beta Gene Polymorphism (rs1143627) in Non-Small Cell Lung Cancer. Yeditepe University, Health Sciences Institute, Department of Molecular Medicine. Master Thesis. Istanbul.

The goal of this written research is to study the role of IL1-β gene SNP rs1146327 in non-small cell lung cancer (NSCLC). Although the etiology and compulsive mechanisms of NSCLC are unknown, and it is a multifaceted and complex illness, age and inherited factors are supposed to have a part in its growth. Nonetheless, smoking, age, family history, and genetic variables are the most commonly cited factors related with NSCLC. We tested the rs1146327 polymorphism in patients with NSCLC and comparison groups using real-time PCR, and found no statistically big differences in findings between the two groups of affected and control groups. Our data show that the patients' dominant form (GG), heterozygote (GA), and recessive form (AA) rates are 33.3 percent, 43.8 percent, and 22.9 percent, respectively. However, the dominant form (GG), heterozygote (GA), and recessive form (AA) percentages in the comparative group are 16.7 percent, 59.5 percent, and 23.8 percent, respectively. According to our findings, the homozygote mutant type (AA) ratio was larger in controls than in affected persons, despite the change was not significant (p-value = 0.091). The homozygote wild type (GG) ratio was also greater in controls than in patients, with a p-value of 0.951, which was not major.

Key Words: IL1Beta, NSCLC, rs1143627

ÖZET

Shehab L., (2022). Küçük Hücreli Dışı Akciğer Kanseriyle IL 1-Beta Geni (rs1143627) Polmorfizmi Rolü. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Moleküler Tıp Anabilim Dalı. Yüksek lisans Tezi. İstanbul.

Bu yazılı araştırmanın amacı, küçük hücreli dışı akciğer kanserinde IL1-β gen polimorfizmi rs1l436 'nin rolünü incelemektir. KHDAK'nin etiyolojisi ve patolojik mekanizmaları bilinmemekle birlikte, çok yönlü ve karmaşık bir hastalık olmasına rağmen, gelişiminde yaş ve kalıtsal faktörlerin rolü olduğu düşünülmektedir. Bununla birlikte sigara kullanımı, yaş, aile öyküsü ve genetik değişkenler KHDAK ile en çok ilgili ilişkilendirilen etkenlerdir. rs1143627 polimorfizmini KHDAK olan hastalarda ve karşılaştırma gruplarında gerçek zamanlı PCR kullanarak test ettik ve bu hasta grubu ile karşılaştırma grupları arasındaki bulgularda istatistiksel olarak anlamlı bir fark bulamadık. Verilerimiz, hastaların homozigot doğal tip (GG), heterozigot (GA) ve homozigot mutant tip (AA) oranlarının sırasıyla yüzde 33,3, yüzde 43,8 ve yüzde 22,9 olduğunu göstermektedir. Bununla birlikte, karşılaştırmalı grupta homozigot doğal tip (GG), heterozigot (GA) ve homozigot mutant tip (AA) yüzdeleri sırasıyla yüzde 16.7, yüzde 59.5 ve yüzde 23,8'dir. Bulgularımıza göre, homozigot mutant tip (AA) oranı kontrollerde hastalara göre daha yüksekti, ancak fark anlamlı değildi (p-değeri = 0.091). Homozigot tip (GG) oranı da kontrollerde hastalardan daha yüksekti ve p değeri 0.951 idi ve bu anlamlı değildi.

Anahtar Kelimeler: IL1Beta, NSCLC, rs1143627

1. INTRODUCTION AND PURPOSE

Lung cancer has one of the highest fatality rates between all cancers globally [1]. It is a complex disease with various subtypes that have pathologic and clinical implications [2]. LC is classified into two major sets, small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC) representing 15% and 85% of all cases, correspondingly. NSCLC categorises into three forms "the adenocarcinoma (AC), squamous cell carcinoma (SCC), and large cell undifferentiated carcinoma (LCC),"[3,4].

About 25–30% of all incidences are SCC, which is highly related to cigarette smoking. It develops in the core of the lung from its precursors specifically in the bronchial tubes [5]. while AC is the largest prevalent type accounts for 40% of all cases, and generated from small mucus secretion cells which are lining the internal surface of the airway called type II alveolar cells [6].

It is more probable easy for AC to be diagnosed before it extends outside the lungs because it develops more slowly than other types [7]. The third type of NSCLC is known as LCC and represents about 5–10% of all incidence. It is usually developing in the core part of the lung and extends to the neighbouring organs, and detected by ruling out alternative ways since this kind of cancer lacks indications of squamous or glandular development [8].

Many studies revealed that there is a significant relationship between prolonged inflaming and NSCLC, also shows that congenital respiratory failure, nicotine, smog, lung conditions, and environmental dirt are all examples of respiratory diseases have all been linked to NSCLC development [9]. Inflammation is a physiological process that immune cells trigger in order to fight infections. Long-term inflammation caused by a persistent infection, however, can cause ongoing tissue damage and cellular growth, leading to tumorigenesis [10].

However, tumor development and migration are known to be induced by cytokines, growth factors, and adhesion molecules which are secreted from tumor cells, immune cells, and stromal cells.

All these chemicals have a considerable relationship with inflammation and tumor development, taking into consideration the fact that 15% of all tumors are caused by inflammation [11]. Under normal conditions when tissue is damaged by mechanical stimuli, pathogenic agents, or exposed to hazard chemicals, the body naturally starts an inflammatory response. However, within prolonged inflammation different types of cells like fibroblasts and stromal cells communicate with each other via cytokines and chemokines.

The sustained exposure to inflammatory cytokine, has the potential to encourage sarcoma development, causing nucleic acid disruption and other events that favour cancer initiation [12].

Cytokines control stromal and tumor cell proliferation, transport, signaling, and differentiation. Additionally, cytokines released by sarcomas promote ideal development within cancer, whereas stromal cells emit cytokines that may influence the behaviour of malignant cells [13].

Inflammation and cancer are related via an internal process in which genetic events coordinate the formation of an inflammatory milieu because of cancer, and an extrinsic pathway in which non-resolving inflammation drives carcinogenesis [14].

Prolonged inflaming might be damaging the tissue and lead to disease. Moreover, chronic inflammation has been associated with cytokines, chemokines, and adhesion molecules [15]. Under circumstance conditions, cytokines facilitate the cross talk among deffirent kind of cells within the tumor, for instance, previous study revealed that there is cross talk between malignant cells and immune responses in lung adenocarcinoma [16].

Cytokines also called interleukins have significant roles in the tumorigenesis. The effect of these interleukins in cancer developments is detected by many factors including its biological source, receptors, and signaling pathways, as well as dose-dependency. While interleukin activity can be cell specific, also it can affect cancer start, tumor growth, and tumor control [17]. The genes cluster of interleukin 1 family located on human chromosome 2 contains IL1-α, IL1-β, and IL1-RA and concentrated within a 430-kb

The structure of IL 1-β gene contains a TATA box along with 7 exons and 6 introns, and is monitored by the sense strand which is condensed within 7.5-kb [19]. During inflammation development, the IL 1-β has kay function in controlling the transcriptional activity of various genes [20].

Sustained stimulation of IL 1-β increases the inflammatory response's severity and establishes an inflammatory microenvironment favorable to tumor start and/or promotion [21]. Interestingly, Cancer risk, symptoms, therapy, and outcome are all influenced by genetic differences in the human genome [22]. Recently, the studying of cytokine gene sequences detected various SNPs and a minor quantity of microsatellite polymorphisms which are mostly found in their promoter regions [23]. SNPs are, in fact, the most prevalent variations in the genome [24].

Within the sense strand that control the IL 1-β, different SNPs have been found, including -31 (rs1143627) and -511 (rs16944). However, various research showed that there is a relation between IL 1-β gene and lung cancer, especially the -31 (rs1143627) SNP [25]. Once mutation occurs, it mediates the change from T to C (TATAAA to CATAAA) and a probable interruption of the promoter region by the rs1146327 polymorphism [26]. Thus, despite the lack of a molecular mechanism, the widespread agreement is that the -31 (rs1143627) SNP is actually an important variant in this site. [27]

The fundamental goal of this research is to study; the role of IL1-β gene polymorphism (rs1146327) in non-small cell lung cancer.

2. LITERATURE REVIEW

2.1. The lung

One of the most crucial organ between all organs is the lung. Their primary role is to make O2/CO2 transmission from adjacent atmosphere to bloodstream and vis versa, where the alveoli pass oxygen to the capillary network and neighbouring arterial system. Nearly 300 million alveoli extend from the internal lobes of the lungs, while the respiratory system mainly consists of nose, oropharynx, larynx, trachea, bronchi, and bronchioles. The diaphragm is the key muscle in the respiratory system, and the peripheral intercostal muscles play critical roles during body exercise and respiratory discomfort [28-31].

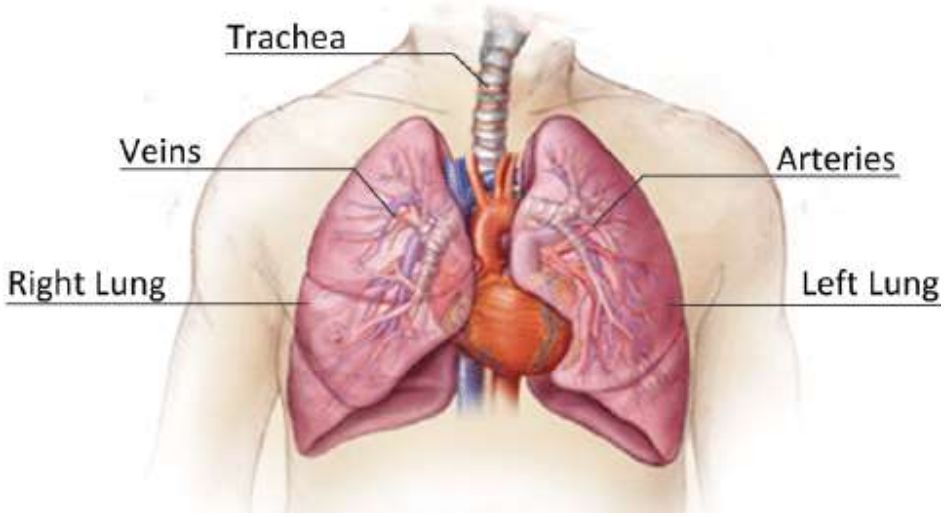

Figure 1: Anatomy of the human lung [32]

2.2. Non-small cell lung cancer (NSCLC)

Basically, there are three main kinds of NSCLC containing adenocarcinoma (AC), squamous cell carcinoma (SCC), and large cell undifferentiated carcinoma (LCC) [3,4].

SCC represent about 30% of all incidences. It develops from the primary membranous cells that covers internal surface of the airway tubes in the lungs core, and is usually related to cigarette smoking [5]. AC is the main frequent kind of lung cancer, representing about 40% of all patients. Its formed from type II alveolar cells, which are microscopic epithelial cells that secret phlegm. [6] AC grows in a slow way which helps to be diagnosed earlier which will help to prevent the tumor development [7].

LCC represent roughly 10% of all patients. Since no evidence have been shown of squamous or glandular maturation in this form, it is frequently diagnosed by default to exclude alternative ways. LCC Typically, it begins in the core of the lungs. while spreading to surrounding lymph nodes, the chest walls, and distant organs [8].

2.3. Epidemiology of lung cancer

As per GLOBOCAN data, there were 2,094,000 reported incidences globally in 2018, putting it between one of the highest sarcoma universally. Over 1,369,000 instances, LC is considered the 2nd frequent cancer between males after prostate cancer, and the 2nd frequent disease between femails after breast cancer, with approximately 725,000 incidences.

Males have a 3.8% incidence rate of lung cancer while women have a 1.77% [33,34]. Poor countries where cigarette smoking is most common have 20-fold higher incidence of LC compared with other countries. Whereas prostate cancer is the most prevalent disease between males in 104 countries, In 37 countries, this kind of sarcoma is the highly frequent between women. (Figure.2)

In North Korea, there is considerable prevalence of LC between women. Micronesia/Polynesia has the most prevalent cases in the world, with 52.2/100,000 patients all are men, whereas Hungary registered the most number of cases between men with 77.4/100,000 patients. While North America and Northern and Western Europe register the most number of cases between women all over the world. And the lowest number of cases were between Men and women in Western, Central, and Eastern [33].

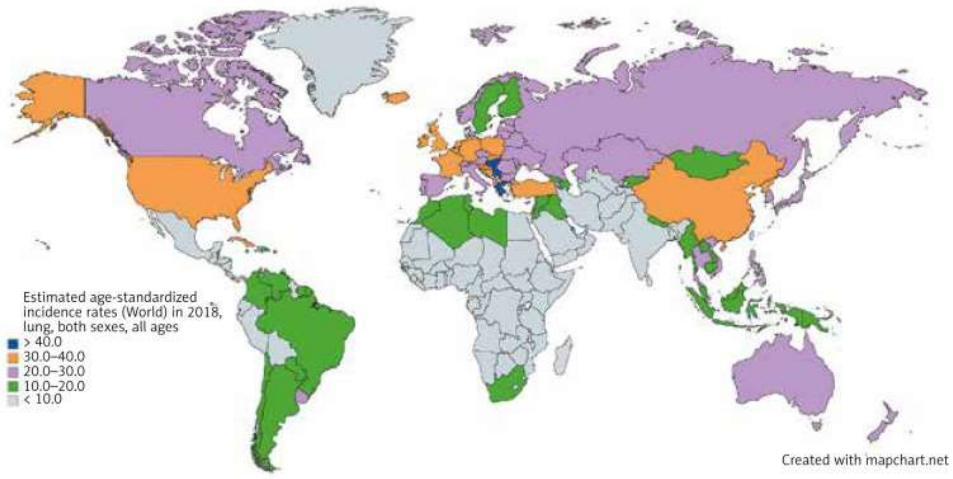

Figure 2: Map depicting the approximate age-standardized prevalence rate of lung cancer in both genders and at all ages in 2018. Made with mapchart.net. Globocan 2018 [34]

2.4.. Risk factors of NSCLC

Many factors found to be linked with NSCLC, although the main reason is still under investigation, some of these factors include smoking, radon exposure, genetic factors, air pollution, and radiation [35].

2.4.1. Cigarette smoking

Cigarette smoking is without a doubt to blame for the onset of the lung cancer epidemic in the twentieth century. Pipe and cigar smoking has a similar influence on LC threat as mild cigarette smoking. [36, 37] Because of the modest success in smoking cessation in the US and the UK, lung cancer rates are expected to plateau in the coming 20 years. Unless smoking prevalence is drastically reduced, for many years LC will still the major cause of death [38].

2.4.2. Air pollution

The prolonged exposure to the air pollutant, especially those containing the hydrocarbon chemicals, is considered a major risk factor that leads [39,40].

2.4.3. Radon Exposure

Every year about 21,000 patients with lung cancer die due to radium emanation. It is considered the 2nd leading factor linked to NSCLC and the leading cause between smokers globally [41].

2.5. Interleukin 1 beta

Basically, IL1-β family is secreted from monocytes, like monocytes, macrophages, and dendritic cells. The production of IL1-β promoted by two main stimuli known as pathogen-associated molecular pattern molecules (PAMPs) and damage-associated molecular pattern molecules (DAMPs)

PAMPs, such as lipopolysaccharide (LPS), lipoteichoic acid, or flagellin, are used to identify invading microbes. Endogenous ligands such as adenosine triphosphate, waste product, and a family of calcium-binding cytosolic proteins are secreted by injured or damaged cells [42].

The synthesis and processing of IL1-β is tightly regulated, including two main mechanisms known as "priming" and "activation". During priming the IL1-β gene transcribed while activation leads to inflammasome complexes formation resulting in the mature form of IL1-β [43].

IL1-β is generated and secreted by different types of cells within the sarcoma, among them are immune cells and fibroblasts. The processes of IL1-β production, on the other hand, have been examined extensively in macrophages. However, as previously stated, two signals are required for IL1-β production: "priming" and "cleavage" (Figure 3).

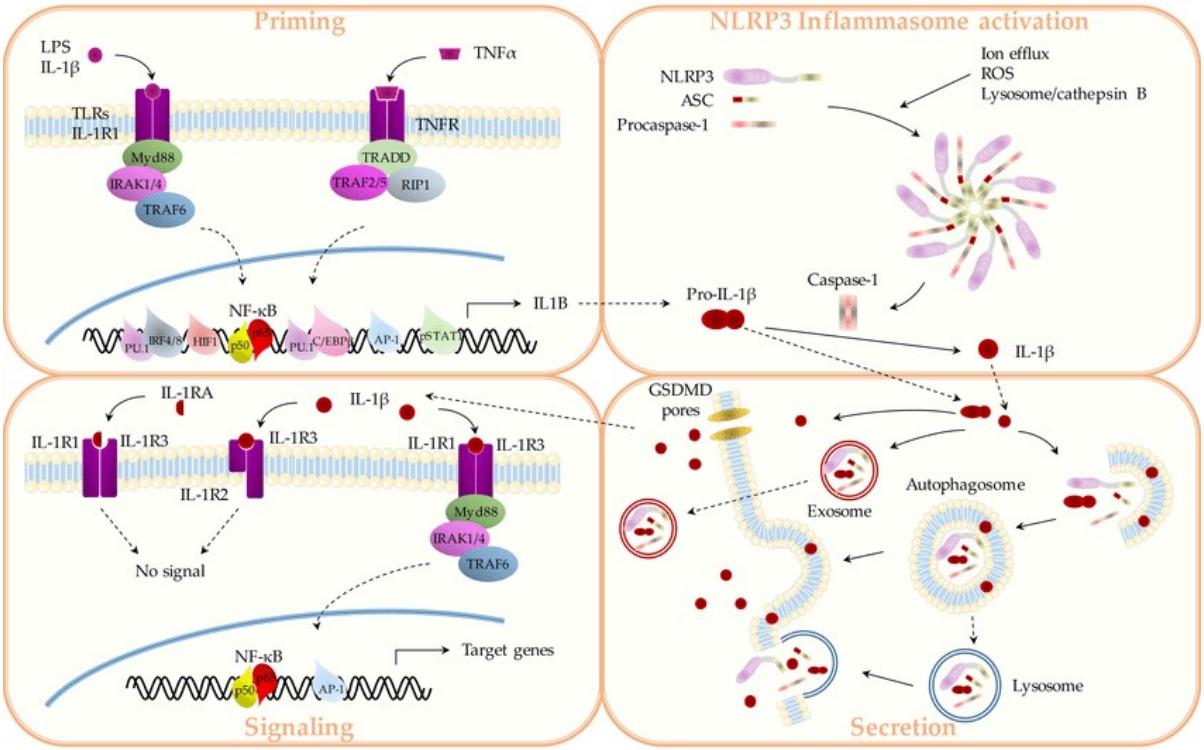

Figure 3: Steps of IL-1β production [44]

2.5.1. Priming

The stimulation of toll-like receptors (TLRs), particularly by lipopolysaccharides (LPS) or IL1-β itself, or the tumor necrosis factor (TNFα) by the TNF receptor (TNFR), will induce the transcription of the IL1-β gene (Figure 4).

TLRs and interleukin 1 receptor type 1 (IL-1R1) both stimulate the interleukin-1 receptor-associated kinase 1/4 (IRAK1/4) and TNFR-associated factor 6 (TRAF6) pathways by using the same adaptor protein, myeloid differentiation primary response 88 (Myd88), which binds to the receptor internal part. On other hand, the stimulation of TNFR by TNFα leads to activation of the TNFR1-associated death domain (TRADD), which in turn triggers the TRAF2/5 and the receptor interacting protein 1 (RIP1) signaling pathway. Both mechanisms as a result are able to trigger B-cell nuclear factor kappa-light-chain-enhancer (NF- kB) [45,46]. During these cascades, many transcription factors are involved, including hypoxia-induced factor 1 (HIF1), CCAAT/enhancer binding protein (C/EBP-) β, interferon response factors 4/8 (IRF4/8) and PU.1 or protein kinase C (PKC)/activator protein-1 (AP-1) [45].

Furthermore, the STAT1 pathway has also been demonstrated to be essential for IL1-β generation in macrophages [47].

Finally, SNPs in the IL1-β gene can affect IL1-β synthesis by limiting or permitting the fixing of accessory proteins such as those mentioned above. Pro-IL1-β is created as an inactive 31kDa protein after transcription/translation, and it must be spliced into 17kDa IL1-β to be mature.

2.5.2. Inflammasomes

Proteases are required to cleave the immature IL1-β before it can be activated. Caspases are the only proteases that can produce the active form of IL1-β, despite the fact that it has been shown to cleave the IL1-β with cathepsin G or elastase in neutrophils, resulting in low-activity IL1-β. Under circumstance conditions Caspases-8 has been shown to cleave IL1-β. But the most significant enzyme associated with IL1-β production is caspase-1, which has been stated to be triggered by a variety of sources [48].

Caspase-1 is activated when it is recruited to inflammasomes, which are multi-protein structures. These cytoplasmic components all have a binding site and an adaptor, making it possible to recruit and activate pro-inflammatory caspases [49].

2.5.3. Secretion

Both Pro- IL1-β and IL1-β are found in the cytoplasm, with a portion of them secreted in membrane-bound cell organelle that contains digestive enzymes an unknown process. Part of this IL1-β is exposed for disruption, while the other part is retained for continued releasing by fusing with the plasma membrane [50,51].

To avoid destruction, IL1-β may be kept safe from the lysosome's acidic pH. Which is made by autophagy, a mechanism that engulfs the ruptured product within a membranous structure called an auto-phagosome. Auto-phagosomes usually combine with lysosomes to destroy their contents. Interestingly, it has been observed that IL1-β can be found between the two layers of auto-phagosomes, which explains why it is not destructed. [52].

2.5.4. IL1-β Signaling

The TLR/IL-1R superfamily is identified by the intracellular TIR (TLR/IL-1R) portion, to which IL1-β binds (Figure 4). IL1-β linked with the extracellular Ig associated proteins of IL-1R1 and IL-1R2, which are linked to the similar IL-1R protein (IL-1RAcP or IL-1R3).

After IL1-β binds to its receptors, signal transmission is indeed not that simple. The absence of a TIR portion in IL-1R2 makes it a decoy protein. IL-1RA might linked to IL-1R1 lacking an initiator trigger, and effectively replacing IL1-R1. [45, 53].

A signal can only be transmitted if IL-1/IL-1R1/IL-1RAcp is present. This expresses concern about the amount of IL1- produced, as well as the expression of IL-1Rs on concern cells and IL-1RA levels. When IL-1R/IL1-RAcP is activated, TIR part in the intracellular part of IL-1R and on the C-terminal portion of Myd88 help IL-1R/IL1-RAcP to recruit Myd88. As a result, MyD88 is linked to IRAK 4, IRAK 1, and/or IRAK 2. The phosphorylation of IRAK1 and IRAK2 by IRAK4 allows them to easily interact with TRAF6. TRAF6 stimulate the transforming growth factor-activated kinase 1 (TAK1).

TAK1 can trigger the IKK (inhibitor of NF-kB kinase) complex, which includes IKK-, IKK-, and IKK-, as well as p38 and JNK (c-Jun N-terminal kinase), resulting with induction of AP-1 the catalytic subunit. The IKK complex phosphorylates I-kB before being disrupted, allowing NF- kB to move from the cytoplasm to the nucleus and activate NF- kB [54].

2.6. Interleukin 1 beta gene polymorphism

The 5' and 3'–UTR site control the IL1-β gene, which is approximately 7.5 kb long and has seven exons. [55,56]. The IL1- gene has a lot of variations in its sequence, including over 144 SNPs [57]. In the IL1-β gene, there are multiple (SNPs). Two of them are found in the sense strand at -511 (rs16944) and -31. (rs1143627). Promoter region with the C allele is interrupted by the T/C alteration, which occurs 31 bp upstream the starting point. This alteration shown to be related with stomach sarcoma and inflammatory disorders. [58,59].

The presence of -511 (rs16944) and -31 (rs1143627) SNP in the IL1-β gene will affect its production. SNP -31 (rs1143627) has been shown to interrupt the promoter region upstream of the starting site, causing abnormal gene expression. [55]. Furthermore, within lipopolysaccharide-activated monocytes, the T allelomorph of this SNP linked to increased nuclear protein affinity than the -31C one. [60].

Previous examination on A549 LC cells revealed that the -31 T allelomorph demonstrated considerably higher transcriptional activation than the -31 C allele [61]. The impact of IL1-β on cancer formation or progression is the first consideration. This assessment can be carried out by evaluating gene polymorphisms that may influence IL1-β mRNA or protein expression, or by quantifying IL1-β mRNA or protein expression (Table 1).

Table 1. Effects of IL1-β polymorphisms on cancer [62].

Heading	Type of Cancer	Pro (+) or Anti (−) Tumor
High IL-1β IHC staining	Nasopharyngeal carcinoma	(−)
High IL-1β blood level	NSCLC	(+)
High IL1B mRNA	Breast cancer	(−)
High IL1B mRNA	Cervical cancer	(−)
High IL1B-signature	Gliomas	(+)
IL1B-511 C > T (rs16944) T allele	Ovarian cancer	(+) or (−)
	Lung cancer	(+) or (−)
	Gastric cancer	(+) or (−)
	Cervical cancer	(+)
	Acute myeloid leukemia	(+)
	Chronic myeloid leukemia	(+)
IL-1β-31 C > T (rs1143627) T allele	Breast cancer	(+)
	Lung cancer	(+)
	Cervical cancer	(+)
	Hepatocellular carcinoma	(+)
	Osteosarcoma	(+)

SNPs in IL1-β gene affect the level of IL1-β expression. For instant, the IL1-β -511 C > T (rs16944) and IL1-β -31 C > T (rs1143627) T allelomorphs, have been linked to elevate the level of IL1-β in people with cervix and gastric sarcoma [63-65] as well as in the supernatant of cells carrying rs1143627 [66]. While the affected people have a greater level of IL1-β in their broncho alveolar lavage than those with benign lung disease [67].

2.7. Chromosomal Localization

The family members of IL1-β, IL-1R and IL-1α gene clusters are shown, located in the human chromosome-2 [68].

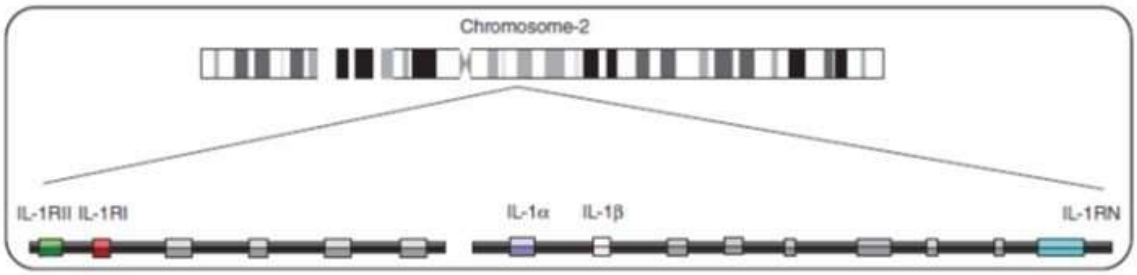

Figure 4: Gene cluster for IL-1 family

2.8. Symptoms and Diagnosis of NSCLC

NSCLC is usually neglected until it has progressed to an advanced stage [69]. Hemoptysis, chest distress, and dyspnea are the most typical signs, which affect 50% to 75% of patients. [70] Laboratory abnormalities or paraneoplastic syndromes are two more less common signs. Histologic confirmation is required for the diagnosis. The size of the tumor must also be detected during diagnosis in order to determine the disease in which phase. Which will eventually determine therapy choices. During the initial screening, the possibility of mutation detection and personalized treatment has influences for all suspected lung malignancies. [71].

2.9. Staging of NSCLC

Stage 0 disease is referred to as "in situ," which indicates the tumor is still in its location and has not extended from where it began. [72].

Stage I: The tumor has only blow-out to one lung and hasn't progressed or moved to any lymph nodes. Stage I tumors have a longest size of less than 4 cm [73].

Stage II: sarcoma has blow-out to adjacent lymph nodes, and not to other area of the body. Tumors in stage II can grow up to 5cm in length. They could have also expanded to nearby areas such as the chest wall, nerves connecting the heart and lung, or the lining of the heart and caused lung implosion or edema [72].

Stage III: The disease has expanded throughout the chest and not the rest of the body. These sarcomas are commonly referred to as progressive lung cancer. Stage III tumors can

grow up to 7 cm in diameter and have spread to lymph nodes. At stage III, numerous sarcoma might be located in the same lung. The diaphragm, heart, windpipe, or esophagus, among other tissues in the chest, may have been affected by stage III cancers. Stage III tumors, like stage II tumors, may have triggered lung failure or edema. [72].

Stage IV This is a progressive level of the disease and is also known as progress cancer. In stage IV, the tumor would migrate to the neighbouring organs including the liver or other parts. [72].

2.10. Treatment of NSCLC

2.10.1. Surgery

Surgical treatment is the frequent reliable option for people with LC. To be viable, the cancer must be fully reliable, and the person have to accept the suggested surgical technique.

Respectability refers to the consideration of patient variables and surgical techniques that reduce surgical risk prior to surgery, such as imaging assessments and biopsy, whereas operability refers to the consideration of patient variables and surgical techniques that reduce threat and death rates. The detection of disease stage is critical because the prognostic information helps to determine the best treatment course. [74].

2.10.2. Chemotherapy

Over 70% of people suffering from this disease have regionally, progress or migrate at the time of evaluation. Chemotherapy for pain control is most valuable [75].

2.10.3. Adjuvant

People who have had lung cancer, combination treatment may be valuable in resection surgery. to reduce their chances of relapse. This kind of therapy includes radiation, chemotherapy, and targeted therapy. Chemotherapy is normally given to people with stage IIA, IIB, or IIIA NSCLC after surgery to eliminate any cancerous tissue and enhance their livelihoods. [76].

2.10.4. Radiotherapy

In radiotherapy, high-energy beams are used to kill cancer cells by damaging their DNA. Tumors in specific areas of the body can be reduced or even eliminated with this treatment.

Radiation could be useful for patients with NSCLC that has spread. Radiotherapy could be a part of therapeutic options to enhance life satisfaction in people with NSCLC who have not benefited to surgery or chemotherapy [77].

3. MATERIAL AND METHOD

3.1. Sample Selection and definition

Patients with NSCLC (n=48) and healthy people as a control group (n=42) are included in this study. Patients diagnosed by the pulmonary medicine department made up the patient group, whereas healthy people made up the control group. The project also received ethical approval from the Yeditepe University Ethics Committee.

3.1.1. Patient Group

This study included all NSCLC patients from Yeditepe University Hospital, ranging from the ages from 42 to 83 years.

3.1.2. Control Group

The control group consists of persons without non-small-cell lung cancer. Ages between 45 to 86 year.

3.2. Materials and Devices Used in Experiment

3.2.1. Material Used in DNA Extraction

DNA was pull out from blood samples taken from the periphery. The blood samples were kept at -80°C in EDTA-containing tubes until they were used. Because it prevents coagulation, EDTA is used. A DNA Isolation Robot (iPrep pure link, Invitrogen, and Thermo Fisher Scientific Inc) equipment was also employed to isolate DNA. In addition, the DNA isolation mixture included 1.12 mg/ml Proteinase K, 8 M Guanidine hydrochloride, 10.5 nM EDTA, 10.5 nM NaCl, and 10.5 nM Tris-Cl at pH 8.8.

3.2.2. The Equipment used in Experiment

The following equipment was used in the experiment: DNA Isolation Robot (iPrep Purelink, Invitrogen, Thermo Fisher Scientific Inc), Nanodrop 2000 (Thermo Fisher Scientific Inc), 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 Fisher Scientific Inc).

3.3. Methods

3.3.1. Genomic DNA isolation from Blood

All blood samples from both the patient and the control group were obtained in 5 mL EDTA-containing tubes. All blood samples were kept in the refrigerator at +4°C until DNA isolation began. DNA was isolated using an iPrep DNA extraction robot (Invitrogen) and a genomic DNA isolation kit with iPrep. DNA can be extracted from 350L of peripheral blood with this technology, allowing 13 blood samples to be processed at once. Each sample is placed in its own cartridge, which is shaken for a few seconds to ensure that the magnetic beads attach efficiently to the DNA before the samples are placed in their cartridge. The iPrep robot uses ChargeSwitch® (CST®) technology to perform a calibrated extraction technique. This method allows for the extraction of a large amount of genomic DNA from the samples. Using paramagnetic particles, this method can extract large amounts of pure genomic DNA from materials. These particles are encased in a DNA-binding surface. When compared to other extraction procedures, such as silica-based DNA extraction, the ChargeSwitch® (CST®) extraction procedure is truly unique.

The charge of beads is affected by the pH of the enclosing particle buffer. The DNA backbone becomes negatively charged at low pH and bonds to positively charged beads. The DNA backbone becomes negatively charged at low pH and bonds to positively charged beads. Those charged beams were neutralized by using a low salt buffer with a higher pH for DNA elution. The DNA samples are ready for the experiment once the nucleic acids molecules have passed through the washing buffer. Finally, soluble deoxyribonucleic acid samples were collected, and kept at +4°C in the refrigerator.

3.3.2. DNA Purity Measurement

NanoDrop is a UV spectrophotometer that measures UV (ultraviolet) light absorption of nucleic acids at 260 nm. To determine DNA purity, the OD260/OD280 and OD260/OD230 ratios are calculated. NanoDrop can also detect DNA concentrations. Because.

Nanodrop has no cuvettes or capillaries, samples are evaluated directly away from external influences, yielding more realistic findings than conventional UV spectrometers. Nanodrop 2000 from Thermo Fisher Scientific was used in this experiment to detect the aggregation of DNA. Each individual, both NSCLC patients and the control group, had 0.5-1 l of DNA sample taken. As a control sample, distilled water was used. The sample placement stage was cleaned every 15-20 measures with distilled water. The Nanodrop 2000 was then cleaned with distilled water at the conclusion of all sample measurements.

3.3.3. Genotyping with Real Time PCR

In this investigation, genotyping analysis was carried out using a R-T PCR instrument, the 7500 Fast-RT- PCR (Applied Biosystems).

Single nucleotide polymorphisms (SNPs) were detected using fluorescence probe dyes. Fluorescent signal detection is plainly visible. Fluorescent dye probes with two different wavelengths unique to mutant and wild type alleles are utilized in the Real-Time PCR

procedure. To detect, two distinct TaqMan probes were used. FAM was one of them, and VIC was the other. FAM can identify wild type allele variants. VIC can also detect mutant allele variants. Each allele's fluorescent dye-linked probes bind to the amplified area.

The primer was created using the sequencing of the human IL 1- β. The SNP of IL 1- β (rs1143642) was resolute by the TaqMan Genotyping Assay and TaqMan Genotyping Master Mix on an Applied Biosystems 7500 Fast R-T PCR (Applied Biosystems, Foster City, CA, USA) (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA). The polymorphism-containing region was amplified utilizing the 5' - CCAGTTTCTCCCTCGCTGTTTTTAT[G/A]GCTTTCAAAAGCAGAAGTAGGAGGC 3' method.

Allele frequencies considered as G wild type and T mutant form based on Minor allele frequency (MAF) study. Probes are hydrolyzed by using the Taq polymerase enzyme.

3.3.4. Real Time PCR Protocol

The following ingredients were used: 5 microliters of Master Mix, 0.25 microliters of Taqman genotyping assay, 3.75 microliters of DNAse, RNAse-free water, and 1 microliter of template DNA. (Table 2) also shows the total volume of reagents used in Real-Time PCR and reaction mixtures. The combination was first produced with Master Mix, DNAse, RNAse-free water, and the TaqMan genotyping test, and then it was separated into each of the 96-well plates. Then, one by one, each template DNA sample was added to the mixture. The 96-well plate was then gently covered. 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 2. 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

Stages for real-time PCR were created. The denaturation procedure was then repeated for 15 seconds per cycle at 92 °C, followed by elongation to 1 minute for each cycle at 60 °C. Denaturation and annealing/extension processes were applied in 40 cycles, as illustrated in (Table 3)

Table 3.: 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

3.3.5. Statistical Analysis

The test was designed to obtain numerical values in this investigation. All data was entered into the SPSS 26.0 program after the PCR assays were completed and allelic discrimination data was received. Furthermore, the other clinical parameters had previously been gathered and recorded in the SPSS document.

(Tables 4) indicate clinical parameters. The data was then analysed using Fisher's Exact Testing and Chi-square tests in SPSS 26.0. The genotype distribution and alleles were statistically evaluated using Chi-square and Fisher's Exact Tests. P values ≤ to 0.05 are reflected important result, allowing the evaluation to identify and assess potential risk factors for NSCLC.

4. RESULTS

As a consequence, after testing the IL 1 BETA gene rs1146327 polymorphism in 90 patients with NSCLC and control group samples. Statistically, There were no important differences between the two groups, found in this work. The quantity of people with NSCLC (n=48) and the control groups (n=42) in particular.

Table 4. Demographic data and explanations

Parameter	NSCLC (n=48)	Control (n=42)	p-Value
Age (years), mean±SD	60.53±9.03	59.86±11.3	0.793
Gender (Female/Male)	7/41	14/28	0.047*
Age			
<60	n=23 (47.9%)	n=22 (52.4%)	0.297
≥60	n=25 (52.1%)	n=20 (47.6%)	
Smoking Status			
Active Smokers	n=21 (43.8%)	n=4 (9.5%)	<0.0001*
Non-smokers	n=27 (56.2%)	n= 38 (90.5%)	

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

(Table 5) shows that homozygote mutant genotype (GG) has a value of 0.091, heterozygote genotype has a value of 0.146, and homozygote wild type genotype has a value of 0.951. Our data show that the patients' homozygote wild type (GG), heterozygote (GA), and homozygote mutant type (AA) rates are 33.3 percent, 43.8 percent, and 22.9 percent, respectively.

However, the dominant (GG), heterozygote (GA), and recessive form (AA) percentages in the comparative group are 16.7%, 59.5 percent, and 23.8 percent, respectively. According to our findings, the ratio of homozygote mutant type (GG) was larger in controls than in patients, as shown in (Table 5), although with a p-value of 0.091, it is not a significant value.

The homozygote wild type (AA) ratio was also higher in healthy people than in illness, with a p-value of 0.951, which was not important.

With a p-value of 0.146, the ratio of heterozygote genotype (GA) was more in the patient group than in the healthy one.

Further reveals that the patient group's value of G allele (53%) is greater than the comparison group's value of (43%). As a result, unlike the G allele (p=0.999), the (A) allele value is not statistically significant among groups (Table 5).

The natural allele (A) was found in 45 percent of the controls, whereas the mutant allele (G) was found in 39%. The wild allele (A) was found in 43% of the patients, whereas the mutant allele (G) was found in 53%.

When compared to heterozygous (GA) and homozygous (AA) genotypes, homozygous (GG) genotypes showed weaker protective behavior (OR=2.5) in patients with NSCLC. In other words, having the G allele confers no benefit to patients with NSCLC. Furthermore, statistical analysis findings reveal that heterozygous type (GA) (OR: 0.529; p: (0.146) does not increase the risk of NSCLC, as shown in the Table below. Furthermore, having the (G) allele increases the risk. Even having the (G) allele in the heterozygous form (GA) is a risk factor.

Table 5. Comparison of IL1B genotype between NSCLC patients and healthy individuals

IL1-Beta Genotypes	Control (n=42)	NSCLC (n=48)	*P-Value*	Odd Ratio (OR)	95% Confidence Interval
			χ^2=3.533; p=0.171		
GG Genotype	16.7% (n=7)	33.3% (n=16)	0.091	2.500	0.911- 6.859
GA Genotype	59.5% (n=25)	43.8% (n=21)	0.146	0.529	0.228-1.225
AA Genotype	23.8% (n=10)	22.9% (n=11)	0.951	0.951	0.358-2.531
Allelic Distributions					
G Allele	39	53	0.999	1.051	0.395-2.796
A Allele	45	43	0.091	0.400	0.146-1.097

*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 important differences*

4.1. Statistical Assessment of Real-Time PCR Findings

In both the patient and control groups, allele types were analyzed using the 7500 Fast-Real Time PCR apparatus. Below is a representation of the allelic discrimination plot obtained from this gadget.

The allelic discrimination was found automatically using this tool's software. The contribution of dyes contained in the probes was used in the analysis and readings of fluorescence irradiation. Despite this, several samples were unable to be differentiated and interpreted. Two different dyes, corresponding to FAM color blue and VIC color green, were employed in this experiment.

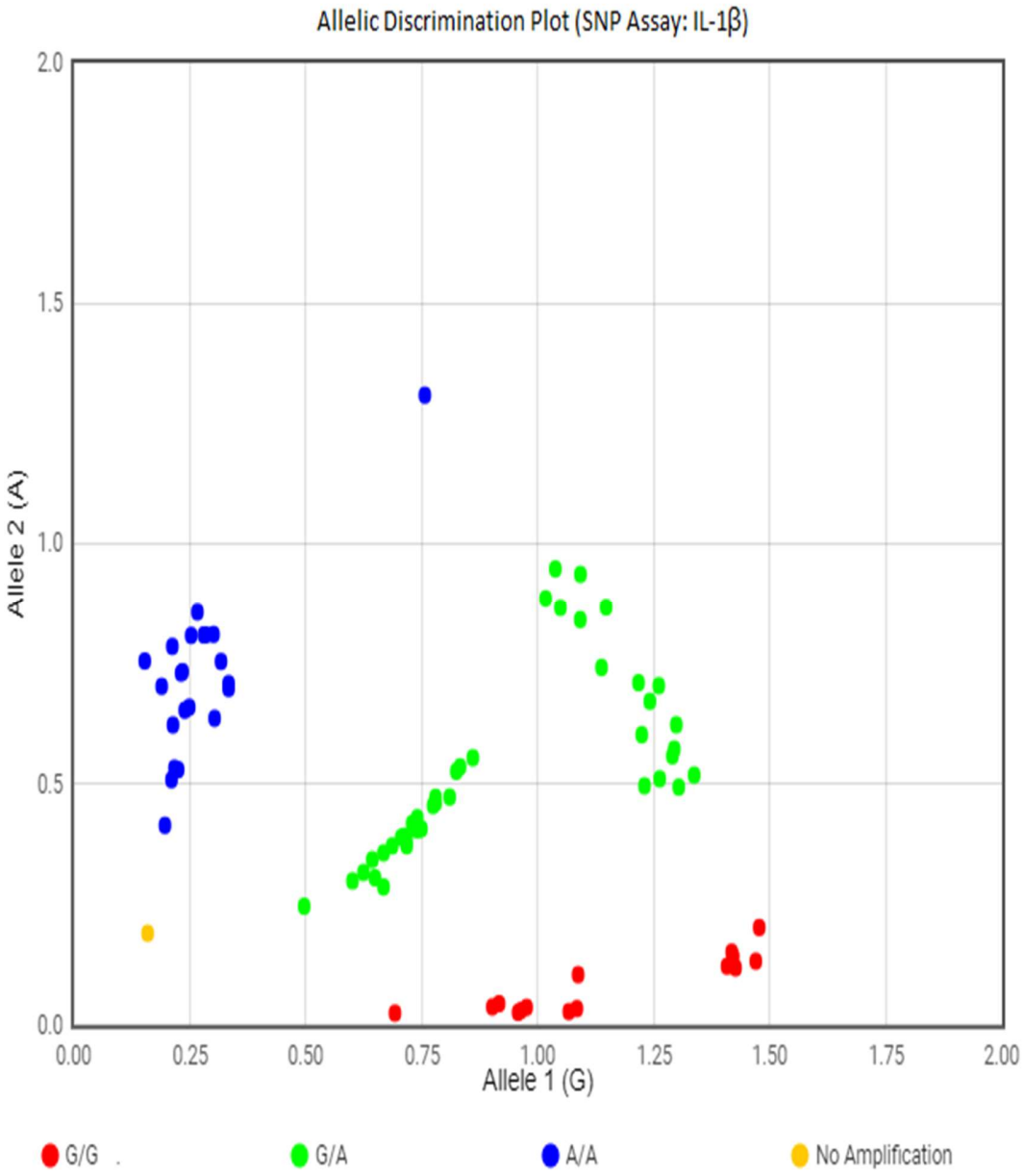

Figure 5: Findings on Allele Discrimination GG stands for Homozygote Wild Type. GA: Heterozygote Mutant Type AA: Homozygote Mutant Type

The figure below represented above shows amplification plot of Allele A. The yellow line is corresponding to Threshold value (0.0248)

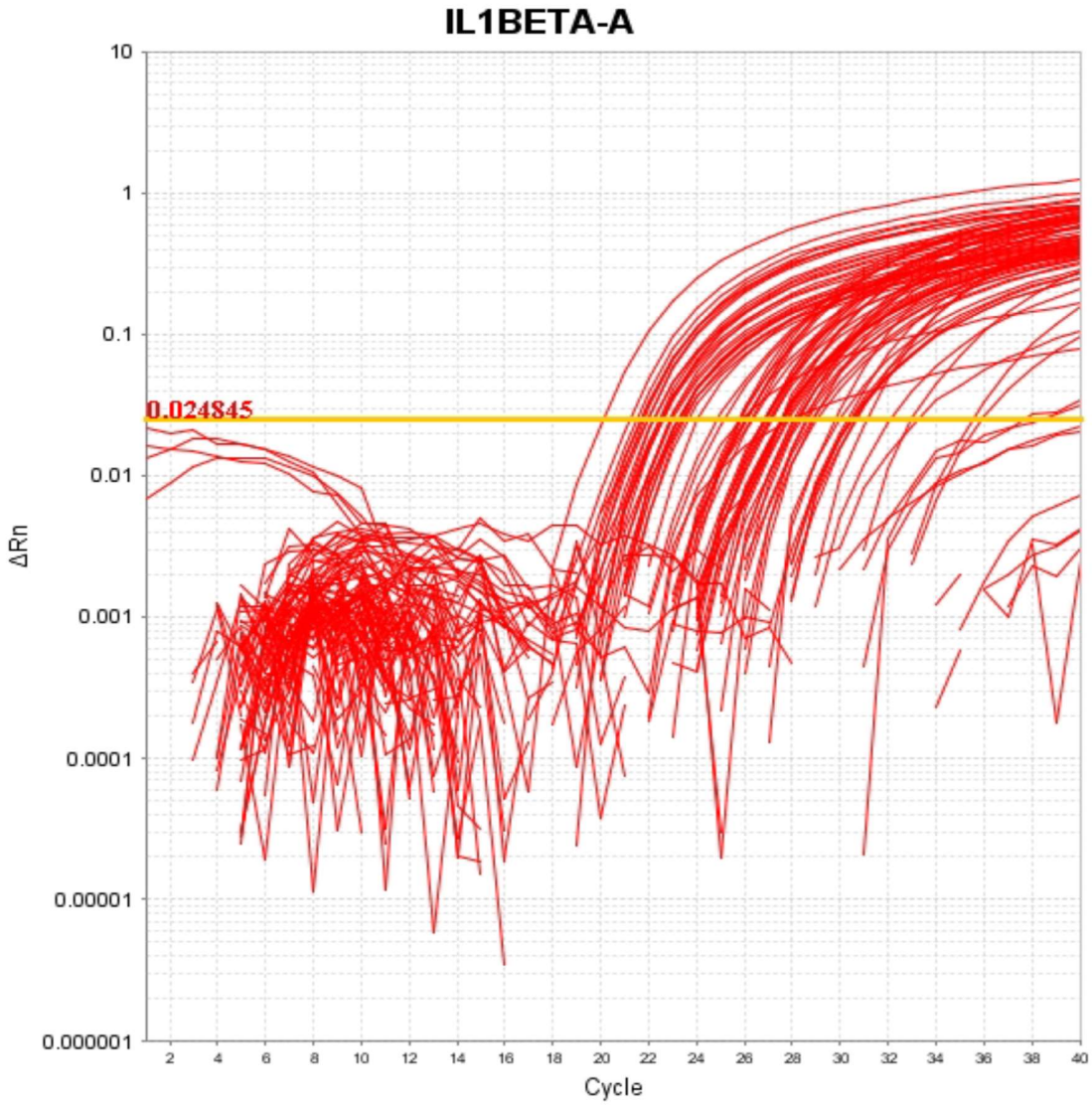

Figure 6: Amplification plot display of Allele A

The figure represented above shows amplification plot of Allele G. The yellow line is corresponding to Threshold value (0.0225).

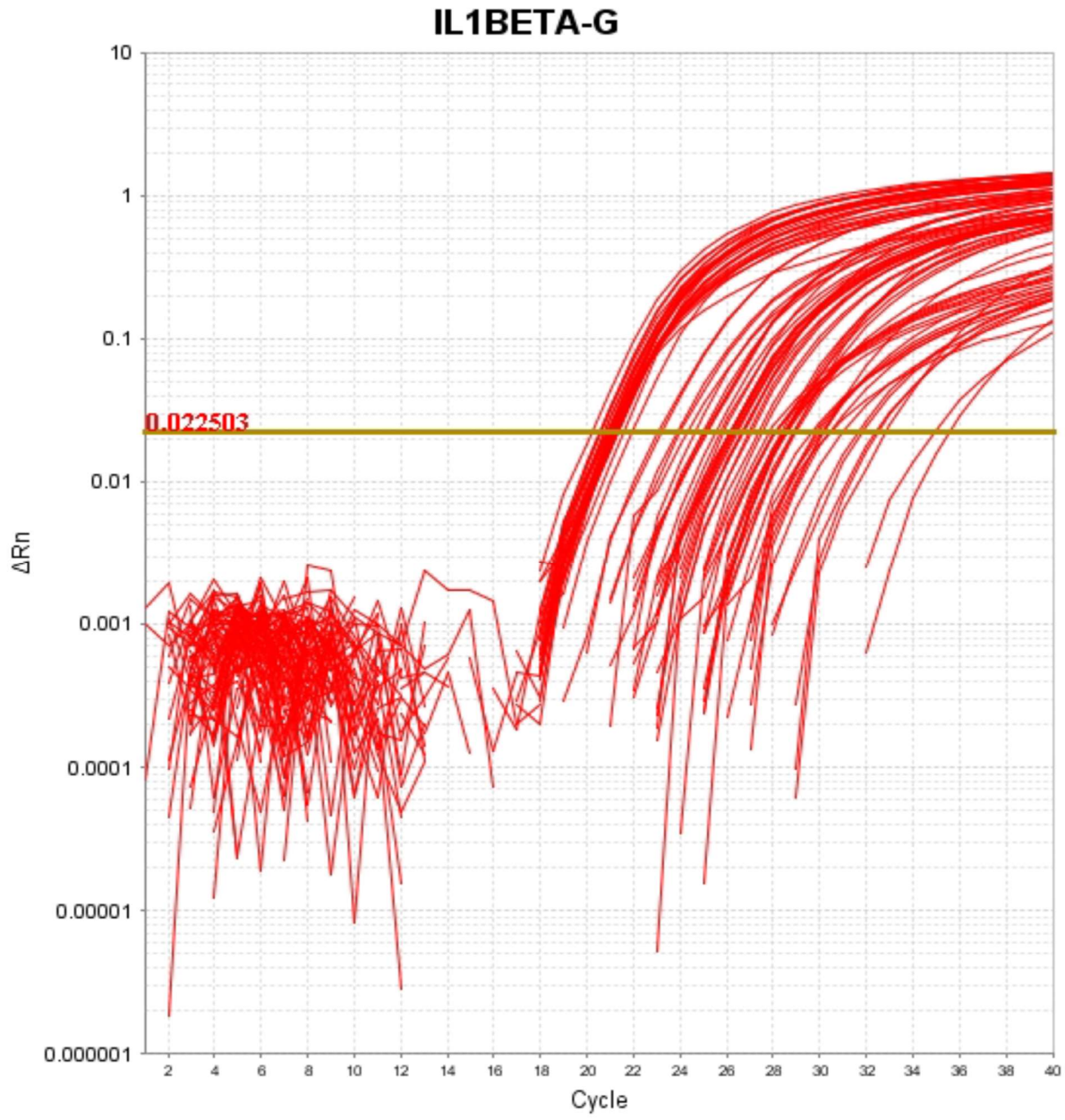

Figure 7: Amplification plot display of Allele G

5. DISCUSSIONS AND CONCLUSIONS

In adults, chronic inflammation commonly induces the development of lung cancer., according to epidemiological research [78].

Many prior investigations have found cytokines to be significantly linked to cancer pathogenesis, and there is growing indication that they contribute to tumor initiation, development, and metastasis [79].

Numerous investigations on cytokine gene polymorphisms have been carried out to study their links to a variety of inflammatory and cancer illnesses [80]. IL1-β expression was shown to be raised in a variety of malignancies, and individuals with elevated expression had a poor prognosis [81]. Recent research has found that based on allele frequency, many polymorphisms in the IL1-β promoter region regulate the transcription of IL1-β gene. [82,83].

Zienolddiny's group conducted a meta-analysis that revealed how the process of IL1-β gene controlling is not that simple. Recognizing the role of individual SNPs, however, is likely needed to comprehend their interaction. As per Zienolddiny's findings, IL1-β rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511), and rs1143627C/T (-31) comprised a danger allele frequency (GGCT) which is linked with NSCLC in European societies. [84].

The C allele rs1143627C/T (-31) disrupts the promoter region, which is thought to influence IL1-β regulation. In A549 cells, the T- allelomorph of the sense seqment, rs1143627 (-31) displayed increased transcription (p 0.001) than the C-allele [85].

Ecological causes that influence a genetically susceptible person are largely acknowledged as necessary for lung cancer development. Because NSCLC is known to be caused by smoking. Furthermore, such SNPs in inflaming DNA sequence, for example, may influence lung inflammation. Researchers may be able to better understand the interpersonal differences in lung cancer risk due to the presence of these SNPs.

The polymorphism of a gene can affect gene products, mRNA consistency, or the amount and behavior of the resulting protein. As a result, inspection and risk assessment are critical for cancer identification and control. As a result, we looked into whether the IL1-β rs1146327 polymorphism SNP and smoking had any interaction.

In conclusion, the IL1-β rsl146327 polymorphism in people with NSCLC and healthy controls found no high variations between the two groups. In our investigation, we identified no statistically significant differences in IL1-β gene polymorphism genotype between patients and controls based on alleles.

We urge that these investigations be expanded by increasing the sample size of the study groups to advance more precise identification of NSCLC, given the influence of the IL1-β gene polymorphism differ from person to person.

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7. APPENDIX

7.1. Raw data

Crosstabs

Notes

Comments		
Input	Data	C:\Users\busra\Desktop\Genotyping NSCLC\NSCLC-Luay-Raw Data.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	203
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 Genotype_IL1Beta_rs1143627 rs1143627_GG rs1143627_GA rs1143627_AA rs1143627_G rs1143627_A Aktif_Smoker Sigara_Hiç_İçmemiş /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Grup * Genotype_IL1Beta_rs1143627	90	44,3%	113	55,7%	203	100,0%
Grup * rs1143627_GG	90	44,3%	113	55,7%	203	100,0%
Grup * rs1143627_GA	90	44,3%	113	55,7%	203	100,0%
Grup * rs1143627_AA	90	44,3%	113	55,7%	203	100,0%
Grup * rs1143627_G	90	44,3%	113	55,7%	203	100,0%
Grup * rs1143627_A	90	44,3%	113	55,7%	203	100,0%
Grup * Aktif_Smoker	163	80,3%	40	19,7%	203	100,0%
Grup * Sigara_Hiç_İçmemiş	96	47,3%	107	52,7%	203	100,0%

Grup * Genotype_IL1Beta_rs1143627

Crosstab

			Genotype_IL1Beta_rs1143627			
			GG	GA	AA	Total
Grup	Kontrol	Count	7	25	10	42
		% within Grup	16,7%	59,5%	23,8%	100,0%
		% within Genotype_IL1Beta_rs1143627	30,4%	54,3%	47,6%	46,7%
		% of Total	7,8%	27,8%	11,1%	46,7%
	Hasta	Count	16	21	11	48
		% within Grup	33,3%	43,8%	22,9%	100,0%
		% within Genotype_IL1Beta_rs1143627	69,6%	45,7%	52,4%	53,3%
		% of Total	17,8%	23,3%	12,2%	53,3%
Total		Count	23	46	21	90
		% within Grup	25,6%	51,1%	23,3%	100,0%
		% within Genotype_IL1Beta_rs1143627	100,0%	100,0%	100,0%	100,0%

% of Total	25,6%	51,1%	23,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	3,533[a]	2	,171
Likelihood Ratio	3,613	2	,164
Linear-by-Linear Association	1,398	1	,237
N of Valid Cases	90		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,80.

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 * rs1143627_GG

Crosstab

			rs1143627_GG		
			GA+AA	GG	Total
Grup	Kontrol	Count	35	7	42
		% within Grup	83,3%	16,7%	100,0%
		% within rs1143627_GG	52,2%	30,4%	46,7%
		% of Total	38,9%	7,8%	46,7%
	Hasta	Count	32	16	48
		% within Grup	66,7%	33,3%	100,0%
		% within rs1143627_GG	47,8%	69,6%	53,3%
		% of Total	35,6%	17,8%	53,3%
Total		Count	67	23	90
		% within Grup	74,4%	25,6%	100,0%
		% within rs1143627_GG	100,0%	100,0%	100,0%
		% of Total	74,4%	25,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,271[a]	1	,071		
Continuity Correction[b]	2,453	1	,117		
Likelihood Ratio	3,352	1	,067		
Fisher's Exact Test				,091	,058
Linear-by-Linear Association	3,234	1	,072		
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,73.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	2,500	,911	6,859
For cohort rs1143627_GG = GA+AA	1,250	,982	1,591
For cohort rs1143627_GG = GG	,500	,228	1,097
N of Valid Cases	90		

Grup * rs1143627_GA

Crosstab

			rs1143627_GA		
			GG+AA	GA	Total
Grup	Kontrol	Count	17	25	42
		% within Grup	40,5%	59,5%	100,0%
		% within rs1143627_GA	38,6%	54,3%	46,7%
		% of Total	18,9%	27,8%	46,7%
	Hasta	Count	27	21	48
		% within Grup	56,3%	43,8%	100,0%
		% within rs1143627_GA	61,4%	45,7%	53,3%
		% of Total	30,0%	23,3%	53,3%
Total		Count	44	46	90
		% within Grup	48,9%	51,1%	100,0%
		% within rs1143627_GA	100,0%	100,0%	100,0%
		% of Total	48,9%	51,1%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,230[a]	1	,135		
Continuity Correction[b]	1,644	1	,200		
Likelihood Ratio	2,241	1	,134		
Fisher's Exact Test				,146	,100
Linear-by-Linear Association	2,206	1	,138		
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 20,53.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,529	,228	1,225
For cohort rs1143627_GA = GG+AA	,720	,462	1,121
For cohort rs1143627_GA = GA	1,361	,906	2,043
N of Valid Cases	90		

Grup * rs1143627_AA

Crosstab

			rs1143627_AA		
			GA+GG	AA	Total
Grup	Kontrol	Count	32	10	42
		% within Grup	76,2%	23,8%	100,0%
		% within rs1143627_AA	46,4%	47,6%	46,7%
		% of Total	35,6%	11,1%	46,7%
	Hasta	Count	37	11	48
		% within Grup	77,1%	22,9%	100,0%
		% within rs1143627_AA	53,6%	52,4%	53,3%
		% of Total	41,1%	12,2%	53,3%
Total		Count	69	21	90
		% within Grup	76,7%	23,3%	100,0%
		% within rs1143627_AA	100,0%	100,0%	100,0%
		% of Total	76,7%	23,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,010[a]	1	,920		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,010	1	,920		
Fisher's Exact Test				1,000	,558
Linear-by-Linear Association	,010	1	,921		
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,80.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,951	,358	2,531
For cohort rs1143627_AA = GA+GG	,988	,786	1,243
For cohort rs1143627_AA = AA	1,039	,491	2,199
N of Valid Cases	90		

Grup * rs1143627_G

Crosstab

			rs1143627_G		
			AA	GG+GA	Total
Grup	Kontrol	Count	10	32	42
		% within Grup	23,8%	76,2%	100,0%
		% within rs1143627_G	47,6%	46,4%	46,7%
		% of Total	11,1%	35,6%	46,7%
	Hasta	Count	11	37	48
		% within Grup	22,9%	77,1%	100,0%
		% within rs1143627_G	52,4%	53,6%	53,3%
		% of Total	12,2%	41,1%	53,3%
Total		Count	21	69	90
		% within Grup	23,3%	76,7%	100,0%
		% within rs1143627_G	100,0%	100,0%	100,0%
		% of Total	23,3%	76,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,010[a]	1	,920		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,010	1	,920		
Fisher's Exact Test				1,000	,558
Linear-by-Linear Association	,010	1	,921		
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,80.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	1,051	,395	2,796
For cohort rs1143627_G = AA	1,039	,491	2,199
For cohort rs1143627_G = GG+GA	,988	,786	1,243
N of Valid Cases	90		

Grup * rs1143627_A

Crosstab

			rs1143627_A		
			GG	AA+GA	Total
Grup	Kontrol	Count	7	35	42
		% within Grup	16,7%	83,3%	100,0%
		% within rs1143627_A	30,4%	52,2%	46,7%
		% of Total	7,8%	38,9%	46,7%
	Hasta	Count	16	32	48
		% within Grup	33,3%	66,7%	100,0%
		% within rs1143627_A	69,6%	47,8%	53,3%
		% of Total	17,8%	35,6%	53,3%
Total		Count	23	67	90
		% within Grup	25,6%	74,4%	100,0%
		% within rs1143627_A	100,0%	100,0%	100,0%
		% of Total	25,6%	74,4%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,271[a]	1	,071		
Continuity Correction[b]	2,453	1	,117		
Likelihood Ratio	3,352	1	,067		
Fisher's Exact Test				,091	,058
Linear-by-Linear Association	3,234	1	,072		
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,73.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,400	,146	1,097
For cohort rs1143627_A = GG	,500	,228	1,097
For cohort rs1143627_A = AA+GA	1,250	,982	1,591
N of Valid Cases	90		

Grup * Aktif_Smoker

Crosstab

			Aktif_Smoker		
			Yok	Var	Total
Grup	Kontrol	Count	35	32	67
		% within Grup	52,2%	47,8%	100,0%
		% within Aktif_Smoker	30,7%	65,3%	41,1%
		% of Total	21,5%	19,6%	41,1%
	Hasta	Count	79	17	96
		% within Grup	82,3%	17,7%	100,0%
		% within Aktif_Smoker	69,3%	34,7%	58,9%
		% of Total	48,5%	10,4%	58,9%
Total		Count	114	49	163
		% within Grup	69,9%	30,1%	100,0%
		% within Aktif_Smoker	100,0%	100,0%	100,0%
		% of Total	69,9%	30,1%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	16,951[a]	1	,000		
Continuity Correction[b]	15,552	1	,000		
Likelihood Ratio	16,911	1	,000		
Fisher's Exact Test				,000	,000
Linear-by-Linear Association	16,847	1	,000		
N of Valid Cases	163				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 20,14.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	,235	,116	,479
For cohort Aktif_Smoker = Yok	,635	,496	,813
For cohort Aktif_Smoker = Var	2,697	1,638	4,441
N of Valid Cases	163		

Grup * Sigara_Hiç_İçmemiş

Crosstab

			Sigara_Hiç_İçmemiş		
			Yok	Var	Total
Grup	Hasta	Count	95	1	96
		% within Grup	99,0%	1,0%	100,0%
		% within Sigara_Hiç_İçmemiş	100,0%	100,0%	100,0%
		% of Total	99,0%	1,0%	100,0%
	Total	Count	95	1	96
		% within Grup	99,0%	1,0%	100,0%
		% within Sigara_Hiç_İçmemiş	100,0%	100,0%	100,0%
		% of Total	99,0%	1,0%	100,0%

Chi-Square Tests

	Value
Pearson Chi-Square	.[a]
N of Valid Cases	96

a. No statistics are computed because Grup is a constant.

Risk Estimate

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

a. No statistics are computed because Grup is a constant.

T-Test

Notes

Output Created		07-JUN-2022 11:52:59
Comments		
Input	Data	C:\Users\busra\Desktop\Genotyping NSCLC\NSCLC-Luay-Raw Data.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	203
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Grup(0 1) /MISSING=ANALYSIS /VARIABLES=Yaş Sigara_Hiç_İçmemiş Aktif_Smoker /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,02

Group Statistics

	Grup	N	Mean	Std. Deviation	Std. Error Mean
Yaş	Kontrol	59	61,71	10,709	1,394
	Hasta	95	60,82	8,310	,853
Sigara_Hiç_İçmemiş	Kontrol	0[a]	.	.	.
	Hasta	96	,01	,102	,010
Aktif_Smoker	Kontrol	67	,48	,503	,061
	Hasta	96	,18	,384	,039

a. t cannot be computed because at least one of the groups is empty.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Yaş	Equal variances assumed	7,070	,009	,578	152	,564	,891	1,541	-2,155	3,936
	Equal variances not assumed			,545	100,795	,587	,891	1,634	-2,351	4,133
Aktif_Smoker	Equal variances assumed	46,632	,000	4,323	161	,000	,301	,070	,163	,438
	Equal variances not assumed			4,123	117,041	,000	,301	,073	,156	,445

Grup * Cinsiyet

Crosstab

			Cinsiyet		
			Kadın	Erkek	Total
Grup	Kontrol	Count	14	28	42
		% within Grup	33,3%	66,7%	100,0%
		% within Cinsiyet	66,7%	40,6%	46,7%
		% of Total	15,6%	31,1%	46,7%
	Hasta	Count	7	41	48
		% within Grup	14,6%	85,4%	100,0%
		% within Cinsiyet	33,3%	59,4%	53,3%
		% of Total	7,8%	45,6%	53,3%
Total		Count	21	69	90
		% within Grup	23,3%	76,7%	100,0%
		% within Cinsiyet	100,0%	100,0%	100,0%
		% of Total	23,3%	76,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4,402[a]	1	,036		
Continuity Correction[b]	3,416	1	,065		
Likelihood Ratio	4,442	1	,035		
Fisher's Exact Test				,047	,032
N of Valid Cases	90				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,80.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Grup (Kontrol / Hasta)	2,929	1,049	8,176
For cohort Cinsiyet = Kadın	2,286	1,020	5,124
For cohort Cinsiyet = Erkek	,780	,612	,996
N of Valid Cases	90		

Grup * YAŞARALIĞI

Crosstab

			YAŞARALIĞI		
			<60	>EŞİT60	Total
Grup	Kontrol	Count	13	8	21
		% within Grup	61,9%	38,1%	100,0%
		% within YAŞARALIĞI	38,2%	25,0%	31,8%
		% of Total	19,7%	12,1%	31,8%
	Hasta	Count	21	24	45
		% within Grup	46,7%	53,3%	100,0%
		% within YAŞARALIĞI	61,8%	75,0%	68,2%
		% of Total	31,8%	36,4%	68,2%
Total		Count	34	32	66
		% within Grup	51,5%	48,5%	100,0%
		% within YAŞARALIĞI	100,0%	100,0%	100,0%
		% of Total	51,5%	48,5%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,331[a]	1	,249		
Continuity Correction[b]	,791	1	,374		
Likelihood Ratio	1,342	1	,247		
Fisher's Exact Test				,297	,187
Linear-by-Linear Association	1,311	1	,252		
N of Valid Cases	66				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,18.

b. Computed only for a 2x2 table

7.2. Plate Layout

7.3. Ethical committee approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2253
Konu: Klinik Araştırmalar Etik Kurul Başvurusu hk.

25/11/2021

İlgili Makama (Luay Shehab)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, **"Küçük Hücreli Dışı Akciğer Kanserinle IL 1 – Beta Geni (rs1143627) Polymorfizminin Rolü"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2313)** 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: 1533)**

Prof. Dr. Turgay ÇELİK

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

7.4. Curriculum Vitae

Personal Informations

Name	Luay	**Surname**	Shehab

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate			
Master			
University			
High school	Medıcal scıence -	**Phıladelphıa unıversıty**	2007

Languages	Grades (#))
Englısh	6.5 ıelts

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

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
Field scientific officer	Mıylteny bıotech	2010-2020
		-

Computer Skills

Program	Level
word	Excellent
Excel-power poınt	Excellent

***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)

Flow cytometer trainıng –Germany 2013
Basic Cellular trainıng – Germany 2013