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YEDITEPE UNIVERSITY
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DEPARTMENT OF MOLECULAR MEDICINE

DETERMINATION of IDH 1, IDH2, MGMT, TERT, ATRX and H3F3A GENE MUTATIONS in GLIAL TUMORS

PHD THESIS

CUMHUR KAAN YALTIRIK, M.D.

İSTANBUL, 2021

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THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Molecular Medicine

Title of the Thesis : Determination of IDH 1, IDH2, MGMT, TERT, ATRX and H3F3A Gene Mutations in Glial Tumors

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Examination Date : 22.10.2021

This study have approved as a Master/Doctorate Thesis in regard to content and quality by the Jury.

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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 04.11.2021 and numbered 2021/11-01

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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.

17/11/2021

Cumhur Kaan Yaltırık

DEDICATION

I dedicate my thesis to my wife Nazlı,
my daughter Defne Naz and to my lovely Family.

ACKNOWLEDGEMENTS

Firstly, I want to deliver my regards and gratitude to Prof.Dr. Turgay İSBİR for helping me on my way to becoming a PhD, by training me how to do study and leaving his personal imprint on my life. Professor Dr. Turgay İSBİR is a motivation for me to become an educator and scientist.

I would like to acknowledge the support that Prof. Dr. M. Gazi YAŞARGİL had provided me and to thank him for helping me to pursue my studies in molecular dynamics of malignant glial tumors and to apply for the PhD program in Molecular Medicine.

I am very thankful to Prof.Dr. Uğur TÜRE and Prof.Dr. Başar ATALAY for being kind to help me meet Prof.Dr. Turgay İSBİR and my application for the PhD course.

Special thanks to my dear friend and colleague Associated Professor Selçuk ÖZDOĞAN for checking and checking my work several times during this process. Also another special thanks to my friend Associated Professor Seda Güleç Yılmaz for the support in my Phd program.

I am so happy to have met you all and I will always be thankful for your kindness.

Cumhur Kaan Yaltırık

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LIST OF SYMBOLS AND ABBREVIATIONS

Akg	Alpha Ketoglutarate
ATRX	X-linked helicase II
BRAF	B-Raf Proto-Oncogene
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2
CNS	Central Nervous System
CST	Charge Switch Technology
DNA	Deoxyribonucleic Acid
EGFR	Epidermal Growth Factor Receptor
GBM	Glioblastoma
HIF	Hypoxia Inducing Factor
IDH	Isocitrate Dehydrogenase
LOH	Loss Of Heterozygozygozity
MDM2	Murine Double Minute 2
MGMT	O6-Methylguanine-DNA Methyltransferase
NGS	Next-Generation Sequencing
OS	Overall Survival
PCR	Polymerase Chain Reaction
PDGFRA	Platelet-Derived Growth Factor Receptor A
PHD	Proline Hydroxylase
PTEN	Phosphatase and tensin homolog
RB1	Retinoblastoma 1
TERT	Telomerase Reverse Transcriptase
TP53	Tumor Suppressor Protein 53
WHO	World Health Organisation

ABSTRACT

Yaltırık, C.K. (2021). Determination of IDH 1, IDH2, MGMT, TERT, ATRX and H3F3A Gene Mutations in Glial Tumors. Yeditepe University, Institute of Health Science, Department of Molecular Medicine, PhD thesis, İstanbul.

Glial tumors make up almost half of all malignant brain tumors. The most popular and dangerous kind of primary brain tumor is Glioblastoma Multiforme(GBM). The survival rates of GBM are very short and it could be predictable with molecular markers. The aim of our study is to evaluate IDH1, IDH2, TERT, MGMT and ATRX genes with next-generation sequencing to find potential pathological mutations and their effect on survival. Thirty patients who had a craniotomy and diagnosed with high-grade glioma were evaluated for this study. Peripheral blood samples were obtained from all participants. IDH1, IDH2, TERT, MGMT and ATRX genes were evaluated with next-generation sequencing from the samples. To evaluate the effects of all these mutations on survival we made survival analysis by SPSS. The median age of the patients was 58.5 (11-74), and 56.7% (n=17) were under 60 years of age. According to gender, the rate of male patients was 66.7% and the rate of female patients was 33.3%. Eighty percent of the patients had IDH, 83.3% MGMT, and 70% had EGFR positivity. After the targeted gene sequencing performed on the patients, 21 mutations were detected. Three of these mutations were found in IDH 1, 2 in IDH2, 6 in TERT, 7 in MGMT, and 3 in ATRX. When female gender status was taken as reference, the overall survival of female gender status was 4.63 times, and when more than three chromosome anomaly was accepted as a reference, it was determined from the available data that anomaly in three or less chromosomes negatively affected overall survival 2.83 times. Targeted NGS enables glial tumor categorization that is both accurate and therapeutically meaningful, providing better prognostic information than conventional histology. There is evidence to suggest that these cancers should be reclassified using molecular markers, although how this should be done in clinical practice is still up for debate. However, it's obvious that incorporating molecular diagnostics into our standard-of-care routine will help us better understand our patients' outcomes.

Key Words: Next Generation Sequencing, Glioblastoma, Molecular Biology, IDH1/2, MGMT, TERT, ATRX

ÖZET

Yaltırık, C.K. (2021). Glial Tümörlerde IDH 1, IDH2, MGMT, TERT, ATRX ve H3F3A Gen Mutasyonlarının Belirlenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD., Master Tezi. İstanbul.

Glial tümörler tüm malign beyin tümörlerinin neredeyse yarısını oluşturur En popüler ve tehlikeli primer beyin tümörü türü Glioblastoma Multiforme'dir (GBM). Gbm'nin hayatta kalma oranları çok kısadır ve moleküler belirteçlerle tahmin edilebilir. Çalışmamızın amacı, potansiyel patolojik mutasyonları ve sağkalım üzerindeki etkilerini bulmak için IDH1, IDH2, TERT, MGMT ve ATRX genlerini yeni nesil dizileme ile değerlendirmektir. Bu çalışma için kraniyotomi yapılan ve yüksek dereceli glioma tanısı alan otuz hasta değerlendirildi. Tüm katılımcılardan periferik kan örnekleri alındı. Örneklerden yeni nesil dizileme ile IDH1, IDH2, TERT, MGMT ve ATRX genleri değerlendirildi. Tüm bu mutasyonların sağkalım üzerindeki etkilerini değerlendirmek için SPSS ile sağkalım analizi yapıldı. Hastaların ortanca yaşı 58.5 (11-74), %56.7'si (n=17) 60 yaşın altındaydı. Cinsiyete göre erkek hasta oranı %66.7, kadın hasta oranı %33.3 idi. Hastaların yüzde sekseninde IDH, % 83.3 MGMT ve% 70'inde EGFR pozitifliği vardı. Hastalar üzerinde hedeflenen gen dizilimi yapıldıktan sonra 21 mutasyon tespit edildi. Bu mutasyonların üçü IDH 1'de, 2'si IDH2'de, 6'sı TERT'de, 7'si MGMT'de ve 3'ü ATRX'te bulundu. Kadın cinsiyet durumu referans olarak alındığında, kadın cinsiyet durumunun genel sağkalımı 4.63 kat, üçten fazla kromozom anomalisi referans olarak kabul edildiğinde, mevcut verilerden üç veya daha az kromozomdaki anomalinin genel sağkalımı 2.83 kat olumsuz etkilediği belirlenmiştir. Hedeflenen NGS, hem doğru hem de terapötik olarak anlamlı olan glial tümör kategorizasyonunu mümkün kılarak konvansiyonel histolojiye göre daha iyi prognostik bilgi sağlar. Klinik uygulamada bunun nasıl yapılması gerektiği hala tartışmaya açık olmasına rağmen, bu kanserlerin moleküler belirteçler kullanılarak yeniden sınıflandırılması gerektiğini gösteren kanıtlar vardır. Bununla birlikte, moleküler teşhisin standart bakım rutinimize dahil edilmesinin hastalarımızın sonuçlarını daha iyi anlamamıza yardımcı olacağı açıktır.

Anahtar Kelimeler: Next Generation Sequencing, Glioblastoma, Molecular Biology, IDH1/2, MGMT, TERT, ATRX

1. STATEMENT AND PURPOSE

Central nervous system tumors account for 2.3 percent of cancer deaths and 1.9 percent of newly diagnosed cancer cases, and they affect three out of every ten thousand individuals every year. The majority of these lesions are high-grade astrocytomas or glioblastomas becoming the most dangerous diffuse glioma of the astrocytic lineage The most popular and dangerous kind of primary brain tumor is GBM. Gliblastoma is divided into two subtypes: primary and secondary. People are afflicted at various ages and by various hereditary mechanisms. Primary and secondary GBM tissues have clinical and cytogenetic differences. There is growing interest in using DNA genome-wide methylation-based categorization of central nervous system cancers, which has been shown to have diagnostic sensitivity and clinical utility in a recent series. IDH1, IDH2, BRAF, ATRX, p53, TERT including promoter area, H3F3A, EGFR amplification or mutation, CDKN2A loss, and copy number data on chromosomes 1, 7, 10, and 19 are some of the key markers that may help characterize a glioma's genetic profile. To evaluate all important genes, a comprehensive characterisation may now involve several tests (e.g., FISH, IHC, methylation-specific PCR, NGS, etc.). Each of the more wide molecular diagnostic panels has the benefit of evaluating several molecular features, resulting in more detailed diagnoses. The World Health Organization classification of CNS tumors has been revised immensely as molecular criteria are integrated in 2016 WHO CNS classification. Especially low grade and high-grade variations have been affected greatly by the modified classification. Glioblastomas may present abnormal expression of various growth-control genes and their proteins Genes such as IDH 1, IDH2, p53, MGMT, TERT, ATRX are crucial in diagonsing the tumor and the assessment of possible response to treatment methods such as radiotherapy and chemotherapy.

The aim of our study, was to evaluate 5 genes in the patients with GBM. We evaluate IDH1, IDH2, MGMT, TERT and ATRX by next generation sequencing.

2. LITERATURE REVIEW

2.1. Overview of the Brain Tumors

Central nervous system tumors account for 2.3 percent of cancer deaths and 1.9 percent of newly diagnosed cancer cases, and they affect three out of every ten thousand individuals every year (1). Glial tumors make up almost half of all malignant brain tumors and are the most prevalent form of primary CNS cancer. Human gliomas are classified into four groups (I–IV) according to the World Health Organisation (WHO). The majority of these lesions are high-grade astrocytomas or glioblastomas (GBM) becoming the most dangerous diffuse glioma of the astrocytic lineage (2). The most popular and dangerous kind of primary brain tumor is GBM. Gliomas account for 54% of all gliomas and 16% of adult brain tumors (3).

On a molecular level, glioblastomas are classified into two subgroups: primary and secondary. The differentiation between primary and secondary glioblastoma was first rendered by German neuropathologist Hans-Joachim Scherer in 1940 (4). Males are more likely than females to develop primary glioblastoma, although females are more likely to develop secondary glioblastoma (5). Gliblastoma is divided into two subtypes: primary and secondary. People are afflicted at various ages and by various hereditary mechanisms. Primary and secondary GBM tissues have clinical and cytogenetic differences (6). Primary glioblastoma accounts for the majority of instances (> 90%), progresses quickly and spontaneously, and has little clinical or histological manifestations as comparison to malignant precursor lesions. These are cancers that are most often seen in older people (60 years of age) and are identified as glioblastoma after the initial histopathological review. Since they progress quickly, clinical signs appear during the first three months, and the overall recovery period is 4.7 months. Single-step transition is not advised since, like other neoplasms, it leads to the acquisition of many genetic abnormalities. It is distinguished by the lack of 10q (70%), EGFR amplification (36%), P16INK4A deletion (31%), and PTEN mutation (25%) of the genome (6). Secondary glioblastoma arises from low-grade diffuse astrocytoma or anaplastic astrocytoma and progresses slowly. Low-grade astrocytomas require an average of 4-5 years to progress to GBM. Secondary GBM usually develops in people between the ages of 30 and 40, with an estimated life period of 7-8 months. Clinical or histological observations are used to diagnose secondary glioblastoma. According to genetics, the

TP53 mutation is commonly found, and buuanomal may be identified at an early stage . The most prominent genetic changes in primary (7). GBM are mutations in the epidermal growth factor receptor (EGFR) and cyclin-dependent kinase inhibitor 2 (CDKN2A) genes, as well as lack of heterozygozity in chromosome 10q23. MDM2 protein overexpression is found in more than half of all primary GBMs. Loss of heterozygozygozity (LOH) in chromosome 10q23 occurs in 60-80% of cases, and this area contains the tumor suppressor gene phosphate and tensin homoglogous (PTEN) (8). The EGFR family of receptors and their ligands play an important role in CNS production and primarytenance, including cell migration, proliferation, differentiation, and survival. It's an essential signalling pathway in the formation of primary glioblastoma and plays a vital role in gliomagenesis . In GBM, there is EGFR gene amplification (40-80 percent) and EGFR overexpression (10-60 %). In primary GBM, EGFR amplification and overexpression are normal, but less so in secondary GBM. The magnitude of the EGFR gene is linked to structural variations in the gene. The EGFR gene has seven large mutant forms, the most popular of which is variant III (EGFRvIII), also known EGFR. EGFRvIII is produced as a consequence of the deletion of exon 2-7, and irregular transcript expression and protein are observed at the genomic stage (9). The extracellular ligand binding site is deficient as a consequence of this genomic deletion, and the receptor is structurally autophosphorylated, resulting in enhanced tumor development, proliferation, migration, and neovascularization. Chemotherapy tolerance is linked to this damaged receptor. In both primary and secondary glioblastoma, the P16INK4A / RB1 pathway is essential. Primary GBM has a higher prevalence of homozygous P16INK4 deletion than secondary GBM. RB1 signaling pathway abnormalities were stated to be 78 percent in the last TCGA pilot project, with the following anomalies: P16INK4A homozygous deletion (52 %), P15INK4B homozygous deletion (47 %), P18INK4c homozygous deletion (2 %), CDK4 amplification (18 %), cyclin D2 (CCD2) amplification (2 %), CDK6 amplification (11 %). The genes for platelet-derived growth factor receptor A (PDGFRA), PDGFRA ligand, and tumor suppressor protein 53 (TP53) are both altered in secondary GBM. In secondary GBM, mutations in the p16INK4A and pRB1 genes, amplification of the cyclin-dependent kinase 4/6 (CDK) and murine double minute 2 (MDM2) genes, and chromosome 10 deletions were also found (10). The TP53 gene produces a protein that is found on chromosome 17p31.1 and is involved in a variety of cellular processes, including cell cycle, cell death, and cell differentiation. Secondary GBM has a large

prevalence of P53 mutations (> 65%) (11). Although 57% of mutations in secondary GBMs are found in codon 248 and 273, which are two hotspot areas, mutations in primary GBMs (10%) are evenly spread among all exons (12, 13). p14ARF expression loss is normal in GBMs (76%) and is linked to homozygous deletion or methylation of the p14ARF promoter. While the frequency of p14ARF modifications is similar in primary and secondary GBM, secondary GBM has a higher rate of p14ARF promoter methylation. In GBM, the RB LOH or point mutation is observed at a frequency of 30-40%. Secondary GBM has a higher rate of RB1 promoter methylation (43%) than primary GBM (14 %). In addition to both of these abnormalities, chromosome 10 loss and tumor suppressor PTEN gene deletion are typical genetic changes seen in both primary and secondary GBM (6). This grouping, on the other hand, has no definite boundaries. As a result, both forms of GBM may have genetic modifications including TP53 mutations and CDK4 amplification. The genetic anomaly of loss of 10q heterozygozity (60-80%) is seen in primary and secondary glioblastoma with a similar frequency, 10q25-qter. Deletions of three related loci have been found in several LOH experiments. Many tumor suppressor genes, including 10p14-p15, 10q23-24 (PTEN), and 10q25-qter, have been shown to play a part in the pathogenesis of glioblastoma (14).

Primary and secondary subtypes of GBM exist, each of which progresses by distinct genetic mechanisms, influencing people of various ages and, as a result, resulting in different symptoms and life expectancy following diagnosis (15). For newly diagnosed glioblastoma patients, standard treatment requires maximum healthy tumor resection, 60 Gy of radiotherapy, concurrent temozolomide (75 mg/m2 daily) administration for 6 weeks, and 6 cycles of adjuvant temozolomide (150-200 mg/m2, for 5 days of each 28-day cycle) administration (16, 17). Clinicians all around the world prefer this procedure, also known as the "Stupp protocol," and it greatly improves total recovery when opposed to radiotherapy alone. Recurrent glioblastoma, on the other side, has no approved therapeutic plan. Surgical debulking to provide a tissue sample is the first care choice (18). However, literature indicates that owing to the infiltrative nature of GBMs, a single surgical resection is insufficient, with median survivals ranging from three to six months (19-22). As a consequence of advanced technical advancement and the use of adjuvant radiotherapy in the last 60 years, the life span after surgical resection has risen to about one year. Thanks to recovery with anesthesia,

radiotherapy, and chemotherapy, the life span after surgery has now increased to 14-16 months. Temozolamide became the first chemotherapy medication choice (16, 17). Patients with late stage GBMs have a grim prognosis, but advances in treatment approaches such as chemotherapy, adjuvant radiotherapy, and surgical resection have improved the five-year survival rate from two to ten percent. As a consequence, early detection of malignant gliomas is critical in order to produce improved outcomes and a better prognosis (23).

2.2. Molecular Bases of GBM

The World Health Organization classification of CNS tumors has been revised immensely as molecular criteria are integrated in 2016 WHO CNS classification. Especially low grade and high-grade variations have been affected greatly by the modified classification. Glioblastomas may present abnormal expression of various growth-control genes and their proteins (24). Genes such as IDH 1, IDH2, p53, MGMT, TERT, ATRX are crucial in diagonsing the tumor and the assessment of possible response to treatment methods such as radiotherapy and chemotherapy.

2.2.1. Isocitrate dehydrogenase (IDH1 and IDH 2)

IDH 1 and 2 are metabolic enzymes encoded by the IDH 1 and 2 genes. These enzymes have many different functions inside the cell, and one of the most important of these is their place in the Krebs cycle, which plays a role in energy production. This cycle is an important chemical process that enables living cells to extract energy from food.

The compound alpha ketoglutarate (aKG) is formed during the Krebs cycle, and is used to generate steam. aKG achieved by Isocitrate dehydrogenase (IDH), isocitrate oxidative decarboxylation. NADP+ is used as a cofactor and as a generator of NADPH during this catalysis. Proteins IDH1 and IDH2 have multiple genes coding and these genes have a significant sequence similarity degree (IDh2, 2q33, IDH2, 15q26) (70 percent in humans). IDH1 is situated in peroxisomes and cytosol; IDH2, while immensely similar, lies in mitochondria and catalyzes unmistakable and variable reactions. Homodimeric enzymes IDH1 and IDH2 consist of two active locations per dimer. IDH subunits consist of three domains (large area, limited area and clasp area),

while IDH2 comprises an additional targeting sequence of mitochondria. Per active site contains NADP(H) and isocitrate binding sites (25).

IDH1 and IDH2 contribute to glucose metabolism, redox regulation, lipogenesis and glutamine synthesis. It is also supposed that they have separate locations in each function and a catalytic flux that varies between forward and reverse behavior (26).

IDH1 helps to generate acetyl-CoA for lipid synthesis during hypoxia by catalyzing reductory aKG carboxylation (27). Transgenic mice caused obesity, fatty liver, and hyperlipidemia due to overexpression of IDH1 of the liver and adipose tissues (28). In the formation of mouse astrocytes, IDH1 also regulates phospholipid metabolism (29). IDH2 can play a minor or tissue-specific role in reductional glutaminolysis (27).

In the human brain, IDH1 is the main storage site for NADPH (30). In other tissues, it serves as a vital source of NADPH as well (31). By reducing oxidized glutathione and thioredoxin, NADPH plays a critical role in oxidative damage protection. IDH1 inhibits lipid peroxidation and oxidative DNA impairment by producing NAPDH by oxidative decarboxylation of isocitrate (32). IDH1/2 action also reduces oxidative DNA damage and lipid peroxidation, preventing replicative senescence (33). UVB-induced phototoxicity is avoided since IDH1 produces NAPDH (34), reduces the amount of reactive oxygen species and prevents kidney damage caused by ischemia and reperfusion (31)

The evidence therefore indicates that wild-type IDH1 and IDH2 are essential to the regulation of oxidative stress in reaction to different cellular insults. IDH1 and IDH2 coding genes also mutate into different forms of human cancer. There are two conflicting hypotheses as to how this mutation is causing cancer. The first view is that the 2-hydroxyglutarate (2HG) is the enzyme proline hydroxylase (PHD). Therefore, PHD is responsible for asking the cell whether there is sufficient oxygen for oxidative phosphorylation through hydroxylating Hypoxia Inducing Factor (HIF). As 2HG blocks the reaction of PHD, so PHD is transferred to the nucleus. There, it is thought to abnormally trigger gene transcription, contributing to cancerous conditions (35).

As the activity of IDH1 / 2 enzymes is altered as a consequence of a mutation, KG is converted to another intermediate chemical, alpha hydroxyglutaric acid (2-HG).

The buildup of 2-HG in the body disrupts the activities of enzymes involved in cellular processes, changes cellular metabolism and epigenetic control, and leads to tumor development by inhibiting the DNA repair process. It also disrupts the cell division cycle by inhibiting the activity of demethylase enzymes, which extract methyl groups from histone and DNA. It also speeds up the development of new blood vessels, which lead to tumor formation, and stimulates genes that help cells survive in low-oxygen environments.

As a result of genetic studies, it has been determined that IDH1 / 2 genes are observed in 70-80% of low-grade gliomas and a large part of secondary glioblastomas. These data add a new dimension to the GBM classification and divide it into two classes as IDH-mutated GBM (usually representing secondary GBMs) and IDH-mutated GBM (including primary GBMs).

Many tumors, including grade 2 and 3 astrocytomas, oligodendrogliomas, oligoastrocytomas, secondary glioblastomas, chondrosarcomas, angioimmunoblastic t-cell lymphomas, intrahepatic cholangioplasia syndromes, and acute myeloid lymphoma, have IDH1 and IDH2 mutations. It may be found in tumors of the colon and lungs.

Tumors with IDH1 and IDH2 mutations often have abnormal histone and DNA methylation patterns, which may lead to stem cell alterations and tumorigenesis. This unique characteristics of IDH1 and IDH2 mutations are crucial for identifying new drug targets and interpreting them as accurate biomarkers. In approximately 8.8% of the patients, unselected GBMs and IDH mutants were found (35). Albeit exceptional in primary GBM, *IDH* mutations occurred in almost 90% of secondary GBM (6). As a result, *IDH* mutations are accepted as standard identifiers for better prognosis in GBM (36).

2.2.2. O6-Methylguanine-DNA methyltransferase (MGMT)

MGMT (O6-Methylguanine-DNA Methyltransferase) is a DNA methyltransferase that extracts promutagenic alkyl groups from guanine's O6 location. As a result, it prevents cells from alkylating agents. The MGMT gene, which is located on the chromosome 10q26, covers a protein of DNA reparation which removes a critical location of DNA alkylation alkyl groups from O6 position of guanine. Prompt cytotoxicity and apoptosis may be the product of chemotherapy-restored lesions, in

particular O6-methylguanine (37). The methyl group on the O6 site of the guanine is removed by MGMT, which is irreversibly attached to it. The MGMT complex is degraded by ubiquitin as it is combined with the methyl group. The cell's life cycle begins as normal. When the cell lacks the MGMT molecule, O6-methylguanine attaches to thymine during replication, resulting in incorrect base pair pairing. The DNA mismatch repair pathway proteins identify this base pair (MLH1, MSH2, MSH6, and PMS2). DNA is healed or the cell enters apoptosis at the conclusion of this step. Methylation of promoter CpG islands may cause MGMT expression to be lost. In 45-75 percent of glioblastomas, the MGMT promoter is methylated. Secondary glioblastomas have a higher rate of promoter methylation than primary glioblastomas. Harris et al. demonstrated the suppressive function of the p53 molecule in MGMT control (38-40). Hypoxia induces MGMT expression through hypoxia inducing factor (HIF)-1, according to research.

High MGMT expression in cancer cells allows cells to form a harmful phenotype that hinders the therapeutic effectiveness of alkylating agents (37). MGMT gene epigenetic mutilation caused by promoter methylation is linked to MGMT expression loss and decreased DNA repair activity (41). Since this technique disrupts the function of tumor suppressor genes, DNA methylation gene silencing is an early, critical mechanism in the tumor phase (42).

Increased activity of MGMT in glial tissue was linked to the marginal benefits of alkylating-agent chemotherapy before 2005, according to reports. In a limited group of patients who received radiotherapy, bis-chloroethylnitrosourea, and platinum injection. It was reported that methylation of the MGMT gene's promoter area was related to better outcomes. Centered on the temozolomide reference list of newly diagnosed glioblastoma patients, a polymerase chain reaction (PCR)-based simulatenous study uncovered a subpopulation of patients who underwent and benefited from both radiotherapy and temozolomide. Since then, the neuro-oncology group has generally agreed on two points: MGMT promoter methylation, as measured by PCR, is the most important prognostic predictor in all malignant gliomas, and no other test is accurate for safely forecasting therapeutic benefits. A centralized assessment of MGMT status using methylation-specific PCR revealed major shortcomings in the phase III trial for the integrin antagonist cilengitide, confirming that MGMT research is difficult to standardize. Finally, the beneficial benefits of temozolomide alone over radiotherapy

were measured using the successful benefit of MGMT promoter methylation in 2012 glioblastoma patients (43, 44). The methylation of the MGMT promoter is common in oligodendrogliomas and low-grade astrocytomas. MGMT have a complex association with IDH mutations and 1p19q codeletion. Furthermore, several experiments have shown that the methylation status of the MGMT promoter has prognostic and predictive value. MGMT promoter methylation was seen in 51-66% of people with glioblastoma, and it inhibits the MGMT gene's function while still promoting chemosensitivity and longevity in glioblastoma patients (22).

2.2.3. Telomerase reverse transcriptase (TERT)

Telomerase reverse transcriptase, along with the telomerase RNA portion, is a catalytic subset of the telomerase enzyme as the most essential unit in the telomerase complex. Telomerases are part of a distinct RNA-related polymerase subgroup. Telomerase causes senescent cells to become post-mitotic, pass over the Hayflick limit and become perhaps immortal, often like cancer cells, through the elonization of telomeres in DNA strings. In particular, TERT is responsible for catalyzing the connection of nucleotides to the ends of the telomeres of the chromosome in the TTAGGG chain. This insertion of repeated DNA sequences stops chromosome ends from becoming disturbed after many replication cycles. TERT gene codes for a particularly specialized reverse transcriptase that adds hexamer repeats to chromosomes' 3' ends (45, 46). Cancer patients have higher telomerase function, which preserves telomeres and allows tumors to avoid senescence (47). Conflicts over TERT promoter mutations have been identified at the work of a number of cancers (48-50), most notably in glioma (51). The two most common TERT mutations are C228T and C250T. They are located _124 and _146 bp upstream of the TERT ATG site, respectively (52). The impact of TERT heterogeneity on cancer susceptibility is being more widely debated and observed. GBM is linked to the existence of SNP rs2736100, which is more common in IDH1 wild-type GBM (53). In oligodendroglioma and primary glioblastoma multiforme, pTERT mutations are common. pTERT mutations were seen in 78 percent of oligodendrogliomas and 83 percent of GBMs IDH-wildtype in a sample of more than 200 glioma specimens (54, 55). The occurrence of the pTERT mutation in both grade II and III oligodendrogliomas was confirmed in a wider sample of over 1000 gliomas. However, IDH-mutant pTERT mutations were seen in just 10% of astrocytomas and GBMs (51).

2.2.4. Alpha-thalassemia/Mental retardation, X-linked (ATRX)

The ATRX gene was found in a survey of patients with the x-linked mental retardation condition, which includes -thalassemia, extreme psychomotor impairments, urogenital anomalies, and facial dysmorphism patterns. The ATRX gene allows for the production of a protein that is critical to normal growth and development. As far as we know, ATRX protein does not serve any particular function; yet, studies indicate that it regulates the expression of other genes by chromatin remodeling processes. One way gene expression is controlled during growth is by chromatin remodeling. At high levels of packing, the expression of genes is low. This protein seems to control the HbA1 and HbA2 genes in an important way.

Although there is no co-occurrence of 1p/19q codeletion and ATRX failure, there is a close association between IDH canonical and ATRX mutations. In diffuse astrocytoma and anaplastic astrocytoma, mutations in IDH1 and ATRX are common, but uncommon in pilocytic astrocytoma and glioblastoma (56, 57). This notable aspect is that ATRX also causes mutations in secondary GBMs but not in primary GBMs (58). ATRX mutations are frequently linked with IDH and TP53 mutations in GBMs.

3. MATERIALS AND METHODS

3.1. The Study Population and Protocol

We examined thirty patients in this study. The majority of the participants were chosen from Yeditepe University's Department of Neurosurgery in Istanbul, Turkey. All of the patients had a craniotomy and were diagnosed with high-grade glioma. The removed tumours were studied and histopathologically examined according to the World Health Organization CNS Tumor Classification. Signed informed consent agreements were obtained from all parties. Patients' demographic profiles have been recorded and reviewed for future use. The lesions were classified by their magnitude and their location. Peripheral blood samples were obtained from all participants who gave informed consent. The approval for the study was obtained from the Yeditepe University Faculty of Medicine Ethics Committee, and blood samples were taken in line with the 1975 Declaration of the Ethical Guidelines of Helsinki (file no: ...). We contributed clinical, demographic, and survival data to the current assessment of this diagnostic tool. The date of the first (diagnostic) operation was chosen as the date of diagnosis, and survival was evaluated from this date. The study's main goals were to evaluate IDH1, IDH2, TERT, MGMT and ATRX genes with NGS to find potential pathological mutations and their effect on survival.

3.2. Genomic DNA Isolation From Blood

Venous blood samples of the patients were collected in 5 cc EDTA tubes and stored at +4C° until DNA was extracted. The iPrep DNA extraction robot (Invitrogen) and the iPrep genomic DNA isolation kit (Invitrogen) were used to extract DNA from samples (iPrep gDNA Blood kit, Invitrogen, Thermo Fisher Scientific Inc). This device can isolate DNA from up to 13 samples in a single run. For each sample, 350l of peripheral blood was utilized in a robotic system termed magnetic bead-based technology, which relied on the surface charge over the pH of the buffer in the environment to extract DNA. The negatively charged nucleic acid backbone bonds to the positively charged CST® (ChargeSwitchTechnology®) at low pH. As a result, proteins and other impurities are unable to bind, and the liquid wash buffer is used to wash them away. To clean nucleic acids, the charge on the bead surface is neutralized by employing a low salt elution buffer to raise the pH to 8.5. The isolated nucleic acid

goes straight into the wash buffer and is ready to use in experiments. After being placed in appropriate containers, the isolated DNA samples were maintained in a +4C° refrigerator.

3.3. DNA Purity Measurement

The optical density of 50ng/l (g/ml) double-stranded DNA at 260 nm wavelength was found to be 1. The Nanodrop2000 device was used to determine the purity of DNA samples. The OD260/OD280 ratio was used to evaluate the purity of DNA samples. Clean samples were those with an OD260/OD280 ratio of 1.7 to 1.9, which were recognized as acceptable for genotyping.

3.4. Qiaseq Targeted DNA Panel

When using Illumina NGS systems, Qiaseq DNA targeted libraries require a custom sequence primer.

The panel was created for the genes and regions of interest that can be enriched with 10-40 ng Fresh DNA or 40-250 ng FFPE DNA.

There are four basic stages in this system:

1. Fragmentation: The genomic DNA sample is fragmented with a controlled multi-enzyme reaction, end-repair is done, and A-tail is added. Ready DNA fragments are connected to the sequence platform to be used at 5 ends with specific adapters (including UMI and sample index).

2. UMI assignment: Each DNA molecule is assigned a unique sequence or index known as the UMI before the target enrichment and Library creation stages. This assignment is achieved by ligating a 12-base random sequence adapter (59) with fragmented DNA. This process provides statistically 412 distinct markings per adapter, and each DNA molecule in the sample takes a unique UMI sequence. Moreover, this adapter that is connected also contains the first sample index.

3. Target enrichment and final library creation: The stage in which DNA molecules containing UMI are adequately enriched is the target enrichment stage. For enrichment, UMI-linked DNA molecules are amplified by Targeted PCR with Adaptor

complementer 1 site-specific primer and 1 universal primer. Finally, to amplify the library and add the platform-specific adaptor sequence and extra sample indices, Universal PCR is used.

4. Next Generation Sequencing: Qiaseq Targeted DNA panels are compatible with Illumina NGS systems (MiniSeq®, MiSeq® Personal Sequencer, NextSeq® 500, HiSeq® 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500 and GAIIx) and Thermo Fisher Scientific systems (Ion Personal Genome Machine® (PGMTM), Ion ProtonTM and Ion S5TM). (Figures 1-2)

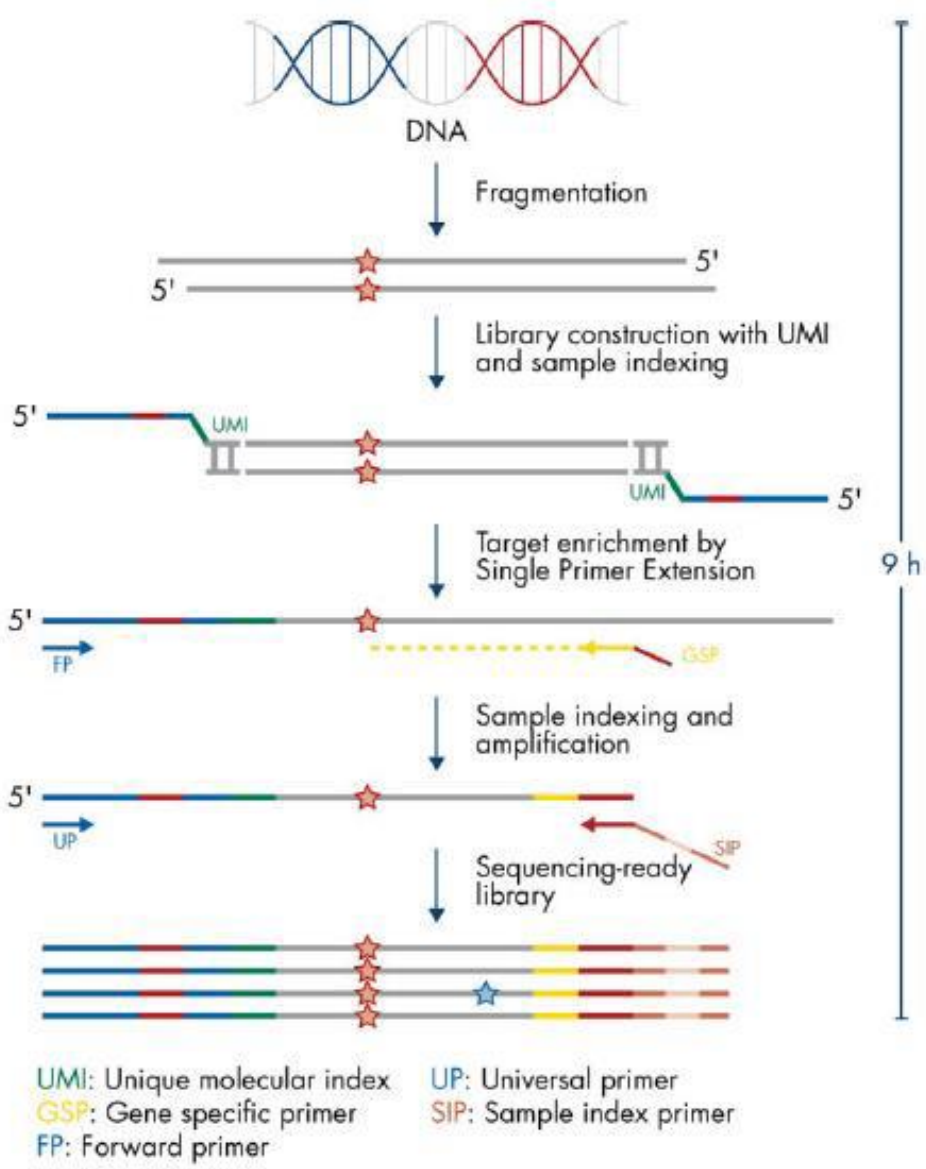

Figure 1. Qiaseq Targeted DNA Panel Procedure Schematic Summary- 1

Figure 2. Qiaseq Targeted DNA Panel Procedure Schematic Summary-2

3.4.1. Fragmentation, end-repair, and addition

1. After ensuring that the Thermocycler had reached a temperature of 4 degrees, the fragmentation, End-repair, A-addition mix was made according to the table below and pipetted 7-8 times. Brief centrifugation was performed. Work was carried out on cold block or ice (Table 1).

Table 1. Fragmentation, End-Repair, and Addition

	Standard DNA, FFPE DNA, cfDNA	**CfDNA Contaminated by Cellular DNA**
Contents	Volume (µL)	Volume (µL)
DNA	Various	Various
Fragmantation Buffer, 10x	2,5	2,5
Nuclease- Free Water	Various	Various
FERA solution	0,75	0,75
FG solution	-	1,25
TOTAL	20	20

2. 5 µl of Fragmentation Enzyme Mix was added to each reaction and mixed by pipetting 7-8 times. Care was taken to ensure that the tubes were on the cold block during the reaction setup.
3. They were organized according to the following PCR program.
4. The PCR program was started, and paused when it reached 4 C.
5. The device was loaded with samples, and the application was started.
6. The samples were taken on the ice after the program was completed and the block had reached 4 C.
7. The adapter ligation phase was started immediately.

3.4.2. Adapter ligation of samples

1. The reaction mix was made according to the table below. By softly pipetting 10-12 times, all contents were mixed (Table 2).

The ligation solution was not included in the following mix because it is quite viscous, but it was added to each reaction thereafter. The residual ligation solution on the pipette tip was scraped off while adding the ligation solution.

Table 2. Adapter Ligation of Samples

	Standard DNA	**FFPE DNA**	**cfDNA**
Content	Volume (µL)	Volume (µL)	Volume (µL)
DNA	25	25	25
Ligation Buffer x5	10	10	10
IL-N7 Adapter	2,8	2,8	0,5
DNA Ligase	5	5	5
Ligation Solution	7,2	7,2	7,2
Nuclease Free Water	-	-	2,3
TOTAL	50	50	50

2. Thermocycler was set to 20 C and incubated for 15 minutes.

3. After the reaction was finished, it was placed on ice and the QIAseq Beads cleaning process began.

3.4.3. Clearance of adapter-bound DNA with QIAseq Beads

1. 50µl (standard-FFPE DNA) sample from the ligation reaction was transferred to a 1.5 ml LoBind tube. It was made up to 100 µl by adding 50 µl of nuclease-free water.
2. 100 µl of QIAseq Beads was added to the standard-FFPE DNA study. In the cfDNA study, on the other hand, 112 µl of QIAseq Beads were added. By pipetting it numerous times, it was thoroughly mixed.
3. It was incubated for 5 minutes at room temperature.

4. It was put on a magnetic rack, and the beads were left to separate from the supernatant for 10 minutes. The supernatant was carefully extracted and discarded after the solution became clear. Care was taken not to touch the beads containing the target DNA.
5. 200µl of freshly prepared 80% ethanol was added on the magnetic rack. The beads were washed by turning the tube. The supernatant was extracted and discarded delicately.
6. The ethyl alcohol washing stage was repeated. ETOH should be discarded entirely at this stage. For this purpose, a 200 µl pipette was used to remove the residual liquid, and a 10 µl pipette was used to remove the rest.
7. After removing the ethanol from the magnetic rack, the beads were allowed to dry completely for 10 minutes.
8. DNA was eluted to target beads outside the magnetic rack with 52 µl of nuclease-free water. Pipetting was done thoroughly, and the solution was put on the magnetic rack again until it became clear.
9. 50µl of the supernatant was transferred into a clean 1.5 ml LoBind tube or PCR plate well.
10. For standard-FFPE samples, 50µl of QIAseq Beads was added onto 50µl of DNA solution; and for cfDNA samples, 70µl of QiAseq Beads was added onto the DNA solution. By pipetting it numerous times, it was thoroughly mixed.
11. It was incubated for 5 minutes at room temperature.
12. It was taken on a magnetic rack, and the beads were left to separate from the supernatant for 5 minutes. The supernatant was extracted and discarded delicately after the solution became clear.
13. 200µl of freshly prepared 80% ethanol was added on the Magnetic Rack. Beads were washed by turning the tube or moving the plate. The supernatant was extracted and discarded delicately.
14. Ethyl alcohol washing stage was repeated 1 more time.

15. Using a 200l pipette tip first, then a 10l pipette tip, the ethanol was fully extracted. While remaining on the magnetic rack, the beads were dried for 15 minutes.

    It is critical that the beads are completely dried before elution. Ethanol carried to the next Target Enrichment PCR stage will affect PCR efficiency.

16. The DNA target beads were eluted with 12µl of nuclease-free water after being removed from the magnetic rack. It was mixed thoroughly by pipetting. The solution was maintained on the rack until it turned transparent.

17. The target enrichment PCR setup was started with 9.4 µl of the supernatant transferred to a clean PCR tube or PCR plate well.

3.4.4. Target enrichment PCR

1. The reaction was prepared according to the following table. After a brief centrifugation, it was pipetted 7-8 times and centrifuged again (Table 3).

Table 3. Target Enrichment

Contents	**Volume (µL)**
DNA library (in item 28)	9.4
TEPCR Buffer, 5x	4
QIAseq Targeted DNA panel	5
IL-Forward primer	0.8
HotStar Taq DNA Polymerase	0.8
Total	20

2. The thermocycler was set up
3. After the reaction was finished, it was placed on ice and the QIAseq Beads cleaning process began.

3.4.5. Clearance of the target enrichment PCR with QIAseq Beads

1. 20µl of the PCR reaction was transferred to a 1.5 ml LoBind tube. To make it 100 µl, 80 µl of nuclease-free water was added. For the cfDNA sample, 70 µl of nuclease-free water was added to 20 µl of sample and it was completed to 90 µl.
2. 100µl (1.0x volume) of QIAseq Beads was added to 100µl of diluted PCR solution. By pipetting it numerous times, it was thoroughly mixed. It was incubated for 5 minutes at room temperature.
3. The sample was taken on the magnetic rack and the beads were left to separate from the supernatant for about 5 minutes. The supernatant was extracted and discarded delicately after the solution became clear.
4. 200µl of freshly prepared 80% ethanol was added (while still on the magnetic rack). Beads were washed by turning the tube or moving the plate. The supernatant was extracted and discarded delicately.
5. Ethyl alcohol washing stage was repeated 1 more time.
6. Using a 200l pipette tip first, and then a 10l pipette tip, the ethanol was entirely extracted. While still on the magnetic rack, the beads were dried for 10 minutes.
7. DNA beads were eluted with 16µl of nuclease-free water. It was mixed thoroughly by pipetting. The solution was left on the rack until it turned transparent. 13.4 µl of supernatant was transferred to a clean PCR tube and the Universal PCR amplification setup was began.

3.4.6. Universal PCR amplification

1. Universal PCR preparation was carried out using the table below. It was briefly centrifuged and pipetted 7-8 times (Table 4).

Table 4. Universal PCR Amplification

GRADIENT	**Volume (µl)**
Enriched DNA	13.4
UPCR Buffer, 5x	4
Hot Start Taq DNA polimeraz	1
Nuclease Free Water	1.6
TOTAL	**20**

2. The Thermocycler's cycle settings were determined using the Tables.
3. The PCR reaction was put on ice after completion, and the cleaning process with QIAseq beads was continued.

3.4.7. Clearance of the universal PCR with QIAseq beads

1. 20 µl of the PCR reaction was transferred to a 1.5ml LoBind tube. 80 µl of Nuclease-free water was added to it (to make up to 100 µl).
2. 100µl (1.0x volume) of QIAseq Beads was added to 100µl of diluted PCR solution. By pipetting it numerous times, it was thoroughly mixed. It was incubated for 5 minutes at room temperature.
3. The tube was placed on the magnetic rack for 5 minutes to allow the beads to separate from the supernatant. The supernatant was extracted and discarded delicately after the solution became clear.
4. 200µl of freshly prepared 80% ethanol was added (while still on the magnetic rack). Beads were washed by turning the tube or moving the plate. The supernatant was extracted and discarded delicately.
5. Ethyl alcohol washing stage was repeated 1 more time.
6. Ethanol was removed. For this purpose, first, it was drawn with a 200 µl pipette and the rest with a 10 µl pipette, and the beads were allowed to dry for 10 minutes (on the magnetic rack).

7. DNA library beads were eluted with 30µl of nuclease-free water outside the magnetic rack. Pipetting was done thoroughly, and the solution was put on the magnetic rack again until it became clear. 28 µl of supernatant was transferred into a clean 1.5ml LoBind tube or PCR tube.

3.5. Qiaseq Quant Assay Protocol

1. The ILMN DNA standard was thawed on ice.
2. The ILMN DNA standard was diluted with 5 serial dilutions as seen in the table below (Table 5). For the ILMN standard dilution, five tubes were prepared. 45 µl of Dilution buffer was added to each tube. 5 µl of ILMN standard stock was added to the first tube and mixed by pipetting. Then, 5 µl of solution taken from the first tube was transferred to the second tube. For the remaining tubes, the same procedure was followed.

Table 5. Qiaseq Quant Assay Protocol

Standard	**Ion Torrent or Illuina DNA Standard**	**Dilution buffer**
Std1	5 µl undiluted	45 µl
Std2	5 µl Std1	45 µl
Std3	5 µl Std2	45 µl
Std4	5 µl Std3	45 µl
Std5	5 µl Std4	3 µl

3. Three dilutions were prepared for the samples (First dilution: 1:50, Second dilution:1:5,000, Third dilution: 1:50,000). The first dilution's goal is to get samples ready for the second and third dilutions.
4. Preparing the PCR mix (the prepared mix is kept on the cold block)
    a. SYBR Green ROX FAST Mastermix was centrifuged for 10-15 seconds.
    b. For 5 standards and 1 NTC, 3 copies were prepared (non-template control). Hence, the first 18 of RotorGene tubes would contain the standards and NTC. One copy of each sample's dilutions ((1:5,000) and (1:50,000)) was filled into RotorGene tubes.

5. The real-time PCR device (RotorGene) was set up in the following cycle. Data for fluorescence irradiation was obtained at the conclusion of 30 cycles.
6. The relevant values of the samples were obtained and entered into the "Illumina Data Analysis Program" after the PCR cycle was completed.

3.6. QiaSeq Upload Protocol

1. Before beginning the dilution, the Illumina Experiment Manager was used to prepare the SampleSheet.
2. The code starting with MSxxx was written in the Reagent Cartridge Barcode section.
3. The 'custom primer for read 1' section on the right was marked.
4. 1N stock NaOH was diluted to 0.2N with nuclease-free water (800µl water + 200µl NaOH).
5. Based on the results of the Quant assay protocol, all of the samples were calibrated to be 4 molars.
6. To generate a mega pool, adequate volumes were added into the 0.2 µl PCR tube based on the number of primers from each library.
7. 5 µl was taken from the tube containing the mega pool and added into a separate tube. 5 µl of NaOH, which we diluted to 0.2N, was added to it. By gently vortexing and spin down, it was allowed to denaturate for 5 minutes at room temperature.
8. Meanwhile, the 1.5 ml tube was labelled "20 pM pool" and 990 µl of the yellow-capped Hyb buffer solution, which came out of the cartridge, was added into it.
9. As soon as 5 minutes were up, 10 µl of the solution in the tube containing NaOH and our sample was added to the 1.5ml tube containing 990ul Hyb, which we labelled as "20 pM pool".
10. The dilution of the samples was maintained according to the cartridge and library used.
11. We labelled another 1.5 ml tube as "Forward primer". 597 µl of HT1 was added to this tube. 3 µl of primers labelled "Qiaseq A Read1 Custom Primer1" with an orange cap from the index box were added to it.

12. Both tubes were kept on the cold block until they were loaded.
13. The device was powered off via MiSeq Control Software before starting the sequence. After a while, it was turned on again from the button on the back and the device was expected to turn on.
14. Our samples, which had been brought to the desired concentration, were loaded into well 17 as 600 µl, as shown in the photo below. 600 µl was drawn and placed into well number 18 from the tube labelled "Forward primer."
15. Before beginning the run, examine the run settings and conduct a pre-run check after loading the flow cell and reagents. The Sequencing panel displays run progress, intensities, and quality ratings during the run. Before continuing, we examine the Sequencing screen's findings. We proceeded to a post-run wash once the run was over. At certain times throughout a run, run stats show on the Sequencing screen.

3.7. Patient Demographics

All patients included in the study noted as their age, gender, tumour localization, pathology results, IDH1, IDH2, MGMT, TERT, ATRX results in pathology examination, and survival time examined. The effects of the sequencing results and the data received on survival were assessed.

3.8. Statistical Analysis Method

While evaluating the findings of the study, SPSS (Statistical Package for the Social Sciences) version 25.0 (IBM Corp., Armonk, NY, USA) program was used for statistical analysis. Descriptive statistical methods (number, percentage, median, etc.) were used while evaluating the study data. Survival calculations were made using the Kaplan-Meier analysis method. The effects of various prognostic factors related to tumor and patient characteristics on overall survival were investigated by log-rank test. In addition, the effects of multiple prognostic factors on overall survival were investigated using the multivariate Cox regression test. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

4. RESULTS

The median age of the patients was 58.5 (11-74), and 56.7% (n=17) were under 60 years of age. According to gender, the rate of male patients was 66.7% and the rate of female patients was 33.3%. Eighty percent of the patients had IDH, 83.3% MGMT, and 70% had EGFR positivity. Ten of the patients had Neocortex, 9 Limbic, 4 Multicentric, 4 Deep Nuclei, 2 Corpus Callosum and 1 Cerebellar localization. The demographic information of the patients is summarized in Table 6 and Table 7. After the targeted gene sequencing performed on the patients, 21 mutations were detected. Three of these mutations were found in IDH 1, 2 in IDH2, 6 in TERT, 7 in MGMT, and 3 in ATRX (4). Among the 21 chromosomes examined in the patients and given in Table 8 and , the highest rate of anomaly was found in C915GA with 73.3% and C2671CG with 63.3%. To evaluate the effects of all these mutations on survival we made survival analysis by SPSS.

Overall Survival

The mean follow-up period of the patients was 22.77±15.55 months, and the median follow-up period was 19.5 (3-70) months. In the first 3-year follow-up, 25 (83%) patients died due to the disease. According to Kaplan-Meier survival analysis, 1, 2, and 3-year overall survival rates of patients were calculated as 73.3%, 30%, and 16.7%, respectively. In the log rank test comparisons;

- Males have a lower 3-year overall survival rate than females (5% vs 40%; p=0.002),
- The survival of EGFR positive patients is lower than negative patients (EGFR→9.5% vs 33.3%; p=0.007),
- It was determined from the available data that the 3-year survival rate of patients with 3 or less chromosome anomalies as a result of 21 gene analysis was worse (5% vs 40%; p=0.033) compared to patients with more than 3 chromosome anomalies. (Figure 4, 5, 6 7) (Table 9- 10).

According to the results of multivariate Cox Regression analysis in which factors with sufficient cases in variable subgroups were included, male gender status of the independent factors negatively affecting overall survival [HR: 4.627(1.654-

12,948)); p=0.004] and 3 or less chromosome anomalies [HR: 2.831(1.004-7.981); p=0.049]. When female gender status was taken as reference, the overall survival of male gender status was 4.63 times, and when more than three chromosome anomaly was accepted as a reference, it was determined from the available data that anomaly in three or less chromosomes negatively affected overall survival 2.83 times. (Table 11).

Table 6. Patient Demographic Biochemical and Genetic Test Characteristics

Variables	Category	n(%)	Median(range)
Age	**All**	30(100)	58.5(11-74)
Age group	<60	17(56.7)	
	≥60	13(43.3)	
Gender	Male	20(66.7)	
	Female	10(33.3)	
IDH	Positive	24(80)	
	Negative	6(20)	
MGMT	Positive	25(83.3)	
	Negative	5(16.7)	
EGFR	Positive	21(70)	
	Negative	9(30)	
C532GA	Positive	1(3.3)	
	Negative	29(96.7)	
C315CT	Positive	1(3.3)	
	Negative	29(96.7)	
C1234CT	Positive	1(3.3)	
	Negative	29(96.7)	
C3324GA	Positive	1(3.3)	
	Negative	29(96.7)	
C3039C	Positive	9(30)	
	Negative	21(70)	
C2775CT	Positive	1(3.3)	
	Negative	29(96.7)	
C2751GA	Positive	1(3.3)	
	Negative	29(96.7)	
C1590GA	Positive	1(3.3)	
	Negative	29(96.7)	
C915GA	Positive	22(73.3)	
	Negative	8(26.7)	
C28CT	Positive	6(20)	
	Negative	24(80)	
C159CT	Positive	8(26.7)	
	Negative	22(73.3)	
C250CT	Positive	7(23.3)	
	Negative	23(76.7)	
C392GA	Positive	1(3.3)	
	Negative	29(96.7)	
C427AG	Positive	6(20)	
	Negative	24(80)	
C526TC	Positive	1(3.3)	
	Negative	29(96.7)	
C533AG	Positive	5(16.7)	
	Negative	25(83.3)	
C996CT	Positive	3(10)	
	Negative	27(90)	
C419GA	Positive	1(3.3)	
	Negative	29(96.7)	
C7140CT	Positive	1(3.3)	
	Negative	29(96.7)	
C5465AG	Positive	1(3.3)	
	Negative	29(96.7)	
C2671CG	Positive	19(63.3)	
	Negative	11(36.7)	

Table 7. Patient Demographic Data

Patient ID	Age	Gender	Localization	Pathology	P53	EGFR	MGMT	IDH	Last Control Status	Survival Month
1	41.00	male	Multicentric	GBM	Positive	Positive	Positive	Positive	exitus	12.00
2	31.00	male	Postcentral gryrus	GBM	Positive	Positive	Positive	Positive	exitus	14.00
3	36.00	male	Cerebellar	GBM	Positive	Positive	Positive	Positive	exitus	14.00
4	71.00	male	Multicentric	GBM	Positive	Negative	Positive	Negative	exitus	5.00
5	26.00	male	Thalamic	GBM	Positive	Positive	Positive	Positive	exitus	9.00
6	68.00	male	Corpus callosum	GBM	Positive	Positive	Positive	Negative	exitus	19.00
7	60.00	male	Hipokampus+ Parahipokampal gyrus	GBM	Positive	Positive	Positive	Positive	exitus	20.00
8	40.00	male	Thalamic	GBM	Positive	Positive	Negative	Positive	exitus	22.00
9	40.00	female	Temporal glial tumor	GBM	Positive	Negative	Positive	Positive	exitus	32.00
10	74.00	male	Multicentric	GBM	Positive	Positive	Positive	Positive	exitus	4.00
11	11.00	male	Thalamic	GBM	Positive	Positive	Positive	Positive	exitus	15.00
12	70.00	female	Superior temporal gyrus (T1)	GBM	Positive	Positive	Positive	Positive	exitus	12.00
13	61.00	male	Hippocampus+ Cingulate	GBM	Positive	Positive	Positive	Positive	exitus	16.00
14	48.00	male	Hipokampus+ Parahipokampal gyrus	GBM	Positive	Positive	Positive	Positive	exitus	17.00
15	58.00	female	Temporal pole	GBM	Negative	Positive	Negative	Negative	exitus	49.00
16	59.00	female	Mediobasal temporal region	GBM	Positive	Negative	Positive	Positive	alive	38.00
17	67.00	male	Corpus callosum	GBM	Positive	Positive	Positive	Positive	exitus	4.00
18	59.00	male	Occipital neocortex	GBM	Positive	Positive	Positive	Negative	exitus	22.00
19	62.00	female	Superior Frontal Gyrus (F1)	GBM	Positive	Negative	Positive	Positive	exitus	35.00
20	71.00	male	Cingulate gyrus	GBM	Positive	Negative	Positive	Positive	exitus	24.00

Table 7. (Continuing)

Patient ID	Age	Gender	Localization	Pathology	P53	EGFR	MGMT	IDH	Last Control Status	Survival Month
21	33.00	female	Right precuneus	GBM	Positive	Negative	Negative	Positive	alive	32.00
22	60.00	female	Corpus callosum	GBM	Positive	Positive	Positive	Positive	exitus	23.00
23	55.00	male	MTR+ Thalamic	GBM	Positive	Positive	Positive	Positive	exitus	3.00
24	36.00	male	Superior temporal gyrus (T1)	GBM	Positive	Positive	Negative	Negative	exitus	70.00
25	60.00	female	Mediobasal temporal region	GBM	Positive	Negative	Positive	Positive	exitus	44.00
26	68.00	Male	Superior Frontal Gyrus (F1)	GBM	Positive	Positive	Positive	Positive	exitus	7.00
27	31.00	Female	Thalamic	GBM	Positive	Negative	Positive	Positive	exitus	38.00
28	60.00	Male	Superior temporal gyrus (T1)	GBM	Positive	Positive	Positive	Positive	exitus	20.00
29	40.00	female	Paracentral lobule	GBM	Positive	Positive	Positive	Negative	exitus	19.00
30	28.00	Male	Inferior Frontal Gyrus (F3)	GBM	Positive	Negative	Negative	Positive	exitus	34.00

Table 8. Summary of 21 Chromosome Anomalies detected with NGS

Gene Symbol	Chromosome	Transcript Variant	Protein Variant	Normal	Population Frequency	Chromosome	Gene Region	Translation Impact	Classification	Phenotype	Transcripts	Patient ID
IDH1	2	c.532G>A	p.V178I	Normal	8.7%	209,108,317	Exonic	missense	Benign	Cancers and Tumors	NM_005896.4	7
IDH1	2	c.315C>T	p.G105G	Normal	10.53%	209,113,192	Exonic	synonymous	Benign	Cancers and Tumors	NM_005896.4	7
IDH1	2	c.123-4C>T		Normal	2.96%	209,113,388	Intronic		Likely Benign	Hereditary Disorder	NM_005896.4	16
TERT	5	c.3324G>A	p.P1108P	Normal	3.02%	1,253,918	Exonic; ncRNA	synonymous	Likely Benign	Dyskeratozis Congenita	NM_198253.3	4
TERT	5	c.3039C>	p.H1013H	Normal	24.36%	1,255,520	Exonic; ncRNA	synonymous	Benign	Dyskeratozis Congenita	NM_198253.3	5, 11, 13, 16, 17, 21, 24, 28, 30
TERT	5	c.2775C>T	p.H925H	Normal	1.98%	1,264,587	Exonic; Intronic	synonymous	Likely Benign	Dyskeratozis Congenita	NM_198253.3	18
TERT	5	c.2751G>A	p.T917T	Normal	0.02%	1,264,611	Exonic; Intronic	synonymous	Uncertain Significance	Dyskeratozis Congenita	NM_198253.3	17
TERT	5	c.1590G>A	p.P530P	Normal	0.002%	1,282,723	Exonic; ncRNA	synonymous	Uncertain Significance	Dyskeratozis Congenita	NM_198253.3	17
TERT	5	c.915G>A	p.A305A	Normal	47.88%	1,294,086	Exonic; ncRNA	synonymous	Benign	Familial Pancreatic Cancer	NM_198253.3	2, 3, 4, 5, 10, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30
MGMT	10	c.-28C>T	p.R22R	Normal	7.82%	131,265,545	5'UTR; Exonic	synonymous	Benign	Familial Pancreatic Cancer	NM_002412.5	1, 3, 10, 16, 24, 25
MGMT	10	c.159C>T	p.L53L	Normal	22.80%	131,506,192	Exonic	synonymous	Benign	Cancers and Tumors	NM_002412.5	1, 10, 16, 18, 19, 21, 25, 30

Table 8. (Continuing)

Gene Symbol	Chromosome	Transcript Variant	Protein Variant	Normal	Population Frequency	Chromosome	Gene Region	Translation Impact	Classification	Phenotype	Transcripts	Patient ID
MGMT	10	c.250C>T	p.L84F	Normal	23.17%	131,506,283	Exonic	missense	Benign	Cancers and Tumors	NM_002412.5	1, 10, 16, 18, 21, 25, 30
MGMT	10	c.392G>A	p.G131E	Loss	0.001<%	131,557,583	Exonic	missense	Uncertain Significance	Familial Pancreatic Cancer	NM_002412.5	25
MGMT	10	c.427A>G	p.I143V	Normal	12.67%	131,565,064	Exonic	missense	Benign	Familial Pancreatic Cancer	NM_002412.5	4, 12, 21,22,25,30
MGMT	10	c.526T>C	p.L176L	Normal	0.28%	131,565,163	Exonic	synonymous	Likely Benign	Familial Pancreatic Cancer	NM_002412.5	19
MGMT	10	c.533A>G	p.K178R	Normal	12.81%	131,565,170	Exonic	missense	Benign	Cancers and Tumors	NM_002412.5	4, 12, 21,25,30
IDH2	15	c.996C>T	p.S332S	Normal	20.87%	90,628,591	Exonic	synonymous	Benign	Cancers and Tumors	NM_002168.4	8, 16, 30
IDH2	15	c.419G>A	p.R140Q	Gain	0.01%	90,631,934	Exonic	missense	Pathogenic	Cancers and Tumors	NM_002168.4	12
ATRX	X- Linked	c.7140C>T	p.Y2380Y	Normal	0.04%	76,764,054	Exonic	synonymous	Likely Benign	X- linked Alpha Thalassemia	NM_138270.5	15
ATRX	X- Linked	c.5465A>G	p.N1822S	Normal	1.03%	76,856,021	Exonic	missense	Likely Benign	X- linked Alpha Thalassemia	NM_138270.5	14
ATRX	X- Linked	c.2671C>G	p.E891E	Normal	87.73%	76,937,963	Exonic	missense	Benign	X- linked Alpha Thalassemia	NM_138270.5	2, 3, 4, 8, 9, 10, 11, 13, 16, 18, 19, 21, 22, 23, 24, 25, 26, 27, 30

Table 9. Number of Anomalies in Chromosome

	Category	n(%)	Median (range)
Number of chromosome anomalies	*All*	*30(100)*	*2.5(0-8)*
	0	1(3.3)	
	1-3	19(63.3)	
	4-6	7(23.3)	
	≥7	3(10)	

Table 10. Three -Year Survival Rates of Patients

			3-Year Overall Survival(OS)		
Variables	***Category***	**n**	**%**	***χ2***	***p-value***
Age group	*<60 vs ≥60*	17 vs 13	23.5% vs 7.7%	1.647	0.199
Gender	*male vs female*	20 vs 10	5% vs 40%	**9.687**	**0.002***
IDH	*positive vs negative*	24 ve 6	12.5% vs 33.3%	0.764	0.382
MGMT	*positive vs negative*	25 vs 5	8% vs 60%	**6.087**	**0.014***
EGFR	*positive vs negative*	21 vs 9	9.5% vs 33.3%	**7.181**	**0.007***
C532GA	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C315CT	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C1234CT	*positive vs negative*	1 vs 29	100% vs 13.8%	-	-**
C3324GA	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C3039C	*positive vs negative*	9 vs 21	33.3% vs 9.5%	1.178	0.278
C2775CT	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C2751GA	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C1590GA	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C915GA	*positive vs negative*	22 vs 8	18.2% vs 12.5%	0.075	0.785
C28CT	*positive vs negative*	6 vs 24	50% vs 8.3%	1.963	0.161
C159CT	*positive vs negative*	8 vs 22	37.5% vs 9.1%	3.491	0.062
C250CT	*positive vs negative*	7 vs 23	42.9% vs 8.7%	2.889	0.089
C392GA	*positive vs negative*	1 vs 29	100% vs 13.8%	-	-**
C427AG	*positive vs negative*	6 vs 24	33.3% vs 12.5%	1.132	0.287
C526TC	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C533AG	*positive vs negative*	5 vs 25	40% vs 12%	1.246	0.264
C996CT	*positive vs negative*	3 vs 27	33.3% vs 14.8%	1.273	0.259
C419GA	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C7140CT	*positive vs negative*	1 vs 29	100% vs 13.8%	-	-**
C5465AG	*positive vs negative*	1 vs 29	0% vs 17.2%	-	-**
C2671CG	*positive vs negative*	19 vs 11	21.1% vs 9.1%	1.589	0.207
Number of chromosome anomalies	*≤3 vs >3*	20 vs 10	5% vs 40%	**4.522**	**0.033***

*:p<0.05, Kaplan Meier Analysis (Log rank test)
**:Alt grup sayı yetersizliğinden karşılaştırma yapılmadı

Table 11. Results of Multivariate Cox Regression analysis

		Multivariate- **Overall Survival Analysis**	
Factors**	**Category**	**HR (95%CI)**	***p-value***
Age	-	1.009(0.982-1.036)	0.528
Gender	Female	Reference (1)	
	Male	4.627(1.654-12.948)	**0.004***
Number of chromosome anomalies	>3	Reference (1)	
	≤3	2.831(1.004-7.981)	**0.049***

***:**p<0.05**,** Cox Regression analysis, (*Method=Enter),* **HR:**Hazard ratio, **CI:**Confidence interval
**: Variables with 10 or more patients in the subgroups were included in the modeling.

Gene Symbol	Transcript Variant
IDH1	c.532G>A
IDH1	c.315C>T
IDH1	c.123-4C>T
IDH2	c.996C>T
IDH2	c.419G>A
TERT	c.3324G>A
TERT	c.3039C>
TERT	c.2775C>T
TERT	c.2751G>A
TERT	c.1590G>A
TERT	c.915G>A

Gene Symbol	Transcript Variant
MGMT	c.-28C>T
MGMT	c.159C>T
MGMT	c.250C>T
MGMT	c.392G>A
MGMT	c.427A>G
MGMT	c.526T>C
MGMT	c.533A>G
ATRX	c.7140C>T
ATRX	c.5465A>G
ATRX	c.2671C>G

Figure 3. Summary of 21 Chromosome Anomalies Detected by The NGS System

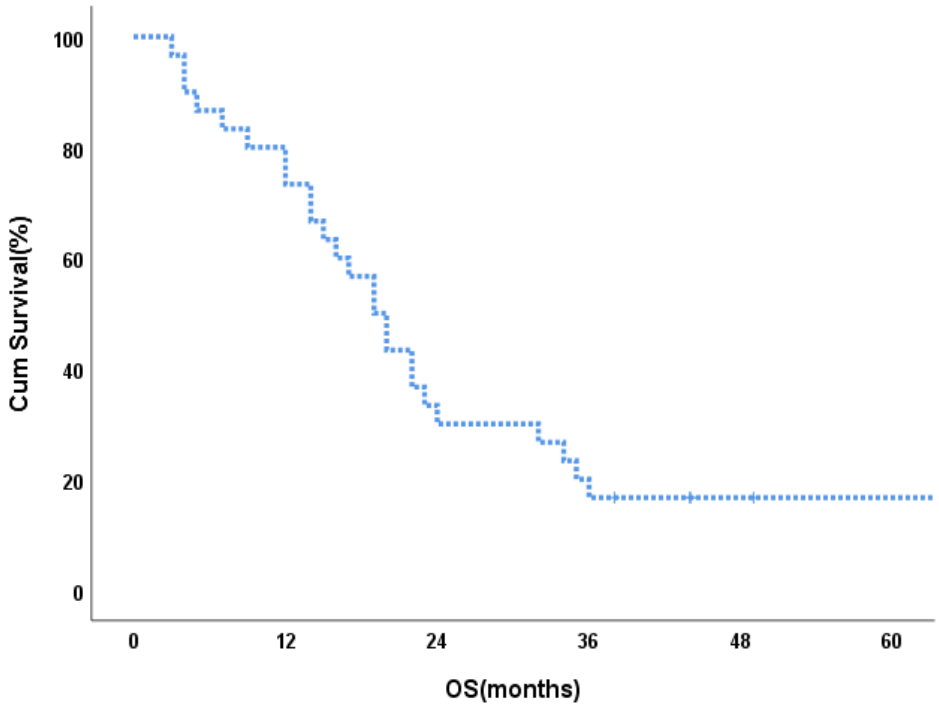

Figure 4. Kaplan-Meier Survival Analysis of 3 Year Overall Survival

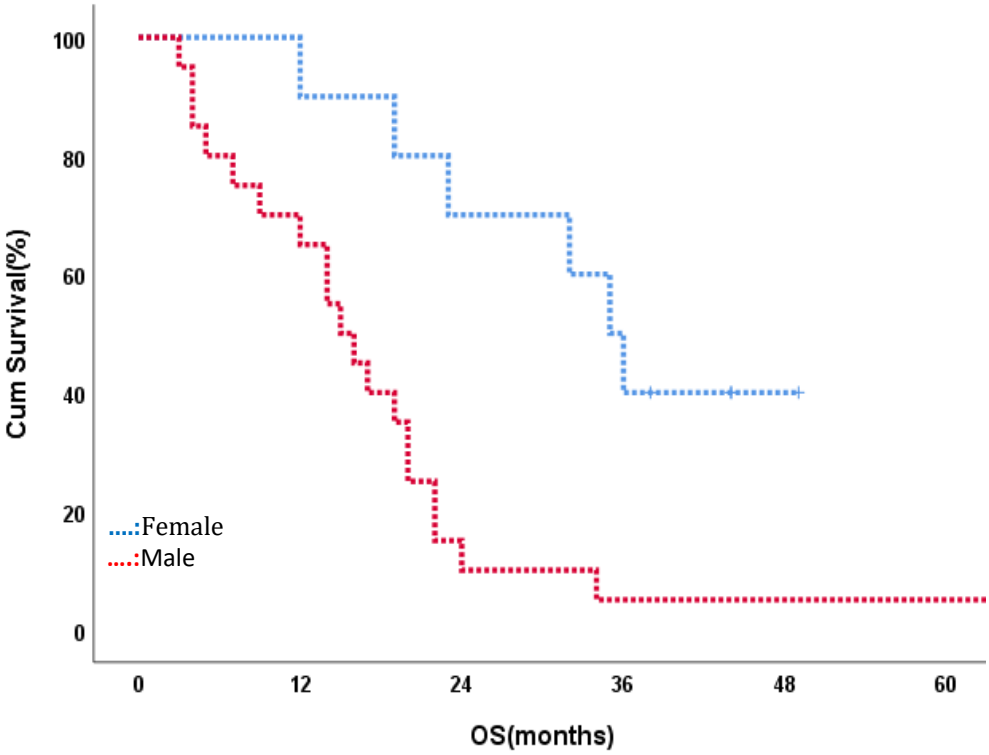

Figure 5. Gender Kaplan-Meier Survival Analysis of 3 Year Survival

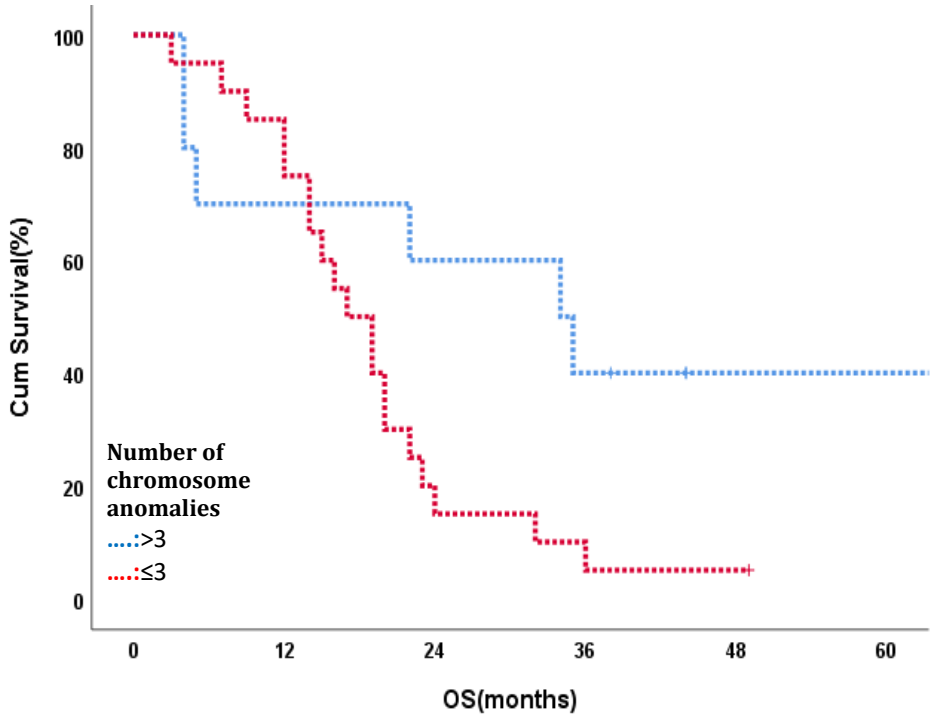

Figure 6. Number of Chromosome Anomalies Kaplan-Meier Survival Analysis of 3 Year Survival

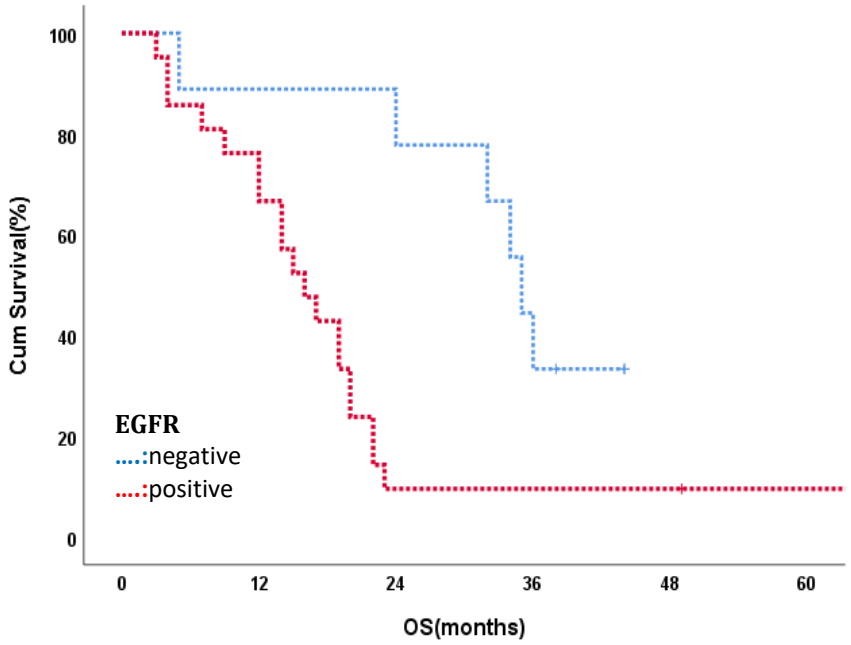

Figure 7. EGFR Kaplan-Meier Survival Analysis of 3 Year Survival

5. DISCUSSION

The histomorphologic examination of brain tumors is still an important initial step in the diagnostic process. However, particularly in light of the revised WHO 2016 classification, molecular investigations are often required for the final correct categorization. Many of the mutations discussed may be found in various gliomas as well as other cancers, thus individual recurrent mutations and copy number aberrations identified in glial tumors are not completely selective or sensitive. A technique with a panel of markers that results in a more comprehensive molecular profile or fingerprint is considerably more robust (60).

To evaluate all important genes, a comprehensive characterisation may now involve several tests (e.g., FISH, IHC, methylation-specific PCR, NGS, etc.). Previous studies demonstrated that a simple, focused NGS test can identify many of the frequent and important GBM changes, including both point mutations and CNVs (61). When compared to traditional methods, next-generation sequencing (NGS) or massively parallel sequencing, a method of simultaneously sequencing millions of fragments of DNA has been quickly adopted in the clinical laboratory due to its ability to analyze multiple genes or gene regions with a single test (62). NGS is mainly used to detect mutations, but it may also detect copy number variations or fusion genes, depending on the technique employed. There is growing interest in using DNA genome-wide methylation-based categorization of central nervous system cancers, which has been shown to have diagnostic sensitivity and clinical utility in a recent series. Each of the more wide molecular diagnostic panels has the benefit of evaluating several molecular features, resulting in more detailed diagnoses. Any new version of these diagnostic panels, of course, must be thoroughly verified before being used in regular clinical diagnosis. At all phases of the process, pathologists, molecular biologists, and physicians should work together as closely as possible.

Acute myeloid leukemia, cholangiocarcinoma, chondroid tumors, and especially lower grade gliomas and secondary glioblastomas (>80%), are among the malignancies in which mutations in the IDH1 and IDH2 genes are infrequent. Citrate metabolism is regulated by IDH1 (cytosol) and IDH2 (mitochondria). Gain-of-function mutations occur in single amino acid changes in arginine at position 132 of IDH1 or the similar hotspots R172 and R140 of IDH2.

IDH1, IDH2, BRAF, ATRX, p53, TERT including promoter area, H3F3A, EGFR amplification or mutation, CDKN2A loss, and copy number data on chromosomes 1, 7, 10, and 19 are some of the key markers that may help characterize a glioma's genetic profile. The majority of them are well-known and are linked to or define WHO organizations (59). The NGS panel utilized evaluates mutations in MGMT, TERT, ATRX, CIC, EGFR, FUBP1, NOTCH1, PTEN; H3F3A, IDH1/2, PIK3CA and BRAF; EGFR, MDM2 and MET amplifications; and CNAs of chromosome 1p, 19q, 10, and 7 as reported previously (63, 64). IDH1 and IDH2 gene sequencing is therefore a more sensitive technique for detecting these critical alterations (60).

Despite the fact that NGS results in diffuse glioma had previously been reported, just few of these prior studies included data on the diagnostic usefulness of the NGS panel and provide survival data. Synhaeve et al. Reported that the diagnostic strength of the platform they created is confirmed on a large cohort of patients, with a clear separation of glioma subgroups with varied outcomes. It shows the diagnostic usefulness and effectiveness of glioma-targeted NGS for routine glioma diagnostics, enabling a glioma diagnosis to be made in the vast majority of patients with a single test. They also include survival data on this study. Using an NGS pattern that allows for the simultaneous evaluation of multiple important markers is a reliable and efficient method of diagnosing brain cancers, allowing for a fast diagnosis according to the WHO 2016 classification of brain tumors (65). Several studies have shown that NGS panels have a high sensitivity for detecting genetic changes that are known to be present using traditional methods (66-68). In addition, Zacher et al. utilized a 20-gene panel for an integrated histological and molecular diagnosis of 111 diffuse gliomas, allowing for the categorization of oligoastrocytoma and glioblastoma based on IDH-status and the identification of tumors with H3F3A mutations (69). Ballester et al. utilized a large NGS panel in 381 brain cancers and discovered that IDH1, IDH2, TP53, PIK3CA, BRAF, EGFR, PDGFRA, and FGFR1/2/3 were the most clinically relevant genes for brain tumor categorization in their panel (70). In a prospective cohort, Sahm et al. discovered that information obtained from their NGS procedure revealed promising targets for experimental treatment (EGFR, BRAF, PTEN) in glioblastomas, pilocytic astrocytomas, and medulloblastomas (68). NGS can be performed with blood sample, frozen tissue or formalin fixed tissue (71, 72). All of these investigations used formalin-

fixed tissue for sequencing. In our study we use blood samples of the GBM patients. In our study we perform sequencing with blood samples.

IDH1, IDH2, BRAF, ATRX, p53, TERT including promoter area, H3F3A, EGFR amplification or mutation, CDKN2A loss, and copy number data on chromosomes 1, 7, 10, and 19 are some of the key markers that may help characterize a glioma's genetic profile. We select 5 genes to perform sequencing: IDH1, IDH2, TERT, ATRX, MGMT. We found 3 mutations in IDH 1, 2 in IDH2, 6 in TERT, 7 in MGMT, and 3 in ATRX.

In glioblastoma multiforme, a recurrent heterozygous somatic mutation in the gene encoding cytosolic isocitrate dehydrogenase 1 (IDH1) changes residue R132 in the enzyme's active region (36). The enzyme loses its capacity to convert isocitrate to 2-ketoglutarate (2-KG) and gains the ability to convert 2-KG to D-2-hydroxyglutarate (D-2-HG) as a result of this mutation (73). Other tumor forms, such as acute myeloid leukemia and gliomas, have been shown to contain heterozygous mutations affecting mitochondrial isocitrate dehydrogenase 2 (IDH2) residues R140 and R172, the latter matching to R132 of IDH1. These mutations also cause abnormal D-2-HG production (74, 75).

The most common mutations in invasive glioma were TP53, TERT, IDH1, PTEN, ATRX, and EGFR (76, 77) in literature. IDH mutations are common in diffuse gliomas of the astrocytic and oligodendroglial lineages. IDH mutations are often seen in astrocytic gliomas with mutations in the tumor protein p53 (TP53) and the ATRX genes (66, 78). In contrast, IDH mutations are linked with chromosomal arms 1p and 19q codeletion, as well as common mutations in the Drosophila homolog of capicua transcriptional repressor (79) gene and the telomerase reverse transcriptase (TERT) promoter (66, 78). IDH mutations were discovered in a younger age group and were linked to MGMT methylation. There were two different group of molecular profiles in IDH Mutant DGs. Grade II–III AC and GBM both contain genetic abnormalities such TP53 mutations and ATRX deletion, while GBMs have a higher rate of other genetic alterations. The findings back with the hypothesis that IDH, TP53, and ATRX mutations occur early in the genesis of AC, with tumors developing as additional events occur (80, 81) In Grade II–III ODs, TERT mutation and 1p/19q-codeletion were identified, but no TP53 mutation or ATRX loss. Mutations in Notch1 and ARID1A

were also more prevalent in ODs than in ACs, supporting previous findings that these genes were commonly altered in a subset of ODs. EGFR amplification or combined whole chromosome seven gain and whole chromosome 10 loss or TERT promoter mutation in IDH wild type diffuse astrocytic glioma with an aggressive clinical course (66, 81). H3F3A, IDH1/2, and pTERT multiplexed dPCR tests, according to Appay et al, may be especially helpful in the setting of high-grade diffuse gliomas, when screening for the targeted molecular change is required for an appropriate diagnosis (82).

The current dataset on anaplastic oligodendroglioma locally diagnosed with long follow-up data demonstrates the power of targeted NGS for the routine molecular categorization of diffuse glioma in regular practice. In a previous study tumor samples were effectively examined despite the lengthy period spent in paraffin, with 95% of them being recognized molecularly as one of three types of cancer: type I (oligodendroglioma), type II (astrocytoma), or type III (glioblastoma) (83). There were significant differences in survival rates between patients with molecularly defined tumor classes, but in this small group, median survival of 3.1 years and 5-year survival of 47% in the type II group were not statistically significant from median survival of 9.5 years and 5-year survival of 71% found in the type I group. Although there have been sporadic reports, the most recent investigations consistently indicate that genuine mixed glioma tumors do not occur at the molecular level (84-86).

Aside from encouraging genetic research, the clinical significance of identifying a mixed phenotype is unknown. Molecular classification was shown to have more prognostic value than histological classification in predicting survival. Neither the overall prognosis of 1p/19q codeleted tumors nor the response to PCV treatment was affected by the presence of CIC, FUBP, NOTCH, TP53, or other abnormalities in type I tumors. The existence of contrast enhancement, necrosis, and microvascular proliferation are all linked with chromosomal copy number changes rising in 1p/19q codeleted oligodendrogliomas (87, 88).

The fact that various criteria have been suggested for separate molecularly defined subtypes of diffuse glioma has been blamed for the difficulty in identifying the illness. For example, it's unclear whether the loss of 1p/19q codeletion is sufficient for the molecular diagnosis of type I, or oligodendroglioma; whether ATRX mutations are

required for the diagnosis of type II, or astrocytoma; and whether type III, or glioblastoma, is best defined as gain of Chr 7 and loss of 10 or the presence of TERT mutations in the absence of 1p/19q codeletion. Only IDH and TERT gene mutations, as well as IDH mutations, 1p/19q, and LOH 10q with Chr 7 imbalance, provide prognosis categories that are extremely similar.

Examining additional mutations and copy number variations has the benefit of increasing molecular diagnostic confidence by discovering consistent molecular results in a tumor. Indeed, the mutual exclusivity of certain findings (such as ATRX and TERT mutations, IDH mutations, and EGFR amplifications) and the presence of findings that usually occur together (such as CIC mutations in 1p/19q codeleted cases, TP53 mutations in IDH mutated but 1p/19q intact tumors, and TERT and IDH mutations in 1p/19q codeleted tumors) contribute to the strengthening of the classification of individual tumors. Identifying 1p/19q codeleted tumors lacking oligodendroglial characteristics has lately come up in discussions. Evidence suggests that in 1p/19q codeleted instances, the lack of TERT and IDH mutations may be more important, since they are rare. Similarly, TP53 mutations were found in almost all IDHmut cancers except for 1p/19q intact tumors, and an ATRX mutation was found in the lone TP53wt tumor. This group had a lower percentage of ATRX mutations (65 percent), which is in line with previous research (58, 89).

A greater proportion of tumors had a 1p/19q codeletion, compared to earlier FISH results, and there was a significant connection between this finding and other anomalies (TERT mutations, IDH mutations). IDH and TERT mutations have been identified in cancers with 1p/19q codeletion at equally high rates elsewhere (52, 79, 90). These findings indicate that NGS is more sensitive than our prior diagnoses, with FISH. Better prognosis and treatment outcome prediction are two possible benefits of molecular categorization.

PCV treatment seems to be beneficial to individuals with malignancies other than those with 1p/19q codeletion in the North American RTOG 9402 trial and the EORTC 26951 research. According to the RTOG 9402 trial, patients with IDH mutations benefited with PCV in 74% of instances, which was confirmed by testing. Preliminary findings from the EORTC 26951 study suggested that CIMP—and specifically, MGMT promoter methylation as assessed with the Illumina H450

Beadchip—were highly predictive of improved outcome following PCV, whereas IDH status was insufficient to identify patients who benefited from PCV chemotherapy (91).

A distinction between RTOG 9402 and EORTC 26951 is that in the EORTC dataset, only 50 percent of patients had IDH mutations, and in the current study 41 percent of the tumors were categorized as type III, or glioblastoma. In RTOG 9402, 74 percent of the studied patients exhibited an IDH mutation (92).

Taken together, the results indicate that the greatest predictive predictor for benefit is MGMT promoter methylation, although 95 percent of IDH mutant tumors with MGMT status available had MGMT promoter methylation. This seems to reconcile the observed discrepancies between the North American and European research (92, 93).

Notably, both EORTC 26951 and RTOG 9402 studies of IDH and MGMT are retrospective in nature. The current CATNON trial, which is evaluating temozolomide treatment in 1p/19q intact grade III malignancies, will provide further insight into the best prognostic marker. Notably, 41% of the sequenced tumors were glioblastoma from a molecular viewpoint and would have been excluded if molecular criteria had been employed. Recent research on molecular profiling of low-grade and grade III gliomas revealed a strikingly similar finding, with 15%–20% of grades II and III gliomas exhibiting a glioblastoma-like profile (66, 94).

One of the first recognized carcinogenic genes discovered in GBM is the EGFR gene. EGFR amplification was not linked with prolonged progression-free survival in Carter's cohort, but amplifications were enriched in a group with slightly longer overall survival despite radiographic signs of disease progression, according to Carter et al. (80). In our study, we found that EGFR positive patients had a lower survival than negative patients. This data was found to be similar to the literature.

The lower the frequency of the genetically determined mutation in the population, the more likely it is to be a pathogen. As an example the rate of occurrence of the missence c419 G>A mutation detected on the exon of chromosome 15 on the IDH2 gene in the population was determined as 0.01% and is is cited as pathogenic. Previously it was reported in D-2-hydroglutaric aciduria, type 2 disease (95-97). We also perform a survival analysis to evaluate the effect the number of mutations on

survival. We found that patients with 3 or less mutations had a shorter survival time. Another example the rate of occurrence of the missence c392 G>A mutation detected on the exon of chromosome 10 on the MGMT gene in the population was determined as less than 0.001%. This mutation was described in familial pancreatic cancer previously. In our sequencing process we found c7140 C>T mutation on ATRX. This X linked mutation was described on X linked alpha thalassemia. We also find a TERT mutation on chromosome 5. C2751 G>A change was discribed on Dyskeratosis Congenita.

A liquid biopsy is a blood test that looks for cancer cells from a tumor circulating in the bloodstream or fragments of DNA from tumor cells in the bloodstream. A liquid biopsy may aid in the detection of cancer at an early stage. It may also be used to aid in treatment planning, as well as to determine how well therapy is working and if cancer has returned. The ability to collect numerous blood samples over time may also aid physicians in determining what molecular alterations are occurring in a tumor. The goal of our research was to utilize NGS as a liquid biopsy to assess molecular alterations and predict patient survival. It is the first publication that the samples which were used in NGS were studied from blood tissue. The data we obtained were compatible with the literature. The importance of molecular data has increased in the WHO classification of CNS tumors. Targeted NGS makes enables to have detailed information about the diagnosis and survival in glioma patients. The limitation of this study is the small number of patients and the absence of a control group. Therefore, studies with larger populations including the control group are needed in the near future.

6. CONCLUSION

Targeted NGS enables glial tumor categorization that is both accurate and therapeutically meaningful, providing better prognostic information than conventional histology. There is evidence to suggest that these cancers should be reclassified using molecular markers, although how this should be done in clinical practice is still up for debate. However, it's obvious that incorporating molecular diagnostics into our standard-of-care routine will help us better understand our patients' outcomes.

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7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2227
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

14/10/2021

İlgili Makama (Cumhur Kaan Yaltırık)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, Beyin ve Sinir Cerrahisi AD.Dr. Öğr. Üyesi Cumhur Kaan Yaltırık'ın sorumlu araştırmacı olduğu **"Glial Tümörlerde IDH1 , IDH2 , MGMT, TERT , ATRX ve H3F3A Gen Mutasyonlarının Belirlenmesi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2288)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **13.10.2021** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir **(KAEK Karar No: 1512)**

Prof. Dr. Hasan AYDIN

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkan Yrd.

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7.2. CIRRUCULUM VITAE

Personal Informations

Name	CUMHUR KAAN	**Surname**	YALTIRIK
Place of Birth	Adana		
Nationality	T.C.		
Email			

Educational Informations

	Name of Institution	**Year**
Doctorate	Yeditepe University Health Institute Molecular Medicine	**2017- 2021**
Medical Expertise	**Yeditepe University School of Medicine, Department of Neurosurgery, İstanbul**	**2006-2012**
Medical School	**İstanbul University, İstanbul School of Medicine**	**1998- 2004**

Work Experience

Neurosurgeon	İstanbul Training and Research Hospital	2020- ...
Neurosurgeon (Ass. Proffesor)	Yeditepe University School of Medicine	2015- 2020
Neurosurgeon	Yeditepe University School of Medicine	2013- 2015
Neurosurgeon	Çaycuma State Hospital	2012- 2013

Language	Reading*	Speaking*	Writing*	KPDS/UDS Score	(17) Score
English	Good	Good	Good		YOKDIL 92,5

*****Very Good, Good, Basic

Computer skills

Program	Ability to use
Microsoft Office	Very Good

Publications / Notices Certificates / Awards

A –International Articles

A.1 Serra Carlo, Türe Hatice, Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Türe Ugur (2020). Microneurosurgical removal of thalamic lesions: surgical results and considerations from a large, single-surgeon consecutive series. Journal of Neurosurgery, 1-11., Doi: 10.3171/2020.6.JNS20524

A.2 Özdogan Selçuk, Yaltirik Cumhur Kaan, Yilmaz Seda Güleç, Akdeniz Fatma Tuba, Sümerkent Kadir, Düzkalir Ali Haluk, Türe Ugur, Isbir Turgay (2020). Investigation of the Effects of MicroRNA-221 Expression Levels in Glioblastoma Multiforme Tumors. Anticancer Research, 40(6), 3265-3270., Doi: 10.21873/anticanres.14308

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A.4 Tüysüz Emre Can, Güllüoglu Sükrü,Yaltirik Cumhur Kaan, Özbey Utku, Kuskucu Aysegül, Çoban Esra Aydemir, Sahin Fikrettin, Türe Ugur, Bayrak Ömer Faruk (2019). Distinctive role of dysregulated miRNAs in chordoma cancer stem-like cell maintenance. Experimental Cell Research, 380(1), 9-19., Doi:10.1016/j.yexcr.2019.03.039

A.5 Yaltirik Cumhur Kaan, Kahraman Özlem Timirci, Yilmaz Seda Güleç, Özdogan Selçuk, Atalay Basar, Isbir Turgay (2019). The Evaluation of Proteoglycan Levels and the Possible Role of ACAN Gene (c.6423T C) Variant in Patients with Lumbar Disc Degeneration Disease. In Vivo, 33(51), 413-417., Doi: 10.21873/invivo.11488

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A.7 Lovato Renan Maximilian, Araujo Joaoluiz Vitorino, Paiva Aline Lariessy Campos, Pesente Francisco
Spessatto, Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Esteves Veiga José Carlos (2019). The use of osirix for surgical planning using cranial measures and region of interest tools: Technical note. Asian Journal of Neurosurgery, 14(3), 762-766., Doi: 10.4103/ajns.AJNS_63_19

A.8 Kuskucu Aysegül, Tüysüz Emre Can, Gürkan Sezin, Demir Zeynel, Yaltirik Cumhur Kaan, Özkan Ferda, Ekici Isin Dogan, Bayrak Ömer Faruk, Türe Ugur (2019). Co-polysomy of 1p/19q in glial tumors: Retrospective analysis of 221 cases from single center. GENE, 701, 161- 168., Doi: 10.1016/j.gene.2019.02.073

A.9 Bakirezer Selvi Duman, Yaltirik Cumhur Kaan, Kaya Ahmet Hilmi, Yilmaz Seda Güleç, Özdogan Selçuk, Billur Deryanaz, Isbir Turgay (2019). The Evaluation of Glutathione Reductase and Malondialdehyde Levels in Patients With Lumbar Disc Degeneration Disease. In Vivo, 33(3), 811-814., Doi: 10.21873/invivo.11543

A.10 Güllüoglu Sükrü, Tüysüz Emre Can, Sahin Mesut, Yaltirik Cumhur Kaan, Kuskucu Aysegül, Özkan Ferda, Dalan Altay Burak, Sahin Fikrettin, Türe Ugur, Bayrak Ömer Faruk (2019). The role of TNF-alpha in chordoma progression and inflammatory pathways. Cellular Oncology, 42(5), 663-677., Doi: 10.1007/s13402-019-00454-y

A.11 Topçuoglu Osman Melih, Yaltirik Cumhur Kaan, Firat Zeynep, Sarsilmaz Aysegül, Harput Mehmet Volkan, Sarikaya Basar, Türe Ugur (2019). Limited positive predictive value of diffusion tensor tractography in determining clinically relevant white matter damage in brain stem cavernous malformations: A retrospective study in a single center surgical cohort. Journal Of Neuroradiology, Doi: 10.1016/j.neurad.2019.07.005

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A.13 Özdogan Selçuk, Yaltirik Cumhur Kaan, Yilmaz Seda Güleç, Kaya Mustafa, Düzkalir Ali Haluk, Demirel Nail, Kafadar Ali, Isbir Turgay (2018). Tumor Necrosis Factor-Alpha (TNFalpha-308G A) Polymorphism in High-grade Gliomas. In Vivo, 32(51), 287-289., Doi: 10.21873/invivo.11236

A.14 Güllüoglu Sükrü, Tüysüz Emre Can, Sahin Mesut, Kuskucu Aysegül, Yaltirik Cumhur Kaan, Türe Ugur, Küçükkaraduman Bails, Akbar Muhammad Waqas, Güre Ali Osmay, Bayrak Ömer Faruk, Dalan Altay Burak (2018). Simultaneous miRNA and mRNA transcriptome profiling of glioblastoma samples reveals a novel set of OncomiR candidates and their target genes. Brain Research, 1700, 199-210., Doi: 10.1016/j.brainres.2018.08.035

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A.16 Yaltirik Cumhur Kaan, Özdogan Selçuk, Atalay Basar (2017). Thoracic Epidural Hematoma Complicating Vertebroplasty. American Journal of Case Reports, 18, 1229-1232., Doi: 10.12659/AJCR.907385

A.17 Yaltirik Cumhur Kaan, El Tecle Najib E., Pierson Matthew J., Puryear Aki, Atalay Basar, Elbabaa Samer K. (2017). Management of concomitant scoliosis and tethered cord syndrome in non-spina bifida pediatric population. Childs Nervous System, 33(11), 1899-1903., Doi: 10.1007/s00381-017-3504-0

A.18 Özdogan Selçuk, Sayman Ceyhun, Yaltirik Cumhur Kaan, Düzkalir Hanife Gülden, Kaya Mustafa, Düzkalir Ali Haluk, Sarikaya Basar, Aktekin Berrin (2017). Encephalocraniocutaneous Lipomatosis: Haberland Syndrome. American Journal of Case Reports, 18, 1271-1275., Doi: 10.12659/AJCR.907685

A.19 Güllüoglu Sükrü, Sahin Mesut, Tüysüz Emre Can, Yaltirik Cumhur Kaan, Kuskucu Aysegül, Özkan Ferda, Sahin Fikrettin, Türe Ugur, Bayrak Ömer Faruk (2017). Leukemia Inhibitory Factor Promotes Aggressiveness of Chordoma. Oncology Research, 25(7), 1177-1188., Doi: 10.3727/096504017X14874349473815

A.20 Özdogan Selçuk, Kaya Mustafa, Demirel Nail, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan (2017). Isolated C5 Vertebrae Dislocation with Trauma: An Extremely Rare Case of Isolated C5 Dislocation. American Journal of Case Reports, 18, 1256-1260., Doi: 10.12659/AJCR.907396,

A.21 Dalan Altay Burak, Güllüoglu Sükrü, Tüysüz Emre Can, Kuskucu Aysegül, Yaltirik Cumhur Kaan, Öztürk Oguz, Türe Ugur, Bayrak Ömer Faruk (2017). Simultaneous analysis of miRNA-mRNA in human meningiomas by integrating transcriptome: A relationship between PTX3 and miR-29c. BMC Cancer, 17(207), Doi: 10.1186/s12885-017-3198-4

A.22 Yaltirik Cumhur Kaan, Ashour Ahmed M., Reis Conner R., Özdogan Selçuk, Atalay Basar (2016). Vertebral augmentation by kyphoplasty and vertebroplasty: 8 years experience outcomes and complications. Journal of Craniovertebral Junction and Spine, 7(3), 153-160., Doi: 10.4103/0974-8237.188413

A.23 Yaltirik Cumhur Kaan, Özdogan Selçuk, Ekici Isin Dogan, Atalay Basar (2016). Cauda equina cavernous hemangioma: very rare pediatric case. Childs Nervous System, 32(12), 2289- 2291., Doi: 10.1007/s00381-016-3286-9

A.24 Is Merih, Yaltirik Cumhur Kaan (2007). Mydriasis as a falsely localizing sign in epidural hematoma - A case report. Neurosurgery Quarterly, 17(1), 74-75., Doi: 10.1097/WNQ.0b013e318033a663

B-National Articles

B.1 Yaltirik Cumhur Kaan, Yilmaz Seda Güleç, Akdeniz Fatma Tuba, Sümerkent Kadir, Özdogan Selçuk, Isbir Turgay (2020). Lomber Dejeneratif Disk Hastalıgında Glutatyon Peroksidaz (GSH-Px) Düzeylerinin Prognostik Önemi. Experimed, 10(51), 67-71., Doi: 10.26650/experimed.2020.0011

B.2 Yaltirik Cumhur Kaan,Yüceli Sahin (2019). Spinal Arachnoid Cysts. Journal Of Turkish Spinal Surgery, 30(3), 207-210

B.3 Yüceli Sahin, Yaltirik Cumhur Kaan (2019). Cervical Spinal Alignment Parameters. Journal of Turkish Spinal Surgery, 30(3), 181-186

B.4 Yaltirik Cumhur Kaan, Kaya Mustafa (2018). Thoracolumbar Fracture Surgeries: Analysis With Thoracolumbar Injury Classification and Severity Score (Tlics). Journal of Turkish Spinal Surgery, 29(51), 107-113

B.5 Yaltirik Cumhur Kaan (2018). Spinal Anesthesia for Elective Lumbar Spine Surgery: Is it effective?. Journal of Turkish Spinal Surgery, 29(3), 177-181

B.6 Yilmaz Seda Güleç, Yaltirik Cumhur Kaan, Altinkiliç Emre Murat, Isbir Turgay (2018). Relation Between Lumbar Degenerative Disc Disease and Human 8-hydroxydeoxyguanosine (8- OHDG) Serum Level. Journal of Turkish Spinal Surgery, 29(1), 1-3

B.7 Yaltirik Cumhur Kaan, Atalay Basar (2018). Osteoporotik Omurga Kırıklarında Vertebroplasti ve Kifoplasti. Türkiye Klinikleri Nörosirürji - Özel Konular, 8(1), 30-25

B.8 Özdogan Selçuk, Köken Murat, Düzkalir Hanife Gülden, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan, Öztürk Erek, Savrunlu Eyüp Can, Civelek Erdinç, Kabatas Serdar (2017). Brief Review on Fundamentals of Cervical Spine Alignment. The Journal of Turkish Spinal Surgery, 28(1), 53-60

B.9 Yaltirik Cumhur Kaan, Atalay Basar (2017). Nörodejeneratif Hastalıklar ve Fiziksel Aktivite. Türkiye Klinikleri Farmakoloji - Özel Konular, 5(1), 43-49

B.10 Özdogan Selçuk, Köken Murat, Düzkalir Hanife Gülden, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan, Öztürk Erek, Savrunlu Eyüp Can, Civelek Erdinç, Kabatas Serdar (2017). Measurement of Cervical Lordosis with Different Methods. Journal of Turkish Spinal Surgery, 28(1), 21-26

B.11 Yaltirik Cumhur Kaan, Fard Ayda Parnian, Kontaytekin Kumsal Bihter (2017). Classification of Metastatic Spinal Tumors. Journal of Turkish Spinal Surgery, 28(4), 277-282

B.12 Demirel Nail, Gül Alptekin, Gergin Sinem, Düzkalir Hanife Gülden, Basaran Recep, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan, Özdogan Selçuk (2016). Craniovertebral Junction Morphometic Evaluation With Reconstructive Computed Tomography. Journal of Turkish Spinal Surgery, 27(1), 13-17

B.13 Güdü Burhan Oral, Yaltirik Cumhur Kaan, Çiftçi Engin, Fard Ayda Parniayan, Atalay Basar (2016). Open and Endoscopy Assisted Trigonocephalus Surgery: Report of Two Cases. Yeditepe Medical Journal, 37, 993-998., Doi: 10.15659/yeditepemj.17.01.379

B.14 Özdogan Selçuk, Gür Erdal, Süslü Hüsnü, Tiryaki Mehmet, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan (2015). Failed Back Surgery Syndrome. Journal of Turkish Spinal Surgery, 26(3), 239-244

B.15 Özdogan Selçuk, Yaltirik Cumhur Kaan, Atalay Basar, Tiryaki Mehmet, Düzkalir Ali Haluk, Süslü Hüsnü (2015). Unilateral Approach for Bilateral Spinal Microdecompression in Lumbar Spinal Stenosis: Short Term Results. Journal of Turkish Spinal Surgery, 26(3), 201-206

B.16 Düzkalir Ali Haluk, Özdogan Selçuk, Tiryaki Mehmet, Alptekin Gül, Yaltirik Cumhur Kaan, Köken Murat (2015). Complications Of The Percutaneous Vertebroplasty. Journal of Turkish Spinal Surgery, 26(3), 245-252

B.17 Süslü Hüsnü, Yaltirik Cumhur Kaan, Özdogan Selçuk, Senol Özgür, Tatarli Necati, Düzkalir Ali Haluk, Düzkalir Hanife Gülden (2015). Epiduroscopy Usage for Pain Management of Failed Back Surgery Syndrome. Journal of Turkish Spinal Surgery, 26(51), 128- 134

B.18 Düzkalir Ali Haluk, Özdogan Selçuk, Demirel Nail, Yaltirik Cumhur Kaan (2015). Complications of Anterior Cervical Spine Surgery: Review of The Literature. Journal of Turkish Spinal Surgery, 26(4), 305-312

B.19 Süslü Hüsnü, Düzkalir Hanife Gülden, Özdogan Selçuk, Senol Özgür, Tatarli Necati, Düzkalir Ali Haluk, Yaltirik Cumhur Kaan (2015). Fluoroscopy Guided Transforaminal Steroid Injection On Cervical Radicular Pain. Journal of Turkish Spinal Surgery, 26(51), 121-128

B.20 Atalay Basar,Yaltirik Cumhur Kaan (2015). Kraniyovertebral Bileske Biyomekanigi ve Stabilizasyon Prensipleri. Türk N.rosirürji Dergisi, 25(51), 96-103

B.21 Türe Hatice, Keskin Özgül, Köner Özge, Bilgen Sevgi, Uztüre Neslihan, Yaltirik Cumhur Kaan, Tekin Kivilcim, Türe Ugur (2014). Anesthetic management of Awake craniotomy Our Standardised Protocol and Review of The Literature. Yeditepe Medical Journal, 31, 794-806., Doi: 10.15659/yeditepemj.15.10.139

B.22 Koner Özge, Haliloglu Murat, Yaltirik Cumhur Kaan, Atalay Basar (2012). Trapping of the Epidural Catheter Between Lumbar Facet Joints: Identification By Ct and A Simple Removal Technique. Yeditepe Medical Journal, 23, 551-554., Doi: 10.15659/yeditepemj.15.11.275

B.23 Atalay Basar, Yaltirik Cumhur Kaan, Harput Mehmet Volkan (2011). Patient-Physician Relationship and The Informed Consent. Yeditepe Medical Journal, 17, 349-352., Doi: 10.15659/yeditepemj.15.10.194

C – International Oral Presentations

C.1 Bilmez Banu Saçli,Firat Zeynep,Topçuoglu Osman Melih,Yaltirik Cumhur Kaan,Türe Ugur,Isik Esin Öztürk (2020). Identifying Overall Survival in Glioblastoma Patients Using VASARI Features at 3T. 2020 ISMRM SMRT Virtual Conference Exhibition

C.2 Yilmaz Seda Güleç, Yaltirik Cumhur Kaan (2018). Investigation of 8-hydroxydeoxyguanosine (8-OHdG) levels as the marker of oxidative stress in Glial Tumors x. 2nd International Congress on Advances in Bioscience and Biotechnology (ICABB)

C.3 Yaltirik Cumhur Kaan, Atalay Basar, Ekici Isin Dogan (2015). Cauda Equina Cavernous Hemangioma: Technical Note. 15th Interim Meeting of The World Federation of Neurosurgical Societies

C.4 Atalay Basar, Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Tekin Kivilcim, Türe Ugur (2015). Unilateral Microscopic Approach For Intradural Spinal Tumors. ISMISS Turkey 2015

C.5 Yaltirik Cumhur Kaan, Özdogan Selçuk, Atalay Basar (2015). Complications After Percutaneus Vertebral Augmentation Procedure. 15 th Interim Meeting of The World Federation of Neurosurgical Societies

C.6 Atalay Basar,Harput Mehmet Volkan,Yaltirik Cumhur Kaan,Koner Özge (2012). Minimally Invasive Vertebral Augmentation in High Risk Patients. 28 th Annual Meeting of the AANS/ CNS Section

C.7 Atalay Basar, Yaltirik Cumhur Kaan, Türe Hatice (2010). Minimal Invasive Vertebral Augmentation in Geriatric Patients with High Comorbidity. 26 th Annual Meeting of the AANS/CNS Section on Disorders of the SPINE And Peripheral Nerves

C.8 Atalay Basar, Yaltirik Cumhur Kaan, Karlikaya GüLden, Türe Hatice, Türe Ugur (2010). Craniocervical Surgery of the Complex Medullary Compression. 26 th Annual Meeting of the AANS/CNS Section on Disorders of the SPINE And Peripheral Nerves

D – National Oral Presentations

D.1 Yaltirik Cumhur Kaan, Atalay Basar (2019). Geriatrik hastalarda lomber spinal stenozun cerrahi tedavisinde unilateral yaklasımla bilateral dekompresyonun yeri. Yaslanan Omurga Sempozyumu

D.2 Yaltirik Cumhur Kaan (2019). Yeni Tanı Almıs Glioblastomlu Hastalarda Cerrahi Rezeksiyon Miktarının Sagkalım Süresi Üzerine Etkisi. 2nd International Hippocrates Congress on Medical and Health Sciences

D.3 Yaltirik Cumhur Kaan, Atalay Basar (2017). Vertebral Augmentation by Kyphoplasty and Vertebroplasty: 8 year experience Outcomes And Complications. 12. International Turkish Spine Congress

D.4 Türe Hatice, Keskin Özgül, Yaltirik Cumhur Kaan, Uztüre Neslihan, Köner Özge, Türe Ugur (2016). Nörosirurjide Oturur Pozisyonda Venöz Hava Embölisinin Saptanmasında Transzefajial Ekokardiyografi ve Prekordiyal Dopplerin Etkinliklerinin Karsılastırılması. Türk Anesteziyoloji Dernegi 50. Ulusal Kongresi

D.5 Türe Hatice, Keskin Özgül, Köner Özge, Bilgen Sevgi, Sancar Nurcan, Yaltirik Cumhur Kaan, Türe Ugur (2014). Sagdan Sola Kardiyak Santı Olan Hastalarda

Transözefajial Ekokardiyografi Monitorizasyonu Ile Oturur Pozisyonda Kraniyotomi Tecrübelerimiz. Türk Anesteziyoloji ve Reanimasyon Dernegi 48. Ulusal Kongresi

D.6 Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Tekin Kivilcim, Atalay Basar (2012). Lomber Füzyonun korkulu rüyası: Psödoartroz. Türk N.rosirürji Dernegi, 26. Bilimsel Kongresi,

D.7 Harput Mehmet Volkan, Yaltirik Cumhur Kaan, Atalay Basar, Köner Özge, Türe Ugur (2011). Ko-Morbiditesi Yüksek Geriatrik Hastalarda Minimal Invasiv Vertebral Güçlendirme. Türk Nörosirurji Dernegi, 25. Bilimsel Kongresi

D.8 Harput Mehmet Volkan, Yaltirik Cumhur Kaan, Atalay Basar, Türe Ugur (2010). Servikal Pedikül Vidalama Tekniginin Anatomik Çalısması. Türk Nörosirurji Dernegi 24. Bilimsel Kongresi

D.9 Yaltirik Cumhur Kaan, Karlikaya Gülden, Harput Mehmet Volkan, Atalay Basar, Türe Ugur (2009). Spinal Cerrahide Nöromonitörlemenin Önemi. Türk Nörosirurji Dernegi 23. Bilimsel Kongresi

D.10 Yaltirik Cumhur Kaan, Tekin Kivilcim, Türe Hatice, Atalay Basar, Türe Ugur (2009). Oksipitoservikal Fiksasyon Deneyimlerimiz. Türk Nörosirurji Dernegi 23. Bilimsel Kongresi

D.11 Karlikaya Gülden, Yaltirik Cumhur Kaan, Çiftçi Beyza, Bingöl Canan, Türe Ugur (2009).
Nörofizyolojinin Yeni Bir Kullanım Alanı: Intraoperatif Kortikospinal Traktus Lokalizasyonu. 25. Ulusal Klinik Nörofizyoloji EEG-EMG Kongresi

D.12 Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Imer Pinar, Atalay Basar (2009).
Vertebroplasti ve Kifoplasti Uygulamalarımız. Türk Nörosirurji Dernegi 23. Bilimsel Kongresi

D.13 Karlikaya Geysu, Yaltirik Cumhur Kaan, Çitçi Beyza, Davulcu Ezgi, Türe Ugur, Bingöl Canan Aykut (2009). Postoperatif Fasial Paralizinin Engellenmesi Amacıyla Fasiyal Sinir Monitorizasyonu. 25. Ulusal Klinik Nörofizyoloji EEG-EMG Kongresi

D.14 Türe Ugur, Yaltirik Cumhur Kaan, Harput Mehmet Volkan, Türe Hatice, Imer Pinar, Atalay Basar,Güçlü Bülent (2008). Serebrovasküler Cerrahi Girisimlerde ”Indocyaninne Green” Anjiografisi Deneyimlerimiz. Türk Nörosirurji Dernegi 22. Bilimsel Kongresi

E – National Book Chapters

E.1 Temel Spinal Cerrahi, Bölüm adı:(Torakal Dar Kanal ve Spondilotik Miyelopatide Tanı ve Tedavi (2020)., Atalay Basar, Yaltirik Cumhur Kaan, Inter Tıp Yayınevi, Basım sayısı:1, ISBN:9786055004170, Türkçe

E.2 Temel Spinal Cerrahi, Bölüm adı:(Omurganın Hareket Segmenti) (2020)., Atalay Basar, Yaltirik Cumhur Kaan, Inter Tıp Yayınevi, Basım sayısı:1, ISBN:9786055004170, Türkçe

E.3 Travma- Acil Servis Yönetimi, Bölüm adı:(Kafa Yaralanmaları) (2017)., Yaltirik Cumhur Kaan, Atalay Basar, Yeditepe Üniversitesi Yayınevi, Basım sayısı:1, ISBN:978-975-307-073-7, Türkçe

E.4 Nöroanestezi, Bölüm adı:(Nöroanatomi) (2017)., Yaltirik Cumhur Kaan, Atalay Basar, Türk Anesteziyoloji ve Reanimasyon Dernegi Yayınları, Basım sayısı:1, ISBN:978-605-67442-2-8, Türkçe

E.5 Dejeneratif Servikal Hastalıklar, Bölüm adı:(Servikal Omurgaya Yaklasım: Cerrahi Anatomi ve Varıs Yolları) (2014)., Atalay Basar, Yaltirik Cumhur Kaan, Türk Nörosirurji Dernegi Yayınları, Basım sayısı:1, ISBN:321132321xx, Türkçe

E.6 Adolesan Idiyopatik Skolyoz, Bölüm adı:(Adolesan Idiyopatik Skolyoz Epidemiyolojisi ve Patogenezi) (2017)., Atalay Basar, Yaltirik Cumhur Kaan, Türk Omurga Dernegi Yayınları, Basım sayısı:1, ISBN:9789789789, Türkçe

E.7 Principles of Spine Surgery Vol.1, Bölüm adı:(Motion Segment of the Spine) (2016)., Atalay Basar, Yaltirik Cumhur Kaan, Inter Tıp Yayınevi, Basım sayısı:1, ISBN:9786055004118, Ingilizce

E.8 Principles of Spine Surgery Vol.2, Bölüm adı:(Thoracic Stenosis and Myelopathy: Methods for Diagnosis and Therapy) (2016)., Atalay Basar, Yaltirik Cumhur Kaan, Inter Tıp Yayınevi, Basım sayısı:1, ISBN:9786055004118, Ingilizce

E.9 Osteoporotik Omurga, Bölüm adı:(Osteoporoz Etiyolojisi) (2016)., Yaltirik Cumhur Kaan, Atalay Basar, Türk Omurga Dernegi Yayınları, Basım sayısı:1, ISBN:978978978978, Türkçe

E.10 Klinik Durumlarda Klinik Uygulama Rehberi, Bölüm adı:(Spinal Travmalı Hastaya Yaklasım) (2016)., Yaltirik Cumhur Kaan, Atalay Basar, Yeditepe Üniversitesi Yayınları, Basım sayısı:1, ISBN:9789753070676, Türkçe

E.11 Spinal Deformiteler, Bölüm adı:(Eriskin Tip Skolyoz Cerrahisinde Erken ve Geç Komplikasyonlar) (2015)., Atalay Basar,Yaltirik Cumhur Kaan, Türk Nörosirürji Dernegi Yayınları, Basım sayısı:1, ISBN:9789789782, Türkçe

E.12 Spinal Deformiteler, B.lüm adı:(DeNovo Skolyozda Cerrahi Sınırlar) (2015)., Atalay Basar,Yaltirik Cumhur Kaan, Türk Nörosirürji Dernegi Yayınları, Basım sayısı:1, ISBN:9789789782, Türkçe

F –Invited Speaker

F.1 International Scientific Conference “Life Threating Emergency“, Emergency situation in neurology and neurosurgery, Batumi, Konusmalarım, 03.08.2018 - 03.08.2018 (Uluslararası)

F.2 Türk N.rosirürji Dernegi 32. Bilimsel Kongresi, Normal basınçlı hidrosefali, Antalya, 20.04.2018 -24.04.2018 (Ulusal)

G –Awards

En Iyi Ikinci Poster Ödülü, Türk Anesteziyoloji ve Reanimasyon Dernegi 50. Ulusal Kongresi, 2016