T.C.
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ANALYZING MICRORNA-221 EXPRESSION LEVELS IN GLIAL TUMORS

PHD THESIS

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

Selçuk ÖZDOĞAN

DEDICATION

I dedicate to my thesis

to my wife Esra, my son Burak Hakan, my daughter Alara Nehir

and

to my lovely Family

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Firstly, I want to announce my best regards and thanks for Prof.Dr. Turgay İSBİR for supporting me on the way to be Associated Professor of Neurosurgery and PhD, teaching me how to make researches and giving magic touches to my family in social life. Prof.Dr. Turgay İSBİR is my inspiration to become an academician and a scientist in the academic life.

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Selçuk ÖZDOĞAN

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LIST OF SYMBOLS AND ABBREVIATIONS

$\Delta\Delta C_T$: Delta Delta C_T

ΔC_T : Delta C_T

ADAM22 : A disintegrin and metalloprotease-22

AUC : Area Under Curve

BCNU : Carmustine

BHQ1 : Black hole quencher 1

cDNA : Complementary DNA

CDK : Cyclin-dependent kinase

CNS : Central Nervous System

CT : Computed Tomography

C_T : Cycle Threshold

DGCR8 : DiGeorge Syndrome Critical Region-8

DIABLO : Direct Inhibitor of Apoptosis Protein-Binding Protein with Low PI

DISC : Death Inducing Signaling Complex

DNA : Deoxyribonucleic Acid

DOXO MB : doxorubicinconjugated molecular beacon

EGFR : Epidermal Growth Factor Receptor

Exp5 : Exportin-5

GABA : Gamma-aminobutyric acid

GBM : Glioblastoma Multiforme

IDH-1 : Cytosolic $NADP^+$ related isocitrate dehydrogenase

In(2-($\Delta\Delta$CT)) : Fold change

Lin : Lineage deficient

LNA :Locked nucleic acid

IncRNA : Long noncoding RNA

LOH : Loss of Heterozygosity

µl :microliter

mg :milligram

miRNA : Micro RNA

MRI : Magnetic Resonance Imaging

mRNA : Messenger RNA

NADP+ : Nicotinamid Adenin Dinucleotide Phosphate

ng : Nanogram

OR : Odds Ratio

ORF : Open reading Frame

PIP : phosphotidylinositol phosphate

PCR : Polymerase Chain Reaction

PDGFA : Platelet-Derived Growth Factor Receptor Alpha

pg : picogram

piRNA : Interacting RNA

pre-miRNA :Precursor-miRNA

pri-miRNA : Primer-miRNA

PTEN : Phosphate and Tensin Homolog

RISC : RNA Induced Silencing Complex

RNA : Ribonucleic Acid

RNAi : RNA interference

RNase III : Ribonuclease III

ROC : Receiver Operating Characteristic

rpm : Rounds per minute

RT-PCR : Real-Time Polymerase Chain Reaction

SD : Standard deviation

shRNA : Short hairpin RNA

siRNA : Small Interfering RNA

SNP : Single Nucleotide Polymorphism

TNF : Tumor Necrosis Factor

TP53 : Protein 53

TRAIL : Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

TRBP : Transactivating Response RNA Binding Protein

UTR : Untranslated Region

WHO : World Health Organization

ABSTRACT

Özdoğan S.Analyzing MicroRNA-221 Expression Levels in Glial Tumors. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. PhD Thesis. Istanbul, 2019.

MicroRNAs (miRNAs) consist of 18–25 nucleotides which a group of endogenous regulatory Ribonucleic Acid (RNA) partipicates as an epigentic mechanism that regulate gene expression by using RNA interference (RNAi) metabolism. miRNAs are found in relationship with pathological processes and begun to be used as biomarker in some diseases. Glial tumors are consisiting 80% of primary brain tumors. The aim of our study is to examine miRNA-221 as a biomarker candidate to define prognosis and/or classification for glial tumors.

The hospital-based prospective case-control study consisted of 39 patients with glial tumor surgery and a 40 healthy individuals as control group. The peripheral blood samples were drawn from patients and control group after getting their "informed consent" were collected into EDTA-tubes. miRNA221 expression is evaluated from all blood samples.

Of the 39 patients we evaluated, 23 were male (59%) and 16 were female (41%). As for the control group which consist of 40 healty individuals, 25 were male (62,5%) and 15 were female (37,5%). The levels of miRNA221 expression were determined by comparing the ΔCT values of miRNAs and the internal control. When the expression levels of miRNA221was compared according to the ΔCT factor, it was determined that the mean expression value of the patient group was 2,98 ± 2,11 while control group had 0,82 ± 2,27, there was statistically significant difference between the groups ($p<0.0001$). The expression levels of both miRNA221 was found to be significantly increased in the patient group than the control group.

In conclusion, we found that miRNA221 expression could be a biomarker for glial tumors. But further researches must be made to make clear for its sensitivity and specifity to use for prognosis of glial tumors.

***Key Words*:** Glial tumors, microRNA, miRNA221

ÖZET

Özdoğan S.Glial Tümörlerde MicroRNA-221 Ekspresyon Seviyelerinin Analizi. Yeditepe Universitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Bölümü. Doktora Tezi. İstanbul, 2019.

MikroRNA'lar (miRNA), RNA etkileşimi (RNAi) yolu ile gen ekspresyonunu düzenleyen endojen düzenleyici bir Ribonükleik Asit molekül sınıfı olan 18-25 nükleotit içeren moleküllerdir. MikroRNAların patolojik süreçlerle ilişkili olduğu anlaşıldı ve bazı hastalıklarda biyo-belirteç olarak kullanılmaya başlandı. Glial tümörler primer beyin tümörlerinin %80' ini oluşturmaktadır. Çalışmamızın amacı mikroRNA-221' in glial tümörlerin prognozunda ve/veya sınıflandırılmasında biyo-belirteç olarak kullanılabilirliğini araştırmaktır.

Hastane bazlı prospektif vaka kontrol çalışmamızda glial tümör nedeniyle opere edilen 39 hasta ve sağlıklı 40 katılımcı kontrol grubu olarak belirlenmiştir. Onam formları alınan hasta ve kontrol grubundan alınan kan örnekleri EDTA' lı tüplere alındı. MikroRNA-221 seviyeleri bu kan örneklerinden çalışıldı.

İncelediğimiz 39 hastanın 23'ü erkek(%59), 16'sı kadın(%41) hasta idi. Kırk kişiden oluşan control grubunun ise 25'i erkek(%62,5) ve 15'ı kadın(%37,5) idi. MikroRNA-221 seviyeleri ΔCT değerleri karşılaştırılarak hesaplandı. Değerler karşılaştırıldığında hasta grubunun ΔCT değeri 2,98 ± 2,11bulunurken kontrol grubununki ise 0,82 ± 2,27 olarak bulundu. Sonuçlara göre iki grup arasında istatistiksel olarak anlamlı fark bulundu ($p<0.0001$). MikroRNA-221 seviyesi gliablastoma tanısı almış hastalarda kontrol gruba göre anlamlı oranda yüksek olarak bulunmuştur.

Sonuç olarak mikroRNA*221 ekspresyon seviyesi glial tümörlerde bir biyobelirteç olarak kullanılabilir. Fakat biyobelirteçin glial tümörlerin prognozunda kullanılabilmesi ve sensitivite - spesifitesinin belirlenmesi için daha ileri araştırmalar gerekmektedir.

***Anahtar Kelimeler*:** Glial tümörler, mikroRNA, miRNA221

1. STATEMENT AND PURPOSE

RNA interference (RNAi) is a epigenetic posttranscriptional regulatory mechanism, in which inhibits (silences) gene expression in post transcrition process. MicroRNAs (miRNAs) consist of 18–25 Ribonucleic Acid (RNA) molecules which are class of endogenous regulatory molecules that controls gene expression via RNAi. MiRNAs targets sequence-specific binding that cause degradation of messenger RNAs (mRNA) via the partipication with RNAi complex. miRNAs as noncoding RNA molecules has enhanced our understanding of the epigenetic mechanisms in the terms of control gene expression at post trancriptional level.

According to liteture, some studies report that miRNA-221 had oncogenic feature and correleated with cell proliferation and migration (1,2,3). MiRNAs are found in relationship with pathological processes and begun to be used as biomarker in some diseases (4,5). Glial tumors are consisiting 80% of primary brain tumors (6). Low grade glial tumors have more survey ratios than high graded tumors. Biomarkers are needed to define the progress of glial tumors. There are only a few studies to investigate the metabolic effect of miRNA-221 in glial tumors. The purpose of our study is to examine miRNA-221 as a biomarker candidate to define prognosis and/or classification for glial tumors. If we can define miRNA-221 as a biomarker, it could show the prognosis of glial tumors earlier so that treatment modalities could be planned earlier and survey rates could increase.

2. LITERATURE REVIEW

2.1. Overview of the Brain Tumors

2.3 % of cancer deaths and 1.9 % of new cancer cases are accounting by Central Nervous System (CNS) Cancers. Moreover, it is reported that newly diagnosed cases in 3.5 of 100,000 people in a year (7). Glioma is the most prevalent type of all primary CNS cancers, which comprises 50 % of human malignant brain tumors. According to the World Health organization (WHO), due to clinicopathological grading criteria for human gliomas could be classified into grades I–IV, and the majority belongs to highgrade astrocytoma or glioblastoma which are grade IV (8,9). Current therapeutic approaches such as, chemotherapy adjuvant radiotherapy, and, surgical resection, have improved 5-year survival rate from 2 to 10 %, unfortunately advanced-staged patients still have poor prognosis (10). Therefore, improving clinical outcomes by early detection of malignant gliomas have great importance (11).

Imageological examinations such as nuclear magnetic resonance imaging and computer tomography are diagnostic screening tools for glioma patients. Owing to the high cost of high tecnology screening examinations unfortunately they could not able to use as a routine diagnosis tools. Histopathologycal examination through micro-surgical resection or stereotactic biopsy, is another alternate for the diagnosis of glioma. However, may be hindered from wide application by its its invasive nature. Recent publications put forward that molecular biomarkers could be account for candidate diagnostic markers insteaed of high cost screening tools in glioma diagnosis. For instance, molecules like tensin homologue(PTEN), phosphatase, and protein 53(TP53) were deregulated in glioma procession, but for further applications in cancer diagnosis, mew methods needs to be improved for detection of biomolecules in serum, blood and tissue samples (12). Therefore, there is an urgent need for a novel, highly efficient and noninvasive biomarker for glioma diagnosis (11).

2.2. MicroRNAs

MicroRNAs(miRNAs) are identified as molecules that are small (a͡pproximately 22nt.), and at the level of post-transcriptional phase miRNAs negatively regulate gene expression (4). Lineage-deficient-4 (lin-4) is the first miRNA that displayed by Lee et al in 1993, lin-14 take part in developmental timing regulation in Caenorhabditis elegans

(13). After more researches, it is understood that there are many miRNAs in C. Elegans and other animals (14).

Reinhart et al (2000) reported that lethal-7 miRNA which regulate the metabolsim of cell growth of C.elegans (15). This miRNA is investigated to supress the fuctions of lin-42, lin-41, lin-28 and daf-12. Later on it is investigated that lethal-7 is found in drosophila and humans; so it is thought that it has an important role in biological function (14).

In 2001 a lot of small RNA molecules found in organisms and miRNA term begun to be said more (16). Researches made to find correlation between miRNAs and cancer and it is reported that approximately 1000 miRNAs are located in human genome in 2002 (17). In 2006 a new database called miRBase is established to collect miRNA datas (18).

The mechanism on regulations of gene expression is beginnig to be understood by the discovery of these transcripts. miRNAs which take part in modulating gene expression with RNA interference(RNAi) pathway in eukaryotic organisms. MiRNAs are described as 18–25 nucleotide long endogenous ribo-regulators that serve as RNAi process. RNAi is known as a posttranscriptional regulatory mechanism that associated with double-stranded RNA. miRNAs induce determination of homologous messenger RNAs (mRNA) and degregates transcipted molecules. However recognition of mRNAs mostly imperfect complementarity, miRNAs are functioning with base-pairing to the target mRNAs at the 3' untranslated regions (UTRs). Binding of target sequence leads mRNA to translational repression by direct cleavage (19,20).

RNA polymerase II complex transcribs 60-70 nucleotide lenght of miRNAs which are originating from longer primary miRNAs (pri-miRNAs) that takes place in the nucleus. pri-miRNAs are processed to mature miRNA intermediates by complex of the RNase III enzyme with Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) which are 60–70-nucleotide lenght. Exportin-5(Exp5), is member of the Ran-transport receptor family, transports the secondary RNA hairpin structure, then pre-miRNAs transported to the cytoplasm. Susequently pre-miRNAs are cleaved by DICER for generating 20–22 nucleotide duplexes, in the cytoplasm. The miRNA duplex(miRNA–miRNA) is unwound in the last phase into mature miRNA with the helicase.

RNA-induced silencing complex is described as the association of mature miRNA into a ribo-nucleoprotein effector complex whereas miRNA is degraded (21). RNA-induced silencing complex (RISC) recognizes target sequence by complementarities between the mRNA and guide miRNA. This complex leads endonucleolytic cleavage of targeted mRNA subsequently followed by post transcriptional repression. Reports in the literature shows that The genome o f mammals could express approximately one thousand of miRNAs that are regulating gene expression with the ratio 30% of genes (17). In humans, there are approximately more than 530 miRNAs and their physological function identified in the pathological mechanism of some diseases such as cancer, atherosclerosis and diabetes. Changes in miRNAs expression levels are determined in the development of cancer and diseases because of their roles in fundamental cellular functions (22). Recent litrature evidences reported that miRNAs could function both as oncogenic and tumor suppressor role (23). Also, differing miRNA expression has been described in many other malignancies (20).

2.2.1. MicroRNABiogenesis

The biosynthesis of miRNAs takes place in three stages. In the first step, primary miRNA (pri-miRNA) transcripted from miRNA genes located on intronic parts of genome. In the second step, pri-miRNAs are converted into precursor mi-RNA (pre-miRNA). In the third step, it has been shown that mature miRNAs occur in the cytoplasm (Figure 2.2.1-1) (24,25).

Pri-mirRNAs are synthesized from genomic DNA by the RNA Polymerase II enzyme. They are approximately 700 nucleotides in length, 5 ’Cap and 3’ Poly A tail-loop structure with the tail (26). The double-stranded pri-miRNA has approximately 70-100 nucleotides in length, and transformed into pre-miRNA, on the side of Drosha and its cofactor DGCR8, an endonuclease of the RNAase II family of enzymes. The complex formed by Drosha and DGCR8 is called Microprocessor Complex (27).

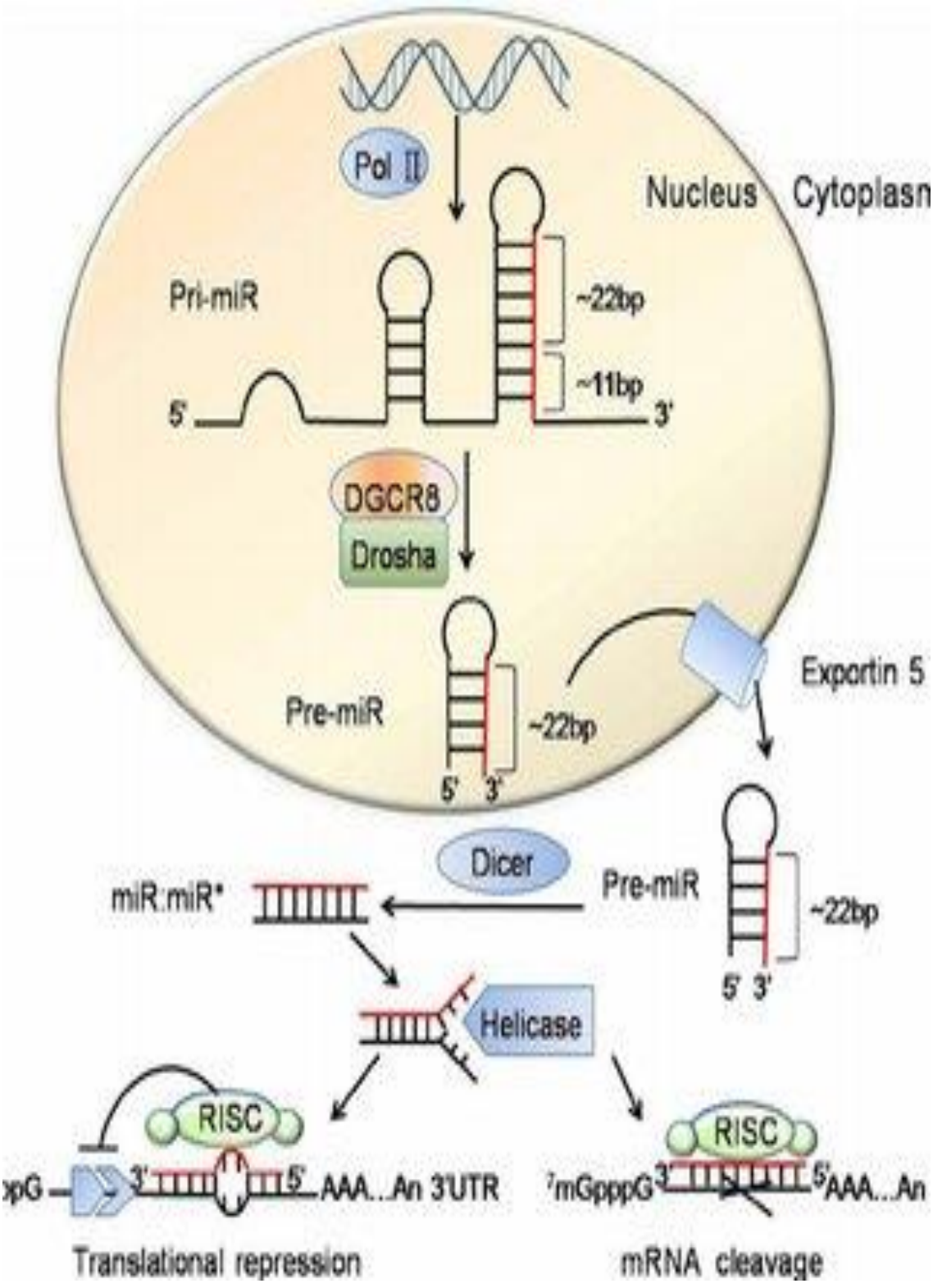


Figure 2.2.1-1. MiRNA Biogenesis (25).

The pre-miRNA is recognized by Exp5, a nuclear transport receptor and transported to the cytoplasm. After this step, an endonuclease from the RNAase III family of enzymes found in the pre-miRNA cytoplasm was cut with the enzyme Dicer 18-24 nucleotide length of the double-chain miRNA miRNA: miRNA duplex has been shown to be converted (28).

A RNA binding protein, Transactivating Response RNA Binding Protein (TRBP), combines with Argonaute and Dicer proteins to initiate the formation of a trimeric ribonucleoprotein complex, RISC. After the stem loop of the pre-miRNA by Dicer is cut, only one of the miRNA-miRNA duplexes is introduced into the RISC complex. This is called a guide strand and interacts with RISC to silence the target mRNA. Meanwhile, the other yarn (passenger strand) is removed and directed to destruction. Steem-loop position and thermodynamic stability are thought to be factors that determine which yarn will be affective (29). MiRNA has been shown to cause target mRNA degradation or suppression of protein translation. Then by the assist of Argonaute protein, complex being integrated into the active RISC complex (30).

miRNAs interact by binding to a highly conserved 6-8 nucleotide (seed-site) site called the target mRNA seed. 2.-8. The matching between nucleotides is important for determining the target. miRNAs can be linked to the target mRNA by showing a partial or exact match (31). The level of mapping between the untranslated 3'UTR (3 R untranslated region) region of the target mRNA and the miRNA seed sequence determines how much and how the miRNA will suppress its target (32). The binding region of miRNAs to the target mRNA is usually 3'UTR, but can also bind to 5'UTR or open reading frame(ORF) and suppress gene expression (33). miRNAs generally perform post-transcriptional regulation by suppressing tranlation or causing destruction of mRNA targets. If the match is too high, the RISC will degrade the target mRNA. This destruction was determined by the destruction of poly A tails and 5'cap structures of mRNAs by means of guide strand in RISC (34). MRNAs with reduced stability due to the disappearance of poly A tails and 5'cap structures are cleaved with enzymes present in the cell. It has been observed that translation is reduced in mammals where pairing is low. Although the mechanism has not been fully elucidated, it has been observed that while the mRNA level of the target gene increases, the protein level does not change, so the repression is thought to be at the translational level. In some studies, it was observed that regulation at the translational level was inhibited at the beginning

and translation stages of translation by affecting various transcription factors of the miRNA-RISC complex at the beginning of translation (35).

2.2.2. MicroRNA-221

MiRNA221 located on the chromosome of the p11.3 arm of the 23 nucleotides of the non-encoded RNA. They were found to suppress especially in the translational phase. 528 contains totally 184 protected areas. It has been shown that there are 496 transcripts of conserved regions(Figure 2.2.2-1) (36,37).

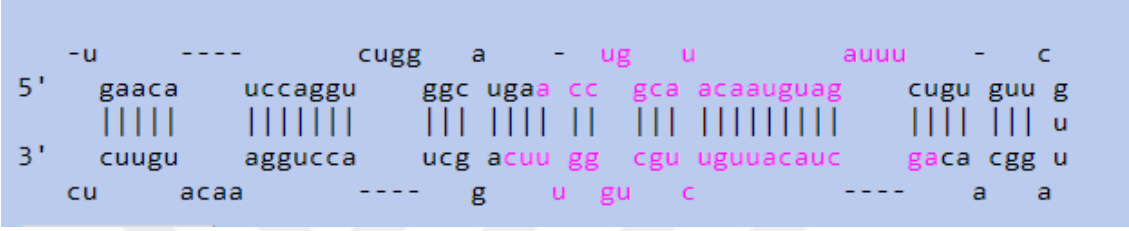


Figure 2.2.2-1 Steem loop and sequence of miRNA221 (37)

The target genes of miRNA221 and miRNA222 are, respectively, Cyclin Dependent Kinase Inhibitor 1B (p27, Kip1), Transcription factor 12, Pantothenate kinase 3, gamma-aminobutyric acid (GABA), KIT gene, MIS12 Kinetecor Komplkes Komponen, Transcription Initiator Protein 5A2, Acyl-CoA dehydrogenase, A Receptor Alpha 1,Chaperone-containing TCP1, Subunit 5 (epsilon), mitochondrial ribosomal Protein S7, Tubulin, alpha 1a, Annexin A3, C-4 to C-12 straight chain, Eukaryotic Translating Initiating Factor 3, Subunit J is protein associated protein 2, which binds to Poly (A), Regulatory Factor X(Figure 2.2.2-2) (38,39).

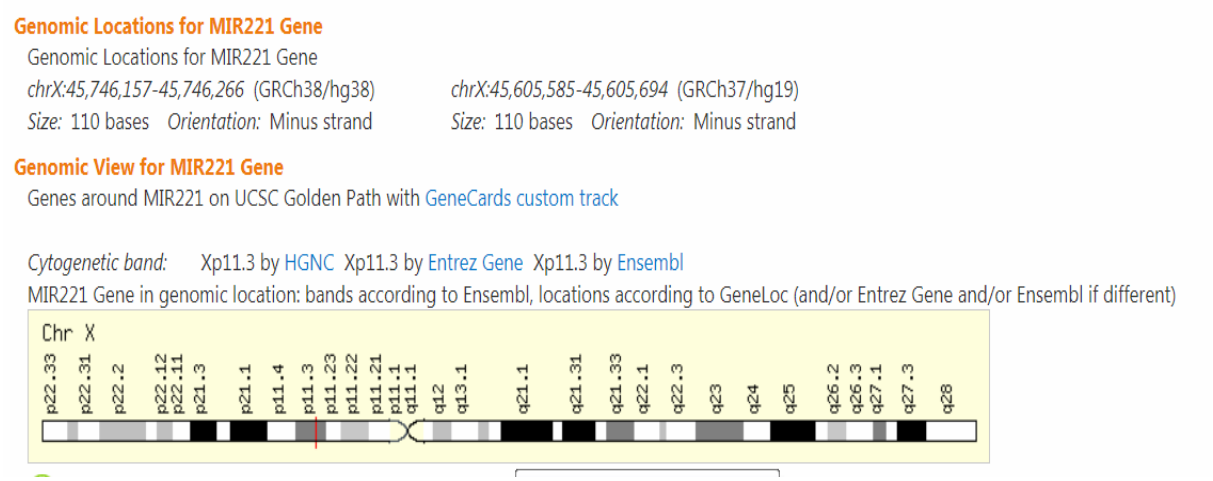


Figure 2.2.2-2 Genomic view of miRNA221 (39)

The effects of microRNA 221/222 on regulation of p27Kip1 was integrated in cell cycle pathway. Medina et al. examined regulation of microRNA 221/222 in the check phase as G1 and Synthesis phase of the cell cycle. In this study they showed that microRNA 221 and miRNA 222 are downregulated in quiescent cells while upregulated during proliferation stage of cell cycle (40).Terasawa et al. have shown that microRNA 221/222 expression levels increased by nerve growth factor, and this biological process regulated by ERK1/2 gene pathway in cell lines of neuroblastomas (41).

Ciafre et al. studied with microarray technique for the analysing in the glioblastoma (GBM) cell lines. The role of miRNA has also been investigated in aggresive cell lines which is the most common and the most aggressive humans brain tumors (42). They showed that expression levels of microRNAs especially miRNA221, significantly variated in tumor cells when while down regulated in peripheral brain cells. They displayed that miRNA221 strongly upregulated in glioblastoma, while some of brain-enriched miRNAs as miRNA128, miR-181a, were down-regulated in glioblastoma.

2.2.3. The role of MicroRNAs in cancer

The fact that miRNAs are composed of genes encoding miRNAs are important in cancer pathogenesis. In recent studies it has been demonstraed that expression level of miRNA is variated in human malignancies. The underling mechanisms as chromosomal abnormalities, alterations in transcriptional process, epigenetic

modifications and defects in the miRNA biogenesis. miRNAs may be effective in carcinogenesis with specific cell types in different cancer types and changes in expression levels were examined.It was determined that the differences between the tissues (Figure 2.2.3-1) (43).

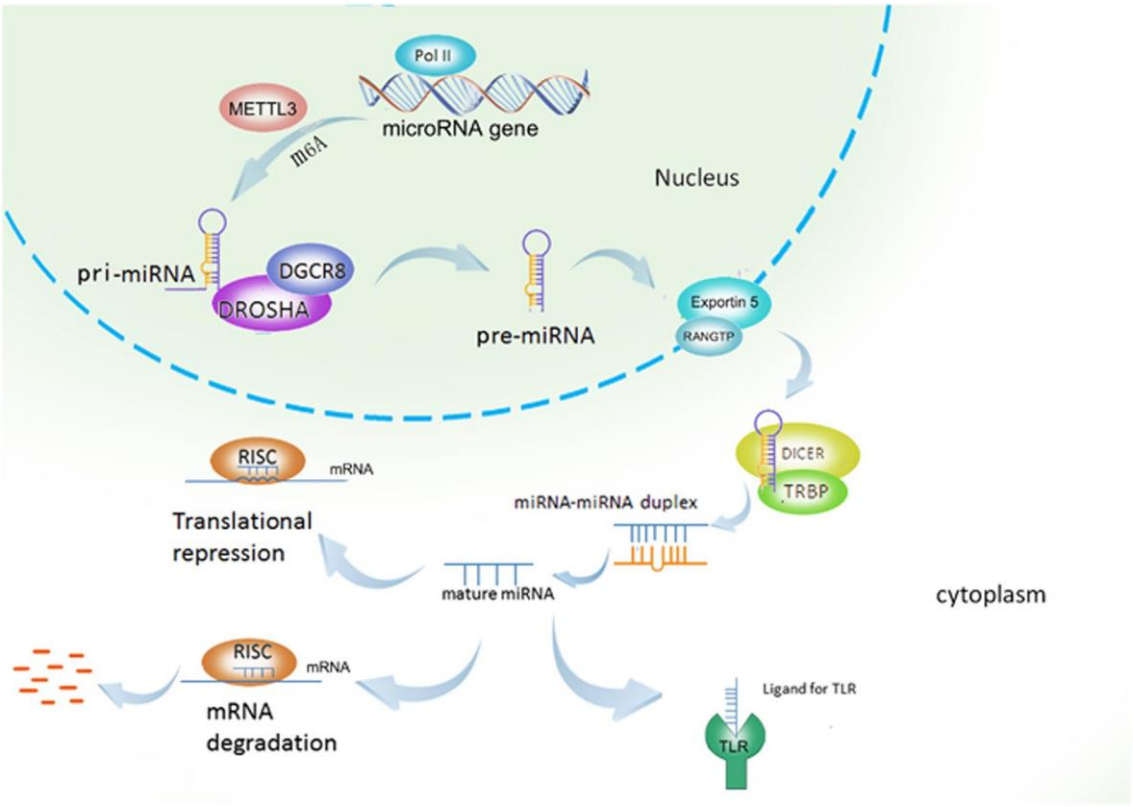


Figure 2.2.3-1 The role of MicroRNAs in human cancer (43)

However microRNAs could have either oncogenic or antioncogenic function, multiple studies have claimed that expression levels of miRNA is altered in tumour cells while it is stabile in normal tissue, this results suggested that miRNA biogenesis associated with cancer (25). Alterations in the microRNA expression levels, triggers the mechanism of biological machinerymolecules such as the Dicer-1, ribonuclease III (RNase III) and Drosha are variated in some human malignancies like gynecological cancers, neuroblastoma, and lung cancer (44). Although recent stuıdies showed that defficiency of regulation and mutations in miRNA posttranscriptional pathway, regulating the pathophysiological mechanism of the miRNA biogenesis machinery in human tumors , the core mechanism of miRNA metabolism remains unclear (44).

3. MATERIALS AND METHODS

3.1. The Patient Population and the Study Protocol

The hospital-based prospective case-control study consited of 39 patients who were operated for glial tumor and 40 healthy subjects were selected for the control group. All partipiciants were recruited from the Neurosurgery Departments of Yeditepe University, Istanbul, Turkey. Demographic characteristics of patients and controls were recorded and followed-up prospectively. The control group examined by cranial magnetic resonance imaging and reported as normal. Tumor classification determined according to their grades, location and sides. Pathological examinations of brain tumors were evaluated according to the World Health Organization Classification of Tumours.

The peripheral blood samples were taken from patients and control group after obtaining informed consent from all individulas, were collected into EDTA-tubes. miRNA221 expression is evaluated from all blood samples. Blood samples collected within the scope of the ethical guidelines of the 1975 Declaration of Helsinki and the study protocol has been approved by the Yeditepe University Medical Faculty Ethics Committee (file no: 23.06.2016 /634)

3.2. Materials and Devices

3.2.1. miRNA Isolation

All serum samples were centrifuged for 10 min at 4500 rpm then supernatant transpotred in a sterilezed tube and frozen at -80°C until miRNA analysis were conducted. Total RNA isolation is made with Trizol and isolated miRNA sample's optical density were measured with NanoDrop2000 (Thermoscientific, Waltham, Massachusetts, USA).

MiRNA isolation from 200 µl serum performed by miRCURY™ RNA Isolation Kit (Exiqon, Qiagen, Hilden, Germany) according to manufacturer's instructions. The isolation of miRNA is based on spin column chromatography using a proprietary resinas the separation matrix. Serum samples are lysed with the provided Lysis Solution then proteins are precipitated by Protein Precipitation Solution. After the precipitation process isopropanol is added to the collected supernatant of samples and the solution is loaded to the spin column. Then solution was purified with Wash Solutions 1 and 2 and the isolated RNA is eluted with RNase free water (Figure 3.2.1)

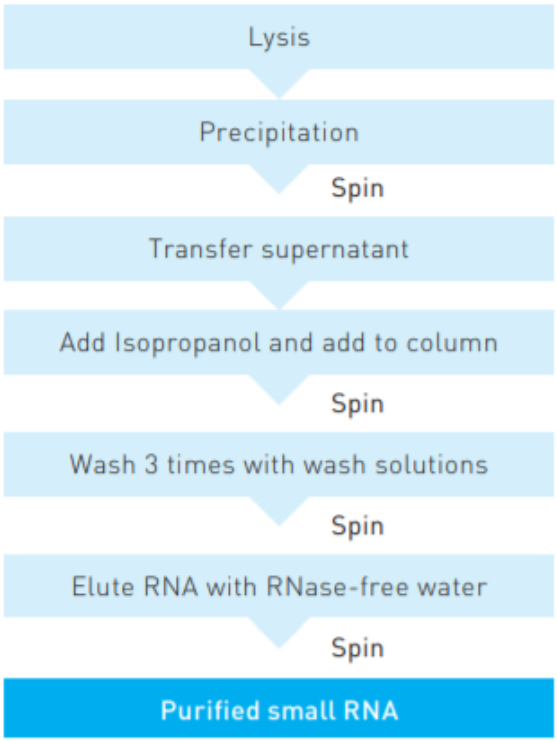


Figure 3.2-1. miRCURY™ RNA Isolation Kit for Biofluidsprocedure

3.2.2. cDNA Synthesis

The isolated miRNA samples exposed to reverse transcription process from to first-strand cDNA synthesis reactions was performed by using the miRCURYLNA RT Kit (Exiqon, Qiagen, Hilden, Germany). The each template RNA sample diluted to 5 ng/µl using nuclease-free water. Then the samples prepared for the reverse transcription reactions on ice according to instructionsshown in Table 3.2.2-1.

Table 3.2.2-1. cDNA mixture for PCR Reaction

Component	Amount
5x miRCURY RTReaction Buffer	2 µl
10x miRCURY RT Enzyme Mix	1 µl
RNA free water	5 µl
miRNA Sample	2 µl
Total volume:	**10 µl**

After cDNA mixture for PCR Reaction prepared samples incubated due tomanufacturer's instructions seen in Table3.2.2-2.

Table3.2.2-2. Reverse transcription reaction temperature cycling protocol.

Step	Time	Temperature
Reverse transcription step	60 min	42°C
Inactivation of reaction	5 min	95°C
Storage	∞	4°C

3.3.3. Measurement of miRNA Purity

The purity of the miRNA samples were measured with the NanoDrop 2000 instrument (Thermoscientific, Waltham, Massachusetts, USA). The purity of the miRNA samples was determined by the OD260/OD280 ratio. The Sample's optical density range greater than 2 were accepted as pure between OD260/OD280 ratio.

3.3.4. Determination of miRNA Levels by Fluorometer

MicroRNA levels were determined flurometrically. miRNA concentration of transcripted samples determined and equalized by Qubit miRNA Assay Kit's standard protocol on the Qubit 3.0 Fluorometer (Thermoscientific, Waltham, Massachusetts, USA). In the first step, 200 µl working solution was prepared by mixing 199 mil miRNA Buffer and 1 mil miRNA Reagent (Qubit microRNA Assay, Invitrogen, Thermo Fisher Scientific Inc.). 10µl standard is added to 190µl working solution on standard 1 and standard 1 and 10µl is added to 190µl working solution on standard 2, standard number 2 is prepared and standards are introduced to the device in turn. For each sample measurement, 2µl sample was added to 198µl working solution and 200 karışıml mixture was obtained in total and microRNA levels were determined by reading in the device (45).

3.3.5 The miRNA Expression Levels Analysed by Real -Time Polymerase Chain Reaction

The target miRNA selected for this study were determined by using the "mirbase" and "targetscan" databases (39,46). miRNA and it targets were analyzed using these data bases regarding molecular mechanisms in glioblastoma. miRNA221 was chosen as the target miRNA for this thesis. Primer sequence of the miRNA221 can be seen in Table 3.3-1 and reaction mix setup for PCR miRNA expression analysis can be seen in Table 3.3-2.

Table 3.3-1. miRNA Primer Sequence

microRNA	Primer Sequence
hsa-miR-221-3p	-AGCUACAUUGUCUGCUGGGUUUC-

Table 3.3-2. Reaction mix setup forPCR miRNA Expression Analysis

Component	Amount
2x miRCURY SYBR Green Master Mix	5 µl
PCR primer mix	1 µl
cDNA template	3 µl
RNase-free water	1 µl
Total volume:	**10 µl**

As shown in Figure 3.3-1 two-part protocol of :the miRCURY LNA™ Universal RT microRNA PCR performed as:

1. First-strand cDNA synthesis
2. Real-time PCR amplifi cation

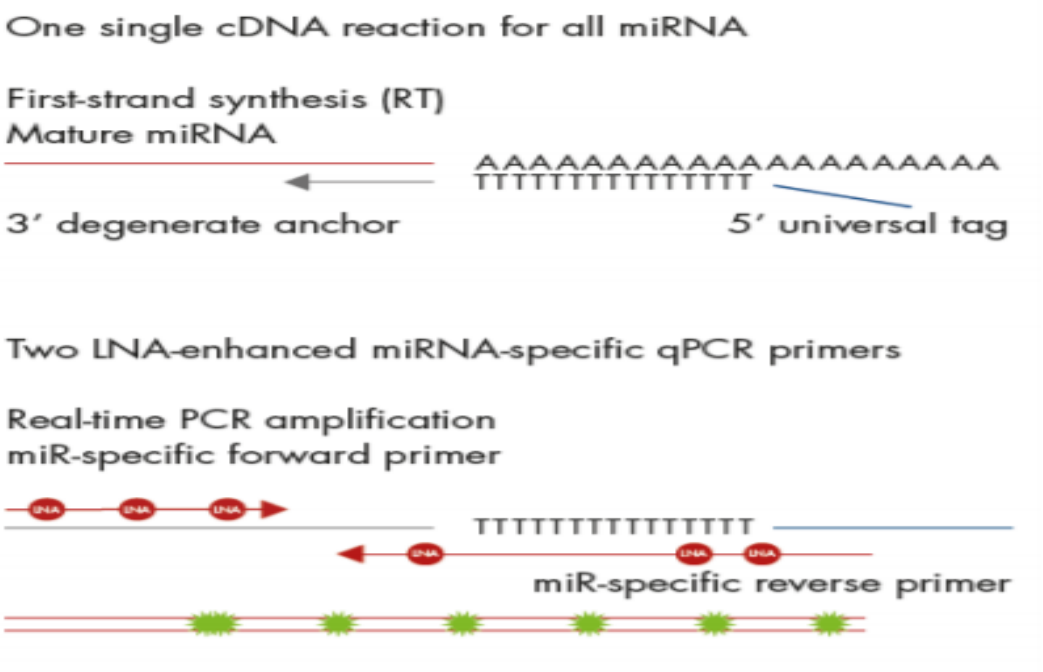


Figure 3.3-1. Schematic outline of the miRCURY LNA miRNA PCR System

Comprehension of miRNA expression levels has been realized by delta CT (ΔCT),

miRNA221 was chosen as the target miRNAs for thesis. Comprehension of miRNA expression levels has been determined by using Ct and delta CT (ΔCT) methods. The internal control (housekeeping assay, RNU6) has been used for calculation of ΔCT

miRNA expression level determinations were accomplished using the delta Ct (ΔΔCT) equations(47).

3.4. Statistical Analyse

All results were evaluated with SPSS 23 statistical analyse program. Pearson and Spearman corelation test were used for corelations. Differences between the patient and control groups were evaluated with student t-test or Mann-Whitney U test. Relationships between variables were determined with Chi-square and Fischer Exact tests.

4. RESULTS

4.1. Demographic Results of Study Groups

We evaluated a total of 39 patients that 23 were male(59%) and 16 were female(41%). Control group were 40 individuals and 25 were male(62,5%) and 15 were female(37,5%). Demographic characteristics of patients and control group were shown in Table 4.1-1.

Table 4.1-1 Demographic characteristics of patients

	Control (n=40)	**Patient (n=39)**	**p value**
Gender	Male / Female 62,5%/ 37,5% (n=25) / (n=15)	Male / Female 59%/41% (n=23) / (n=16)	0,748
Age(years): Mean±SD	42,58±20,21	43,10±20,21	0,883

n=number of sample, X± SD (Mean ± Standard Deviation)*The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.

Tumor classification determined according to their grades, location and sides. Pathological examinations of brain tumors were evaluated according to the WHO Classification of Tumours. Tumors were classified and evaluated according to their type, grades and location as shown at Table 4.1-2 and Table 4.1-3.

Table 4.1-2. Tumor types and grades

Tumor type	n	%
Glioblastoma Grade-4	21	53,9
Oligodendroglioma Grade-2	12	30,8
Oligodendroglioma Grade-3	2	5,1
Astrocytoma Grade-2	2	5,1
Astrocytoma Grade-3	2	5,1
Total	**39**	**100**

Table 4.1-3. Location of tumors

Location of Tumor	n	%
Temporal	16	41
Frontal	5	12,8
Parietal	5	12,8
Occipital	3	7,7
Cerebellum	1	2,6
Thalamus	4	10,3
Cingulate	4	10,3
Bulbus	1	2,6
Total	**39**	**100**

Cytosolic NADP+ related isocitrate dehydrogenase (IDH-1) status of the GBM patient group was determined by usig immunohistochemical methods (Table 4.1-4).As it shown in the table most of the patients with glioblastoma had IDH-1 mutant form (%87,2). IDH-1 mutations, which present in early stage of gliomagenesis, alters the function of the enzymes, causing them to produce 2-hydroxyglutarate that not produce NADPH. Also tumor classification according to their sides given in Table 4.1-5

Table 4.1-4. IDH-1 status of the GBM patients

Tumor type	n	%
IDH-1 Mutant	34	% 87,2
IDH-1 Wild Type	5	% 12,8
Total	39	% 100

Table 4.1-5. Tumor classification according to location of their sides

Tumor side	n	%
Right	18	% 46,2
Left	21	% 53,8
Total	**39**	**% 100**

4.2. microRNA Results

ΔCT values were calculated for analyzing miRNA221expression levels and these values are shown in Table 4.2.-1. The levels of miRNA221 expression were determined by comparing the ΔCT values of miRNAs and the internal control(RNU6). When the expression levels of miRNA221was compared according to the ΔCT factor, it was determined that the mean expression value of the patient group was 2,98 ± 2,11 while control group had 0,82 ± 2,27, there was statistically signifiant difference between the groups ($p<0.0001$) (Table 4.2-1). As it shown in the Figure 4.2.1 the expression levels of both miRNA221 was found to be significantly increased in the patient groupthan the control group.

Table 4.2-1. Comparison of ΔCт levels miRNA expression levels between patients and control groups

MiRNA221-3p	ΔCт	p value	%95 Güven Aralığı
Patient (n=39)	2,98 ± 2,11	0,000*	1,177 – 3,145
Control (n=40)	0,82 ± 2,27		

* ($p<0,05$). X± SD (Mean ± Standard Deviation), n (number of sample) .The difference between the groups was analyzed by the independent sample student t-test.

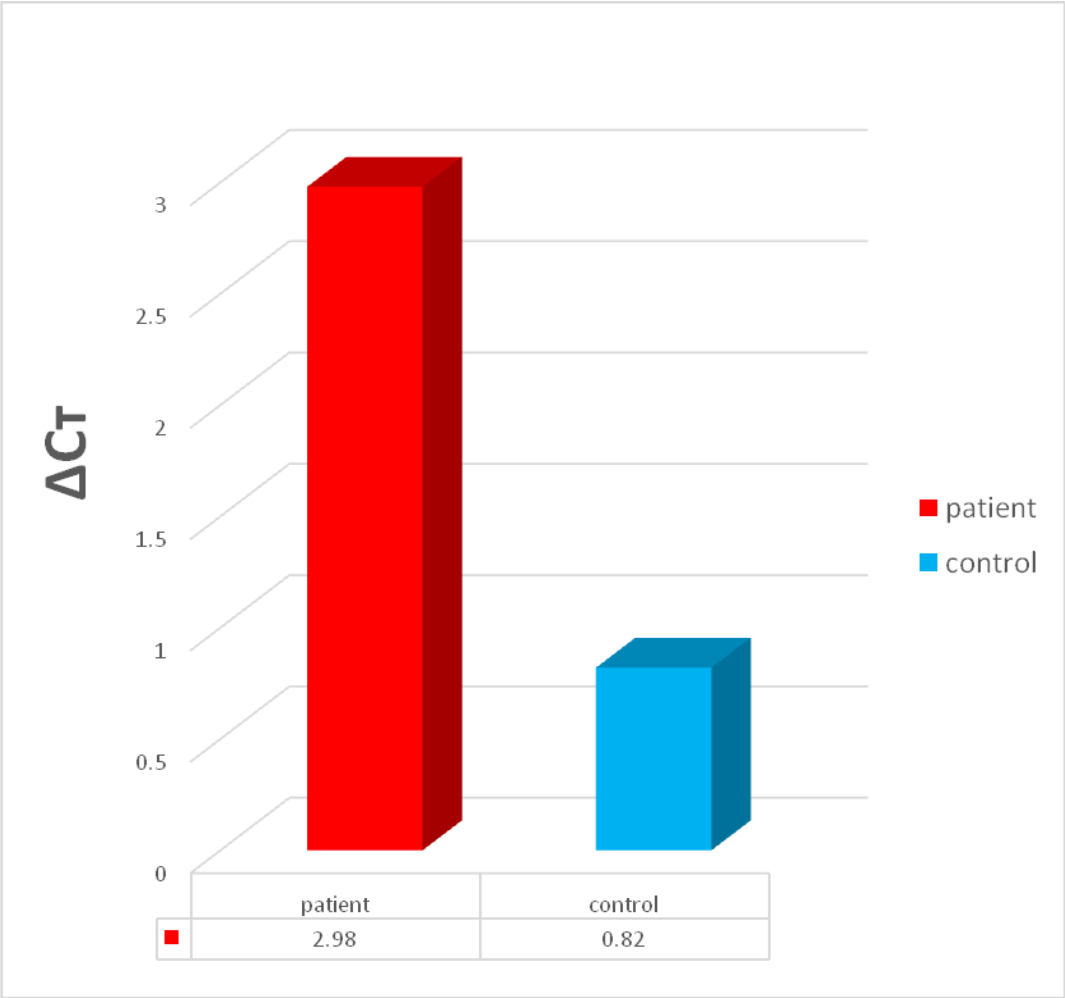


Figure 4.2-1. Comparison of miRNA221 expression levels between patient and control groups according to mean ΔCT value.

However there were no statistical difference regarding ΔCT values of plasma miRNA221 expression levels according to glial tumor type (p=0.693), Asto-3 type tumor had highest expression levels as 4,46 ± 1,01, followed by GBM type as 3,09 ± 2,31, Oligo-2 type 2,94 ± 2,01, Oligo-3 type 2,15 ± 2,18 and Astro -2 type 1,48 ± 1,43 (Table 4.2-2)

Table 4.2-2 Comparison of plasma miRNA221 expression levels as ΔCт according to glial tumor type

MiRNA221-3p	n	ΔCт	p value
Oligo-3	2	2,15 ± 2,18	0,693
GBM	21	3,09 ± 2,31	
Oligo-2	12	2,94 ± 2,01	
Astro-3	2	4,46 ± 1,01	
Astro-2	2	1,48 ± 1,43	

n=number of sample, * (p<0,05). X± SD (Mean ± Standard Deviation), n (number of sample) .The difference between the groups was analyzed by the independent sample student t-test.

There were no statistical difference regarding ΔCT values of plasma miRNA221 expression levels according to glial tumor location (p=0,840). Cerebellum tumor had highest expression levels as 4,64 ± 0,00, followed by patients with Frontal tumor had 3,97 ± 2,53, Temporal located tumors 3,20 ± 2,61, Occipital type 2,99 ± 1,99, Thalamus type 2,57 ± 0,98 , Cingulate type had 2,35 ± 1,63, Bulbus located type 2,30 ± 0,00 and Parietal located type had lowest with 1,93 ± 1,25 (Table 4.2-3)

Table 4.2-3 Comparison of plasma miRNA221 expression levels as ΔCT according to glial tumor location

MiRNA221-3p	n	ΔCT	p value
Thalamus	4	2,57 ± 0,98	0,840
Cingulate	4	2,35 ± 1,63	
Parietal	5	1,93 ± 1,25	
Cerebellum	1	4,64 ± 0,00	
Bulbus	1	2,30 ± 0,00	
Occipital	3	2,99 ± 1,99	
Frontal	5	3,97 ± 2,53	
Temporal	16	3,20 ± 2,61	

n=number of sample, * ($p<0,05$). X± SD (Mean ± Standard Deviation), n (number of sample) .The difference between the groups was analyzed by the independent sample student t-test.

The ΔCT values of miRNA221 was calculated and compared with the IDH-1 types of the GBM patients. As it shown in Table 4.2-4 there was no significant relation between miRNA221 ΔCT expression pattern and type of IDH-1.

Table 4.2-4 Comparison of plasma miRNA221 expression levels as ΔCT according to for IDH-1 Types

MiRNA221-3p	n	ΔCT	p value	%95 Güven Aralığı
IDH-1 Mutant	**34**	3,13 ± 2,17	0,273	-0,921 –3,170
IDH-1 Wild Type	**5**	3,09 ± 2,31		

n=number of sample, X± SD (Mean ± Standard Deviation), n (number of sample) .The difference between the groups was analyzed by the independent sample student t-test.

The ΔCT values of miRNA221 was calculated and compared with the location of tumor. There was no significant relation between miRNA221 ΔCT expression pattern and location type (Table 4.2-5)

Table 4.2-5. Comparison of plasma miRNA221 expression levels as ΔCT according to tumor location

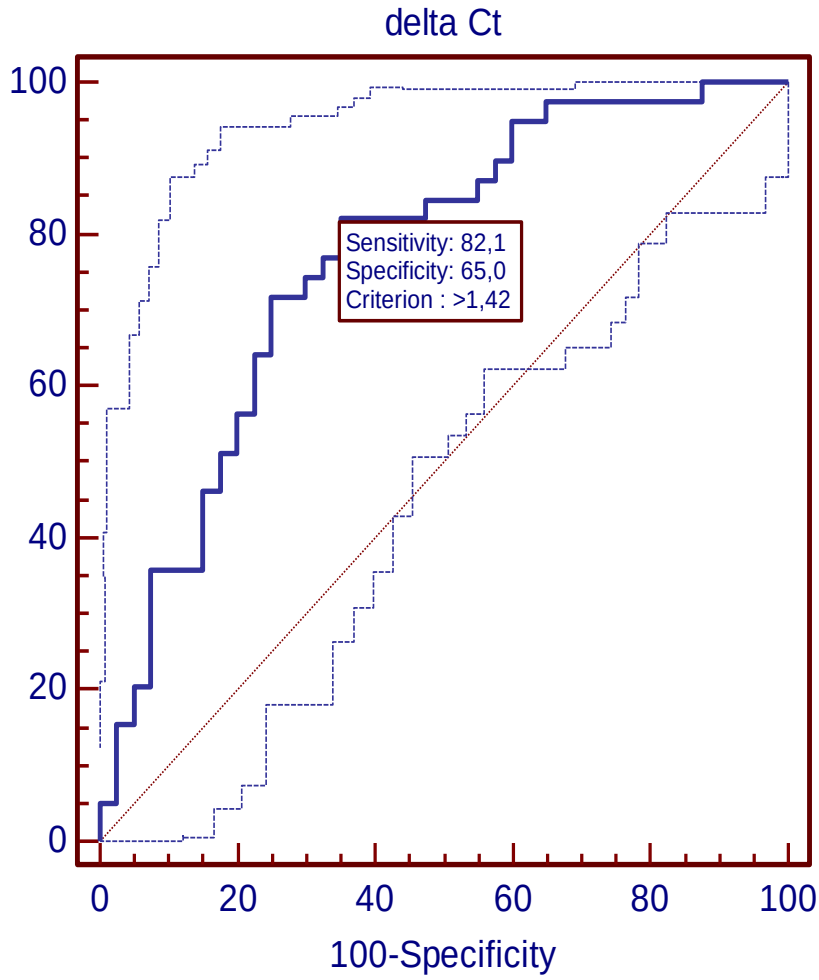
MiRNA221-3p	n	ΔCT	p value	%95 Güven Aralığı
Right sided	**18**	3,21 ± 2,62	0,480	-0,897 – 1,873
Left sided	**21**	2,72 ± 1,32		

n=number of sample, X± SD (Mean ± Standard Deviation), n (number of sample) .The difference between the groups was analyzed by the independent sample student t-test.

4.3. ROC Analysis of microRNA

The Receiver Operating Characteristic (ROC) analysis was performed using the MedCalc Program to determine all serum miRNA levels, and the diagnostic value as well in the patient and control groups. As shown in Figure 4.3-1 it was determined that miRNA221 expression level could be considered as threshold values in patient groups.

The ΔCT parameter of miRNA221 were determined as Area Under Curve (AUC)=0.767 and threshold value > 1,42 (p=0.0001) (Figure 4.3-1).



Sensitivity : 82,1
Specificity : 65.0
Criterion :>1.42

AUC=0,767
%95 Cl=0,659 - 0,855
≤ 1,42
p=0,0001 *

Figure 4.3-1 ROC analysis graph of Plasma mir221 expression levels regarding control and GBM groups * (p<0,05).

5. DISCUSSION AND CONCLUSION

MicroRNAs are defined as RNA sequences that do not encode single chain proteins of approximately 20-25 nucleotides in length, which act as negative gene regulators at the transcriptional and post-transcriptional level andtakes part in the regulation (48,49). MiRNAs regulate gene expression by base matching with specific binding sites located at the 3 ’end of the target mRNAs and located in the non-transcribed region (50). MiRNAs are linked by matching 2-9 bases in the 3’UTR region of target mRNA with the matching parts located at 5-ends. MiRNAs with low specificity binding capacity play crutial role in control of regulation of gene expression. A single miRNA may be the target of more than one mRNA, or may be more than one miRNA targeting an mRNA (50). miRNAs can cause various diseases because of the variations in the genes encoding them, which affect the protein synthesis regulation of which they are associated. In addition, various miRNA biomarkers have been identified to be used in the early diagnosis of various diseases and have been widely used for diagnostic purposes (51).

Zhuang et al investigated that apoptosis are reported as contributing to atherosclerosis and oxidative stress is induced by oxidized low- density lipoprotein (ox- LDL)(52). miRNAs were though as suppressing the target mRNA at posttranscriptional stage. They reported that treatment of ox- LDL seriously decreased the concentration of miR- 221- 3p expression and via controlling cell cycle. Formation of foam cell, lipid biomarkers expression was reduced with transfection of miR- 221- 3p. Also miR- 221- 3p inhibits the macrophages.induced cell apoptosis. Protein named as A metalloprotease- 22 (ADAM22) mRNA degregated because this metalloprotease disintegrin is account for a direct target of miR- 221- 3p. The silencing ADAM22 gene expression resulted in decreased of formation of oxidated foam cell and apoptosis. They concluded that as miR- 221- 3p is blocking formation of oxidated LDL and apoptosis by targeting ADAM22 transcription (52).

In recent studies, it has been shown that miRNA-related dysfunctions are involved in the etymology of so many diseases, especially malignancies (48,53). It has been suggested that various dysfunctions may occur due to other mechanisms and the most important group among them is changes in miRNA expression levels (49).

Genetic-induced expression changes are caused by large-scale genome changes, such as deletion or translocation, and the amount of miRNA decreases due to the change in the coding sequence, while increasing target mRNA and encoded proteins. Point mutations in the genome due to sequence changes miRNA-mRNA interaction can reduce or increase the effect (53). miRNA coding sequences were found to be 9 times higher in the brittle genome regions than in normal regions (50). The presence of miRNA encoding sequences in fragile genome regions where the DNA chain is structurally more vulnerable has been demonstrated by the role of miRNAs in dysfunction. Nowadays, studies explaining the mechanisms of diseases are continuing with great speed (51).

Cancer is one of the leading diseases caused by miRNA-induced dysfunction. In the process of cancer, miRNAs that control events such as growth, proliferation and differentiation have been suggested to have a great effect (54). A part of the miRNA oncogenic tumor suppressing effect is emphasized that some of the important research is being done (50).Oncogenic miRNAs reduce their effects by binding to cell proliferation-suppressing proteins, while tumor suppressor miRNAs target mRNAs of proteins that stimulate cell proliferation, such as growth factor receptors (54).

As miRNAs have a very stable structure in the blood, various researches have been put forward that can be used as an important biomarker in the diagnosis and treatment of clinical diseases. MiRNAs are highly resistant to RNAases, are not affected by pH changes, and are able to be easily detected in serum and plasma samples without the need for invasive methods due to their ability to exhibit resistance to freezing-thawing without deterioration in structure. Because of these advantages, measurement of expression levels of miRNAs can be obtained and treatment information can be obtained as a tumor marker (55).

miRNAs could play role as oncogenic or tumor suppressor properties according to the molecular pathways of the targeted mRNA. In normal tissues, some miRNAs have been reported to inhibit the translation of protooncogenes. These miRNAs whose function is to control the expression of an oncogenThey are expressed as suppressor miRNAs. Therefore, tumor suppressordecreased expression of miRNAs, increased expression of oncogene and tumorformation. In contrast, oncogenic mirRNAsdevelopment these miRNAs are a tumor suppressor. MicroRNAs, oncogen and tumor suppressor mRNAscan see both as a potential target (56).

miR221 / 222 has been reported to play role in cancer process as an oncogen or tumor suppressor gene and had been discovered to be among the most disregulated miRNAs in cancer. miR221 / 222 expression of thyroid cancer,hepatocarcinoma, estrogen receptor negative breast cells and melanoma cells.Cell cycle regulator p27Kip1, miR221 / 222 is one of the target genes. Galardi et al. studied p27Kip1 and miR221 / 222 expression levels in pancreatic cells. There is an inverse correlation between proliferation of miR221 / 222 overexpression speed and regulation of cell cycle phases revealed (57). This result was supported by studies on lung cancer, glioblastoma, breast cancer, thyroid papillary carcinoma and hepatocellular carcinoma in later years.

Many other tumor suppressor genes, the target gene of miR221 / 222, have been identified. Fornari et al. miR221 directly in the liver to target CDKN1C / p57have suggested that oncogenic function in hepatocarcinogenesis(58). They showed that CDKN1C / p57 expression decreased 1.8-fold when miR221 transfected into Hep3B cells, whereas CDKN1C / p57 expression increased 1.3-fold when anti-miR221 was transfected into SNU449 cells (58).

Zhang et al. found that miR221 / 222 regulates apoptosis by targeting PUMA in GBM and described an reverse correlation between PUMA and miR221 / 222 expression (59). MiR221 / 222 overexpresses and regulates tyrosine phosphatase PTPμ expression in patients with advanced glioma. PTPμ of glioma cellsmiR221 / 222 overexpression for glioma tumorformation is important (60).

Zhao et al. found that miR221 / 222 is overexpressed in ER-α (-) breast cancer cells, primary tumors and also inhibits the translation of ER-α by binding to the 3 UT - UTR region (61).

Garofalo et al. in their study of aggressive NSCLC miR-221/222 's overexpresses, PTEN and TIMP3 by suppressing TRAIL resistance induced (62). PTEN is tumor suppressor gene which have crutial role in cell development and apoptosis, and phosphotidylinositol-3,4,5-triphosphate in the signaling pathway.(PIP3) converts phosphotidylinositol- to 4,5-bisphosphate (PIP2), ie PI3 kinase (PI3K)activity. Inactivation of PTEN results in continuous activation of the PI3K / ACT pathway, leading to increased cell cycle progression, protein synthesis and migration. In the same study, MET oncogenin miR-221/222 found that they control their activation via the c-Jun transcription factor. miR-221/222 expression levels have TRAIL sensitivity or TRAIL resistance suggested that it is a prognostic factor in estimating (62).

Pu et al. miR221 plasma levels in colorectal cancer patients and control group is different and suggested that this is an important prognostic factor in determining the survival of colorectal cancer patients (63). Chenet al. have analyzed different thyroid tumors and the papillary thyroid of miR-221/222carcinoma overexpresses have been suggested (64).

Primary malign brain tumors are the important part of neoplasies as the cause of death with 2 years median of survey. GBM, is the most seen and malignant glial tumor of central nervous system. GBM is 80% of malign astrocytomas and has the shortest survey ratios. It has a median survey of 14.2 – 16 months when it is diagnosed (10). Studies showed that surveys of 3 years and more are only 2-3% of these tumors (65).

The cause of malign prognosis is mostly due to the genetic mutations and anomalies as most neoplasies do. As the result of genetic and epigenetic variations-mutations tumor cells signalisation activation increases, angiogenezis, uncontrolled mitosis, invasion ability and resistance to apoptosis ocur. All these processes affect as the histopathologic factors to the survey rates. EGFR and PDGFRA mutations are reported as the cause of seconder GBMs'. Additionally cell cycle regulation defficiencies (p53, CDKN2A, RB) and signalization defects ve (NF1, AKT, p13K, PTEN) also had been reported (66).

Ciafre et al reported miRNAs expression with microarray analysis method in GBM to explain the miRNAs expression in tumors compared to normal brain (42). Additionally they found the upregulation of miR-221, but also confirmed that expression levels of miR-221 increased in subset of glioblastoma samples. The result of our study also supports the hyperexpression of miR-221 in gliomas.

Gillies and Lorimer analyzed the effect of miR-221 and defined a specific function which shows that repressing cell cycle expression regulatory protein p27Kip1 by upregulation of expression levels of miRNA 221 and 222 in glioblastoma cells (67). The p27Kip1 gene, the Cip/Kip family member of cyclin-dependent kinase(CDK) inhibitors, which is taking part in controlling cell cycle progression negatively. Prevention of cell cycle from G1 to S phase is made by protein binding to cyclin E and CDK2 complexes. p27Kip1protein is also acting as a tumor suppressor in human cancers.

Le Sage et al. performed in vitro study that conducted on GBM cell lines. In this study they determined the relation between the high expression levels of miR-221 and miRNA 222 and low levels of p27Kip1 gene expression in GBM cell lines They

showed that high activity of miR-221 and 222 is required for regulation of continuous proliferation that inhibited p27Kip1 gene expression. (68).

Gonzalez et al. had reported that miRNA221 provides connection between these two cell cycle regulatory genes via the inhibition of cdk4 activity enhancing translation of p27Kip1 which (69). Furthermore, Ishii et al. reported that through INK4a/ARF mutations, cdk4 inhibition can be the cause of enhanced miR-221 levels and also reduced p27Kip1 levels. This process was maintained by the 30 UTR of p27Kip1. Also increaed expression level of the cdk4 inhibitor p16Ink4a gene upregulated in the terms of mutated INK4A/ARF gene locus determined in GBM cell lines (70).

Slingerland et al. claimed that because of the oncogenic effect of increaed miRNA-221 expression levels, the up-regulation of miRNA-221 plays very important role in the malformed cell cycle control in high-grade gliomas. Owing to high expression levels of miRNA21 cause enhanced proliferation rate of aggresive tumors could be compared with less prolerative tumors (71). Additionally, up regulation of miRNA-221 is related with poor prognosis in not only GBM but also pancreatic adenocarcinoma, thyroid papillary carcinomas. Enhanced miRNA-221 indeed reinforcing correlation between p27Kip1 and miR-221 in the pathogenesis process of malign glial tumors (72,73,74).

Zhang et al investigated thirty-two publications that includes fifteen types of tumor and 2,693 patients were evaluated in their meta-analysis (75). The results of variation and multivariate analyses reported that miR-221/222 cluster highexpression in many tumors was significantly associated with adverse overall survey (75).

Lee et al reported the diagnosis of cancer with a combined therapy and developed a doxorubicinconjugated miR-221 molecular beacon (miR-221 DOXO MB). This compound is composed of three nucleotides as doxorubicin binding site, binding sequence and miR-221 black hole quencher-1 (BHQ1) (76). Finding expression of miR-221 and miR-221 inhibition function by miR-221 DOXO MB can use as candidate theragnostic probe in several cancers. They suggested that theragnostic probe method could account for promissing therapy which aims to inhibit oncogenic miRNAs affects in many cancers (76).

In conclusion, we found that miRNA221 expression could be a biomarker for glial tumors. But further researches must be made to make clear for its sensitivity and specifity to use for prognosis of glial tumors.

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7. APPENDICES

```
Your temporary usage period for IBM SPSS Statistics will expire in 6178 days.

GET
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DATASET NAME DataSet1 WINDOW=FRONT.
T-TEST GROUPS=grup(1 0)
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  /CRITERIA=CI(.95).
```

T-Test

Notes

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[DataSet1] C:\Users\seda.gulec\Desktop\Selçuk Özdoğan Tez.sav

Group Statistics

	grup	N	Mean	Std. Deviation	Std. Error Mean
yas	hasta	39	43,10	20,218	3,238
	kontrol	40	42,58	10,120	1,600

Independent Samples Test

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2
yas	Equal variances assumed	27,701	,000	,147	77	
	Equal variances not assumed			,146	55,598	

```
CROSSTABS
  /TABLES=grup BY sex
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.

Syntax		CROSSTABS /TABLES=grup BY sex /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,00
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	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
grup * sex	79	77,5%	23	22,5%	102	100,0%

grup * sex Crosstabulation

			sex		
			erkek	kadın	Total
grup	kontrol	Count	25	15	40
		% within grup	62,5%	37,5%	100,0%
		% within sex	52,1%	48,4%	50,6%
		% of Total	31,6%	19,0%	50,6%
	hasta	Count	23	16	39
		% within grup	59,0%	41,0%	100,0%
		% within sex	47,9%	51,6%	49,4%
		% of Total	29,1%	20,3%	49,4%
Total		Count	48	31	79
		% within grup	60,8%	39,2%	100,0%
		% within sex	100,0%	100,0%	100,0%
		% of Total	60,8%	39,2%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,103[a]	1	,748		
Continuity Correction[b]	,008	1	,928		
Likelihood Ratio	,103	1	,748		

Fisher's Exact Test				,820	,464
Linear-by-Linear Association	,102	1	,750		
N of Valid Cases	79				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 15,30.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approxim Significar
Interval by Interval	Pearson's R	,036	,112	,317	
Ordinal by Ordinal	Spearman Correlation	,036	,112	,317	
N of Valid Cases		79			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for grup (kontrol / hasta)	1,159	,470	2,863
For cohort sex = erkek	1,060	,743	1,512
For cohort sex = kadın	,914	,528	1,583
N of Valid Cases	79		

```
CROSSTABS
  /TABLES=grup BY type
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

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	N of Rows in Working Data File	102
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=grup BY type /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,02
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	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
grup * type	79	77,5%	23	22,5%	102	100,0%

grup * type Crosstabulation

			type					
			kontrol	Oligo-3	GBM	Oligo-2	Astro-3	Astro-2
grup	kontrol	Count	40	0	0	0	0	
		% within grup	100,0%	0,0%	0,0%	0,0%	0,0%	
		% within type	100,0%	0,0%	0,0%	0,0%	0,0%	
		% of Total	50,6%	0,0%	0,0%	0,0%	0,0%	
	hasta	Count	0	2	21	12	2	
		% within grup	0,0%	5,1%	53,8%	30,8%	5,1%	
		% within type	0,0%	100,0%	100,0%	100,0%	100,0%	10
		% of Total	0,0%	2,5%	26,6%	15,2%	2,5%	
Total		Count	40	2	21	12	2	
		% within grup	50,6%	2,5%	26,6%	15,2%	2,5%	
		% within type	100,0%	100,0%	100,0%	100,0%	100,0%	10

	% of Total	50,6%	2,5%	26,6%	15,2%	2,5%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	79,000[a]	5	,000
Likelihood Ratio	109,505	5	,000
Linear-by-Linear Association	62,977	1	,000
N of Valid Cases	79		

a. 6 cells (50,0%) have expected count less than 5. The minimum expected count is ,99.

Symmetric Measures

	% of Total	12,8%	87,2%	100,0%
Total	Count	5	34	39
	% within grup	12,8%	87,2%	100,0%
	% within IDH_1	100,0%	100,0%	100,0%
	% of Total	12,8%	87,2%	100,0%

Crosstab

			sag_sol		
			sağ	sol	Total
grup	hasta	Count	18	21	39
		% within grup	46,2%	53,8%	100,0%
		% within sag_sol	100,0%	100,0%	100,0%
		% of Total	46,2%	53,8%	100,0%
Total		Count	18	21	39
		% within grup	46,2%	53,8%	100,0%
		% within sag_sol	100,0%	100,0%	100,0%
		% of Total	46,2%	53,8%	100,0%

Chi-Square Tests

	Value
Pearson Chi-Square	.[a]
N of Valid Cases	39

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

Symmetric Measures

		Value
Interval by Interval	Pearson's R	.[a]
N of Valid Cases		39

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

Risk Estimate

	Value
Odds Ratio for grup (hasta / .)	.[a]

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

```
T-TEST GROUPS=grup(1 0)
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  /VARIABLES=delta_ct
  /CRITERIA=CI(.95).
```

T-Test

Notes

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Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=grup(1 0) /MISSING=ANALYSIS /VARIABLES=delta_ct /CRITERIA=CI(.95).

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Group Statistics

	grup	N	Mean	Std. Deviation	Std. Error Mean
delta_ct	hasta	39	2,9882	2,11515	,33870
	kontrol	40	,8268	2,27213	,35926

Independent Samples Tes

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	S
delta_ct	Equal variances assumed	,402	,528	4,374	77	
	Equal variances not assumed			4,378	76,838	

```
ONEWAY delta_ct BY type
  /STATISTICS DESCRIPTIVES HOMOGENEITY WELCH
  /MISSING ANALYSIS
  /POSTHOC=BONFERRONI T3 ALPHA(0.05).
```

Oneway

Notes

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	N of Rows in Working Data File	102

Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY delta_ct BY type /STATISTICS DESCRIPTIVES HOMOGENEITY WELCH /MISSING ANALYSIS /POSTHOC=BONFERRONI T3 ALPHA(0.05).
Resources	Processor Time	00:00:00,03
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Descriptives

delta_ct

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Mi
					Lower Bound	Upper Bound	
kontrol	40	,8268	2,27213	,35926	,1001	1,5534	
Oligo-3	2	2,1550	2,18496	1,54500	-17,4761	21,7861	
GBM	21	3,0952	2,31495	,50516	2,0415	4,1490	
Oligo-2	12	2,9450	2,01027	,58032	1,6677	4,2223	
Astro-3	2	4,4600	1,01823	,72000	-4,6885	13,6085	
Astro-2	2	1,4850	1,43543	1,01500	-11,4118	14,3818	
Total	79	1,8938	2,43795	,27429	1,3477	2,4399	

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
delta_ct	Based on Mean	,407	5	73	,843
	Based on Median	,360	5	73	,874
	Based on Median and with adjusted df	,360	5	68,293	,874
	Based on trimmed mean	,381	5	73	,860

ANOVA

delta_ct

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102,758	5	20,552	4,158	,002

Within Groups	360,845	73	4,943		
Total	463,603	78			

Robust Tests of Equality of Means

delta_ct

	Statistic[a]	df1	df2	Sig.
Welch	3,644	5	4,246	,110

a. Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: delta_ct

	(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bo
Bonferroni	kontrol	Oligo-3	-1,32825	1,61094	1,000	-6,2172	
		GBM	-2,26849*	,59913	,005	-4,0868	
		Oligo-2	-2,11825	,73178	,075	-4,3391	
		Astro-3	-3,63325	1,61094	,407	-8,5222	
		Astro-2	-,65825	1,61094	1,000	-5,5472	
	Oligo-3	kontrol	1,32825	1,61094	1,000	-3,5607	
		GBM	-,94024	1,64527	1,000	-5,9334	
		Oligo-2	-,79000	1,69808	1,000	-5,9434	
		Astro-3	-2,30500	2,22330	1,000	-9,0524	
		Astro-2	,67000	2,22330	1,000	-6,0774	
	GBM	kontrol	2,26849*	,59913	,005	,4502	
		Oligo-3	,94024	1,64527	1,000	-4,0529	
		Oligo-2	,15024	,80455	1,000	-2,2915	
		Astro-3	-1,36476	1,64527	1,000	-6,3579	
		Astro-2	1,61024	1,64527	1,000	-3,3829	
	Oligo-2	kontrol	2,11825	,73178	,075	-,1026	
		Oligo-3	,79000	1,69808	1,000	-4,3634	

		GBM	-,15024	,80455	1,000	-2,5919	
		Astro-3	-1,51500	1,69808	1,000	-6,6684	
		Astro-2	1,46000	1,69808	1,000	-3,6934	
	Astro-3	kontrol	3,63325	1,61094	,407	-1,2557	
		Oligo-3	2,30500	2,22330	1,000	-4,4424	
		GBM	1,36476	1,64527	1,000	-3,6284	
		Oligo-2	1,51500	1,69808	1,000	-3,6384	
		Astro-2	2,97500	2,22330	1,000	-3,7724	
	Astro-2	kontrol	,65825	1,61094	1,000	-4,2307	
		Oligo-3	-,67000	2,22330	1,000	-7,4174	
		GBM	-1,61024	1,64527	1,000	-6,6034	
		Oligo-2	-1,46000	1,69808	1,000	-6,6134	
		Astro-3	-2,97500	2,22330	1,000	-9,7224	
Dunnett T3	kontrol	Oligo-3	-1,32825	1,58622	,971	-41,3513	
		GBM	-2,26849*	,61988	,011	-4,1898	
		Oligo-2	-2,11825	,68252	,073	-4,3556	
		Astro-3	-3,63325	,80465	,245	-14,1986	
		Astro-2	-,65825	1,07670	,996	-21,1534	
	Oligo-3	kontrol	1,32825	1,58622	,971	-38,6948	
		GBM	-,94024	1,62549	,997	-34,0473	
		Oligo-2	-,79000	1,65039	,999	-30,6022	
		Astro-3	-2,30500	1,70453	,851	-28,6043	
		Astro-2	,67000	1,84858	1,000	-20,1018	
	GBM	kontrol	2,26849*	,61988	,011	,3471	
		Oligo-3	,94024	1,62549	,997	-32,1668	
		Oligo-2	,15024	,76939	1,000	-2,3107	
		Astro-3	-1,36476	,87954	,789	-8,6843	
		Astro-2	1,61024	1,13376	,831	-13,3597	
	Oligo-2	kontrol	2,11825	,68252	,073	-,1191	
		Oligo-3	,79000	1,65039	,999	-29,0222	
		GBM	-,15024	,76939	1,000	-2,6112	
		Astro-3	-1,51500	,92475	,755	-7,9920	
		Astro-2	1,46000	1,16918	,888	-11,5077	
	Astro-3	kontrol	3,63325	,80465	,245	-6,9321	
		Oligo-3	2,30500	1,70453	,851	-23,9943	
		GBM	1,36476	,87954	,789	-5,9548	
		Oligo-2	1,51500	,92475	,755	-4,9620	
		Astro-2	2,97500	1,24444	,524	-10,2060	
	Astro-2	kontrol	,65825	1,07670	,996	-19,8369	
		Oligo-3	-,67000	1,84858	1,000	-21,4418	
		GBM	-1,61024	1,13376	,831	-16,5801	
		Oligo-2	-1,46000	1,16918	,888	-14,4277	
		Astro-3	-2,97500	1,24444	,524	-16,1560	

*. The mean difference is significant at the 0.05 level.

```
ONEWAY delta_ct BY lokasyon
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  /MISSING ANALYSIS
  /POSTHOC=BONFERRONI T3 ALPHA(0.05).
```

Oneway

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY delta_ct BY lokasyon /STATISTICS DESCRIPTIVES HOMOGENEITY WELCH /MISSING ANALYSIS /POSTHOC=BONFERRONI T3 ALPHA(0.05).
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Warnings

Post hoc tests are not performed for delta_ct because at least one group has fewer than two cases.

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
delta_ct	Based on Mean	,657	5	31	,659
	Based on Median	,561	5	31	,729
	Based on Median and with adjusted df	,561	5	23,755	,729
	Based on trimmed mean	,626	5	31	,681

ANOVA

delta_ct

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16,714	7	2,388	,483	,840
Within Groups	153,293	31	4,945		
Total	170,007	38			

Robust Tests of Equality of Means[b]

delta_ct

	Statistic[a]	df1	df2	Sig.
Welch	.	.	.	.

a. Asymptotically F distributed.

b. Robust tests of equality of means cannot be performed for delta_ct because at least one group has the sum of case weights less than or equal to 1.

```
T-TEST GROUPS=IDH_1(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=delta_ct
  /CRITERIA=CI(.95).
```

T-Test

Notes

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	Split File	<none>
	N of Rows in Working Data File	102
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=IDH_1(1 0) /MISSING=ANALYSIS /VARIABLES=delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,01

Independent Samples Tes

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	S
delta_ct	Equal variances assumed	,354	,556	1,113	37	
	Equal variances not assumed			1,468	6,777	

```
T-TEST GROUPS=sag_sol(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=delta_ct
  /CRITERIA=CI(.95).
```

	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	102
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=GBM(1 0) /MISSING=ANALYSIS /VARIABLES=delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,02

Group Statistics

	GBM	N	Mean	Std. Deviation	Std. Error Mean
delta_ct	GBM	21	3,0952	2,31495	,50516
	değil	58	1,4588	2,35131	,30874

Independent Samples Tes

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	S
delta_ct	Equal variances assumed	,209	,649	2,744	77	
	Equal variances not assumed			2,764	35,971	

```
T-TEST GROUPS=temporal(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=delta_ct
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		31-JAN-2019 15:05:49
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\Selçuk Özdoğan Tez.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	102
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=temporal(1 0) /MISSING=ANALYSIS /VARIABLES=delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,02

Group Statistics

	temporal	N	Mean	Std. Deviation	Std. Error Mean
delta_ct	temporal	16	3,2075	2,61265	,65316
	değil	63	1,5602	2,29511	,28916

Independent Samples Test

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	S
delta_ct	Equal variances assumed	,026	,872	2,493	77	
	Equal variances not assumed			2,306	21,258	

```
T-TEST GROUPS=frontal(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=delta_ct
  /CRITERIA=CI(.95).
```

Independent Samples Test

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	S
delta_ct	Equal variances assumed	,003	,958	2,013	77	
	Equal variances not assumed			1,904	4,490	

```
ONEWAY delta_ct BY type
  /STATISTICS DESCRIPTIVES HOMOGENEITY WELCH
  /MISSING ANALYSIS
  /POSTHOC=BONFERRONI T3 ALPHA(0.05).
```

Descriptives

delta_ct

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Mi
					Lower Bound	Upper Bound	
Oligo-3	2	2,1550	2,18496	1,54500	-17,4761	21,7861	
GBM	21	3,0952	2,31495	,50516	2,0415	4,1490	
Oligo-2	12	2,9450	2,01027	,58032	1,6677	4,2223	
Astro-3	2	4,4600	1,01823	,72000	-4,6885	13,6085	
Astro-2	2	1,4850	1,43543	1,01500	-11,4118	14,3818	
Total	39	2,9882	2,11515	,33870	2,3026	3,6739	

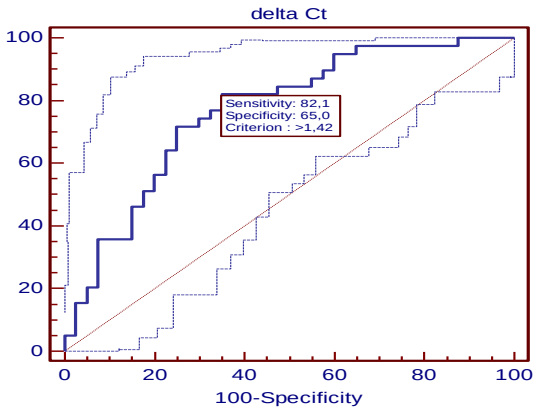
Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
delta_ct	Based on Mean	,356	4	34	,838
	Based on Median	,226	4	34	,922
	Based on Median and with adjusted df	,226	4	30,523	,921
	Based on trimmed mean	,283	4	34	,887

ANOVA

delta_ct

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10,503	4	2,626	,560	,693



ROC curve

Variable	delta_Ct delta Ct
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	39
Negative group	
diagnosis	= 0
Sample size	40

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,767
Standard Error	0,0536
95% Confidence Interval	0,659 to 0,855
z statistic	4,987

Significance level P (Area=0.5)	0,0001

Criterion values and coordinates of the ROC curve[Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>=-4,03	100,00	90,9 - 100,0	0,00	0,0 - 8,9	1,00			
>-4,03	100,00	90,9 - 100,0	2,50	0,4 - 13,2	1,03	0,1 - 7,1	0,00	
>-3	100,00	90,9 - 100,0	5,00	0,8 - 17,0	1,05	0,3 - 4,1	0,00	
>-2,79	100,00	90,9 - 100,0	7,50	1,7 - 20,4	1,08	0,4 - 3,2	0,00	
>-2,64	100,00	90,9 - 100,0	10,00	2,9 - 23,7	1,11	0,4 - 2,8	0,00	
>-2,52	100,00	90,9 - 100,0	12,50	4,2 - 26,8	1,14	0,5 - 2,6	0,00	
>-1,48	97,44	86,5 - 99,6	12,50	4,2 - 26,8	1,11	0,5 - 2,5	0,21	0,03 - 1,4
>-1,47	97,44	86,5 - 99,6	15,00	5,7 - 29,8	1,15	0,5 - 2,4	0,17	0,02 - 1,2
>-1,32	97,44	86,5 - 99,6	17,50	7,4 - 32,8	1,18	0,6 - 2,3	0,15	0,02 - 1,0
>-1,14	97,44	86,5 - 99,6	20,00	9,1 - 35,7	1,22	0,7 - 2,3	0,13	0,02 - 0,9
>-0,67	97,44	86,5 - 99,6	22,50	10,9 - 38,5	1,26	0,7 - 2,2	0,11	0,02 - 0,8
>-0,56	97,44	86,5 - 99,6	25,00	12,7 - 41,2	1,30	0,8 - 2,2	0,10	0,01 - 0,7
>-0,38	97,44	86,5 - 99,6	27,50	14,6 - 43,9	1,34	0,8 - 2,2	0,093	0,01 - 0,7
>-0,23	97,44	86,5 - 99,6	30,00	16,6 - 46,5	1,39	0,9 - 2,2	0,085	0,01 - 0,6
>-0,21	97,44	86,5 - 99,6	32,50	18,6 - 49,1	1,44	0,9 - 2,3	0,079	0,01 - 0,6
>-0,2	97,44	86,5 - 99,6	35,00	20,6 - 51,7	1,50	1,0 - 2,3	0,073	0,01 - 0,5
>0,08	94,87	82,6 - 99,2	35,00	20,6 - 51,7	1,46	1,0 - 2,2	0,15	0,04 - 0,6
>0,25	94,87	82,6 - 99,2	37,50	22,7 - 54,2	1,52	1,0 - 2,3	0,14	0,03 - 0,5
>0,32	94,87	82,6 - 99,2	40,00	24,9 - 56,7	1,58	1,1 - 2,3	0,13	0,03 - 0,5
>0,35	92,31	79,1 - 98,3	40,00	24,9 - 56,7	1,54	1,0 - 2,3	0,19	0,06 - 0,6
>0,39	89,74	75,8 - 97,1	40,00	24,9 - 56,7	1,50	1,0 - 2,2	0,26	0,1 - 0,7
>0,41	89,74	75,8 - 97,1	42,50	27,1 - 59,1	1,56	1,1 - 2,3	0,24	0,09 - 0,6
>0,47	87,18	72,6 - 95,7	42,50	27,1 - 59,1	1,52	1,0 - 2,2	0,30	0,1 - 0,7
>0,52	87,18	72,6 - 95,7	45,00	29,3 - 61,5	1,59	1,1 - 2,3	0,28	0,1 - 0,7
>0,61	84,62	69,5 - 94,1	45,00	29,3 - 61,5	1,54	1,1 - 2,2	0,34	0,2 - 0,8
>0,71	84,62	69,5 - 94,1	47,50	31,5 - 63,9	1,61	1,1 - 2,3	0,32	0,1 - 0,7
>0,85	84,62	69,5 - 94,1	50,00	33,8 - 66,2	1,69	1,2 - 2,4	0,31	0,1 - 0,7
>0,94	84,62	69,5 - 94,1	52,50	36,1 - 68,5	1,78	1,3 - 2,5	0,29	0,1 - 0,7
>1,04	82,05	66,5 - 92,4	52,50	36,1 - 68,5	1,73	1,2 - 2,4	0,34	0,2 - 0,7
>1,08	82,05	66,5 - 92,4	55,00	38,5 - 70,7	1,82	1,3 - 2,5	0,33	0,2 - 0,7
>1,13	82,05	66,5 - 92,4	57,50	40,9 - 72,9	1,93	1,4 - 2,6	0,31	0,1 - 0,7
>1,21	82,05	66,5 - 92,4	60,00	43,3 - 75,1	2,05	1,5 - 2,7	0,30	0,1 - 0,6
>1,41	82,05	66,5 - 92,4	62,50	45,8 - 77,3	2,19	1,7 - 2,9	0,29	0,1 - 0,6
>1,42 *	82,05	66,5 - 92,4	65,00	48,3 - 79,4	2,34	1,8 - 3,1	0,28	0,1 - 0,6
>1,44	76,92	60,7 - 88,8	65,00	48,3 - 79,4	2,20	1,7 - 2,9	0,36	0,2 - 0,7
>1,45	76,92	60,7 - 88,8	67,50	50,9 - 81,4	2,37	1,8 - 3,1	0,34	0,2 - 0,7
>1,62	74,36	57,9 - 86,9	67,50	50,9 - 81,4	2,29	1,7 - 3,0	0,38	0,2 - 0,8
>1,89	74,36	57,9 - 86,9	70,00	53,5 - 83,4	2,48	1,9 - 3,3	0,37	0,2 - 0,7
>2,08	71,79	55,1 - 85,0	70,00	53,5 - 83,4	2,39	1,8 - 3,2	0,40	0,2 - 0,8
>2,1	71,79	55,1 - 85,0	72,50	56,1 - 85,4	2,61	2,0 - 3,4	0,39	0,2 - 0,8
>2,11	71,79	55,1 - 85,0	75,00	58,8 - 87,3	2,87	2,2 - 3,7	0,38	0,2 - 0,8

>2,17	69,23	52,4 - 83,0	75,00	58,8 - 87,3	2,77	2,1 - 3,6	0,41	0,2 - 0,8
>2,3	66,67	49,8 - 80,9	75,00	58,8 - 87,3	2,67	2,0 - 3,5	0,44	0,2 - 0,9
>2,32	64,10	47,2 - 78,8	75,00	58,8 - 87,3	2,56	1,9 - 3,4	0,48	0,2 - 0,9
>2,38	64,10	47,2 - 78,8	77,50	61,5 - 89,1	2,85	2,1 - 3,8	0,46	0,2 - 0,9
>2,47	61,54	44,6 - 76,6	77,50	61,5 - 89,1	2,74	2,0 - 3,7	0,50	0,2 - 1,0
>2,5	58,97	42,1 - 74,4	77,50	61,5 - 89,1	2,62	1,9 - 3,6	0,53	0,3 - 1,1
>2,52	56,41	39,6 - 72,2	77,50	61,5 - 89,1	2,51	1,8 - 3,5	0,56	0,3 - 1,1
>2,54	56,41	39,6 - 72,2	80,00	64,3 - 90,9	2,82	2,1 - 3,9	0,54	0,3 - 1,1
>2,59	53,85	37,2 - 69,9	80,00	64,3 - 90,9	2,69	1,9 - 3,7	0,58	0,3 - 1,2
>2,61	51,28	34,8 - 67,6	80,00	64,3 - 90,9	2,56	1,8 - 3,6	0,61	0,3 - 1,2
>2,62	51,28	34,8 - 67,6	82,50	67,2 - 92,6	2,93	2,1 - 4,1	0,59	0,3 - 1,2
>2,78	48,72	32,4 - 65,2	82,50	67,2 - 92,6	2,78	2,0 - 4,0	0,62	0,3 - 1,3
>2,83	46,15	30,1 - 62,8	82,50	67,2 - 92,6	2,64	1,8 - 3,8	0,65	0,3 - 1,4
>2,88	46,15	30,1 - 62,8	85,00	70,2 - 94,3	3,08	2,1 - 4,4	0,63	0,3 - 1,4
>2,9	43,59	27,8 - 60,4	85,00	70,2 - 94,3	2,91	2,0 - 4,2	0,66	0,3 - 1,5
>2,92	41,03	25,6 - 57,9	85,00	70,2 - 94,3	2,74	1,8 - 4,1	0,69	0,3 - 1,5
>3	38,46	23,4 - 55,4	85,00	70,2 - 94,3	2,56	1,7 - 3,9	0,72	0,3 - 1,6
>3,14	35,90	21,2 - 52,8	85,00	70,2 - 94,3	2,39	1,5 - 3,7	0,75	0,3 - 1,6
>3,28	35,90	21,2 - 52,8	87,50	73,2 - 95,8	2,87	1,9 - 4,4	0,73	0,3 - 1,7
>3,36	35,90	21,2 - 52,8	90,00	76,3 - 97,1	3,59	2,3 - 5,5	0,71	0,3 - 1,9
>3,42	35,90	21,2 - 52,8	92,50	79,6 - 98,3	4,79	3,1 - 7,3	0,69	0,2 - 2,1
>3,64	33,33	19,1 - 50,2	92,50	79,6 - 98,3	4,44	2,8 - 7,0	0,72	0,2 - 2,2
>3,7	30,77	17,0 - 47,6	92,50	79,6 - 98,3	4,10	2,5 - 6,6	0,75	0,2 - 2,3
>3,74	28,21	15,0 - 44,9	92,50	79,6 - 98,3	3,76	2,3 - 6,3	0,78	0,3 - 2,3
>3,79	25,64	13,1 - 42,1	92,50	79,6 - 98,3	3,42	2,0 - 5,9	0,80	0,3 - 2,4
>3,96	23,08	11,2 - 39,3	92,50	79,6 - 98,3	3,08	1,7 - 5,5	0,83	0,3 - 2,5
>4,05	20,51	9,3 - 36,5	92,50	79,6 - 98,3	2,74	1,5 - 5,1	0,86	0,3 - 2,6
>4,16	20,51	9,3 - 36,5	95,00	83,0 - 99,2	4,10	2,2 - 7,6	0,84	0,2 - 3,3
>4,59	17,95	7,6 - 33,5	95,00	83,0 - 99,2	3,59	1,8 - 7,0	0,86	0,2 - 3,4
>4,64	15,38	5,9 - 30,5	95,00	83,0 - 99,2	3,08	1,5 - 6,4	0,89	0,2 - 3,5
>4,65	15,38	5,9 - 30,5	97,50	86,8 - 99,6	6,15	2,9 - 12,9	0,87	0,1 - 6,0
>5,03	12,82	4,3 - 27,4	97,50	86,8 - 99,6	5,13	2,3 - 11,6	0,89	0,1 - 6,2
>5,18	10,26	2,9 - 24,2	97,50	86,8 - 99,6	4,10	1,6 - 10,4	0,92	0,1 - 6,4
>5,22	7,69	1,7 - 20,9	97,50	86,8 - 99,6	3,08	1,0 - 9,1	0,95	0,1 - 6,6
>6,93	5,13	0,8 - 17,4	97,50	86,8 - 99,6	2,05	0,5 - 7,9	0,97	0,1 - 6,7
>7,14	5,13	0,8 - 17,4	100,00	91,1 - 100,0			0,95	
>7,41	2,56	0,4 - 13,5	100,00	91,1 - 100,0			0,97	
>9,57	0,00	0,0 - 9,1	100,00	91,1 - 100,0			1,00	

7.1.FORMS

Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15-1237
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

23/06/2016

İlgili Makama (Selçuk Özdoğan)

Kartal Lütfü Kırdar Eğitim ve Araştırma Hastanesi Beyin ve Sinir Cerrahisi Anabilim Dalı Dr. Selçuk Özdoğan'ın sorumlu olduğu **"Glial Tümörlerde MicroRNA 221 Düzeylerinin Belirlenmesi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1233** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **22.06.2016** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 634**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

7.2CIRRUCULUM VITAE

Personal Informations

Name	SELÇUK	**Surname**	ÖZDOĞAN
Place of Birth	Mersin	**Date of Birth**	15.06.1978
Nationality	T.C.	**ID**	16709173082
Email	drselcukozdogan@hotmail.com	**Phone**	5067637173

Educational Informations

	Name of Institution	**Year**
Doctorate	Yeditepe University Health Institute Molecular Medicine	2013-2019
Medical Expertise	Ufuk University Faculty of Medicine Department of Neurosurgery, Ankara, Turkey	2006 – 2011
University	Gazi University Medical Faculty	1996 – 2005

Work Experience

Responsibilty	**Institution**	**Year**
Chief Asisstant	Sancaktepe Prof.Dr. İlhan Varank Eğitim ve Araitırma Hastanesi	2019
Chief Asisstant	İstanbul Eğitim ve Araştırma Hastanesi	2017-2018
Chief Asisstant	İstanbul Gaziosmanpaşa Taksim Eğitim ve Araştırma Hastanesi–	2016
Specialist	İstanbul Dr.Lütfi Kırdar Kartal Eğitim ve Arağtırma Hastanesi	2015
Specialist	Metin Sabancı Baltalimanı Kemik Hastalıkları Eğitim ve Araştırma Hastanesi	2014
Clinical Fellow	Yeditepe Üniversitesi Hastanesi Beyin Cerrahisi Anabilim Dalı	2013-2014
Specialist	Muş Devlet Hastanesi	2011-2013

Language	Reading*	Speaking*	Writing*	KPDS/UDS Score	(Other) Score
English	Good	Good	Good		YOKDIL 82,5

*****Very Good, Good, Basic

Computer skills

Program	Ability to use
Microsoft Office	Very Good

Publications / Notices Certificates / Awards

A –International Articles

1. Bakirezer SD, Yaltirik CK, Kaya AH, Yilmaz SG, **Ozdogan S**, Billur D, Isbir T. The Evaluation of Glutathione Reductase and Malondialdehyde Levels in Patients With Lumbar Disc Degeneration Disease. In Vivo. 2019 May-Jun;33(3):811-814.
2. Yaltirik CK, Timirci-Kahraman Ö, Gulec-Yilmaz S, **Ozdogan S**, Atalay B, Isbir T. 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. 2019 Mar-Apr;33(2):413-417.
3. Kotil K, **Ozdogan S**, Kayaci S, Duzkalir HG. Long-term Outcomes of a New Minimally Invasive Approach in Chiari Type 1 and 1.5 Malformations: Technical Note and Preliminary Results. World Neurosurg. 2018 Apr 23. pii: S1878-8750(18)30820-9. doi: 10.1016/j.wneu.2018.04.100. [Epub ahead of print]
4. Basaran R, Efendioglu M, Senol M, **Ozdogan S**, Isik N. Morphometric analysis of posterior fossa and craniovertebral junction in subtypes of Chiari malformation. Clin Neurol Neurosurg. 2018 Jun;169:1-11. doi: 10.1016/j.clineuro.2018.03.017. Epub 2018 Mar 20.

5. **Özdoğan S**, Yaltirik CK, Yilmaz SG, Koçak A, Isbir T. Association of rs2228570 Polymorphism of Vitamin D Receptor Gene with Lumbar Degenerative Disc Disease. Turk Neurosurg. 2018 Mar 11. doi: 10.5137/1019-5149.JTN.22275-17.2. [Epub ahead of print]
6. **Özdoğan S**, Yaltırık CK, Düzkalır AH, Demirel N, Kaya M, Atalay B. Spinal Tuberculosis Mimicking Failed Back Surgery. Am J Case Rep. 2018 Mar 6;19:249-253.
7. **Ozdogan S**, Yaltirik CK, Yilmaz SG, Kaya M, Duzkalir AH, Demirel N, Kafadar A, Isbir T. Tumor Necrosis Factor-Alpha (TNF-α-308 G>A) Polymorphism in High-grade Gliomas. In Vivo. 2018 Mar-Apr;32(2):287-289.
8. **Özdoğan S,** Saymaz C, Yaltırık CK, Düzkalır HG, Kaya M, Demirel N, Düzkalır AH, Sarıkaya B, Aktekin B. Encephalocraniocutaneous Lipomatosis: Haberland Syndrome. Am J Case Rep. 2017 Dec 1;18:1271-1275.
9. **Özdoğan S,** Kaya M, Demirel N, Düzkalır AH, Yaltırık CK. Isolated C5 Vertebrae Dislocation with Trauma: An Extremely Rare Case of Isolated C5 Dislocation. Am J Case Rep. 2017 Nov 28;18:1256-1260.
10. Yaltırık CK, **Özdoğan S**, Atalay B. Thoracic Epidural Hematoma Complicating Vertebroplasty. Am J Case Rep. 2017 Nov 21;18:1229-1232.
11. **Ozdogan S,** Kafadar A, Yilmaz SG, Timirci-Kahraman O, Gormus U, Isbir T. Role of Caspase-9 Gene Ex5+32 G>A (rs1052576) Variant in Susceptibility to Primary Brain Tumors. Anticancer Res. 2017 Sep;37(9):4997-5000.
12. Süslü H, Süslü HT, **Özdoğan S**, Güçlü B, Düzkalır AH. Percutaneous Radiofrequency Trigeminal Rhizotomy for the Treatment of Idiopathic Trigeminal Neuralgia: Experience in 106 patients. Journal of Neurological Sciences (Yayın Kabul Edildi Basım Asamasında)
13. Yaltirik K, **Özdoğan S**, Doğan Ekici I, Atalay B. Cauda equina cavernous hemangioma: very rare pediatric case. Childs Nerv Syst 2016 doi:10.1007/s00381-016-3286-9, 2016
14. Yaltirik K, Ashour AM, Reis CR, **Ozdogan S,** Atalay B. Vertebral augmentation by kyphoplasty and vertebroplasty: 8 years experience outcomes and complications. J Craniovert Jun Spine 7:153-60, 2016

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G – National Book Chapters

1. **Özdoğan S,** Yaman O. Toraks ve Torakal Omurganın Biyomekaniği. Servikal ve Torakal Omurganın dejeneratif hastalıkları. Türk Nöroşirurji Derneği Yayınları No:20 s305-309, 2017
2. **Özdoğan S,** Kahraman S. Servikal Dar Kanal ve Spondilotik Myelopatide Tanı/Ayırıcı Tanı. Omurga ve Omurilik Cerrahisinde Tanı El Kitabı. Türk Nöroşirurji Derneği Yayınları No:19 s279-282, 2017
3. **Özdoğan S,** Yaman O. Omurga geometrisi ve Denge Ölçümleri. Omurga ve Omurilik Cerrahisinde Tanı El Kitabı. Türk Nöroşirurji Derneği Yayınları No:19 s112-118, 2017
4. **Özdoğan S,** Düzkalır AH, Kahraman S. Servikal sagittal denge. Omurganın Sagital Dengesinin Klinik Önemi. Türk Omurga Derneği Yayınları No:11 s111-122, 2017
5. **Özdoğan S,** Şenol Ö, Benli T. Vertebroplasti ve Kifoplasti. Osteoporotik Omurga. Türk Omurga Derneği Yayınları No:9 s243-252, 2016

6. **Özdoğan S,** Sabuncuoglu H. Atlantoaksiyel instabilite. Omurganın Sagital Plan Deformiteleri. Türk Omurga Derneği Yayınları No:7 s47-56, 2016
7. **Özdoğan S,** Sabuncuoglu H. Kranioservikal disosiasyon. Omurganın Sagital Plan Deformiteleri. Türk Omurga Derneği Yayınları No:7 s37-46, 2016
8. **Özdoğan S,** Düzkalır AH. Rubinstein-Taybi sendromu. Omurgayı Tutan Sendromlar. Türk Omurga Derneği Yayınları No:6 s397-406, 2016
9. **Özdoğan S,** Düzkalır AH. Gergin kord sendromu. Omurgayı Tutan Sendromlar. Türk Omurga Derneği Yayınları No:6 s237-250, 2016
10. **Özdoğan S**, Ulutaş M. Büyüyen rod tekniği:Endikasyon ve Prensipler. Spinal Deformiteler. Türk Nöroşirurji Derneği Yayınları No:18 s177-185, 2015
11. Düzkalır AH, **Özdoğan S**, Kahraman S. Konjenital deformitelerin doğal seyri ve prognoz. Spinal Deformiteler. Türk Nöroşirurji Derneği Yayınları No:18 s141-153, 2015
12. Düzkalır AH, **Özdoğan S**, Kahraman S. Sagital plan deformite prensipleri. Spinal Deformiteler. Türk Nöroşirurji Derneği Yayınları No:18 s39-51, 2015
13. **Özdoğan S**, Süslü HT, Dalbayrak S. Omurga tümör cerrahisinde stabilizasyon teknikleri ve greft seçimi. Omurga ve Omurilik Tümörleri. Türk Nöroşirurji Derneği Yayınları No:16, 2014
14. **Özdoğan S**, Süslü HT. Metastatik spinal tümör sınıflamaları. Omurga ve Omurilik Tümörleri. Türk Nöroşirurji Derneği Yayınları No:16, 2014
15. **Özdoğan S,** Güçlü B. Siringo-Plevral şant cerrahisi. Nöroşirurjide Şant Cerrahisi. Ömür Günaldı, Erhan Emel (editörler). Selen Yayıncılık s:229-232, 2014
16. Güçlü B, **Özdoğan S**. Hidrosefali. Nöroşirurjide Şant Cerrahisi. Ömür Günaldı, Erhan Emel (editörler). Selen Yayıncılık s:47-51, 2014
17. Tatarlı N, **Özdoğan S**. Hidrosefali Etyolojisi. Nöroşirurjide Şant Cerrahisi. Ömür Günaldı, Erhan Emel (editörler). Selen Yayıncılık, s:77-81, 2014
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H – International Translated Book Chapters

1. **Özdoğan S**, Sabuncuoğlu H. Serebrovasküler cerrahide mikrovasküler doppler. Ameliyat Sırasında Nöromonitorizasyon, çev.ed. Özlü O, Er U, Er S. Pelikan Yayıncılık, Ankara, s127-131, 2016
2. Sabuncuoğlu H, **Özdoğan S**. Nörovasküler cerrahide ameliyat içi arteriografi ve indosiyanin yeşili videoanjiografi. Ameliyat Sırasında Nöromonitorizasyon, çev.ed. Özlü O, Er U, Er S. Pelikan Yayıncılık, Ankara, s131-141, 2016
3. Sabuncuoğlu H, **Özdoğan S**. L5-S1 mikrodekompresif diskektomiye transspinal yaklaşım. Minimal İnvaziv Omurga Cerrahisi Uygulamaları, çev.ed. T. Şatana, M. Özalay, 421-423, Güneş Tıp Kitabevleri, Ankara, 2010
4. Sabuncuoğlu H., **Özdoğan S**. Endoskopi eşliğinde lomber mikrodiskektomi. Minimal İnvaziv Omurga Cerrahisi Uygulamaları, çev.ed. T. Şatana, M. Özalay, 424-427, Güneş Tıp Kitabevleri, Ankara, 2010

I –Invited Speaker

1. En Kötü Günüm Olgu Sunumları: MCA Anevrizması Sıkıntı Büyük. Türk Nörosirurji Dernegi 32. Bilimsel Kongresi, Antalya, 2018
2. Servikal Sagital Denge Parametreleri. 13. Sinir Sistemi Cerrahisi Kongresi, Fethiye, 2017
3. Psödotümör Serebri. Türk Nörosirurji Dernegi 31. Bilimsel Kongresi, Antalya, 2017
4. Sagital Plan Deformiteleri Olgu Tartışmaları. 12. Uluslararası Türk Omurga Kongresi, Antalya, 2017
5. Gergin Omurilik Cerrahisinde Zamanlama. Türk Nörosirurji Dernegi 30. Bilimsel Kongresi, Antalya, 2016

J –Awards

1. 2018 yılı Spinal Grup Sempozyumu Yılın Sözlü Bildirisi İkincilik Ödülü
2. 2018 yılı Türk Nöroşirurji Derneği Prof.Dr. Mahir Tevruz Bilimsel Araştırma Ödülü
3. 2017 yılı Türk Nöroşirurji Derneği Prof.Dr. Hamit Ziya Gökalp Genç Nöroşirurjiyen Teşvik Üçüncülük Ödülü