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INVESTIGATION OF THE EFFECTS OF MIRNA133, MIRNA26 AND MIRNA378 EXPRESSION LEVELS IN LEFT VENTRICULAR HYPERTROPHY

DOCTOR OF PHILOSOPHY THESIS

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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 (2019) 12.12.19 and numbered 2019/19-18

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

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

Zerrin BARUT

DEDICATION

I dedicate my thesis to my precious teacher Prof.Dr. Turgay İSBİR, my lovely sons Ali Ramiz, Sinan, and my dear my family.

Zerrin BARUT

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Zerrin BARUT

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LIST OF SYMBOLS AND ABBREVIATIONS

$\Delta\Delta C_T$: Delta Delta C_T

ΔC_T : Delta C_T ln(2-($\Delta\Delta$CT)) : Fold change

IncRNA : Long noncoding RNA

LVH : Left Ventricular Hypertrophy

µl : microliter

mg : milligram

miRNA : Micro RNA

mRNA : Messenger RNA

ng : Nanogram

OR : Odds Ratio

piRNA : Interacting RNA

pre-miRNA: Precursor-miRNA

pri-miRNA : Primer-miRNA

RISC : RNA Induced Silencing

Complex RNA: Ribonucleic Acid

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

UTR : Untranslated Region

WHO : World Health Organization

XPO5 : Exportin 5

XRN1 : Exonuclease-1

LDL : Low density lipoprotein

HDL : High density lipoprotein

TG : Trigliserid

DM : Diabetes mellitus

HT : Hypertension

KAH : Chronical artery heart disease

VKI : Body mass index

SVKI :Left ventricular mass index

KLF4 : Transcription factor-4

Coll-A1 : Collagen-A1

ABSTRACT

Barut, Z. Investıgatıon Of The Effects Of miRNA133, miRNA26 and miRNA378 Expressıon Levels In Left Ventricular Hypertrophy. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. Doctorate Thesis. Istanbul, 2019.

Left ventricular hypertrophy (LVH) is characterized by the growth in muscular mass of left ventricule due to increased cardiomyocyte size. The microRNAs, a class of endogenously small non-coding RNAs which have approximately 22-nucleotide, play important roles in numerous biological processesare as mediation of cardiac hypertrophy. In present study we aimed to determine roles of miRNA-133, miRNA26, miRNA378 expression levels in left ventricular hypertrophy. In this study we compared left ventricle hypertrophy patient group (n= 70) and control group (n=16) were obtained from the Yeditepe University Hospital Cardiology Clinic. miRNA expression levels determined by realtime polymerase chain reaction (RT-PCR). The analysis of Receiver operating characteristic (ROC) curve was performed to determine the diagnostic capability of miRNAs. The miRNA expression analysis showed that the mean miRNA378 CT (p= 0.029) and ΔCT (p= 0.020) were significantly higher in patients than controls. By the ROC analysis, high expression of miRNA-378 expression maybe highly associated with the left ventricular hypertrophy. The threshold value in which miRNA-133 CT expression level can be evaluated as a diagnosis in patient group has not been demonstrated. According to teh CT values, miRNA was found to be AUC=0.484, 98.6° CI =0.329-0.641, P= 0.860.The mean CT values of miRNA-26 of the control group had highest expression levels (23.12 ± 3.10), it was followed by hypertrophic and eccentric group were 22.43 ± 2.36 and 22.06 ± 3.68 respectively (p=0.782). In conclusion, while there was no statistically significant relationship was found miRNA-133 and miRNA-26 expression levels there was statically significant relationship was found between miRNA-378 expresion levels in LVH group.

Key Words: Left Ventricular Hypertrophy**,** miRNA-26, miRNA-133, miRNA-378

ÖZET

Barut, Z. Sol ventriküler Hipertrofide miRNA133, miRNA26 ve miRNA378 Ekspresyon Düzeylerinin Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Doktora Tezi. İstanbul, 2019.

Sol ventrikül hipertrofisi (LVH), artmış kardiyomiyosit boyutuna bağlı olarak sol ventrikül kas kütlesindeki büyüme ile karakterizedir. Yaklaşık 22-nükleotidi olan, endojen olarak küçük kodlayıcı olmayan bir RNA sınıfı olan mikroRNA'lar, kardiyak hipertrofinin aracı olarak sayısız biyolojik işlemlerde önemli roller oynamaktadır. Bu çalışmada miRNA-133, miRNA26, miRNA378 ekspresyon düzeylerinin sol ventrikül hipertrofisindeki rollerini belirlemeyi amaçladık. Bu çalışmada, sol ventrikül hipertrofi hasta grubu (n = 70) karşılaştırıldı ve kontrol grubu (n = 16) Yeditepe Üniversitesi Hastane Kardiyoloji Kliniğinden alındı. miRNA ekspresyon düzeyleri, gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) ile belirlenir. MiRNA'ların tanısal kabiliyetini değerlendirmek için alıcı işletim karakteristik (ROC) eğrisi analizi yapılmıştır. MiRNA ekspresyon analizi, ortalama miRNA378 CT (p = 0.029) ve ΔCT'nin (p= 0.020) hastalarda kontrollerden anlamlı olarak yüksek olduğunu gösterdi. ROC analiziyle miRNA-378 ekspresyonunun yüksek ekspresyonu, belki de sol ventrikül hipertrofisi ile yüksek oranda ilişkilidir. MiRNA-133 BT ekspresyon seviyesinin hasta grubunda bir teşhis olarak değerlendirilebileceği eşik değeri gösterilmemiştir. BT değerlerine göre miRNA, AUC = 0.484, 98.6 ° CI = 0.329-0.641, P = 0.860 olarak bulundu. Kontrol grubunun miRNA-26'sının ortalama CT değerleri en yüksek ekspresyon seviyelerine (23.12 ± 3.10) sahipti. ardından hipertrofik ve eksantrik grup sırasıyla 22.43 ± 2.36 ve 22.06 ± 3.68 idi (p = 0.782). Sonuç olarak, istatistiksel olarak anlamlı bir ilişki bulunmamasına rağmen, miRNA-133 ve miRNA-26 ekspresyon seviyeleri, LVH grubunda miRNA-378 ekspresyon seviyeleri arasında istatistiksel olarak anlamlı bir ilişki saptanmıştır.

Key Words: Sol ventriküler hipertrofi**,** miRNA-26, miRNA-133, miRNA-378

1. INTRODUCTION AND PURPOSE

Left ventricular hypertrophy (LVH) is identified as an increase in muscular mass of left ventricule (LV) due to increased size of the cardiomyocyte (1). LVH may be considered as a physiological adaptation such as in athletes or pathological (2).

Primary LVH is the result of primary myocardial diseases such as hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM). HCM is a genetic disease in which genes encoding sarcomeric proteins are observed to hold various mutations (3). Asymmetric hypertrophy is seen and abnormal cardiomyocyte calcium cycling and sensitivity are the accused causes of impaired myofibrillar contractile function (4). DCM is usually a result of myocarditis or exposure to cardiotoxic agents. Eccentric hypertrophy is observed. (5).

Secondary LVH; when the heart encounters a hemodynamic problem, such as an increased stress or load against the ventricular wall, it compensates this problem by cardiomyocyte hypertrophy. It is well known that wall stress is directly correlated to LV intracavitary pressure and LV cavity size and inversely related to wall thickness according to Laplace's law (6). Additionally, cytokines and growth factors and also activation of adrenergic and renin-angiotensin-aldosterone (RAAS) systems are other causes of LVH (7,8).

The main causes of pressure overload are arterial hypertension and aortic stenosis whereas the main causes of volume overload that result in LVH are mitral and aortic regurgitation.

Meerson, in 1962 described the prolonged protective state of "compensatory hyperfunction" of the heart by an experimental aortic constriction model (9). It is suggested that if the pressure overload is continued, fibrosis of LV ensues and this results in decompansation of the heart with dilatation and failure (10).

Left ventricle responds to pressure load either with adaptive or maladaptive signals. Pressure load as in arterial hypertension or aortic stenosis cause maladaptive fibrotic hypertrophy and apoptosis leading to heart failure and increased mortality. However exercise induced LV hypertrohy is associated with enzyme Akt (protein kinase B) that leads to myocyte survival and adoptive hypertrophy. Prolonged stretch or pressure results in release of Angiotensin II (A-II) leads to prosurvival of myocytes via extracellular signal-regulated kinase (ERK) or death-promoting pathway via Jun N- terminal kinase (JNK) (11). Tumor necrosis factor-α (TNF-α) is also important in the pathophysiology of LVH that is, if it acts physiologically, it is prosurvival but in excess, it probably acts adversely via nucleat factor κB (NF- κB) (12).

Left ventricle responds to volume load with Akt activation that is prosurvival for myocytes and results in reduced apoptosis, no fibrosis and better left ventricular function with less mortality (11). These different types of responses of LV to pressure or volume load, result in different treatment strategies (13).

In recent years there are studies revealing association of genetic factors and LVH. Kuster et al suggested that levels of gene expression upon cardiac mast cells are different in patients who have cardiac hypertrophy, as well as healthy donors; furthermore, they stated that early stage hypertrophy can be reversible (14). Myocardin is important in inducing cardiomyocyte hypertrophy. Its function is affected by epigenetic modification regulations and it is reported that NF- κB enhances the expression of micro RNA-1 which inhibits smooth muscle cell proliferation and also NF- κB inhibits myocardin and thus myocardin induced cardiomyocyte hypertrophy (15). Since cardiac failure is the main cause of death in over 65 population, new diagnostic and therapeutic strategies are necessary.

Echocardiography is the widely used diagnostic tool to determine LV mass as it is noninvasive and lacks ionizing radiation. The normal values of left and

right ventricular mass in both males and females are known being; 113g/m^2 in males and 95g/m^2 in females (16).

Within a minimum of just one type of cell, 80.4% of the human genome is transcribed (10). That being said, just 1.22% of DNA gathered from the human genome will actually encode for protein-coding exons, which in total make up the 20,687 previously determined protein coding genes (11). Therefore, the majority of the coding responsible by the human genome is specifically for that of non-coding RNAs (ncRNAs) identified as small RNAs such as tRNA, miRNA, snRNA and snoRNA, non-coding long RNAs (lncRNAs) (12). The roles of ncRNAs (especially regarding lncRNAs and miRNAs) are ever-expanding, and they continue to inform a complex and finely nuanced epigenetic regulation model (13, 14).

It has been found that from regular onset and progression to the actual pathogenesis of a disease, miRNAs are involved. To what extent target miRNAs are related to the miRNAs largely determines the end result of those target miRNAs. An estimated modulation of the expression of over 60% of protein-coding genes is accomplished with the participation of miRNAs (15). The majority of mature miRNAs cause either the inhibition of mRNA transcription or the direct degradation of mRNA, and few miRNAs handle the promotion of mRNA transcription (16). In the year 2008, miRNAs were discovered to be present exterior to the cell and circulating within the blood; these newly found extracellular miRNAs have been found to be very stable even under a multitude of extreme simulated conditions like repeated freeze-thaw cycles, long-term storage and even boiling (17). Such findings as these have encourage an increasing amount of investigations looking into the potential of miRNAs to serve as circulating biomarkers in regard to several prospective therapeutic methods for a variety of diseases including HF (18).

miRNAs have been found to hold a great involvement in gene regulation from the basis of regular development to the pathogenesis of various diseases. The result of target miRNAs depends on its complementary miRNA for the most part

(19). The majority of mature miRNAs inhibit mRNA transcription or are responsible for the direct degradation of mRNAs; few miRNAs promote mRNA transcription (20).

Within the scope of this work, it was aimed to determine the roles of miRNA- 133, miRNA-26 and miRNA-378 in regard to expression levels in left ventricular hypertrophy.

2. LITERATURE REVIEW

2.1. MicroRNAs

MicroRNAs (miRNAs) are 21–25 nucleotides (nts) in length, endogenous regulated small RNAs of. In group of biological assets, specifically plants and animals, by aiming at specific mRNAs for posttranscriptional repression or mRNA degradation, their assignment can be describe as a significant compensator. Also, the synthesis pathways and the organizer mechanisms of miRNAs has been expounded by recent scientific improvements in the animals and plants. Due to applicability of the therapy, disease etiology comprises miRNA-based regulation and miRNA-based regulation has been studied. Additionally, so as to miRNA-based therapeutics, various preclinical and clinical studies have been launched (17, 18).

Hairpin structure is precursor form of miRNAs. Transcription process is started after synthesis of miRNA (Figure 2.1). In animal cells, non coding sequences for coding miRNAs are transcribed to primary miRNA (pri-miRNA). In the nucleus of the pri-miRNA, the Drosha, a class 2 RNase III enzyme, is transferred to a parent miRNA (pre-miRNA). Then, exportin-5 (EXP-5) intercedes the bear of pre-miRNAs to the cytoplasm. In the cytoplasm, further processing occurs to become mature miRNAs by means of Dicer, which is an RNase III type protein before amassing themselves onto the Argonaute (AGO) protein in order to give the resulting effector RNA-induced silencing complex (RISC) (19, 20).

There are types of various proteins that comprise in miRNA process. Nucleus is the place where miRNA are first processed in all animals. Pol II's primiRNA is cleaved to the stem of the hairpin framework releasing an estimated 60–70nt hairpin structure, idenfied as the miRNA precursor (pre-miRNA). Drosha completes this step, which further needs the DiGeorge syndrome critical region in gene 8 (DGCR8) in humans. single strand RNA (ssRNA) segment interacts with the DGCR8in order to guide Drosha for slicing of the pri-miRNA. Drosha

separates 11 basepairs away about from the long single strand RNA-stem loop junction, therefore processing the pri-miRNA convert to the pre-miRNA by overhanging of 5′-phosphate group (21).

PremiRNA is transmitted through nuclear pores which are big protein channels integrated into the nuclear membrane. PremiRNA's freight is interceded by the exportin- 5 (EXP5) RanGTP dependent receptor of nuclear transportation. A recommended model for transmitting miRNA indicates that pre-miRNA export is started when EXP5 participates via stem belt of the double stranded RNA (dsRNA) with a 3OH'overhang and connect with both the GTP-liable and the Ran pre-miRNA cofactor in the nucleus. The pre-miRNA bound EXP5 exports out of the nucleus, where hydrolysis of the GTP results in the release of the pre-miRNA. Two strongly related proteins linked ally with the human Dicer, trans-activation triggers protein kinase and RNA-binding protein (TRBP), interferon-mediates double-stranded RNA-dependent activator (PRKRA). Although the dicer-linked proteins do not look to be needed for any transformation activity, they serve as form the RNA-induced silencing complex. Based on the current model, the miRNA duplex is incorporated into the complex Ago family protein after the processşng of an around 22 nucleotide miRNA duplex by Dicer cleavage (22, 23, 24). This produces a complicated effector Majority of the miRNA strand (passenger strand or miRNA) remains unbundled with Ago as mature guide strand miRNA. The Ago protein which has endonucleolytic enzyme activity, has been shown to be liable for removing the miRNA passenger strands. A great part of the miRNAs have a lack of matching action in the center and there are a few Ago proteins that have no slicer activity, which makes the miRNA passenger strand cleavage resistive. The demonstration shows that the unselected sequence of the miRNA duplex mediates with the RNA helicase. The miRNA leads the RISC to its mRNA target after burdening, which is suppressed by degradations or repressive translations (25-30).

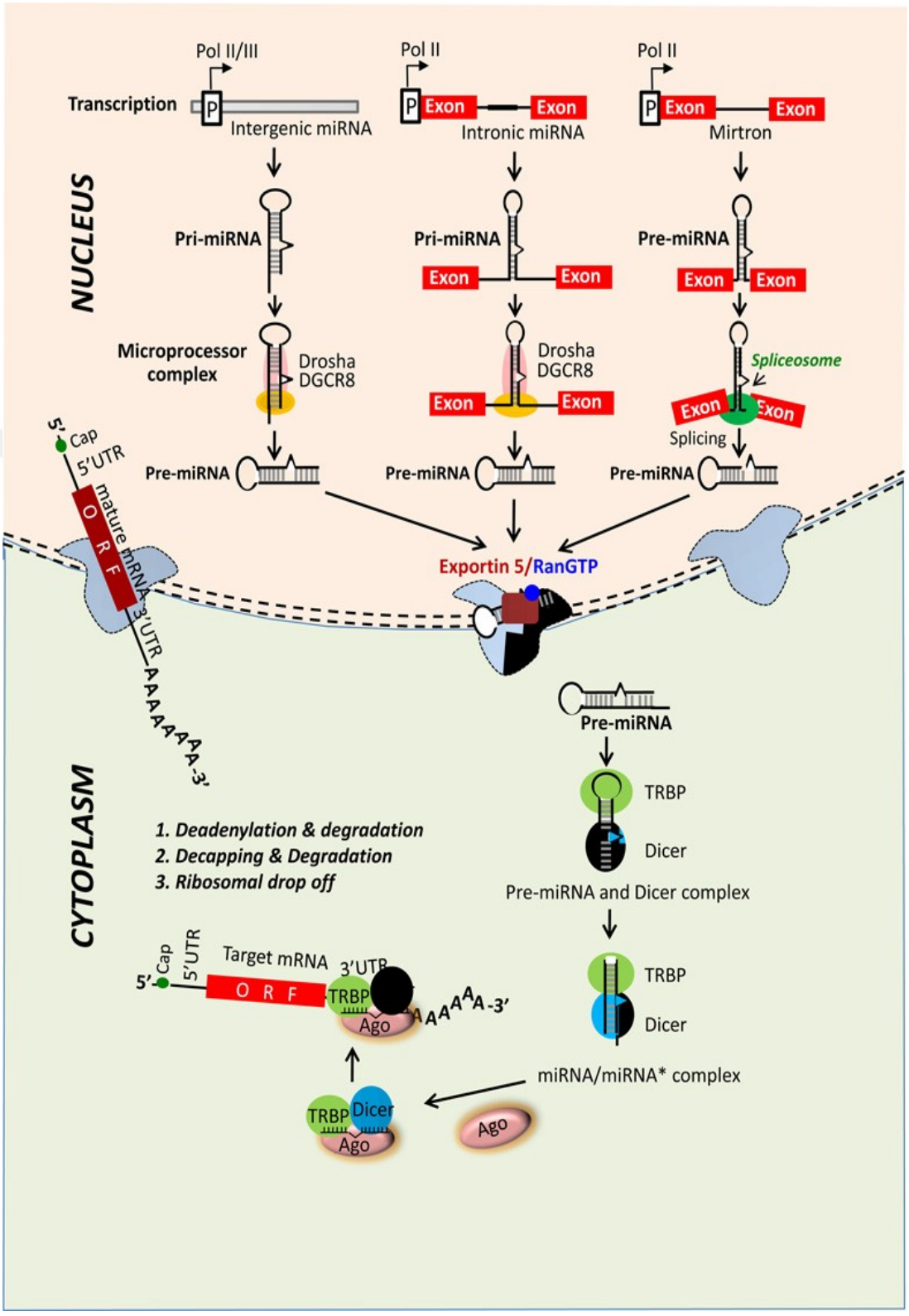

Figure 2.1: General molecular mechanisms of miRNA biogenesis (31).

MiRNAs play an significant position in regulating different mammalian procedures. They offer a main and strong tool in gene control and therefore a potential new kind of therapeutic targets. The evolutionary function of miRNAs and several physiological functions in the animal is evolutionarily preserved. miRNAs are mainly limited in their animal mRNAs, but this is yet adequate to control a variety of physiological mechanisms. It was suggested that the translation process initiation phase should be suppressed and mRNA degradation can follow (31-35).

In scientist's belief, miRNAs are the promissing important class of silencing RNA (sRNA) therapeutic molecules. Due to many therapeutic applications, they have important benefits over siRNAs. Several miRNAs are misregulated in connection with the growth of certain conditions in humans. Restoration of misregulated miRNAs to their ordinary concentrations has proved that illnesses including tumors in animal models can be reduced or even eliminated. Since miRNAs are natural molecules, their implementation as therapeutic agents presents certain benefits. The theory of miRNA replacement, which consists of the introduction of synthetic miRNAs, or miRNA mimetics, in diseased to attempt the regulation of various cellular functions as normal cell proliferation, apoptosis, and cell cycle affected by the altered expression levels of miRNAs is determined by different study groups worldwide. On the other hand, some investigators have used miRNA inhibitors to improve therapeutic protein endogenous concentrations. In theory, the inhibition of a certain miRNA linked with a certain disease may remove a therapeutic protein's block of expression. The administration of a miRNA mimetic, on the other side, could boost the e ndogenous miRNA population and thus eliminate a damaging gene. Reactivation and inhibition of these pathways with miRNA in many instances leads to significant therapeutic reactions (36-40).

2.1.1 miRNA133

There are three kinds of miRNA-133 genes within the human genome: miRNA-133a-1, miRNA- 133a-2 and miRNA-133b. This gene has 1 transcript (splice variant), 147 orthologues and 2 paralogues. miRNA133 gene (Figure 2.1.1) takes place on the chromosome 5.

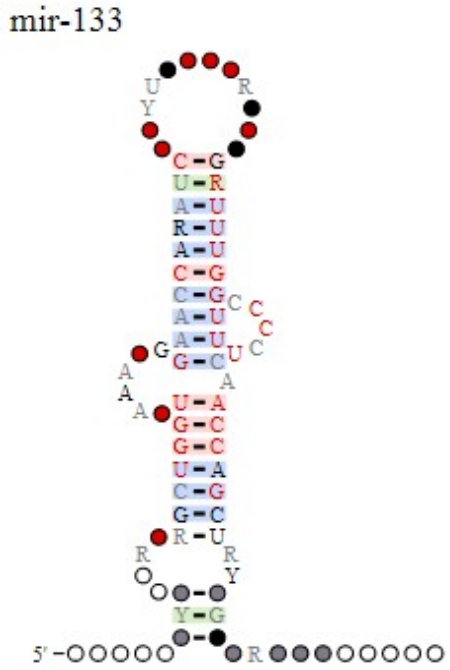

Figure 2.1.1. Secondary Structure of miRNA133 Gene (41)

This miRNA can found most of animal species. According to the Chen et al. in 2006, it can takes roles in proliferation of muscle. Also, they have shown proliferation of myoblast is enhanced by some factor's repression such as erum response factor (SRF) (42).

According to another study which expressed by Kura et al., they have observed that this miRNA can regulates anti-hypertrophic genes. Also, pathology of arhytmias involves niRNA-133. Also, they can regulate channels such as K^+/Na^+ (43).

2.1. 2. miRNA 26

This gene has 1 transcript (splice variant), 106 orthologues and 2 paralogues. miRNA26 gene (Figure 2.1.2) takes place on the chromosome 3.

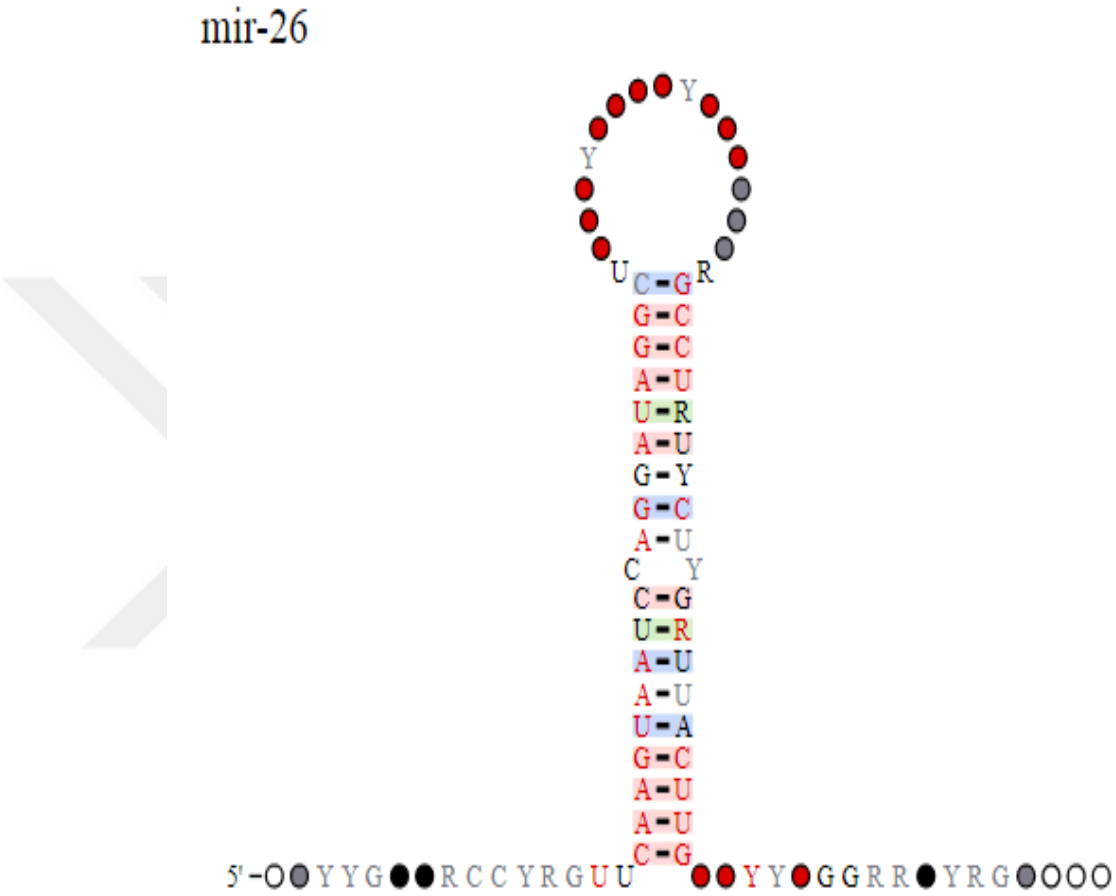

Figure 2.1.2. Secondary Structure of miRNA26 Gene (41)

According to the Jansen et al., this miRNA-26 expressed as one of the vascular miRNAs. miRNA-26 family includes a-1, a-2 and 26-b variants which code in different chromosomes (44). Xiaobin et al. have shown that it can expresses in neurons. In atrial fibrillation, miRNA-26 was found expressed according to the literature information (45).

2.1.2 miRNA378

This gene has 1 transcript (splice variant), 43 orthologues, 2 paralogues and is associated with 1 phenotype. miRNA378 gene (Figure 2.1.3) takes place on the chromosome 5.

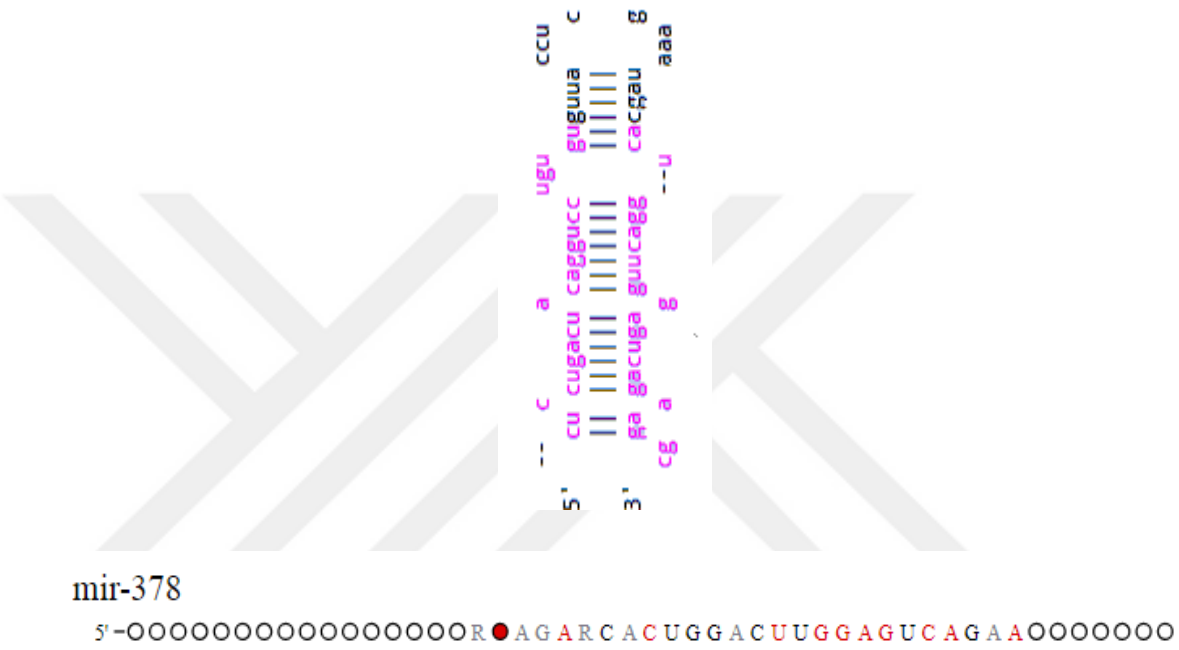

Figure 2.1.3. Secondary Structure of miRNA378 Gene (46)

The presence of several isoforms of miRNA-378 are seen within the microRNA database in humans. These miRNA-378 forms are encoded by different intronic sequencies; however, they hold seed sequences that are the same, therefore they are considered to have common gene targets. miRNA-378 plays potential role in cardiac remodeling; several studies determined chemically modified miRNA-378 mimics as suppressor or inhibiting by antisense oligonucleotides (47,48,49)

3. MATERIALS AND METHODS

3.1. The Patient Population and The Study Protocol

Whole blood and serum samples of the left ventricle hypertrophy patient group (n= 70) and control group (n=16) were obtained from the Yeditepe University Hospital Cardiology Clinic within the scope of the Clinical Research Ethics Committee.

Left ventricular mass $0.8\{1.04[([LVEDD + IVSd + PWd]^3 - LVEDD^3)]\} + 0.6g$.

The given formula is utilized for the evaluation of patients holding no major distortions of LV geometry, for examples those with hypertension. With the use of this formula however, even the smallest of errors may be magnified due to the required cubing of primary measurements. The formula (2PWTd)/(LVIDd) is used for the calculation of relative wall thickness (RWT), which permits the identified as an increased LV mass as either concentric ($RWT \geq 0.42$) or eccentric ($RWT \leq 0.42$) hypertrophies; furthermore it permits the identification of concentric remodeling of a normal LV mass holding an increased RWT.

In this work, patient samples were selected based on those who had presented to the cardiology polyclinic with complaints of masticatory swelling of the temporalis muscle. Exact determination of left ventricle mass serves as a key factor in the successful assessment of left ventricle hypertrophy (Figure 3.1). Left ventricle hypertrophy (LVH) classification can be seen below:

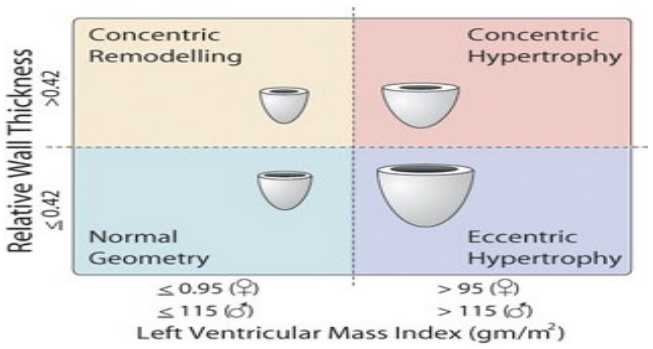

Figure 3.1. Assessment of Left Ventricle Hypertrophy (5)

3.2. Materials and Devices:

3.2.1. Preparation protocols and the separators:

3.2.1.1. miRNA Isolation

miRNA isolation procedure were practiced by using Qiagen's miRNeasy Serum/Plasma Kit. miRNA Isolation Kit (miRNeasy, Qiagen) involves Trizol (Quiazol, Qiagen), Chloroform (Sigma Aldrich), 100% Ethanol (Sigma Aldrich), miRasy Spike in control (miRNA39, Qiagen) and RWT Buffer. RWT Buffer was prepared by adding 44 microliters (µl) ethanol. RPE Buffer is used to eliminate residues which left in colons. Complementary DNA (cDNA) reverse transcription kit (miScript II Kit, Qiagen) comprises miScript HiFlex Buffer, Nucleic Acid Mix miScript Reverse Transcription Mix and RNase free water.

3.2.1.2. Determination of miRNA Levels using a Fluorometer

Working solution was got to ready by using reagent and buffer which peculiar to miRNA. Standard 1 which contains 10 ng/µl and standard 2 which contains 250 pg/µl rRNA which were used for calibration of fluorometer. Measurement was realized by adding working solution and sample RNA into the 500 µl Qubit tubes (Qubit 3.0 microRNA Assay Kit, Invitrogen, Thermo Fisher Scientific Inc.).

3.2.1.3. Detection miRNAs Expression Levels by Real-Time Polymerase Chain Reaction (RT-PCR)

miRNA Universal Primer (Qiagen), miScript PCR Kit SYBR Green (Qiagen), miRNA Primer Assay (miRNA-26, miRNA-133 and miRNA-378, Qiagen), miRNA Housekeeping Assay (RNU6, Qiagen), Strip Tubes and Caps 0.1 ml (Qiagen) and DNase-RNase free 18m ohm water were used.

3.2.2. The Equipments:

+4ºC Refrigerator (Haier), -20ºC Refrigerator (Haier), -80ºC Refrigerator (Haier), Vortex (V.I Plus Biosan), Automatic Pipette Kit (Thermo Fisher Scientific Inc.), Centrifuge (Centrifuge 22R, Beckman Coulter), Ultra-pure water device (Purelab option Q, Elga), Fluorometer (Qubit 3.0, Invitrogen, Thermo Fisher Scientific Inc), Rotor Gene-Q Series (Qiagen).

3.3. Methods:

3.3.1. miRNA Isolation

miRNA isolation from serum samples procedure were practiced by using Qiagen's miRNeasy Serum/Plasma Kit. Blood samples which belong to the patients and healthy volunteers were collected into EDTA containing tubes. By centrifugation of the blood samples at 4500rpm for 15 min serum samples were had. Serum samples were stored at -80ºC. Before the isolation of the target miRNAs, melting process of serum samples were realized by incubation at 37 ºC. 200 µl of serum samples were set into the sterile ependorfs and 1000µl lysis solution (Qiazol Lysis Solution) was joined and mixed. Then, samples were placed in room temperature for 5 minutes for incubation. A fume hood was used for addition process of 200 µl of chloroform and were incubated for 3 minutes. Centrifuged at +4ºC at 12.000g for 15 minutes. After centrifugation, two different phases were obtained. A transparent phase was seen at the top, and 600 µl of it was taken into clean ependorfs. 900 µl of 100% ethanol was added and vortexed. Then, 700 µl of the mixture was run through columns and centrifuged at 8000g for 15 seconds at room temperature. Addition of the 700 µl of RWT buffer to the columns, and the supernatant was discarded. Also, 500 µl of RPE buffer was added and 500 µl of 80% ethanol was added into the same columns and centrifuged for 2 minutes. Finally, discretion of th supernatant. New tubes are used for carrying the columns and centrifuged at a maximum speed for 5 minutes. By adding 14 µl of RNA-free water and following centrifugation the microRNA isolate was obtained.

3.3.2. cDNA Synthesis

The obtained miRNA isolates were converted to cDNA by process which is called reverse transcription (miScript II Kit, Qiagen). Table 3.3.2-1 and Table 3.3.2-2 show cDNA reaction methods and experimental conditions. Samples were stored at -20ºC.

Table 3.3.2-1. cDNA mixture for PCR Reaction

5x miScript HiFlex Buffer	4 µl
10x miScript Nucleic Mix	2 µl
miScript Reverse Transcriptase Mix	2 µl
RNA free water	10,5 µl
miRNA Sample	1,5 µl
Total volume:	**20 µl**

Table 3.3.2-2. Incubation Conditions for cDNA Synthesis

60 min	3
5 min	9
∞	9

3.3.3. Determination of miRNA Levels

Determination of miRNAs expression levels was measured by the fluorometric method. 199 µl of miRNA Buffer and 1µl of miRNA Reagent were used for this procedure (Qubit microRNA Assay, Invitrogen, Thermo Fisher Scientific Inc).

A combination of 198 µl of working solution and 2µl from each sample were used for miRNA measurement.

3.3.4. Detection of miRNA Expression Levels

Selection of miRNAs for this study via the statistical databases. miRNA-26, miRNA-133 and miRNA-378 were chosen as a target miRNAs for this study. Table 3.3.4-1 showing primer sequences of the target miRNAs. Expression status of the miRNAs were calculated by calculation of delta C_T (ΔC_T), and internal control (housekeeping assay, RNU6). These analysis were done by using real-time PCR machine (Rotor Gene-Q, Qiagen). Syber Green dye was used for the determination of the target miRNA primer sequences binding (miRNA-26, miRNA-133 and miRNA 378, Qiagen), (miScript SYBR Green PCR, Qiagen), cDNA sequences and a housekeeping primer (mirRU6, Qiagen) (Table 3.3.4-2).

PCR conditions can be seen in Table 3.3.4-3.

Table 3.3.4-1. miRNA Primer Sequences

microRNA	Primer Sequence
miRNA-26	10 - uucaaguaauccaggauaggcu - 31
miRNA-133	66 - uuuggucccuucaaccagcua - 87
miRNA-378	5 - cuccugacuccagguccugugu - 26

Table 3.3.4-2. Ingredients of the Mixture for miRNA Expression Analysis

SYBR Green PCR Mix	10 µl
miScript Universal Primer	2 µl
miScript Primer Assay	2 µl
RNase free water	4 µl
cDNA	2 µl
Total volume:	**20 µl**

Table 3.3.4-3. miRNA Expression Analysis Cycle Conditions

Denaturation State	95ºC	15 min.	
Cycling State	94ºC	15 sec.	5 Cycle
	55ºC	30 sec.	
	70ºC	30 sec.	
Cycling State	94ºC	15 sec.	45 Cycle
	55ºC	30 sec.	
	70ºC	30 sec.	
Melting Curve	95ºC	30 sec.	

3.3.5. Statistical Analysis

LVH diagnosed patients and control groups together are used for understanding the effect of expression levels of miRNA-26, miRNA-133 and miRNA-378. Chi-square and Fisher's exact tests performed to determine demographic informations of all cases. Student's t-test was used to examine the significance of differences between the patients with LVH and control groups. The miRNA expression levels were calculated with C_T, ΔC_T. Altered miRNA expression levels were determined by student's t-test. Correlations were examined by using Pearson Correlation test. Statistical analysis were performed by the SPSS 22.0 program (SPSS, Inc, Chicago, IL, USA). The diagnostic value of circulating miRNAs were determined by using Receiver Operator Curve (ROC) analysis. MedCalc software program was performed for ROC analysis with 95% confidence interval (CI). Reported *p* values significance level of $p<0.05$ was considered to indicate statistical significance.

4. RESULTS

4.1. Demographic Results of Working Groups

Comprehensive demographic results of 70 left ventricular hypertrophy patients and 16 healthy controls can be seen in Table 4.1-1. The control group consisted of 11 male and 5 female, participants while the patient sample group consisted of 50 male and 20 female participants (Table 4.1-1).

Demographics of left ventricular and control groups were compared in terms of chi-square and student-t test analysis. There was no significant difference between the gender distributions in the atherosclerosis patient group ($p = 0.831$) and when the age distributions were examined, there was no significant difference between the groups ($p= 0.578$). In addition, there was no statistically significant difference was found between the groups in terms of body mass index ($p = 0.687$). (Tables 4.1-1).

Table 4.1-1. Demographic results of Working Groups

	Control (n=16)	**LVH Patient (n=70)**	**p value**
Gender Male / Female	% 68.8 / % 31.2 (n=11) / (n=5)	% 71.4 / % 28.6 (n=50) / (n=20)	0.831
Age (Year)	60.63 ± 12.45	62.21 ± 9.71	0.578
Smoking (Active/ None/ Quit)	% 18.8 / % 56.2 / % 25 (n=3) / (n=9) / (n=4)	% 28.6 / % 47.1 / % 24.3 (n=20) / (n=33) / (n=17)	0.707
Family History Yes / No	% 43.8 / % 56.2 (n=7) / (n=9)	% 41.4 / % 58.6 (n=24) / (n=34)	0.865
Total-K	184.12 ± 50.81	192.07 ± 40.83	0.524
LDL	107.43 ± 45.12	115.67 ± 39.65	0.484
HDL	46.25 ± 11.43	44.58 ± 12.20	0.629
TG	152.43 ± 63.13	175.64 ± 92.86	0.357
DM Yes / No	% 25 / % 75 (n=4) / (n=12)	% 47.1 / % 52.9 (n=33) / (n=37)	0.162
HT Yes / No	% 56.2 / % 43.8 (n=9) / (n=7)	% 72.4 / % 27.6 (n=42) / (n=16)	0.216
KAH Yes / No	% 43.7 / % 56.3 (n=7) / (n=9)	% 45.7 / % 54.3 (n=32) / (n=38)	0.887
VKI	29.24 ± 2.72	28.77 ± 4.40	0.687
SVKI	88.0 ± 13.86	108.94 ± 27.64	0.005*
RDK	0.401 ± 0.021	0.489 ± 0.06	<0.0001*

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

According to distrubition of LVH-Degree of patients with LVH most of the patients had normal LVH degree, while %19 had midly, %12,1 modorate and %15,5 severe degrees. When Left Ventricule Mass (LVM) and Relative Wall Thickness (RWT) are examined, it is seen that there was statistically significant difference between the groups. Patients with LVH had LVM as 108.94 ± 27.64 gr while controls had 88.0 ± 13.86 gr (p=0.005). Control group's RWT values was 0.401±0.021 cm patients with LVH had 0.489 ± 0.06 cm (p<0.0001) (Table 4.1-2).

Table 4.1-2. LVH-Degree of patients with LVH

	LVH Patient	
LVH-Degree	n=70	%
Normal	31	53.5
Midly	11	19
Modorate	7	12.1
Severe	9	15.5

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

4.2. microRNA Results

MicroRNA expression levels evulated as C_T and ΔC_T values and calculated for each of the miRNAs. These values are shown in Table 4.2.-1, Figure 4.2.-1, Figure 4.2- 2.

The C_T values of miRNA26, miRNA133 and miRNA378 are shown in Table 4.2.-1. The mean C_T values of miRNA133 of the patient and control group were 32.57
± 5.65 and 31.62 ± 5.05, respectively (p=0.684). The mean C_T values of miRNA26 of the patient and control group were 23.12 ± 3.10 and 26.59 ± 2.29, respectively (p=0.522). The mean C_T values of miRNA378 of the LVH patients and control group were 21.91 ± 2.36 and 24.69 ± 4.81, respectively (p=0.029) (Table 4.2-2).

By comparing the C_T values, it was found that miRNA133, miRNA26 and miRNA378 were downregulated in the patient group. There were no statistically significant difference due to miRNA133 and miRNA26 C_T values. Only statistically significant difference was found between patient and control group regarding miRNA378 C_T values (p= 0.029). (Figure 4.2-3).

The levels of miRNA expression were determined by comparing the ΔCT values of miRNAs and the internal control (RNU6). By comparing the mean ΔC_T values showed that miRNA378 expression levels had statistically significant difference between the groups. However there were no statistical difference regarding ΔC_T values of miRNA133 or miRNA26. The mean ΔC_T of miRNA133 of the patient and control group were 5.055.51 ± 5.87 and 1.89 ± 11.59, respectively (p=0.398). The mean ΔC_T values of miRNA26 of the patient and control group were -4.34 ± 3.37 and -5.20 ± 3.54, respectively (p=0.438). The mean ΔC_T values of miRNA378 of the LVH patients and control group were -5.55 ± 2.65 and 0.79 ± 7.86, respectively (p=0.020) (Figure 4.2-4)

Table 4.2-1. MicroRNA Analysis

		Control (n=16)	LVH Patient (n=70)	p value
miRNA-133	**CT (X± SD)**	32.57 ± 5.65	31.62 ± 5.05	0.684
	ΔCT (X± SD)	5.51 ± 5.87	1.89 ± 11.59	0.398
miRNA-26	**CT (X± SD)**	23.12 ± 3.10	26.59 ± 2.29	0.522
	ΔCT (X± SD)	-4.34 ± 3.37	-5.20 ± 3.54	0.438
miRNA378	**CT (X± SD)**	21.91 ± 2.36	24.69 ± 4.81	0.029*
	ΔCT (X± SD)	-5.55 ± 2.65	0.79 ± 7.86	0.020*

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

The C_T and ΔC_T values of miRNA26, miRNA133 and miRNA378 were calculated and compared with the LVH types of the patients. No significant relation between miRNA133, miRNA26 and miRNA133 expression pattern and type of LVH (p=0.110 and p=0.782). By comparing the mean C_T values showed that miRNA378 expression levels had statistically significant difference between the groups (p=0.001).

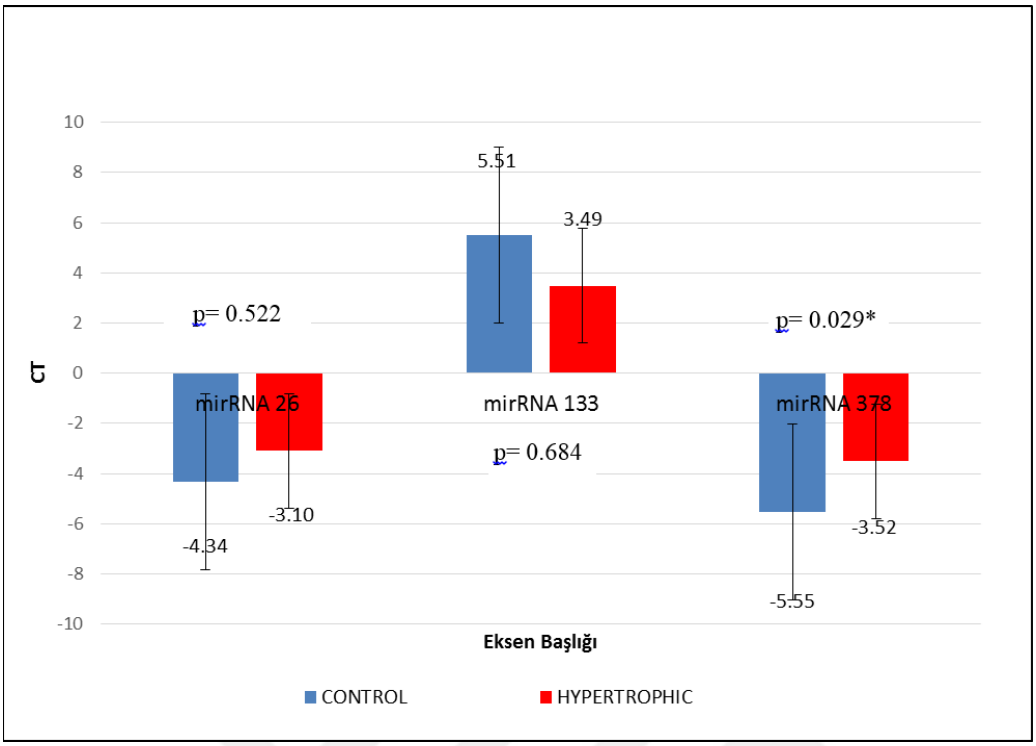

Figure 4.2-1. CT values of miRNA 26, miRNA 133, miRNA 378

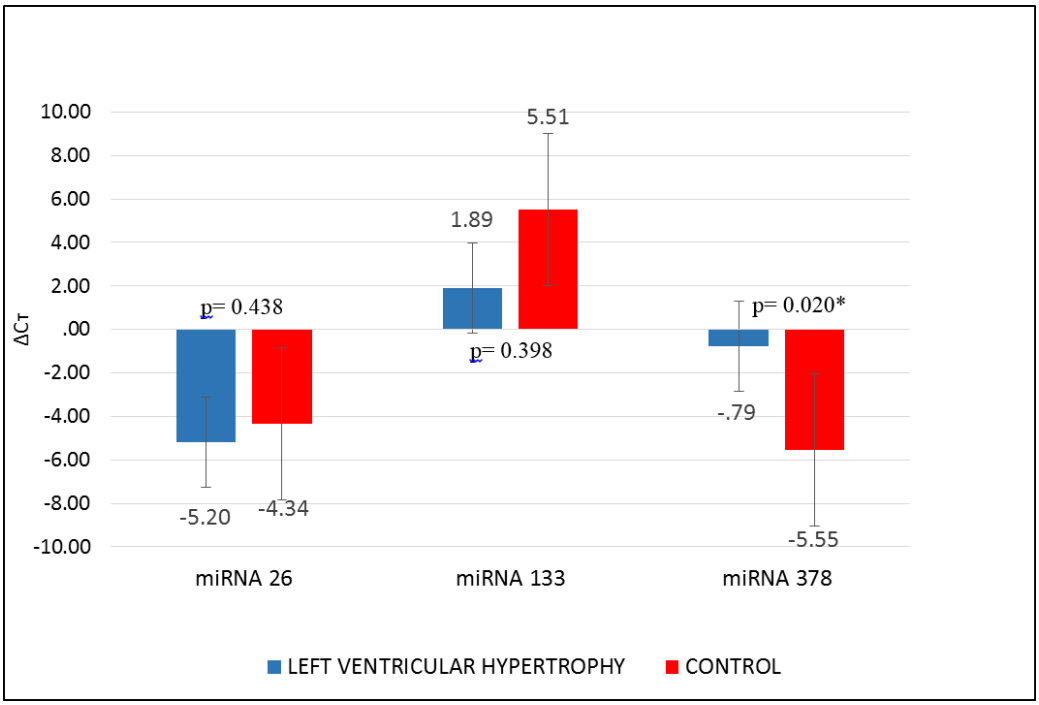

Figure 4.2-2. ΔCT values of miRNA 26, miRNA 133, miRNA 378

Table 4.2-2. Correlation analysis of miRNA C_T , ΔC_T values of patient group

		Control (n=16)	Hypertrophy (n=34)	Remodelling (n=34)	Eccentric (n=2)	p value
miRNA- 133	**CT (X± SD)**	32.57 ± 5.65	30.76 ± 4.20	0	41.83 ± 0	0.110
	ΔCT (X±SD)	5.51 ± 5.87	3.49 ± 7.41	0	-7.66 ±30.48	0.232
miRNA- 26	**CT (X± SD)**	23.12 ± 3.10	22.63 ± 2.26	0	22.06 ± 3.68	0.782
	ΔCT (X±SD)	-4.34 ± 3.37	-5.10 ± 3.62	0	-6.51 ± 2.77	0.641
miRNA- 378	**CT (X± SD)**	21.91 ± 2.36	22.66 ± 3.71	26.44±4.93	27.12±8.36	0.001*
	ΔCT (X±SD)	-5.55 ± -2.65	-3.52 ± 8.21	1.79±6.84	-1.45±7.45	0.003*

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

Due to the LVH type comparing with the C_T values analysis, it was found that miRNA133, miRNA26 and miRNA378 were downregulated in the patient group. There were no statistically significant difference due to miRNA133 and miRNA26 C_T values. Only statistically significant difference was found between patient and control group regarding miRNA378 C_T values (p= 0.029). (Figure 4.2-3).

The mean CT values of miRNA133 of the Eccentric group had highest C_T expression levels (41.83 ± 0), it was followed by control group had 32.57 ± 5.65 and Hypertrophy group had 30.76 ± 4,20 expression levels (p=0.110). The mean C_T values of miRNA26 of the control group had highest expression levels (23.12 ± 3.10), it was followed by Hypertophy and Eccentric groups were 22.63 ± 2.36 and 22.06 ± 3.68, respectively (p=0.782). The mean CT values of miRNA378 of the Eccentric group had highest C_T expression levels (27.12 ± 8.36), it was followed by remodeling group as 26.44± 4.93, Hypertrophy group as 22.66± 3.71, and control group had 21.91 ± 2.36 levels (p=0.001). (Figure 4.2-3)

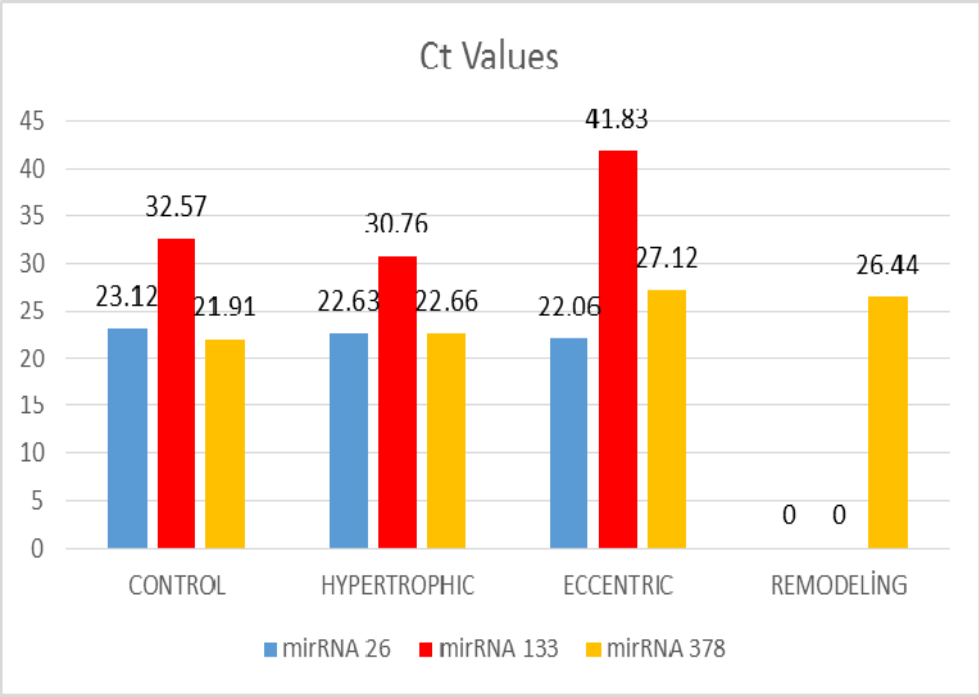

Figure 4.2-3 C_T values of miRNA 26, miRNA 133, miRNA 378 regarding LVH type

According to the LVH types comparing with the ΔC_T values analysis, the levels of miRNA expression were determined by comparing the ΔC_T values of miRNAs and the internal control (RNU6). By comparing the mean ΔC_T values showed that miRNA378 expression levels had statistically significant difference between the groups. However there were no statistical difference regarding ΔC_T values of miRNA133 or miRNA26. The mean ΔC_T values of miRNA378 of the -1.45 ± 7.45 Eccentric LVH patients and control group were -5.55 ± 2.65, Hypertrophy group was -3.52 ± 8.21 and Remodelling 1.79 ± 6.84 (p=0.003) (Figure 4.2-4)

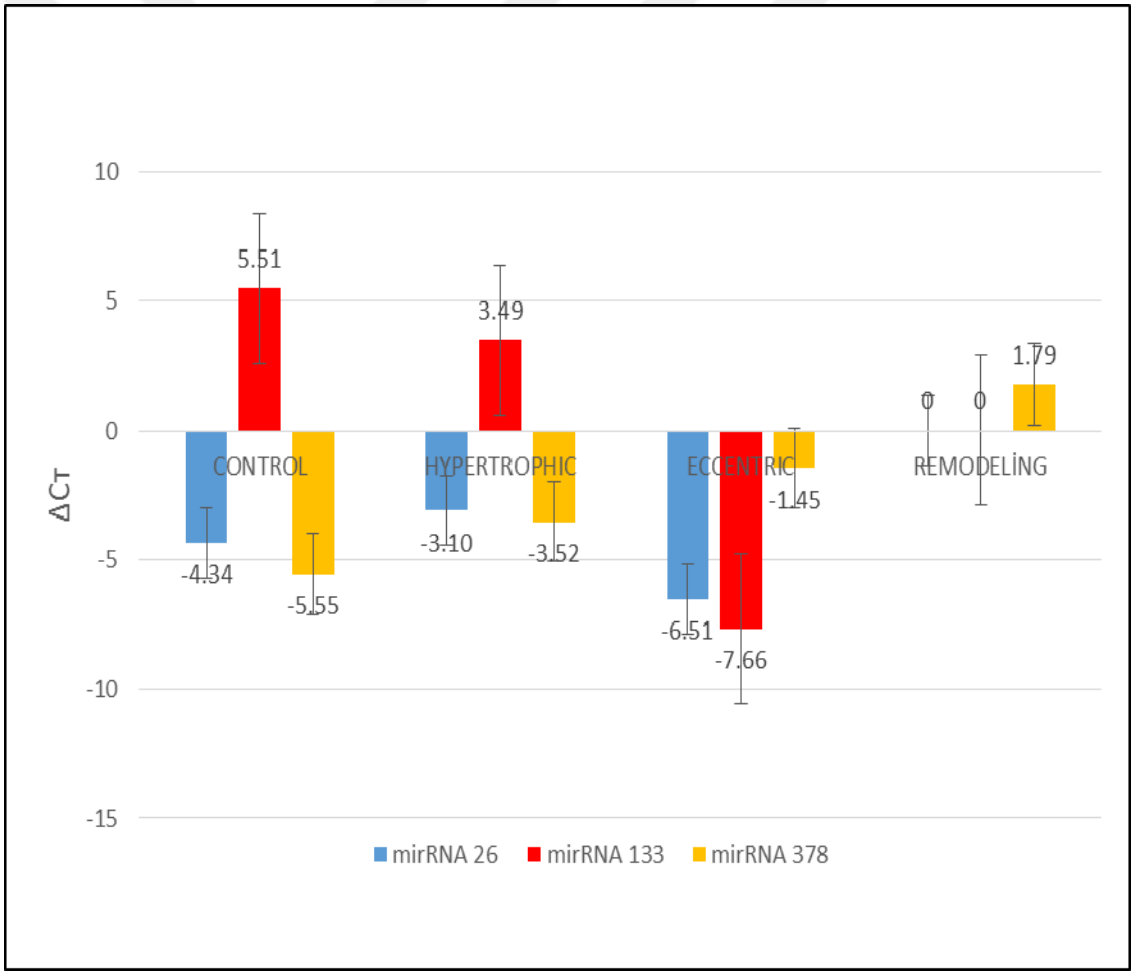

Figure 4.2-4. ΔC_T values of miRNA 26, miRNA 133, miRNA 378 regarding LVH type

4.3. microRNA ROC Analysis

Receiver Operating Characteristic (ROC) method was conducted using MedCalc Program to determine all plasma microRNA levels and diagnostic value in patient with LVH and control groups.

As shown in Figure 4.3-1, it was found that expression levels of miRNA 26 Cт can not be evaluated as a threshold value in patient groups (AUC=0,6885, %95 Cl=0,379 - 0,690, p=0,537). Also according to the miRNA 26 delta Ct values, the threshold value of mirRNA 26 was not determined (AUC=0,577 %95 Cl=0,345 - 0,786, p=0,561) (Figure 4.3-2).

As a result of the analysis, the threshold value in which miRNA133 Ct expression level can be evaluated as a diagnosis in patient groups has not been determined. According to the Ct values, miRNA133 was found to be AUC=0,484, %95 Cl=0,329 - 0,641, p=0,860. (Figure 4.3-3). As shown in Figure 4.3-4, regarding the miRNA133 delta Ct expression level can not be evaluated as a threshold value in patient groups AUC=0,519, %95 Cl=0,294 - 0,739 p=0,885).

There was no threshold for which the expression level of miRNA 378 Ct values could not be considered as a diagnosis in patient groups. According to the miRNA 378 Ct values, miRNA122a was determined as AUC=0,612, %95 Cl=0,482 to 0,732 p=0,179) (Figure 4.3-5).

As shown in Figure 4.3-6, it was found that expression levels of miRNA 378 delta Cт can be evaluated as a threshold value in patient groups (p=0,0013). According to the delta Ct values, the threshold value of miRNA 378 was determined as > -5,38 (AUC=0,484, %95 Cl=0,602 - 0,831 p=0,0013*)

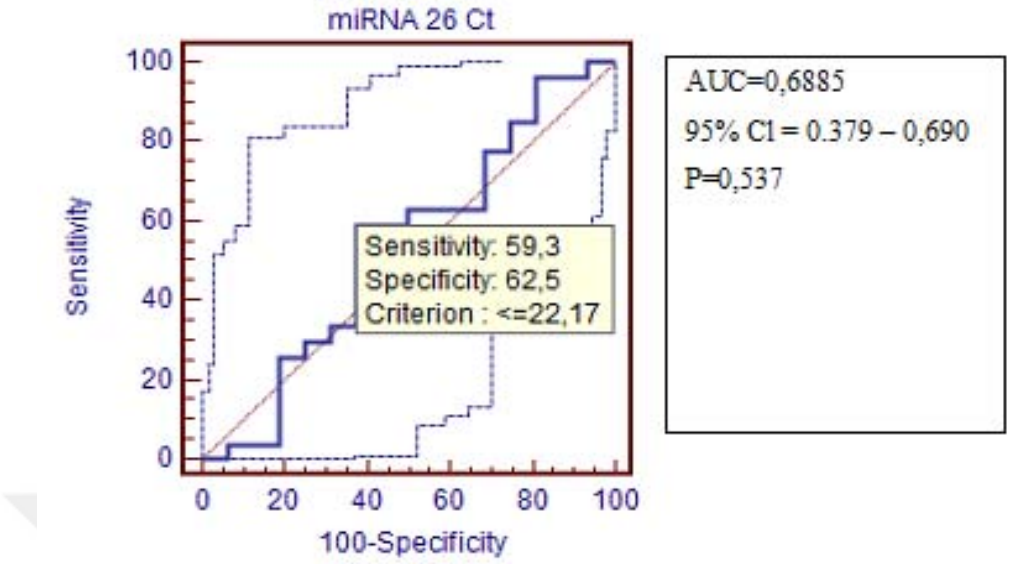

Figure 4.3-1. ROC analysis of miRNA 26 Cт values of patients with LVH

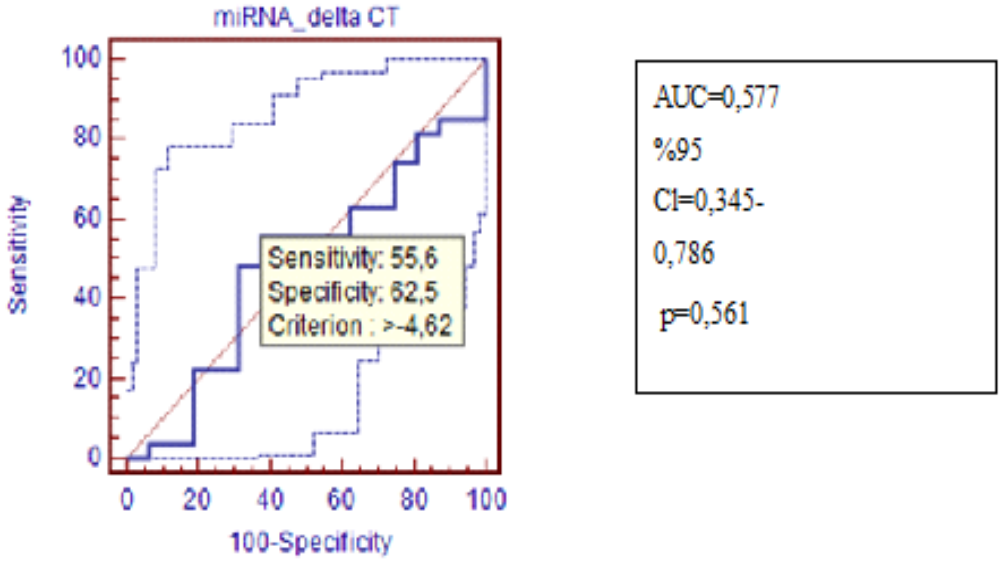

Figure 4.3-2. ROC analysis of miRNA 26 ΔCт values of patients with LVH

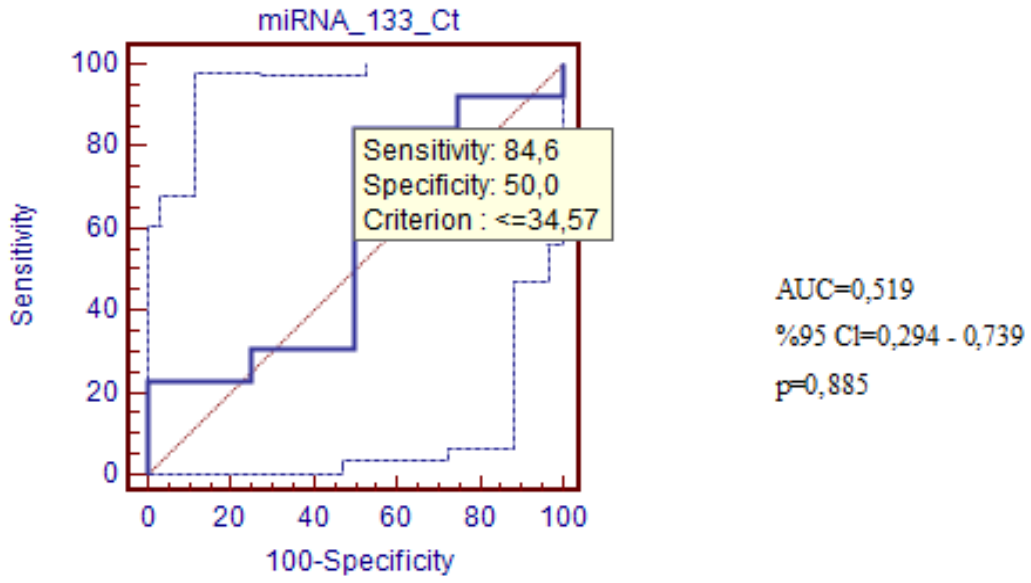

Figure 4.3-3. ROC analysis of miRNA 133 C_T values of patients with LVH

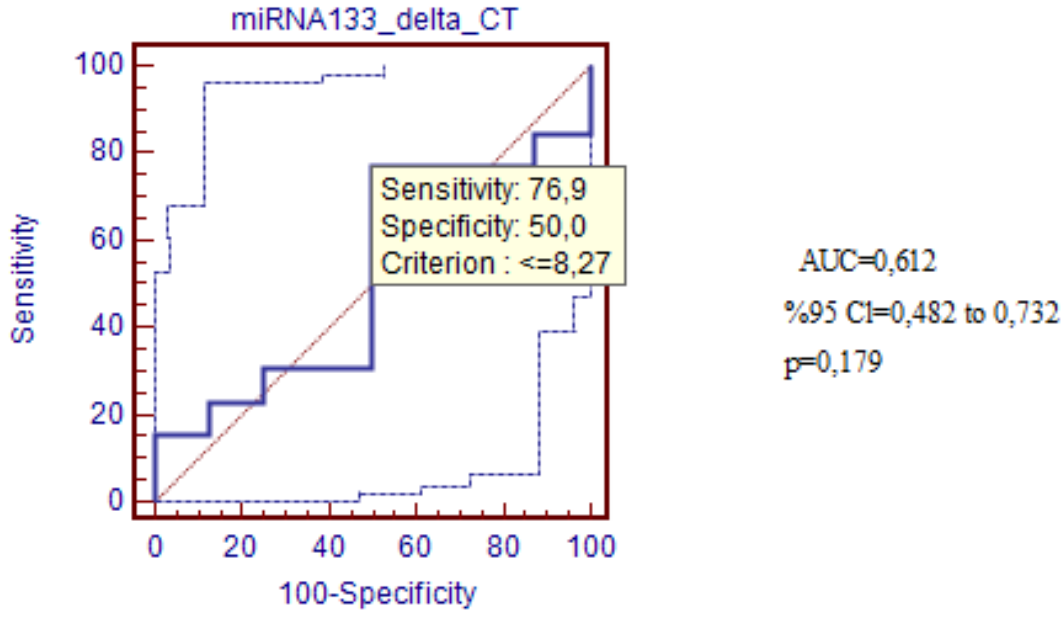

Figure 4.3-4. ROC analysis of miRNA 133 ΔC_T values of patients with LVH

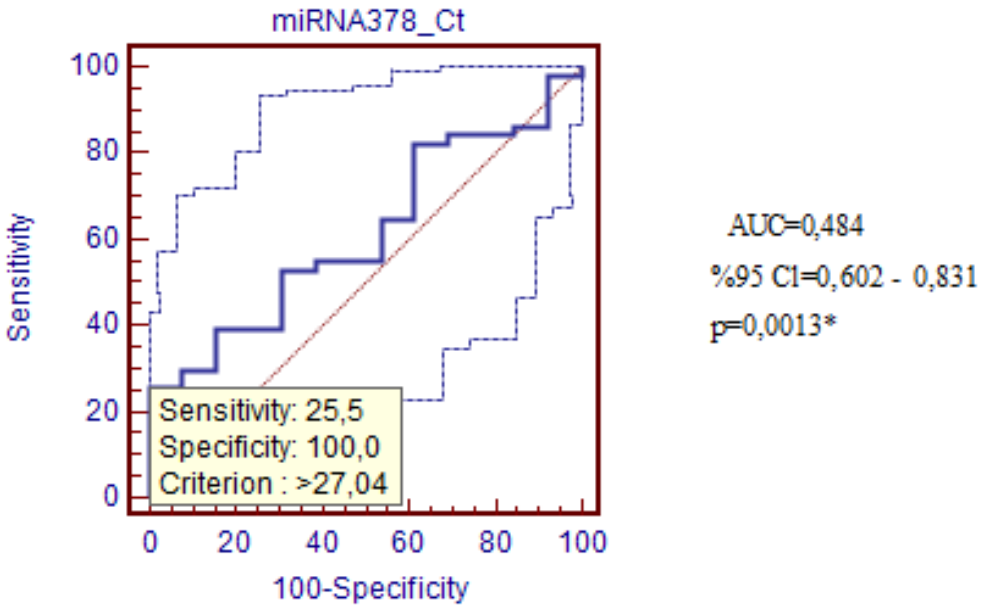

Figure 4.3-5. ROC analysis of miRNA 378 C_T values of patients with LVH

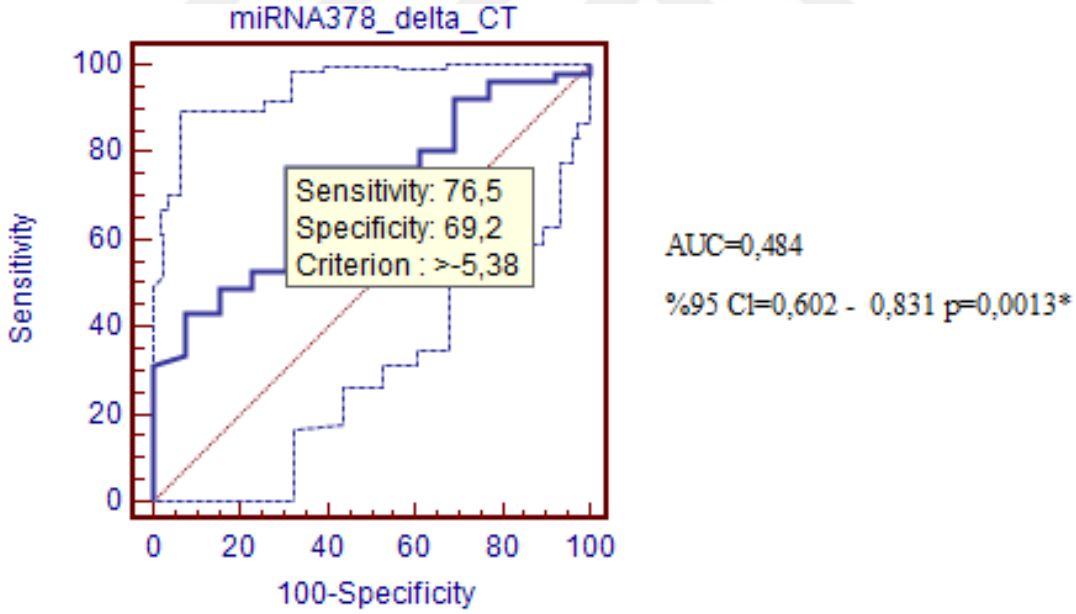

Figure 4.3-6. ROC analysis of miRNA 378 ΔC_T values of patients with

5. DISCUSSION AND CONCLUSION

Heart failure occurs as the heart fails to pump blood at an amount required to fulfill the body's needs. It affects 1% of people over age 50, 5% of people over the age of 75 and 25% of people over the age of 85 (1).

Left ventricular hypertrophy is the most important factor leading to cardiovascular morbidity and mortality. It can be caused by such situations as hypertension, coronary artery disease, valvular pathologies, obesity or a combination of these factors. According to one theory, hypertension may lead to left ventricular dilation, as well as contractile dysfunction. In response to physiological workload, a compensatory mechanism is created, which stimulates changes regarding contractility and ventricular remodeling. However, a response is created in cases where the heart undergoes continuous mechanical overload that can be identified by an increased consumption of energy and oxygen, dilation of the chambers, reduced contractility and eventually overall heart failure. Regarding a myocardium response (adaptive) and pathologic response (maladaptive), physiologic and pathologic hypertrophy involves the activating of various signaling cascades (2).

The heart encounters a hemodynamic problem, it can be controlled in three ways. The first is to increase the number of activated segments between actin and myosin, the other is to increase the muscle mass to bear the excess load and the last one to increase the contractile strength is to operate neurohormonal mechanisms (3). The most important mechanism used by the heart due to increased hemodynamic load is to increase muscle mass. The only way to increase muscle mass is due to hypertrophy of existing myocytes (50). Addition of sarcomeres in parallel to the pressure load resulting from aortic stenosis or hypertension increases myocyte width, resulting in increased wall thickness. This remodeling is called concentric hypertrophy. In cases where the volume load of the heart increases such as chronic aortic insufficiency, mitral insufficiency or anemia, sarcomeres multiply in series and myocyte length increases and ventricular volume increases. This remodeling is defined as eccentric hypertrophy (51)

When the pathophysiology of left ventricular hypertrophy is examined, problems arise at various stages: These are dysfunctions that modulate the formation and regulation of the motor unit, control energy metabolism, and conduction of hormonal pathways is downregulated (52).

Ventricular hypertrophy is mainly due to an increase in the number of sarcomere in the myocyte. Increase in sarcomere number on myocyte can cause conversion of a mechanical input into a biochemical event, and this occurs through the extracellular matrix (ECM). Tyrosine-phosphorylated-kinases and serine-threonine kinases in ECM play a role in signaling mechanisms in hypertrophy (53).

It has been shown by Sadoshima et al. (1993) that angiotensin II plays crucial role in the pathogenesis of hypertrophy via the AT1 receptor (54). This is because the hormone in question directly induces molecular events that cause early cardiac growth (55). Cardiac hypertrophy is known to be associated with expression of various genes. Kuster et al. (2013) (14) showed that gene expression profiles of cardiac mast cells upregulated in cardiac hypertrophy patients when compared with healthy donors. Transcription factors can be inhibited by the nuclear factor of activated B cells (NF-k-light chain enhancer). This factor modulates the altered gene expression profile in cardiomyocytes and inhibits cardiac hypertrophy. Thus Endothelin-1 may cause cardiac hypertrophy through upregulation of atrial natriuretic peptide (ANP) (56). As a result, the identification of new genes that associates with hypertrophy and their biological mechanisms have become increasingly popular in the scientific world, where they are of clinical importance in the diagnosis and treatment of cardiac hypertrophy (56).

In this study, we would like to compare in terms of risk factors for coronary artery disease in patient group and control group. There are many risk factors that related with coronary heart disease or ischemic heart diseases. Effects of various risk factors such as age, sex, ethnicity and family history, and other risk factors as tobacco exposure, high blood pressure (hypertension), high cholesterol, obesity, physical inactivity, diabetes, unhealthy diets and harmful use of alcohol investigated in

patients with CVD (57).

Risk factors for the development of left ventricular hypertrophy (CVD); advanced age, obesity, hypertension, CAD and is identified as low ejection fraction (58). Increased left ventricular mass enhance the risk of sudden cardiac death, but SVH patients can survive for many years without any complaints or symptoms and with normal or near normal exercise reserves. Some patients with CVD

may develop diastolic dysfunction, systolic dysfunction, or both. Because of all these, it is of great importance to make genetic studies for the diagnosis of SVH and to determine treatment strategies (59).

In our study, these risk factors were obtained by questioning and from curriculum vitae from patient and control group. According to the results we found, the age of the patient and control group related to CAD is ($p=0.097$) and not significant.

In many studies, the incidence of CAD and associated death rates are closely related to age. After 40 years of age, the incidence of atherosclerosis and associated CAD is increasing in parallel with the increase in age. The most common age of CAD is 50-60 for males and 60-70 for females. It is also reported that long-term Framingham is an independent risk factor for older age in prospective cardiac studies (60).

Advanced atherosclerotic lesions occur in males about 20 years earlier than females. Men who are exposed to major risk factors for a longer period of time may partly explain the sex difference in CAD. In men, LDL cholesterol and HDL cholesterol decrease with puberty, but this change is not observed in women. This aspect of the gender difference is related to hormonal differences (61).

According to our finding patients with LVH had LVH as 108.94 ± 27.64 gr while controls has 88.0 ± 13.86 gr ($p=0.005$). Control group's RWT values was 0.401 ± 0.021 cm patients with LVH had 0.489 ± 0.06 cm ($p < 0.0001$). (Table 4.1-1., 4.1-2).

Recent studies have shown that miRNAs are located within the human circulation presenting as cell-free forms, which may lend these structures to serve

as blood-based biomarkers (62). Varying plasma or serum levels of different miRNAs have been recorded in several types of cardiovascular disease (63).

As miRNAs have a very stable structure in the blood, it is being demonstrated that it 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 can be easily detected in serum and plasma samples without the need for invasive methods due to their ability to resist 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 (64).

There are many studies showing the relationship between cardiovascular diseases and miRNA. It has been shown that miRNAs have important responsibilities in differentiation of cardiac cells, angiogenesis and myocyte development. In addition, differences in miRNA levels may lead to various dysfunctions such as endothelial dysfunction and proliferation and differentiation of vascular smooth muscle cells that lead to the pathology of cardiovascular diseases (65).

In present study the eccentric group has highest expression levels compare to control and high hyperthyropy group. Our study show that however there is no significance difference between the groups CT values at miRNA-133a there could be a correlation showed divercity.

MiRNA 133a and miRNA133b classified as the same miRNA cluster, skeletal and cardiac muscles are defined as miRNAs have a very important role in the pathophysiology (66). Torella et al (2011) in the in vivo and in vitro studies in the study; miRNA 133 was found to act as a potential inhibitor for DDKH. They found that this effect is due to the reduction of cell proliferation by inhibition of the transcription factor KLF4 and Sp-1, the target gene miRNA133a and miRNA 133b (67). In a study conducted by Wen et al. In 2014, they suggested that miRNA133a may be a biomarker for cardiac hypertrophy. In this study, they found that the level of miRNA133a was significantly lower in patients with left ventricular hypertrophy (SVH) (68). In a study examining

miRNA profile in myocardial cells in the embryonic period, McCarthy et al. (2007) observed that miRNA 133a acts as a regulator in myocardial differentiation (69). In their research in mouse embryos, miRNA133a has been shown to have different expression levels at different development stages of myocardial cells. While myocardial cells proliferated, the expression of miRNA133a increased by 50% and the expression level increased by 2-fold as the cells differentiated. As a result of this study, it is suggested that miRNA133a is a miRNA specific to myocardial cells and may be present at levels of expression specific to the development of cells at different developmental stages (69). In a study by Fichtscherer et al. (2010) on coronary artery disease, miRNA 133a was found to be higher in the patient group. The patients were controlled with troponin levels and it was concluded that there was no destruction of cardiac muscle cells since no elevation was observed. It is suggested that the damage that occurs in patients without muscle destruction may be due to the increase in miRNA133a level, which inhibits cell proliferation by suppressing KLF4 and Sp1 genes (62).

Castoldi et al. had displayed that miRNA-133a, effects CollA1 and plays important role in metabolism of cardiac fibrosis. Their data showed that miRNA-133a could be placed in myocytes and is partipicates cardiac hypertrophy. The metabolism of myocardial fibrosis regulated by not only by expression of miRNAs but also synergistic activities of muscle-specific miRNAs. Furthermore, in cultured fibroblasts miRNA 133a mediates matrix components (70). However, the secretory miRNAs takes place in different mechanism of physiological processes, relation between cardiac myocytes and fibroblasts remained unclear.

The role of miRNA-133a in molecular mechanism of left ventricular hypertrophy disease has nat been fully elucidated. Also, number of the studies which were performed and published by using miRNA-133a is very less, as well as published articles about the roles they play in the mechanism of left ventricular hypertrophy. Therefore, deconstructing the roles of this target miRNA in left ventricular hypertrophy and determining its regulatory status may be useful in providing a noninvasive treatment strategy for individuals with LVH (71).

The present study we displayed that the mean C_T values of miRNA378 expression levels had statistically significant difference between the groups (p=0.001) Our findings correlated with Yuan et al (2018) have demonstrated that in case of cardiac remodelling miRNA-378 is expressed highly. As a result of this information, they proposed that this miRNA regulates cardiac fibrosis (47). Their study showed that miRNA 378 have anti-hypertrophic activity and also plays crutial role in anti-fibrotic effects in the heart muscle cells. Moreover, regarding stress response miRNA-378 knockout mice showed severe cardiac fibrosis when compared with the wild-type mice (72). P38 mitogen-activated protein kinase (p38 MAPK) takes part in a hypertrophy and inflammation signaling pathway which leads cardiac remodeling. Yuan et al showed that p38 MAPK participates regulation of collagens in cardiac fibroblasts and matrix metalloproteinases (MMPs). Mitogen-activated protein kinase kinase 6 (MKK6) phosphorylates, it subsequently activates p38 MAPK for synthesis of inflammatory cytokines. The important role of miRNA-378 in the depression of pressure overload- induced cardiac fibrosis by the metabolism of the p38 MAPK signaling, which targets MKK6 in cardiac fibroblasts directly. Also in this study they showed that miRNA-378 could be secreted by outside of cardiomyocytes after mechanical stimulation and

partipicates cardiac fibroblasts. This data displayed the importance of paracrine signaling between fibroblasts and cardiomyocyte (72).

In a study accomplished by Zhang and colleagues, it was determined that miRNA-378 has a positive effect on osteogenesis and angiogenesis mechanisms. Also, it was found that miRNA-378 is increased following induction of osteoblasts. miRNA378 has been shown to hold various regulatory roles regarding different cellular and organic metabolic processes; it was also found by LEE that it has the ability to promote cell survival, tumor growth, and angiogenesis through targeting SuFu and Fus-1 expression specifically. In that study, osteogenetic markers showed to be significantly higher following miRNA378 transfection, which showed that BMMSCs receive a stronger osteoblastic capacity with the overexpression of miRNA378. These findings were further confirmed with ALP and alizarin red staining procedures. Angiogenic growth factors and angiotensin were important regarding angiogenesis, and VEGF was

believed to be the factor of greatest importance in regard to the promotion of proliferation and vascular endothelial cells undergo tube formation. The enhanced mRNA expression level of ANG1/2 and VEGF displayed that BMMSCs with overexpression of miRNA378 had enhanced angiogenic capacity. miRNA 378 target to accelerate CASP3 translation by activating the PI3K/Akt signaling pathway, the researchers concluded miRNA-378 overexpression weakened high glucose- suppressed osteogenic differentiation through targeting CASP3 and activating (48).

The Receiver Operating Characteristic (ROC) is derived from a radar term that distinguishes correct radio signals from noise. This method is used to determine the discriminating power of the test, to compare the efficacy of various tests, to determine the appropriate positive threshold, to monitor the quality of laboratory results, to compare the effectiveness and characteristics of different practitioners false positives). In binary classification systems, the distinction occurs with the ratio of sensitivity to precision where the threshold value varies. The better the diagnostic test, the more the curve slides up and to the left. After drawing such a curve, the area under the curve is defined as Area Under Curve (AUC). The higher the AUC value, the better the diagnostic test is thought to be capable of differentiation. An analysis above AUC value 0.975 is considered excellent. AUC should be statistically tested to determine if it is different from 0.5 and its p value should also be determined. If the difference between the AUC result of the two different tests is statistically significant, the larger the AUC, the greater the separation power. In short, ROC is also expressed as the fraction of true positives to false positives. It has been shown to be important in the medical field because it provides a new dimension to diagnosis and treatment (73).

Biomarkers are defined as guidelines used for diagnosis and treatment of diseases (65). There are 3 basic criteria for an ideal biomarker. First of all, it should be provided by non-invasive methods such as plasma, serum, urine to be taken for analysis. The second criterion is that the material to be analyzed has a long half-life, is not affected by the time it takes to perform the analysis, and prevents incorrect data acquisition. The final criterion is that the reliability of the analysis to be applied is high and that it has a method that can obtain fast results (65). The most widely used

biomarkers in medicine today are polypeptides and proteins. The first troponin that comes to mind when it comes to cardiovascular disease and biomarker is detected only after infarction. The presence of ideal biomarkers with high sensitivity and selectivity in this area may guide clinicians about the pathogenesis of the disease without risk of life (74). In recent years, circulating miRNAs may be new and potential biomarkers for diagnostic and therapeutic applications in diseases caused by atherosclerosis. The number of biomarker candidate miRNAs in the diagnosis and treatment of cardiovascular diseases, especially cancer, is increasing. The presence of miRNAs that may be biomarker candidates has been demonstrated in a limited number of studies investigating the role of miRNAs and target genes that are thought to be associated with atherosclerosis in the molecular diagnosis of atherosclerosis (75).

In our study, ROC analysis was performed using MedCalc Program to determine plasma microRNA expression levels and diagnostic value in patient and control groups. As a result of the analysis, the expression levels of miRNA 26 Cт can not be evaluated as a threshold value in patient groups (AUC=0,6885, %95 Cl=0,379 - 0,690, p=0,537). Also according to the miRNA 26 delta Ct values, the threshold value of mirRNA 26 was not determined (AUC=0,577, %95 Cl=0,345 - 0,786, p=0,561). The threshold value in which miRNA133 Ct expression level can be evaluated as a diagnosis in patient groups has not been determined. According to the Ct values, miRNA133 was found to be AUC=0,484, %95 Cl=0,329 - 0,641, p=0,860. The miRNA133 delta Ct expression level can not be evaluated as a threshold value in patient groups AUC=0,519, %95 Cl=0,294 - 0,739 p=0,885). There was no threshold for which the expression level of miRNA 378 Ct values could not be considered as a diagnosis in patient groups. According to the miRNA 378 Ct values, miRNA133a expression was determined as AUC=0,612, %95 Cl=0,482 to 0,732 p=0,179) (Figure 4.3-5).

In present study we found that expression levels of miRNA 378 delta Cт can be evaluated as a threshold value in patient groups (p=0,0013). According to the delta Ct values, the threshold value of miRNA 378 was determined as > -5,38 (AUC=0,484, %95 Cl=0,602 - 0,831 p=0,0013*)(Figure 4.3-6).

There are 3 subtypes of the human miRNA-26 family as miRNA26a_1, miRNA26a_2 and miRNA_26b and located on chr 3, chr 12 and chr 2. The mature form of miRNA 26a_1 and miRNA 26a_2 takes place in the same introne, in mature miRNA-26b there are only 2 different nucleotides. The intranuclear and intracytoplasm enzymes modifies Pre-miRNA26 with stem-loop structure and it turns into mature miRNA-26. The mature form of miR-26 was having region of approximately 6–7 nucleotides and 21–22 nucleotides in length. The seed region sequence of miRNA-26, an significant region for binding to target mRNA, have homology in different members of species. Cancer cells and healhty tissues display different miRNA-26 expression levels during tumorigenesis and cell growth. miRNA-26 takes place in different metabolical processes through deficient sequence complementarity via binding main region to 3′UTR of target mRNA. miRNA- 26 effects expression levels of target protein via inhibiting the target gene translation. miRNAs located on their host genes which targets significant genes in regulatory pathways for biological metabolism. They displayed that miRNA-26 expression level is altered in tumors (76).

Gao et al. (2011) performed in vitro studies to reveal that different miRNA26 had altered expression levels in biological physiological processes such as apoptosis, normal cell proliferation and growth, also in pathological conditions like tumorigenesis. The expression level of miRNA26 has been displayed particular to different physiological processes. Especially miRNA26a exhibits abundantly higher expression in tumors that indicates that miRNA26 has crucial roles in formation of oncogenesis. (77). Furthermore Han et al (2012) hypothesized that miRNA26a could play regulatory role in cardiac hypertrophy, they conducted a series of experiments on angiotensin II (Ang II)-induced cardiomyocytes (CMs) and transverse abdominal aortic constriction (TAAC). The expression levels of miRNA26a was upregulated and blocked by different factors as antimirRNA and suppressors. GATA4 is kind of transcription factor that plays important role on cardiac hypertrophy. They displayed that miRNA26 which targets GATA4 gene by recoginizing 3′-UTR seed region of GATA4 and reduce GATA4 mRNA levels post transcriptional level. This study reveals the regulatory role of miRNA26a levels on cardiac hypertrophy (78).

The expression level of miRNA-26 is not only altered in tumorigenesis but also disordered in other diseases. The chronic cholestasis can cause primary billiary cirrhosis (PBC) and it comorbited by autoimmune diseases as scleroderma and rheumatoid arthritis. Zhang *et al* displayed that miRNA-26a expression level is one of the down- regulated miRNAs in miRNA expression profile of 35 independent miRNAs. The altered miRNAs could affect significant targets of the genes not only regulate development of PBC metabolism but also apoptosis, oxidative stress, inflammation and cell proliferation. Much more evidence needed for understanding the role of miRNA-26 on non-tumor diseases (79).

However, the molecule mechanisms of target genes remain to be unclear. The functional effects of miRNA-26 had been investigated and has been indicated roles in different types of tumors. The expression level of miRNA-26 investigated with advanced microarray technique in vitro as in different tumor cells and healty cells. Expression level of miRNA-26 upregulated in Gliobalstoma (GBM) by promote cell proliferation and tumor cell growth. While miRNA-26 is downregulated in normal tissue development and growth with the feature effect on cell differentiation and proliferation. Zhang et al. determined effects of miRNA26 on various potential significant target genes such as EZH2, SMAD1, PTEN, and MTDH. Moreover the expression level of miRNA-26 downregulated in cancer cases as bladder, anaplastic carcinomas, and oral squamous cell carcinoma, breast tumors (80).

The decreased level of miRNA-133 in normal adult cell undergoes cardiac hypertrophy, via inhibiting apoptotic genes (63). This data helps to undertandin role of miRNA-133 on the biological mechanism of pathological hypertrophy that cause size enhancement of myocyte. The experimental modulation of miRNA-133 levels could display different results because of multiple effect of miRNA function (64).

In another study role of miRNA-133 on cardiac hypertroph inestigated and results indicated that miRNAs could have potential for serving as therapeutic targets. They have capacity to regulate the genes which responsible for cellular function the full range of targets that mediate its effects remain to be identified and characterized. Owing to this characteristic of miRNAs, they utilized for therapeutic purposes for

understanding target gene functions and their interactions (81).

In the light of the studies that mentioned above it has been displayed that miRNA26 plays crucial role on cardiovascular disease and pathogenesis of LVH. MiRNA26 mediates biological processes not only controlling intracellular signaling pathways but also endothelial cell growth and angiogenesis.

In conclusion, in this thesis we investigated miRNA expression levels of case and control groups. The results indicated that the mean miRNA378 CT and ΔCT levels were significantly higher in LVH groups than the controls (p= 0.029 and p= 0.020, respectively). In the light of these data, ROC analysis was performed and was determined that expression levels of miRNA-378 could be evaluated as a threshold value and could be a biomarker candidate in the diagnosis of LVH. However there was no statistically significant relationship was found for miRNA-133 and miRNA-26 expression levels, miRNA-378 had statically significant relationship in terms of LVH pathogenesis.

6. REFERENCES

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7.APPENDICES

7.1. ROW DATA

```
T-TEST GROUPS=grup(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=miRNA_26_ct miRNA_26_delta_ct miRNA_133_ct
miRNA_133_delta_ct miRNA_378_ct
    miRNA_378_delta_ct
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		20-JUN-2019 12:02:35
Comments		
Input	Data	C:\Users\seda.gulec\Downloads\Zerri n Barut Doktora Tezi.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	112
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=miRNA_26_ct miRNA_26_delta_ct miRNA_133_ct miRNA_133_delta_ct miRNA_378_ct miRNA_378_delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,01

Group Statistics

	grup	N	Mean	Std. Deviation	Std. Error Mean
miRNA_26_ct	hasta	27		2,29369	,44142
	kontrol	16	23,1281	3,10749	,77687
miRNA_26_delta_ct	hasta	27	-5,2093	3,54895	,68300
	kontrol	16	-4,3481	3,37129	,84282
miRNA_133_ct	hasta	13	31,6200	5,05926	1,40319
	kontrol	9	32,5700	5,65418	1,88473
miRNA_133_delta_ct	hasta	14	1,8986	11,59202	3,09810
	kontrol	9	5,5133	5,87318	1,95773
miRNA_378_ct	hasta	62	24,6960	4,81137	,61104
	kontrol	16	21,9194	2,36705	,59176
miRNA_378_delta_ct	hasta	62	-,7965	7,86654	,99905
	kontrol	16	-5,5569	2,65573	,66393

Independent Samples Test

		F	Sig.	
miRNA_26_ct	Equal variances assumed	1,302	,260	-,646
	Equal variances not assumed			-,598
miRNA_26_delta_ct	Equal variances assumed	,080	,779	-,783
	Equal variances not assumed			-,794
miRNA_133_ct	Equal variances assumed	1,006	,328	-,413
	Equal variances not assumed			-,404
miRNA_133_delta_ct	Equal variances assumed	1,184	,289	-,862
	Equal variances not assumed			-,986
miRNA_378_ct	Equal variances assumed	6,098	,016	2,232
	Equal variances not assumed			3,264

miRNA_378_delta_ct	Equal variances assumed	4,276	,042	2,376
	Equal variances not assumed			3,969

Independent Samples Test

		t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
miRNA_26_ct	Equal variances assumed	41	,522	-,53405
	Equal variances not assumed	24,760	,555	-,53405
miRNA_26_delta_ct	Equal variances assumed	41	,438	-,86113
	Equal variances not assumed	32,967	,433	-,86113
miRNA_133_ct	Equal variances assumed	20	,684	-,95000
	Equal variances not assumed	16,041	,691	-,95000
miRNA_133_delta_ct	Equal variances assumed	21	,398	-3,61476
	Equal variances not assumed	20,217	,336	-3,61476
miRNA_378_ct	Equal variances assumed	76	,029	2,77659
	Equal variances not assumed	50,048	,002	2,77659
miRNA_378_delta_ct	Equal variances assumed	76	,020	4,76042
	Equal variances not assumed	70,700	,000	4,76042

Independent Samples Test

		t-test for Equality of Means		
			95% Confidence Interval of the Difference	
		Std. Error Difference	Lowe	Upper
miRNA_26_ct	Equal variances assumed	,82688	-2,20397	1,13587
	Equal variances not assumed	,89352	-2,37520	1,30710
miRNA_26_delta_ct	Equal variances assumed	1,09	-3,08162	1,35935
	Equal variances not assumed	1,08	-3,06830	1,34603
miRNA_133_ct	Equal variances assumed	2,30	-5,74877	3,84877
	Equal variances not assumed	2,34	-5,93012	4,03012
miRNA_133_delta_ct	Equal variances assumed	4,19	-12,33506	5,10554
	Equal variances not assumed	3,66	-11,25420	4,02467
miRNA_378_ct	Equal variances assumed	1,24	,29866	5,25453
	Equal variances not assumed	,85062	1,06811	4,48508
miRNA_378_delta_ct	Equal variances assumed	2,00	,76969	8,75116
	Equal variances not assumed	1,19	2,36842	7,15243

DATASET ACTIVATE DataSet1.

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SAVE OUTFILE='C:\Users\seda.gulec\Downloads\Zerrin Barut Doktora Tezi.sav'
  /COMPRESSED.DATASET ACTIVATE DataSet1.

SAVE OUTFILE='C:\Users\seda.gulec\Downloads\Zerrin Barut Doktora Tezi.sav'
  /COMPRESSED.
T-TEST GROUPS=hiper(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=miRNA_26_ct          miRNA_26_delta_ct
                                  miRNA_133_ct miRNA_133_delta_ct
```

Notes

Output Created		20-JUN-2019 12:10:23
Comments		
Input	Data	C:\Users\seda.gulec\Downloads\Zerri n Barut Doktora Tezi.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	112
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=hiper(1 0) /MISSING=ANALYSIS /VARIABLES=miRNA_26_ct miRNA_26_delta_ct miRNA_133_ct miRNA_133_delta_ct miRNA_378_ct miRNA_378_delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,02

Group Statistics

	hiper	N	Mean	Std. Deviation	Std. Error Mean
miRNA_26_ct	hiper	25	22,6364	2,26025	,45
	hiper olmayan hasta	2	22,0650	3,68403	2,60
miRNA_26_delta_ct	hiper	25	-5,1048	3,62897	,72
	hiper olmayan hasta	2	-6,5150	2,77893	1,96
miRNA_133_ct	hiper	12	30,7692	4,20197	1,21
	hiper olmayan hasta	1	41,8300	.	.
miRNA_133_delta_ct	hiper	12	3,4925	7,41147	2,13
	hiper olmayan hasta	2	-7,6650	30,48337	21,55
miRNA_378_ct	hiper	29	22,6614	3,71076	,68
	hiper olmayan hasta	33	26,4839	5,00316	,87
miRNA_378_delta_ct	hiper	29	-3,5241	8,21657	1,52
	hiper olmayan hasta	33	1,6006	6,79869	1,18

Independent Samples Test

		Levene's Test for		t-test for Equality of Means
		F	Sig	t
miRNA_26_ct	Equal variances assumed	,777	,387	,333
	Equal variances not assumed			,216
miRNA_26_delta_ct	Equal variances assumed	,241	,628	,533
	Equal variances not assumed			,673
miRNA_133_ct	Equal variances assumed	.	.	-2,529
	Equal variances not assumed			.
miRNA_133_delta_ct	Equal variances assumed		,000	1,292
	Equal variances not assumed			,515
miRNA_378_ct	Equal variances assumed	3,586	,063	-3,377
	Equal variances not assumed			-3,442
miRNA_378_delta_ct	Equal variances assumed	,921	,341	-2,687
	Equal variances not assumed			-2,654

	Equal variances not assumed	1,290	,601	1,41020
miRNA_133_ct	Equal variances assumed	11	,028	-11,06083
	Equal variances not assumed	.	.	-11,06083

miRNA_133_delta_ct	Equal variances assumed	12	,221	11,15750
	Equal variances not assumed	1,020	,696	11,15750
miRNA_378_ct	Equal variances assumed	60	,001	-3,82256
	Equal variances not assumed	58,433	,001	-3,82256
miRNA_378_delta_ct	Equal variances assumed	60	,009	-5,12474
	Equal variances not assumed	54,550	,010	-5,12474

Independent Samples Test

		t-test for Equality of Means		
			95% Confidence Interval of the	
		Std. Error Difference	Lower	U
miRNA_26_ct	Equal variances assumed	1,71509	-2,96089	4,1
	Equal variances not assumed	2,64393	-28,77985	29,9
miRNA_26_delta_ct	Equal variances assumed	2,64459	-4,03643	6,8
	Equal variances not assumed	2,09476	-14,49905	17,3
miRNA_133_ct	Equal variances assumed	4,37354	-20,68694	-
	Equal variances not assumed	.	.	.
miRNA_133_delta_ct	Equal variances assumed	8,63385	-7,65405	29,9
	Equal variances not assumed	21,66092	-251,76306	274,0
miRNA_378_ct	Equal variances assumed	1,13190	-6,08671	-
	Equal variances not assumed	1,11056	-6,04524	-
miRNA_378_delta_ct	Equal variances assumed	1,90741	-8,94013	-
	Equal variances not assumed	1,93098	-8,99523	-

```
T-TEST GROUPS=Remodeling(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=miRNA_26_ct          miRNA_26_delta_ct
                                  miRNA_133_ct miRNA_133_delta_ct
miRNA_378_ct
    miRNA_378_d
        elta_ct
  /CRITERIA=CI(.95).
```

Input	Data	C:\Users\seda.gulec\Downloads\Zerri n Barut Doktora Tezi.sav
	Active Dataset	DataSet1
	Filter	<none>

	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	112
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=Remodeling(1 0) /MISSING=ANALYSIS /VARIABLES=miRNA_26_ct miRNA_26_delta_ct miRNA_133_ct miRNA_133_delta_ct miRNA_378_ct miRNA_378_delta_ct /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,03

Group Statistics

	Remodeling	N	Mean	Std. Deviation	Std. Error Mean
miRNA_26_ct	rem	0[a]	.	.	.
	rem omayan hasta	27	22,5941	2,29369	,44142
miRNA_26_delta_ct	rem	0[a]	.	.	.
	rem omayan hasta	27	-5,2093	3,54895	,68300
miRNA_133_ct	rem	0[a]	.	.	.
	rem omayan hasta	13	31,6200	5,05926	1,40319
miRNA_133_delta_ct	rem	0[a]	.	.	.
	rem omayan hasta	14	1,8986	11,59202	3,09810
miRNA_378_ct	rem	31	26,4426	4,93343	,88607
	rem omayan hasta	31	22,9494	4,05300	,72794
miRNA_378_delta_ct	rem	31	1,7977	6,84003	1,22851
	rem omayan hasta	31	-3,3906	8,07052	1,44951

a. t cannot be computed because at least

Notes

Output Created	20-JUN-2019 12:11:30
Comments	

Group Statistics

	egz	N	Mean	Std. Deviation	Std. Error Mean
miRNA_26_ct	egzant	2	22,0650	3,68403	2,60500
	egz omayan	25	22,6364	2,26025	,45205
miRNA_26_delta_ct	egzant	2	-6,5150	2,77893	1,96500
	egz omayan	25	-5,1048	3,62897	,72579
miRNA_133_ct	egzant	1	41,8300	.	.
	egz omayan	12	30,7692	4,20197	1,21300
miRNA_133_delta_ct	egzant	2	-7,6650	30,48337	21,55500
	egz omayan	12	3,4925	7,41147	2,13951
miRNA_378_ct	egzant	2	27,1250	8,36507	5,91500
	egz omayan	60	24,6150	4,74777	,61293
miRNA_378_delta_ct	egzant	2	-1,4550	7,45998	5,27500
	egz omayan	60	-,7745	7,93862	1,02487

Independent Samples Test

		Means			t
					-
		F	Sig.	t	
miRNA_26_ct	Equal variances assumed	,777	,387	-,333	
	Equal variances not assumed			-,216	
miRNA_26_delta_ct	Equal variances assumed	,241	,628	-,533	
	Equal variances not assumed			-,673	
miRNA_133_ct	Equal variances assumed	.	.	2,529	
	Equal variances not assumed			.	
miRNA_133_delta_ct	Equal variances assumed	2	,000	-1,292	
	Equal variances not assumed			-,515	
miRNA_378_ct	Equal variances assumed	1,125	,293	,723	
	Equal variances not assumed			,422	
miRNA_378_delta_ct	Equal variances assumed	,001	,972	-,119	
	Equal variances not assumed			-,127	

Independent Samples Test

	t-test for Equality of Means		
	df	Sig	Mean Difference

miRNA_26_ct	Equal variances assumed	25	,742	-,57140
	Equal variances not assumed	1,061	,863	-,57140
miRNA_26_delta_ct	Equal variances assumed	25	,599	-1,41020
	Equal variances not assumed	1,290	,601	-1,41020
miRNA_133_ct	Equal variances assumed	11	,028	11,06083
	Equal variances not assumed	.	.	11,06083
miRNA_133_delta_ct	Equal variances assumed	12	,221	-11,15750
	Equal variances not assumed	1,020	,696	-11,15750
miRNA_378_ct	Equal variances assumed	60	,473	2,51000
	Equal variances not assumed	1,022	,745	2,51000
miRNA_378_delta_ct	Equal variances assumed	60	,905	-,68050
	Equal variances not assumed	1,077	,919	-,68050

Independent

```
Your temporary usage period for IBM SPSS Statistics will expire in 6023 days.

GET
  FILE='C:\Users\seda.gulec\Desktop\zerrin barut tez\Zerrin Barut
Doktora Tezi.sav'. DATASET NAME DataSet1 WINDOW=FRONT.
T-TEST GROUPS=grup(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=miRNA_26_ct miRNA_133_ct miRNA_378_ct
  CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		05-JUL-2019 10:53:51
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\zerrin barut tez\Zerrin Barut Doktora Tezi.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	112
Syntax		T-TEST GROUPS=grup(1 0)

	apsed Time	00:00:00,08

[

ROC curve

Variable	miRNA_26_Ct miRNA 26 Ct
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	27
Negative group	
diagnosis	= 0
Sample size	16

Disease prevalence (%)	62,8

Area under the ROC curve (AUC)	0,537
Standard Error	0,0924
95% Confidence Interval	0,379 to 0,690
z statistic	0,401
Significance level P (Area=0.5)	0,6885

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR
< 19,37	0,00	0,0 - 12,9	100,00	79,2 - 100,0			1,00
<=19,37	0,00	0,0 - 12,9	93,75	69,7 - 99,0	0,00		1,07
<=19,46	3,70	0,6 - 19,0	93,75	69,7 - 99,0	0,59	0,09 - 4,1	1,03
<=19,7	3,70	0,6 - 19,0	87,50	61,6 - 98,1	0,30	0,04 - 2,0	1,10
<=19,97	3,70	0,6 - 19,0	81,25	54,3 - 95,7	0,20	0,03 - 1,4	1,19
<=20,28	7,41	1,1 - 24,3	81,25	54,3 - 95,7	0,40	0,1 - 1,5	1,14
<=20,31	11,11	2,5 - 29,2	81,25	54,3 - 95,7	0,59	0,2 - 1,8	1,09
<=20,51	14,81	4,3 - 33,7	81,25	54,3 - 95,7	0,79	0,3 - 2,0	1,05
<=20,55	18,52	6,4 - 38,1	81,25	54,3 - 95,7	0,99	0,4 - 2,3	1,00
<=20,83	22,22	8,7 - 42,3	81,25	54,3 - 95,7	1,19	0,6 - 2,5	0,96
<=20,86	25,93	11,2 - 46,3	81,25	54,3 - 95,7	1,38	0,7 - 2,7	0,91
<=20,93	25,93	11,2 - 46,3	75,00	47,6 - 92,6	1,04	0,5 - 2,1	0,99
<=21,02	29,63	13,8 - 50,2	75,00	47,6 - 92,6	1,19	0,6 - 2,3	0,94
<=21,03	29,63	13,8 - 50,2	68,75	41,4 - 88,9	0,95	0,5 - 1,9	1,02
<=21,3	33,33	16,6 - 54,0	68,75	41,4 - 88,9	1,07	0,6 - 2,0	0,97
<=21,68	33,33	16,6 - 54,0	62,50	35,5 - 84,7	0,89	0,5 - 1,7	1,07
<=21,76	37,04	19,4 - 57,6	62,50	35,5 - 84,7	0,99	0,5 - 1,8	1,01
<=21,78	40,74	22,4 - 61,2	62,50	35,5 - 84,7	1,09	0,6 - 2,0	0,95
<=21,79	44,44	25,5 - 64,7	62,50	35,5 - 84,7	1,19	0,7 - 2,1	0,89
<=21,83	48,15	28,7 - 68,0	62,50	35,5 - 84,7	1,28	0,7 - 2,2	0,83
<=21,95	51,85	32,0 - 71,3	62,50	35,5 - 84,7	1,38	0,8 - 2,3	0,77
<=22,05	55,56	35,3 - 74,5	62,50	35,5 - 84,7	1,48	0,9 - 2,5	0,71
<=22,17 *	59,26	38,8 - 77,6	62,50	35,5 - 84,7	1,58	1,0 - 2,6	0,65
<=22,29	59,26	38,8 - 77,6	56,25	29,9 - 80,2	1,35	0,8 - 2,3	0,72
<=22,36	59,26	38,8 - 77,6	50,00	24,7 - 75,3	1,19	0,7 - 2,1	0,81
<=22,72	62,96	42,4 - 80,6	50,00	24,7 - 75,3	1,26	0,7 - 2,2	0,74
<=22,75	62,96	42,4 - 80,6	43,75	19,8 - 70,1	1,12	0,6 - 2,1	0,85
<=22,84	62,96	42,4 - 80,6	37,50	15,3 - 64,5	1,01	0,5 - 2,0	0,99
<=22,96	62,96	42,4 - 80,6	31,25	11,1 - 58,6	0,92	0,4 - 2,0	1,19
<=23,08	66,67	46,0 - 83,4	31,25	11,1 - 58,6	0,97	0,4 - 2,1	1,07
<=23,15	70,37	49,8 - 86,2	31,25	11,1 - 58,6	1,02	0,5 - 2,2	0,95
<=23,17	74,07	53,7 - 88,8	31,25	11,1 - 58,6	1,08	0,5 - 2,3	0,83
<=23,49	77,78	57,7 - 91,3	31,25	11,1 - 58,6	1,13	0,5 - 2,4	0,71
<=23,59	77,78	57,7 - 91,3	25,00	7,4 - 52,4	1,04	0,4 - 2,5	0,89
<=23,64	81,48	61,9 - 93,6	25,00	7,4 - 52,4	1,09	0,5 - 2,6	0,74
<=24,67	85,19	66,3 - 95,7	25,00	7,4 - 52,4	1,14	0,5 - 2,7	0,59
<=24,72	85,19	66,3 - 95,7	18,75	4,3 - 45,7	1,05	0,4 - 2,9	0,79
<=24,89	88,89	70,8 - 97,5	18,75	4,3 - 45,7	1,09	0,4 - 3,1	0,59
<=25,93	92,59	75,7 - 98,9	18,75	4,3 - 45,7	1,14	0,4 - 3,2	0,40
<=27,28	96,30	81,0 - 99,4	18,75	4,3 - 45,7	1,19	0,4 - 3,3	0,20
<=27,74	96,30	81,0 - 99,4	12,50	1,9 - 38,4	1,10	0,3 - 4,0	0,30
<=28,13	96,30	81,0 - 99,4	6,25	1,0 - 30,3	1,03	0,2 - 6,9	0,59
<=29,57	100,00	87,1 - 100,0	6,25	1,0 - 30,3	1,07	0,2 - 7,1	0,00
<=29,99	100,00	87,1 - 100,0	0,00	0,0 - 20,8	1,00		

ROC curve

Variable	miRNA_26_Ct miRNA 26 Ct
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	27
Negative group	
diagnosis	= 0
Sample size	16

Disease prevalence (%)	62,8

Area under the ROC curve (AUC)	0,537
Standard Error	0,0924
95% Confidence Interval	0,379 to 0,690
z statistic	0,401
Significance level P (Area=0.5)	0,6885

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR
< 19,37	0,00	0,0 - 12,9	100,00	79,2 - 100,0			1,00
<=19,37	0,00	0,0 - 12,9	93,75	69,7 - 99,0	0,00		1,07
<=19,46	3,70	0,6 - 19,0	93,75	69,7 - 99,0	0,59	0,09 - 4,1	1,03
<=19,7	3,70	0,6 - 19,0	87,50	61,6 - 98,1	0,30	0,04 - 2,0	1,10
<=19,97	3,70	0,6 - 19,0	81,25	54,3 - 95,7	0,20	0,03 - 1,4	1,19
<=20,28	7,41	1,1 - 24,3	81,25	54,3 - 95,7	0,40	0,1 - 1,5	1,14
<=20,31	11,11	2,5 - 29,2	81,25	54,3 - 95,7	0,59	0,2 - 1,8	1,09
<=20,51	14,81	4,3 - 33,7	81,25	54,3 - 95,7	0,79	0,3 - 2,0	1,05
<=20,55	18,52	6,4 - 38,1	81,25	54,3 - 95,7	0,99	0,4 - 2,3	1,00
<=20,83	22,22	8,7 - 42,3	81,25	54,3 - 95,7	1,19	0,6 - 2,5	0,96
<=20,86	25,93	11,2 - 46,3	81,25	54,3 - 95,7	1,38	0,7 - 2,7	0,91
<=20,93	25,93	11,2 - 46,3	75,00	47,6 - 92,6	1,04	0,5 - 2,1	0,99
<=21,02	29,63	13,8 - 50,2	75,00	47,6 - 92,6	1,19	0,6 - 2,3	0,94
<=21,03	29,63	13,8 - 50,2	68,75	41,4 - 88,9	0,95	0,5 - 1,9	1,02
<=21,3	33,33	16,6 - 54,0	68,75	41,4 - 88,9	1,07	0,6 - 2,0	0,97
<=21,68	33,33	16,6 - 54,0	62,50	35,5 - 84,7	0,89	0,5 - 1,7	1,07
<=21,76	37,04	19,4 - 57,6	62,50	35,5 - 84,7	0,99	0,5 - 1,8	1,01
<=21,78	40,74	22,4 - 61,2	62,50	35,5 - 84,7	1,09	0,6 - 2,0	0,95
<=21,79	44,44	25,5 - 64,7	62,50	35,5 - 84,7	1,19	0,7 - 2,1	0,89
<=21,83	48,15	28,7 - 68,0	62,50	35,5 - 84,7	1,28	0,7 - 2,2	0,83
<=21,95	51,85	32,0 - 71,3	62,50	35,5 - 84,7	1,38	0,8 - 2,3	0,77
<=22,05	55,56	35,3 - 74,5	62,50	35,5 - 84,7	1,48	0,9 - 2,5	0,71
<=22,17 *	59,26	38,8 - 77,6	62,50	35,5 - 84,7	1,58	1,0 - 2,6	0,65
<=22,29	59,26	38,8 - 77,6	56,25	29,9 - 80,2	1,35	0,8 - 2,3	0,72
<=22,36	59,26	38,8 - 77,6	50,00	24,7 - 75,3	1,19	0,7 - 2,1	0,81
<=22,72	62,96	42,4 - 80,6	50,00	24,7 - 75,3	1,26	0,7 - 2,2	0,74
<=22,75	62,96	42,4 - 80,6	43,75	19,8 - 70,1	1,12	0,6 - 2,1	0,85
<=22,84	62,96	42,4 - 80,6	37,50	15,3 - 64,5	1,01	0,5 - 2,0	0,99
<=22,96	62,96	42,4 - 80,6	31,25	11,1 - 58,6	0,92	0,4 - 2,0	1,19
<=23,08	66,67	46,0 - 83,4	31,25	11,1 - 58,6	0,97	0,4 - 2,1	1,07
<=23,15	70,37	49,8 - 86,2	31,25	11,1 - 58,6	1,02	0,5 - 2,2	0,95
<=23,17	74,07	53,7 - 88,8	31,25	11,1 - 58,6	1,08	0,5 - 2,3	0,83
<=23,49	77,78	57,7 - 91,3	31,25	11,1 - 58,6	1,13	0,5 - 2,4	0,71
<=23,59	77,78	57,7 - 91,3	25,00	7,4 - 52,4	1,04	0,4 - 2,5	0,89
<=23,64	81,48	61,9 - 93,6	25,00	7,4 - 52,4	1,09	0,5 - 2,6	0,74
<=24,67	85,19	66,3 - 95,7	25,00	7,4 - 52,4	1,14	0,5 - 2,7	0,59
<=24,72	85,19	66,3 - 95,7	18,75	4,3 - 45,7	1,05	0,4 - 2,9	0,79
<=24,89	88,89	70,8 - 97,5	18,75	4,3 - 45,7	1,09	0,4 - 3,1	0,59
<=25,93	92,59	75,7 - 98,9	18,75	4,3 - 45,7	1,14	0,4 - 3,2	0,40
<=27,28	96,30	81,0 - 99,4	18,75	4,3 - 45,7	1,19	0,4 - 3,3	0,20
<=27,74	96,30	81,0 - 99,4	12,50	1,9 - 38,4	1,10	0,3 - 4,0	0,30
<=28,13	96,30	81,0 - 99,4	6,25	1,0 - 30,3	1,03	0,2 - 6,9	0,59
<=29,57	100,00	87,1 - 100,0	6,25	1,0 - 30,3	1,07	0,2 - 7,1	0,00
<=29,99	100,00	87,1 - 100,0	0,00	0,0 - 20,8	1,00		

ROC curve

Variable	miRNA_delta_CT miRNA_delta CT
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	27
Negative group	
diagnosis	= 0
Sample size	16

Disease prevalence (%)	62,8

Area under the ROC curve (AUC)	0,484
Standard Error	0,0923
95% Confidence Interval	0,329 to 0,641
z statistic	-0,176
Significance level P (Area=0.5)	0,8607

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR
>=-14,14	100,00	87,1 - 100,0	0,00	0,0 - 20,8	1,00		
>-14,14	96,30	81,0 - 99,4	0,00	0,0 - 20,8	0,96		
>-12,31	92,59	75,7 - 98,9	0,00	0,0 - 20,8	0,93		
>-9,8	88,89	70,8 - 97,5	0,00	0,0 - 20,8	0,89		
>-9,62	85,19	66,3 - 95,7	0,00	0,0 - 20,8	0,85		
>-9,23	85,19	66,3 - 95,7	6,25	1,0 - 30,3	0,91	0,1 - 6,1	2,37
>-8,68	85,19	66,3 - 95,7	12,50	1,9 - 38,4	0,97	0,3 - 3,6	1,19
>-8,48	81,48	61,9 - 93,6	12,50	1,9 - 38,4	0,93	0,3 - 3,4	1,48
>-7,43	81,48	61,9 - 93,6	18,75	4,3 - 45,7	1,00	0,4 - 2,8	0,99
>-7,32	77,78	57,7 - 91,3	18,75	4,3 - 45,7	0,96	0,3 - 2,7	1,19
>-7,29	74,07	53,7 - 88,8	18,75	4,3 - 45,7	0,91	0,3 - 2,6	1,38
>-6,73	74,07	53,7 - 88,8	25,00	7,4 - 52,4	0,99	0,4 - 2,4	1,04
>-6,48	70,37	49,8 - 86,2	25,00	7,4 - 52,4	0,94	0,4 - 2,3	1,19
>-6,35	66,67	46,0 - 83,4	25,00	7,4 - 52,4	0,89	0,4 - 2,2	1,33
>-6,33	62,96	42,4 - 80,6	25,00	7,4 - 52,4	0,84	0,3 - 2,1	1,48
>-5,97	62,96	42,4 - 80,6	31,25	11,1 - 58,6	0,92	0,4 - 2,0	1,19
>-5,86	62,96	42,4 - 80,6	37,50	15,3 - 64,5	1,01	0,5 - 2,0	0,99
>-5,73	59,26	38,8 - 77,6	37,50	15,3 - 64,5	0,95	0,5 - 1,9	1,09
>-5,4	55,56	35,3 - 74,5	37,50	15,3 - 64,5	0,89	0,4 - 1,8	1,19
>-5,31	55,56	35,3 - 74,5	43,75	19,8 - 70,1	0,99	0,5 - 1,9	1,02
>-5,12	55,56	35,3 - 74,5	50,00	24,7 - 75,3	1,11	0,6 - 2,0	0,89
>-4,71	55,56	35,3 - 74,5	56,25	29,9 - 80,2	1,27	0,7 - 2,2	0,79
>-4,62 *	55,56	35,3 - 74,5	62,50	35,5 - 84,7	1,48	0,9 - 2,5	0,71
>-4,55	51,85	32,0 - 71,3	62,50	35,5 - 84,7	1,38	0,8 - 2,3	0,77
>-4,52	48,15	28,7 - 68,0	62,50	35,5 - 84,7	1,28	0,7 - 2,2	0,83
>-4,38	48,15	28,7 - 68,0	68,75	41,4 - 88,9	1,54	0,9 - 2,6	0,75
>-4,28	44,44	25,5 - 64,7	68,75	41,4 - 88,9	1,42	0,8 - 2,4	0,81
>-4,13	40,74	22,4 - 61,2	68,75	41,4 - 88,9	1,30	0,7 - 2,3	0,86
>-3,99	37,04	19,4 - 57,6	68,75	41,4 - 88,9	1,19	0,7 - 2,1	0,92
>-3,85	33,33	16,6 - 54,0	68,75	41,4 - 88,9	1,07	0,6 - 2,0	0,97
>-3,72	29,63	13,8 - 50,2	68,75	41,4 - 88,9	0,95	0,5 - 1,9	1,02
>-3,58	25,93	11,2 - 46,3	68,75	41,4 - 88,9	0,83	0,4 - 1,7	1,08
>-3,3	22,22	8,7 - 42,3	68,75	41,4 - 88,9	0,71	0,3 - 1,5	1,13
>-3,14	22,22	8,7 - 42,3	75,00	47,6 - 92,6	0,89	0,4 - 1,9	1,04
>-2,77	22,22	8,7 - 42,3	81,25	54,3 - 95,7	1,19	0,6 - 2,5	0,96
>-1,79	18,52	6,4 - 38,1	81,25	54,3 - 95,7	0,99	0,4 - 2,3	1,00
>-1,45	14,81	4,3 - 33,7	81,25	54,3 - 95,7	0,79	0,3 - 2,0	1,05
>-1,31	11,11	2,5 - 29,2	81,25	54,3 - 95,7	0,59	0,2 - 1,8	1,09
>-1,28	7,41	1,1 - 24,3	81,25	54,3 - 95,7	0,40	0,1 - 1,5	1,14
>-1,16	3,70	0,6 - 19,0	81,25	54,3 - 95,7	0,20	0,03 - 1,4	1,19
>0,82	3,70	0,6 - 19,0	87,50	61,6 - 98,1	0,30	0,04 - 2,0	1,10
>1,39	3,70	0,6 - 19,0	93,75	69,7 - 99,0	0,59	0,09 - 4,1	1,03
>1,51	0,00	0,0 - 12,9	93,75	69,7 - 99,0	0,00		1,07
>2,17	0,00	0,0 - 12,9	100,00	79,2 - 100,0			1,00

ROC curve

Variable	miRNA_133_Ct
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	13
Negative group	
diagnosis	= 0
Sample size	8

Disease prevalence (%)	61,9

Area under the ROC curve (AUC)	0,577
Standard Error	0,132
95% Confidence Interval	0,345 to 0,786
z statistic	0,581
Significance level P (Area=0.5)	0,5613

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR
< 24,46	0,00	0,0 - 24,9	100,00	62,9 - 100,0			1,00
<=24,46	7,69	1,3 - 36,1	100,00	62,9 - 100,0			0,92
<=24,65	15,38	2,4 - 45,5	100,00	62,9 - 100,0			0,85
<=25,51	23,08	5,3 - 53,8	100,00	62,9 - 100,0			0,77
<=25,53	23,08	5,3 - 53,8	87,50	47,4 - 97,9	1,85	0,7 - 5,2	0,88
<=25,7	23,08	5,3 - 53,8	75,00	35,0 - 96,1	0,92	0,3 - 2,7	1,03
<=27,56	30,77	9,3 - 61,4	75,00	35,0 - 96,1	1,23	0,5 - 3,1	0,92
<=27,71	30,77	9,3 - 61,4	62,50	24,7 - 91,0	0,82	0,3 - 2,2	1,11
<=28,47	30,77	9,3 - 61,4	50,00	16,0 - 84,0	0,62	0,2 - 1,8	1,38
<=29,85	38,46	14,0 - 68,4	50,00	16,0 - 84,0	0,77	0,3 - 2,0	1,23
<=32,33	46,15	19,3 - 74,8	50,00	16,0 - 84,0	0,92	0,4 - 2,3	1,08
<=32,8	53,85	25,2 - 80,7	50,00	16,0 - 84,0	1,08	0,5 - 2,5	0,92
<=32,86	61,54	31,6 - 86,0	50,00	16,0 - 84,0	1,23	0,5 - 2,8	0,77
<=34,07	69,23	38,6 - 90,7	50,00	16,0 - 84,0	1,38	0,6 - 3,0	0,62
<=34,54	76,92	46,2 - 94,7	50,00	16,0 - 84,0	1,54	0,7 - 3,3	0,46
<=34,57 *	84,62	54,5 - 97,6	50,00	16,0 - 84,0	1,69	0,8 - 3,5	0,31
<=34,84	84,62	54,5 - 97,6	37,50	9,0 - 75,3	1,35	0,5 - 3,4	0,41
<=35,58	84,62	54,5 - 97,6	25,00	3,9 - 65,0	1,13	0,3 - 3,8	0,62
<=36,03	92,31	63,9 - 98,7	25,00	3,9 - 65,0	1,23	0,4 - 4,1	0,31
<=37,9	92,31	63,9 - 98,7	12,50	2,1 - 52,6	1,05	0,2 - 6,6	0,62
<=39,33	92,31	63,9 - 98,7	0,00	0,0 - 37,1	0,92		
<=41,83	100,00	75,1 - 100,0	0,00	0,0 - 37,1	1,00		

ROC curve

Variable	miRNA378_Ct
Classification variable	diagnosis

Positive group	
diagnosis	= 1
Sample size	51
Negative group	
diagnosis	= 0
Sample size	13

Disease prevalence (%)	79,7

Area under the ROC curve (AUC)	0,612

Standard Error	0,0836
95% Confidence Interval	0,482 to 0,732
z statistic	1,344
Significance level P (Area=0.5)	0,1790

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR
>=17,51	100,00	93,0 - 100,0	0,00	0,0 - 24,9	1,00		
>17,51	98,04	89,5 - 99,7	0,00	0,0 - 24,9	0,98		
>18,64	98,04	89,5 - 99,7	7,69	1,3 - 36,1	1,06	0,2 - 7,0	0,25
>18,66	96,08	86,5 - 99,4	7,69	1,3 - 36,1	1,04	0,2 - 6,8	0,51
>19,11	94,12	83,7 - 98,7	7,69	1,3 - 36,1	1,02	0,2 - 6,7	0,76
>19,59	92,16	81,1 - 97,8	7,69	1,3 - 36,1	1,00	0,2 - 6,6	1,02
>19,8	90,20	78,6 - 96,7	7,69	1,3 - 36,1	0,98	0,1 - 6,4	1,27
>19,89	88,24	76,1 - 95,5	7,69	1,3 - 36,1	0,96	0,1 - 6,3	1,53
>20,22	86,27	73,7 - 94,3	7,69	1,3 - 36,1	0,93	0,1 - 6,2	1,78
>20,31	86,27	73,7 - 94,3	15,38	2,4 - 45,5	1,02	0,3 - 3,7	0,89
>20,43	84,31	71,4 - 93,0	15,38	2,4 - 45,5	1,00	0,3 - 3,6	1,02
>20,56	84,31	71,4 - 93,0	23,08	5,3 - 53,8	1,10	0,4 - 3,0	0,68
>20,64	84,31	71,4 - 93,0	30,77	9,3 - 61,4	1,22	0,5 - 2,8	0,51
>20,7	82,35	69,1 - 91,6	30,77	9,3 - 61,4	1,19	0,5 - 2,7	0,57
>20,71	82,35	69,1 - 91,6	38,46	14,0 - 68,4	1,34	0,7 - 2,7	0,46
>20,86	80,39	66,9 - 90,2	38,46	14,0 - 68,4	1,31	0,6 - 2,6	0,51
>20,9	78,43	64,7 - 88,7	38,46	14,0 - 68,4	1,27	0,6 - 2,6	0,56
>20,94	74,51	60,4 - 85,7	38,46	14,0 - 68,4	1,21	0,6 - 2,5	0,66
>21,09	72,55	58,3 - 84,1	38,46	14,0 - 68,4	1,18	0,6 - 2,4	0,71
>21,14	70,59	56,2 - 82,5	38,46	14,0 - 68,4	1,15	0,6 - 2,3	0,76
>21,17	68,63	54,1 - 80,9	38,46	14,0 - 68,4	1,12	0,5 - 2,3	0,82
>21,21	66,67	52,1 - 79,2	38,46	14,0 - 68,4	1,08	0,5 - 2,2	0,87
>21,24	64,71	50,1 - 77,6	38,46	14,0 - 68,4	1,05	0,5 - 2,2	0,92
>21,41	64,71	50,1 - 77,6	46,15	19,3 - 74,8	1,20	0,6 - 2,2	0,76
>21,47	62,75	48,1 - 75,9	46,15	19,3 - 74,8	1,17	0,6 - 2,2	0,81
>21,65	60,78	46,1 - 74,1	46,15	19,3 - 74,8	1,13	0,6 - 2,1	0,85
>21,71	58,82	44,2 - 72,4	46,15	19,3 - 74,8	1,09	0,6 - 2,1	0,89
>21,84	56,86	42,2 - 70,6	46,15	19,3 - 74,8	1,06	0,6 - 2,0	0,93
>21,9	54,90	40,3 - 68,9	46,15	19,3 - 74,8	1,02	0,5 - 1,9	0,98
>21,99	54,90	40,3 - 68,9	53,85	25,2 - 80,7	1,19	0,7 - 2,1	0,84
>22,02	54,90	40,3 - 68,9	61,54	31,6 - 86,0	1,43	0,9 - 2,3	0,73
>22,32	52,94	38,5 - 67,1	61,54	31,6 - 86,0	1,38	0,8 - 2,3	0,76
>22,38	52,94	38,5 - 67,1	69,23	38,6 - 90,7	1,72	1,1 - 2,7	0,68
>22,43	50,98	36,6 - 65,2	69,23	38,6 - 90,7	1,66	1,1 - 2,6	0,71
>22,48	49,02	34,8 - 63,4	69,23	38,6 - 90,7	1,59	1,0 - 2,5	0,74
>23,13	47,06	32,9 - 61,5	69,23	38,6 - 90,7	1,53	1,0 - 2,4	0,76
>23,19	45,10	31,1 - 59,7	69,23	38,6 - 90,7	1,47	0,9 - 2,4	0,79
>23,34	43,14	29,4 - 57,8	69,23	38,6 - 90,7	1,40	0,9 - 2,3	0,82
>23,53	41,18	27,6 - 55,8	69,23	38,6 - 90,7	1,34	0,8 - 2,2	0,85
>23,59	39,22	25,9 - 53,9	69,23	38,6 - 90,7	1,27	0,8 - 2,1	0,88
>23,96	39,22	25,9 - 53,9	76,92	46,2 - 94,7	1,70	1,1 - 2,7	0,79
>24,25	39,22	25,9 - 53,9	84,62	54,5 - 97,6	2,55	1,7 - 3,9	0,72
>24,46	37,25	24,1 - 51,9	84,62	54,5 - 97,6	2,42	1,6 - 3,7	0,74
>24,47	35,29	22,4 - 49,9	84,62	54,5 - 97,6	2,29	1,5 - 3,6	0,76
>24,84	33,33	20,8 - 47,9	84,62	54,5 - 97,6	2,17	1,4 - 3,4	0,79
>25,21	31,37	19,1 - 45,9	84,62	54,5 - 97,6	2,04	1,3 - 3,3	0,81
>25,29	29,41	17,5 - 43,8	84,62	54,5 - 97,6	1,91	1,2 - 3,1	0,83
>26,09	29,41	17,5 - 43,8	92,31	63,9 - 98,7	3,82	2,4 - 6,0	0,76
>26,37	27,45	15,9 - 41,7	92,31	63,9 - 98,7	3,57	2,2 - 5,7	0,79
>26,88	25,49	14,3 - 39,6	92,31	63,9 - 98,7	3,31	2,0 - 5,4	0,81
>27,04 *	25,49	14,3 - 39,6	100,00	75,1 - 100,0			0,75

7.2. ETHICAL APPROVAL

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15-1346
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

25/05/2017

İlgili Makama (Zerrin Barut)

Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı Prof. Dr. Turgay İsbir ve Marmara Üniversitesi Hastanesi Kalp ve Damar Cerrahisi Bölümü Prof. Dr. Selim İsbir'in sorumlu olduğu **"Sol Ventriküler Hipertrofide miRNA133, miRNA26 ve miRNA378 Expresyon Düzeylerinin Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (1326 kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **24.05.2017** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 722**).

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

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7.3. CURRICULUM VITAE

Personal Informations

Name	Zerrin	**Surname**	Barut
Place of Birth	İzmir	**Date of Birth**	23.04.1960
Nationality	Turkish	**ID**	25039202676
Email	zerrinbarut@hotmail.co	**Phone**	05322743005

Educational Informations

	Name of Institution	**Year**
Doctorate	Ege Universitesi Diş Hek.Fak. Pedodonti A.D.	1989-1994
Master		
University	Ege Universsitesi Diş Hekimliği Fakültesi	1977-1983
High School	İzmir Özel Türk Koleji	1971-1977

Language	**Reading***	**Speaking***	**Writing***	**TOEFL Score**	**(Other)**
English				81	

*Very Good, Good, Basic

	Quantitative	**Equal Weight**	**Literature**
ALES Score		75.58	
(Other) Score			

Computer Skills

Program	**Ability to use**
Microsoft Office	Good