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YEDITEPE UNIVERSITY
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THE RELATIONSHIP BETWEEN LEPTIN, MIRNA221 AND THE CORONARY ARTERY DISEASE AND THE SEVERITY OF THE DISEASE

DOCTOR OF PHILOSOPHY THESIS

AYÇA TÜRER CABBAR

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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 ..... and numbered .....

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DECLARATION

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

01.07.2022

Ayça TÜRER CABBAR

DEDICATION

I dedicate to my thesis to my lovely family; my husband Fatih CABBAR, my daughter Tuvana CABBAR, my son Talu CABBAR, my mother Ferde TÜRER, my father Sedat TÜRER and my sisters Begüm & Ceren.

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LIST OF SYMBOLS AND ABBREVIATIONS

CAD	Coronary Artery Disease
MI	Myocardial Infarction
DM	Diabetes Mellitus
CVD	Cardiovascular Disease
sOB-R	Soluble Leptin Receptor
FLI	Free Leptin Index
miRNAs	Micro Ribonucleic Acids
eNOS	Nitric Oxide Synthase
siRNA	Small Interfering RNA
FHS	Framingham Heart Study
BMI	Body Mass Index
OB-Rs	Leptin Receptors
LIF	Leukemia Inhibitory Factor
IL	Interleukin
GCSF	Granulocyte Colony-Stimulating Factor
JAK	Januskinase
aa	Amino Acids
STAT	Signal Transducer and Activator of Transcription
MAP	Mitogen-Activated Protein
OB-Rfl	Long form of Leptin Receptor
PTP1B	Protein Tyrosine Phosphatase 1B
SOCS3	Suppressor of Cytokine Signaling 3
ER	Endoplasmic Reticulum
sIL-6R	Soluble IL-6 Receptor
RISC	RNA Induced Silencing Complex
3'UTR	3' Untranslated Region
mRNA	Messenger RNA
pH	Power of Hydrogen
GABA	Gamma-Aminobutyric Acid
HUVEC	Human Umbilical Vein Endothelial Cells
NO	Nitric oxide
TNF	Tumor necrosis factor

WHO	World Health Organization
ELISA	Enzyme Linked Immunosorbent Assay
EDTA	Ethylene Diamine Tetra Acetic Acid
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ΔCT	delta CT
HRP	Horseradish Peroxidase
SPSS	Statistical Package for the Social Sciences
SD	Standard Deviation
n	Number of individuals
X2	Chi-square test
C	Cholesterol
ROC	Receiver Operating Characteristic
AUC	Area Under Curve
CI	Confidence Interval

ABSTRACT

Türer Cabbar, A. (2022). The Relationship Between Leptin, miRNA-221 and the Coronary Artery Disease and the Severity of the Disease. Yeditepe University, Health Science Institute, Molecular Medicine Department, PhD thesis, İstanbul.

Coronary artery disease (CAD) has probably the most critical and most widespread type of cardiovascular disease (CVD) threatening people's health, worldwidely. High leptin levels in obese individuals participate to low-graded systemic inflammation, which makes this patients more sensitive for CVDs. The soluble leptin receptor (sOB-R) is the primary protein for leptin binding, so it is able to pivotally modifying its bioavailability. The degree of leptin's decrease may be a gauge for remaining leptin sensitivity. The status of leptin resistance and action has a biomarker called the free leptin index (FLI) and it is one of a fraction of total leptin and the sOB-R concentrations. In cardiovascular biology, micro ribonucleic acids (miRNAs) regulate many diverse processes including the maintenance of cardiac function and development of the heart. Also miRNAs are found in relationship with pathological processes and begun to be used as biomarker in some diseases. Within the scope of the study, it was aimed to search the relationship between leptin, sOB-R, FLI and miRNA221 and the CAD and the severity of the CAD.

The prospective case-control study consisted of 78 individuals who had an indication for coronary angiography except acute coronary syndrome. 40 patients whose coronary lesion needed invasive treatment and 38 individuals who had normal coronary artery as the control group. The peripheral blood samples were drawn from patients and control group after performing coronary angiography. Coronary angiograms also were evaluated by quantification of the lesions according to the SYNTAX score. sOB-R and leptin levels in serum samples were determined by an enzyme immunoassay method, a two-site sandwich Enzyme Linked Immunosorbent Assay (ELISA) to quantitate sOB-R and leptin in samples. miRNA-221 expression levels determined by realtime polymerase chain reaction (PCR). The analysis of Receiver operating characteristic (ROC) curve was performed to determine the diagnostic capability of miRNA-221.We evaluated a total of 40 patients that 23 were male (57,5%) and 17 were female (42,5%). Control group included 38 individuals and 17 were male (44,7%) and 21 were female (55,3%).The results indicate that sOB-R levels were statistically higher ($p<0,001$*) and FLI results were significantly lower ($p<0,001$*) in patients than the control group. In addition, leptin level differences were not found significant between groups. As a result

of the subgroup analyzes performed, the relationship between CAD and sOB-R levels and also with FLI results were independent from obesity. Also we found a significant positive correlation between l sOB-R and SYNTAX score (p<0,001*) and significant negative correlation between FLI and SYNTAX score (p<0,001*). Beside these findings miRNA-221 levels was found significantly three-folds higher in the patient group than the controls (P= 0,024). Furthermore, there was a statistically significant correlation between miRNA-221 and sOB-R (p=0,029) but no correlation with leptin, FLI and SYNTAX score.In conclusion, the results showed that sOB-R level is statistically higher and FLI is statistically lower in CAD group than the normal coronary artery group. miRNA-221 levels statistically higher in CAD group than the normal coronary artery group. Also sOB-R levels and FLI are correlated with the severity of CAD. Further, if these results are confirmed in other studies, sOB-R levels, FLI and miRNA-221 may be useful to detect significant CAD before performing coronary angiography.

Key words: miRNA221, Leptin, Soluble Leptin Receptor, Coronary Artery Disease

ÖZET

Türer Cabbar, A. (2022). Leptin ve miRNA-221'in Koroner Arter Hastalığı ve Yaygınlığı ile İlişkisinin İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD. Doktora Tezi. İstanbul.

Koroner arter hastalığı (CAD), muhtemelen dünya çapında insan sağlığını tehdit eden en önemli ve en yaygın kardiyovasküler hastalık (CVD) tipidir. Obez bireylerde yüksek leptin seviyeleri, düşük dereceli sistemik inflamasyonuna neden olur ve bu da hastaları CAD'a karşı daha duyarlı hale getirir. Leptinin azalma derecesi, leptin duyarlılığı için bir gösterge olabilir. Çözünür leptin reseptörü (sOB-R), leptin için birincil bağlayıcı proteindir, bu nedenle biyoyararlanımını etkileyebilir. Leptin direncinin durumu, serbest leptin indeksi (FLI) adı verilen bir biyobelirteç ile değerlendirilebilir. FLI, toplam leptin ve sOB-R konsantrasyonlarının oranıdır.Kardiyovasküler biyolojide, mikro ribonükleik asitler (miRNA'lar), kalp fonksiyonunun korunması ve kalbin gelişimi dahil olmak üzere birçok farklı süreci düzenler. Ayrıca miRNA'lar patolojik süreçlerle ilişki içinde bulunmuş ve bazı hastalıklarda biyobelirteç olarak kullanılmaya başlanmıştır.Bu kapsamda çalışmada leptin, leptin reseptörü, FLI ve miRNA-221 ile CAD arasındaki ilişkinin ve CAD şiddetinin araştırılması amaçlanmıştır.Prospektif vaka-kontrol çalışması, akut koroner sendrom dışında koroner anjiyografi endikasyonu olan 78 kişiden oluşturuldu. Koroner lezyonu invaziv tedavi gerektiren 40 hasta ve koroner arterleri normal olan 38 kişi kontrol grubu olarak alındı. Koroner anjiyografi yapıldıktan sonra hastalardan ve kontrol grubundan periferik kan örnekleri alındı. Koroner anjiyogramlar ayrıca lezyonların SYNTAX skoruna göre değerlendirildi. Serum numunelerindeki sOB-R ve leptin seviyeleri, numunelerdeki sOB-R ve leptini ölçmek için bir sandviç enzim immünoanaliz yöntemi (ELISA) ile belirlendi. miRNA-221 ekspresyonu gerçek zamanlı polimeraz zincir reaksiyonu yöntemi (PCR) ile belirlendi. miRNA-221'in tanısal kapasitesini belirlemek için ROC eğrisinin analizi yapıldı.Hasta grubu, 23'ü erkek (%57,5), 17'si kadın (%42,5) olmak üzere toplam 40 bireyden meydana gelmekteydi. Kontrol grubu, 38 kişi olup 17'si erkek (%44,7), 21'i kadın (%55,3) idi.Çalışmamızda, kontrol grubuna göre hastalarda sOB-R düzeylerinin istatistiksel olarak daha yüksek ($p<0,001$*) ve FLI sonuçlarının istatistiksel olarak daha düşük ($p<0,001$*) olduğu saptandı. Ayrıca leptin düzeyleri gruplar arasında anlamlı farklılık göstermedi. Yapılan alt grup analizleri sonucunda CAD ve sOB-R düzeyleri ile FLI sonuçları arasındaki ilişki obeziteden bağımsızdı. Ayrıca SYNTAX skoru ile

leptin reseptörü arasında istatistiksel olarak anlamlı pozitif korelasyon (p<0,001*) ve SYNTAX skoru ile FLI arasında negatif korelasyon (p<0,001*) saptandı. Bu bulguların yanında miRNA-221 düzeyleri hasta grubunda kontrollere göre anlamlı olarak 3 kat daha yüksek bulundu (P=0,024). Bunun yanında miRNA-221 ile sOB-R arasında istatistiksel olarak anlamlı bir korelasyon saptandı (p=0,029) ve leptin ve FLI, SYNTAX skoru ile korele saptanmadı.Sonuç olarak, CAD grubunda normal koroner arter grubuna göre sOB-R düzeyinin istatistiksel olarak daha yüksek ve FLI'nin istatistiksel olarak daha düşük olarak saptandı. miRNA-221 seviyeleri CAD grubunda normal koroner arter grubuna göre istatistiksel olarak daha yüksek saptandı. Ayrıca sOB-R seviyeleri ve FLI, CAD şiddeti ile ilişkili bulundu. Mevcut sonuçların başka çalışmalarla doğrulanması halinde, sOB-R, FLI ve miRNA-221 değerlendirilmesi, koroner anjiyografi yapılmadan invaziv tedavi gerektiren CAD saptamak için faydalı olabilir.

Anahtar Kelimeler: miRNA-221, Leptin, Leptin Reseptör, Koroner Arter Hastalığı

1.INTRODUCTION and PURPOSE

Changes in nutritional attitude for food with high calories and a reduced tendency to exercise have led to rising of the incidence of metabolic syndrome both in developing and developed countries. Besides, increased risk for CAD and myocardial infarction (MI) it is highly related with metabolic disorders, such as hypertension, obesity, diabetes mellitus (DM) and, lipid metabolism disorders [1]. CAD can cause to total obstruction of blood flowing to the heart therefore can lead to heart attack. For its high mortality and morbidity ratio, CAD has probably the most critical and most widespread type of CVD threatening people's health, worldwidely [2].

However, the detection of significant CAD before performing coronary interventions in patients with suspected CAD is a challenging task in everyday clinical practice [3]. A lot of studies over the past two decades have shown that the adipocyte-derived peptide hormone leptin plays an important role linking CVDs, metabolic syndrome, obesity and inflammation. However, the role of leptin in the cardiovascular system remains controversial. Although it is contentious some researches shows that leptin interferes with the chronic inflammation on pathogenetic basis. Its believed that high leptin levels in obese individuals participate to low-graded systemic inflammation, which makes this patients more sensitive for CVDs [4]. Leptin resistance's pathophysiological status is particularly characterized by lower leptin impacts on the adjustment of energy expenditure and food uptake [1, 5].

sOB-R is the primary binding protein for leptin [6], so it is able to pivotally modifying its bioavailability. As serum sOB-R concentration is suggested to project the amount of membraneanchored receptors [7], the degree of its decrease may be a gauge for the remaining leptin sensitivity [1]. The status of leptin resistance and action has a biomarker called the FLI and it is a fraction of total leptin and sOB-R concentrations [8].

In cardiovascular biology, miRNAs regulate many diverse processes including the maintenance of cardiac function and development of the heart [9]. miRNA-222 and miRNA-221 present anti-angiogenic effects [10]. miRNA-222 and miRNA-221 overexpression decreases the expression of the endothelial nitric oxide synthase (eNOS) in the Dicer small interfering RNA (siRNA) - transfected cells, indirectly [11].

Adipose miRNA-221 evidence is positively related with increasing BMI withal indicating that adipose miRNA-221 is up setting in obesity [12-14].

Within the scope of the study, it was aimed to search the relationship between leptin, soluble leptin receptor, FLI and miRNA221 and the CAD and the severity of the CAD.

2. LITERATURE REVIEW

2.1. Overview about coronary artery disease

CAD both non-obstructive or obstructive is a pathological process described by atherosclerotic plaque collection in the epicardial arteries, [15]. Of late years, even though there have been important a development in atherosclerotic CVD outcomes, atherosclerotic CVD stays major reason of mortality and morbidity [16-18].

Epidemiological study results, particularly the Framingham study, have been very important for our valid information about CVD. Defining the risk factors, evaluation of their predictive capability, and their effect for disease prevention are the main points. The Framingham heart study (FHS), which published its findings in 1957, invented the notion of "risk factors" in CAD. FHS showed the epidemiologic connection of blood pressure, cholesterol levels, and cigarette smoking to incidence of CAD. What was found was avant-garde, as it brought about a change in the way medicine was practiced. There is a lot of risk factors for CAD, modifiable risk factors are: DM, overweight, high blood presure, smoking, lack of physical activity, high blood cholesterol levels, stress, obesity, and unhealthy diet and the risk factors which cannot be modified are: Family past, sex (men are at higher risk), race, age (as the age increases the risk increase).

Kannel et al. [19] first described the connection between CAD and obesity 50 years ago. Obesity, a metabolic disease associated with comorbidities such as type-II DM, sleep apnea, CAD, and hypertension, is also a risk factor for the all-cause mortality because as extra adipose tissue accumulates, changes in function and metabolic profile and various adaptations in cardiac structure take place. [20]. The current research showed that greater body mass index (BMI) during childhood is relevant with an higher risk of CAD in adulthood [21]. The main factor to prevent the CAD, is to control and avoid obesity and overweight in childhood and adulthood. [22, 23].

Invasive coronary angiography well situated as the gold standard identification to CAD, onward the first accidentally selective coronary angiography in 1958 performed by pediatric cardiologist Dr. Mason Sones, in the course of ventriculography and aortography [24](Sones et al., 1959), [25].

Coronary angiography stays default way to describe coronary anatomy and characterize the intensity of coronary artery stenosis. A visually approximated diameter stenosis solemnity of ≥50% for left main disease and ≥70% for non–left main disease has been for defining significant stenosis and guiding revascularization strategies [26]. Expected accuracy of revascularization, the anatomical complication of the lesions, the estimated death risk, negative results are critical elements to determine in defining the type of revascularization in individuals with CAD. [26].

A lot of factors conduce to the prediction of the complexity of CAD. The SYNTAX score was generated from the SYNTAX study prospectively to back up in this decision-making process by providing an objective gauge to rate the anatomic complexity of CAD in individuals with multivessel disease [27].

2.2. Leptin and Leptin Receptors

Leptin is an adipocytokine also a polypeptide hormone that is formed by folding of 167 amino acid long polypeptide chain as 4 α-helix structures. By the cleavage of signal peptide, leptin passes into the circulation as 146 amino acid length, 16Kda weight functioning active form. After its synthesis in white adipose tissue, leptin transports in the circulation to its target tissue hypothalamus [28].

Leptin is arrange peripheral metabolic processes [29-31], influences reproductive function [32-34] contributed in the central regulation of food uptake [35, 36], and as well as the immune response [37-41].

Obese people's leptin concentrations in the serum, are clearly higher from the normal-weight individuals [41, 42]. Amounts of leptin produced by adipose tissues and whole body fat mass are correlated. Signal-transducing receptors of leptin (OB-Rs) and the leptin binding proteins affect the impact of leptin sensitivity and adipocytokine of tissues, besides the amount of expression of membrane-anchored and spread of leptin synthesis by adipose tissue. OB-R mediates leptin actions [43].

The OB-R is concerned to the gp130 subunit of the leukemia inhibitory factor (LIF)-receptor, interleukin (IL)-6-receptor-complex and the granulocyte colony-stimulating factor human (GCSF) [43] and owns a single transmembrane domain.

The OB-R has been designated to class-I cytokine receptor family, because of this correlation. So far, 4 different membrane-anchored OB-R isoforms have been defined in humans (OB-R219.1, OB-Rfl, OB-R219.2, and OB-R219.3). They differ in the length of the intracellular domain but have same transmembrane, ligand-binding and extracellular domains (Figure 2.2-1).

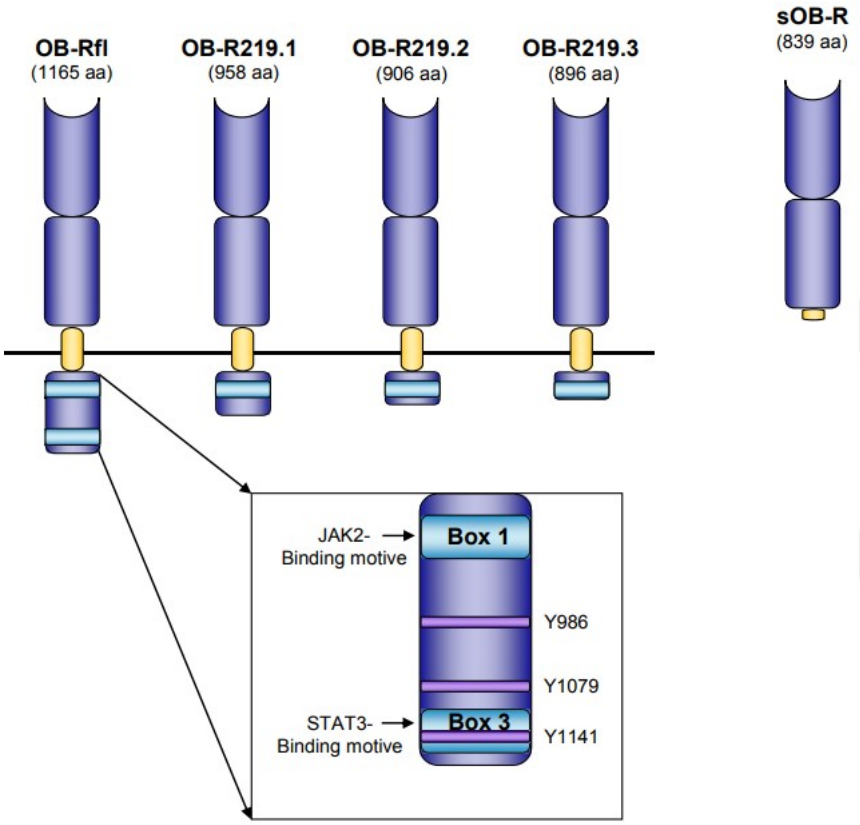

Figure 2.2-1 sOB-R and human membrane-anchored leptin receptor isoforms.

JAK2, Januskinase 2; aa, amino acids; sOB-R, soluble leptin receptor; STAT3, activator and signal transducer of transcription 3[1].

Although last study results shows that, wıth the pull of mitogen-activated protein (MAP) kinase pathway, the short isoforms are likely to included in leptin signaling [44], what is known is the long form (OB-Rfl) solely own Box 3 motif with Tyr1141, and for this reason it is aforethought to carry out the signaling capability of OB-R via the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway [5]. 4:4 stoichiometry lays down as a condition for leptin signal transduction, with respect to last study, where 4 molecules of leptin create a compound with 2 pre-formed leptin receptors dimers [45].

Not contributing leptin receptor versions can be created by the mutations in leptin receptor gene. The broken down signal transduction of leptin leads to induce morbid obesity, moreover, delayed onset of puberty, growth retardation, a hypogonadism and hyperthyroidism are also being relevant with impairments in leptin signaling [46]. The significance of the functional leptin-receptor axis as a regulator of endocrine and hypothalamic regulatory circuits has been shown by this.

The primary leptin binding protein is sOB-R [6]. Therefore, sOB-R can modulate bioavailability and, as a result, the cellular impacts of peptide hormone leptin [47]. The existence of a soluble receptor form [48, 49], additionally to these four membraneanchored receptors, was indicated in peripheral blood that shows the key leptin-binding activity [6] also shows the membrane OB-R density [7].

2.3. Leptin Resistance

So far, the pathomechanism of leptin resistance not exactly come out. Like the insulin resistance, higher numbers of leptin only provide decreased biological effects of ligand via membrane-anchored receptor isoforms. Differences in food uptake and energy consumption, leptin resistance [5] pathophysiologically portrayed by decreased leptin effects. Obese people with no defined mutation of the leptin receptor, can suffer from this. Illogically, leptin resistance is unrelated with decreased leptin synthesis, cause the overweight and also obese people has higher amount of concentration [41, 42]. Besides, animal studies prove that the suppressor of cytokine signaling 3 (SOCS3) and receptor-associated protein tyrosine phosphatase 1B (PTP1B) may be related. Higher concentrations of these proteins were seen in diet-induced obese mice [50-56], and these proteins are accepted to end the activation of the OB-R.

Moreover, a troubled endoplasmic reticulum (ER) homeostasis because of disturbed transport of leptin through the blood-brain barrier and increased ER stress is very critical for the improvement of leptin resistance [57-62]. Besides, the sOB-R is importantly lengthened in normal weight individuals comparison with obese individuals [41, 63].

Serum sOB-R concentration is suggested to mirror the amount of membraneanchored receptors [7], the spread of its shortness might be a gage for the residuary leptin sensitivity.

Inversely to the positive energy balance; states of energy deficiencies (i.e. during starvation and anorexia nervosa or) are defined by meaningfully reduced serum sOB-R concentrations [41, 64, 65], recommended a recovery of the decreased leptin action by an higher OB-R density. Conversely, tissue culture researches and animal studies show an inhibitive impact of clearly higher sOB-R amounts on leptin signal transduction. So individuals who are underweight and normal weight both are significantly affected by the leptin resistance, which is likely to be directed by the sOB-R. As a result, the sOB-R is directly included to the case of the formation of the leptin-resistant conditions.

Moreover, estimating the serum sOB-R might be a helpful scale to evaluate the extent of a present leptin resistance. The sOB-R is the primary binding protein for leptin [6], so it is able to pivotally modifying its bioavailability. Normally, membrane-anchored receptors soluble form, yet the latter seems to overpower, can present both antagonistic and agonistic properties. Soluble IL-6 receptor (sIL-6R) is the leading agent of these agonistic receptors. While binding the sIL-6R, IL-6 may interplay with the gp130, which is signal-transducing subunit of IL-6R membrane combined. Interplay of these receptors eventually cause the activation of intracellular signaling pathways, and is called trans-signaling [66].

In the literature antagonistic effects of soluble receptor isoforms are told much more often. By binding its ligand, membrane-anchored receptor activation broken by the soluble form of the receptor for advanced glycation endproducts [67]. Division of its extracellular domain cause to downregulation of the membrane-anchored receptor for the transferrin receptor [68].

On the contrary the way sOB-R treat on leptin, antagonistic or agonistic is continuing. Co-incubation researches carried out in tissue culture. to interpret the impact of higher amounts of sOB-R on leptin signaling, these researches showed that sOB-R amounts of two-folds molar excess over leptin have a mainly restrictive impact on leptin signal transduction [69].

A restrictive impact of the sOB-R on the leptin action has shown in the literature. For example High sOB-R amounts, neutralizes the activator of transcription 3 (STAT3) activation and leptin-mediated signal transducer, and for this reason the anorexigenic impact of leptin in rats [70].

Furthermore, auxiliary findings revealed that sOB-R reducing leptin sensitivity, by restricting the transport of the leptin throughout the blood-brain barrier, [71, 72]. A different research in mice showed, increased levels of leptin and the sOB-R were related to mouse endotoxaemia and the cecal ligation puncture models of the sepsis. Even though applied externally, leptin-increased mortality in the endotoxaemia cecal ligation puncture models of the sepsis, the direction of sOB-R probably as a recovery anti-inflammatory mechanism, leads to advantages of survival.

These results might be translational attention oncoming, as the obesity is related with higher sepsis-induced mortality and morbidity [39]. Normally, higher sOB-R amounts may be based on under conditions of energy shortage like eating disorders such as bulimia and anorexia or the apparition of type-I DM [64, 65, 73-75]. sOB-R eventually induces energy intake and food by decreasing leptin functions. In the first ages of life after birth, this mechanism is significant, as this ages is followed by a need for enough energy supply and speed up growth. With later periods, it's also related with clearly increased sOB-R amounts. Together with age sOB-R amounts constantly reduces to the point of puberty start [76, 77], in order to the negative impact of leptin on onset of puberty are not broken by an excess of the sOB-R to leptin. Lastly, negative impacts of the sOB-R also significant in bone metabolism. The extra sOB-R in mouse could backward the leptin-mediated decrease in the bone mass [78, 79]. Lesser endogenous amount of leptin which is mostly seen in individuals who have lipodystrophy, described by higher bone density, bone age and mass [78-80] compared to the normal patients, is demonstrate parallel affect.

Overexpression of the sOB-R, are the counter finding in studies with mice. Investigations showed that animals, defined by two-fold increased serum sOB-R amounts, found to have higher energy expenditure and reduced body weight compared to wild-types [81]. Resultantly, it was recommended that mouse with the overexpression of the sOB-R has increased leptin sensitivity, due to the higher serum sOB-R concentrations. Different study revealed without determining negative impact on leptin sensitivity, that liver-specific insulin receptor found seriously higher sOB-R amounts in the blackout animals compared to the wild-type mouse [82]. Investigators come up with the idea of the higher concentrations of sOB-R might slow down renal clearance and leptin disruption, leading to preserve or heal leptin sensitivity [1].

2.4. Free Leptin Index

The fraction of the total leptin concentrations to the sOB-R gives us the FLI, which we profit by a status of leptin action and a biomarker of leptin resistance. In obesity individuals, leptin circulates mainly in free form because of the small concentrations of sOB-R whereas in lean subjects, it circulates mainly in bound form [83].

FLI is related with metabolic abnormalities and could be much strong predictive of type-II DM than other biomarkers [84]. So, describing the FLI as the relations between leptin levels and sOB-R multiplied by 100 is gaining diagnostic worth [70, 85, 86]. Sadly, FLI's reference range not specifically determined so it restricts broader usage.

2.5. Micro RNAs

miRNAs act a part in post-transcriptional gene regulation, average are 22 nucleotide long, single stranded, and small non-coding RNA molecules. miRNAs has a role in RNA Induced Silencing Complex (RISC), and binds to 3' untranslated region (3'UTR) of messenger RNA (mRNA) consequently translational arrest and/or mRNA degradation [87].

Studies on miRNA expression in different tissues have shown that miRNAs also exists in body fluids. Also it is shown that transport of miRNAs in circulation occurs by serum proteins, lipoproteins, exosomes or by free [88]8. Besides differing expression profiles of miRNAs by the developing pathologies in different tissues and organs, changes in serum or exosomal miRNA profiles creates the idea of using miRNAs as diagnostic and/or prognostic biomarkers [88-90]. Onward they are much specific and sensitive for early diagnosis, risk assessment and monitoring disease progression, miRNAs seems to be helpful for pre-clinical diagnosis [24].

miRNAs shows significant stability under hard circumstances like: temperature, power of hydrogen (pH), multiple freeze-thaw cycles and storage[91, 92].

miRNAs works in different pathways like metabolism [93-95]. Abnormal process of miRNAs causes irregular metabolic work and is confused in the development of type-II DM, metabolic syndrome, CVD [93].

Various researches reveal that a statistically significant variance happens between people with metabolic disease and the control groups. miRNAs: 27a-3p, 197-3p, 320a, 221-3p, 130a-3p and 23-3p shows changed levels correlating with pathophysiological components of metabolic syndrome [91, 92, 94, 96-100]. Levels of, miR-27a-3p, miR-130a-3p, miR-320a, miR-221 and miR-23-a changes in the circulation of patients with metabolic syndrome [94, 99]. Researches clarified that levels of miR-197, -221, -23-a and -130a-3p comply with the states of obesity [94, 99, 101].

2.6. Relationship between miRNA and Coronary Artery Disease

miRNAs organize various events bearing the care of cardiac function and evolution of the heart in cardiovascular biology. Irregular miRNAs connected with different cardiovascular pathologies [102-104].

miRNAs role have bring to light potential therapeutic and diagnostic targets and have maintained new aspect on disease mechanisms [105]. Specific miRNAs have been seen to be quantitatively changed in CVDs including fibrosis, atrial fibrillation, heart failure, cardiac hypertrophy, and CAD [104]. Researches indicated that cardiac-specific miRNAs play a role in CAD pathogenesis, [106] with some indicating dissimilarities in miRNA expression between stable ACS and CAD [107].

2.7. miRNA221

MiRNA221 located on the chromosome of the p11.3 arm of the 23 nucleotides of the non-encoded RNA. They were found to suppress especially in the translational phase. 528 contain totally 184 protected areas. It has been shown that there are 496 transcripts of conserved regions (Figure 2.7-1) [108, 109].

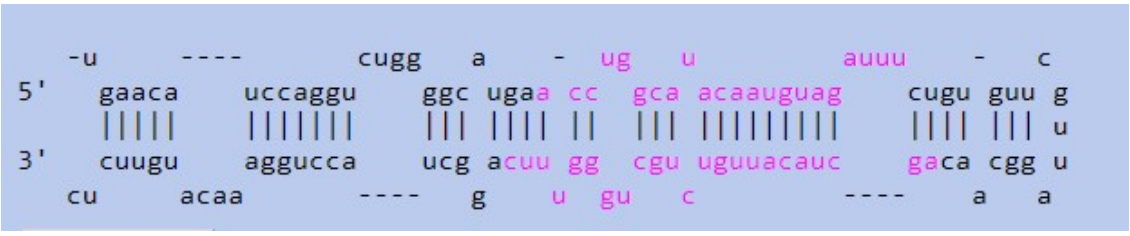

Figure 2.7-1 Steem loop and sequence of miRNA-221 [109]

The target genes of miRNA221 and miRNA222 are, respectively, Cyclin Dependent Kinase Inhibitor 1B (p27, Kip1), Transcription factor 12, Pantothenate kinase 3, gamma-aminobutyric acid (GABA), KIT gene, MIS 12 Kinetecor Komplkes Komponen, Transcription Initiator Protein 5A2, Acyl-CoA dehydrogenase, A Receptor Alpha 1, Chaperone-containing TCP1, Subunit 5 (epsilon), mitochondrial ribosomal Protein S7, Tubulin, alpha 1a, Annexin A3, C-4 to C-12 straight chain, Eukaryotic Translating Initiating Factor 3, Subunit J is protein associated protein 2, which binds to Poly (A), Regulatory Factor X (Figure 2.7-2) [110, 111].

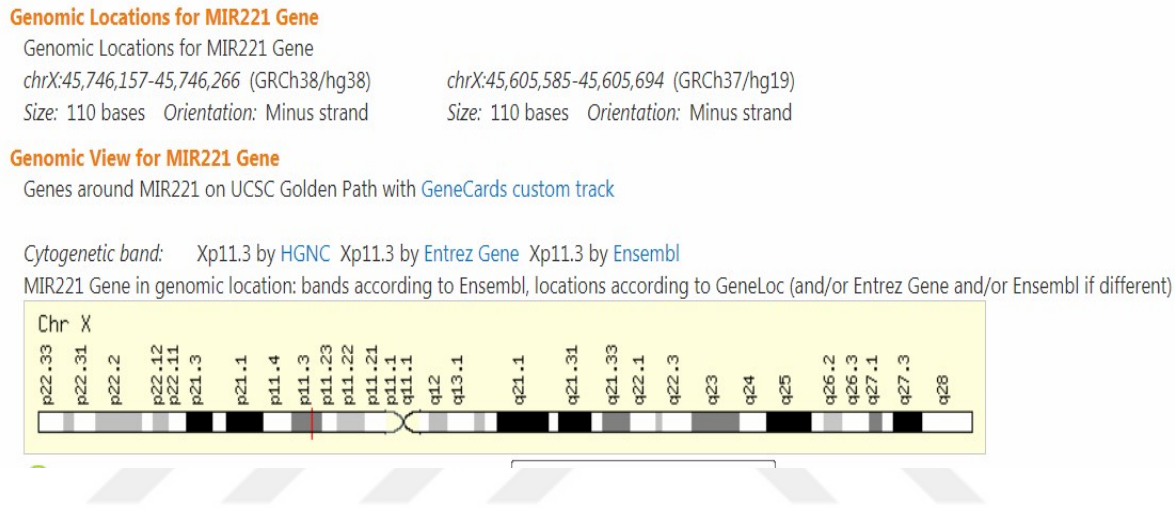

Figure 2.7-2 Genomic view of miRNA-221[111]

miR-222 and miR-221, between the highly expressed miRNAs in Human Cells of Umbilical Vein Endothelial shows anti-angiogenic effects (HUVEC) [10]. So, transfection of the endothelial cells with miR-222 and miR-221 restricts healing of the endothelium, migration, tube formation in vitro [10]. Everlastingly, a study revealed the anti-angiogenic properties of miR-222/221 on endothelium [11]. The underlying mechanism proposes a posttranscriptional adjustment and includes the downregulation of the protein expression of foretell target c-kit, which is the receptor for stem cell factor, without affecting mRNA levels [10]. The miR-222/221 declines c-kit expression and as a result cell proliferation occurs, in the cells of hematopoietic progenitor [108]. C-kit is prognosticating miR-222 and miR-221 can be related with cardiac stem cell differentiation or function, as it is an crucial marker [112, 113] But, miR-222 and miR-22's act for cardiac differentiation or function still couldn't understood.

miR-222 and miR-221 indirectly declines the expression of the eNOS in Dicer siRNA-transfected cells when its appears too much [11].

Nitric oxide (NO)'s broken bioavailability landmark for individuals with ischemic cardiomyopathy and atherosclerosis not just a main regulator for migration, endothelial cell vascular remodeling, growth and angiogenesis [114-117]. Newly it's shown that eNOS also have a critical role for the functional activity and mobilization of progenitor and stem cells [118-120]. Because of this, miRNAs targeting eNOS may not just arrange angiogenesis, could be also included in vasculogenesis (Figure 2.7-3).

Consumption of miR-222 and miR-221 changed miRNA signature of the HUVEC51, shows miRNAs check on coming out of other miRNAs. 23 miRNAs were downregulated and 9 miRNAs were upregulated as a result of miR-222/ 221 consumption. 1/3 of the regulated miRNAs, supringly, are expected to target c-kit 30 UTR, recommending a link between the miRNAs that interest parallel duty. [121].

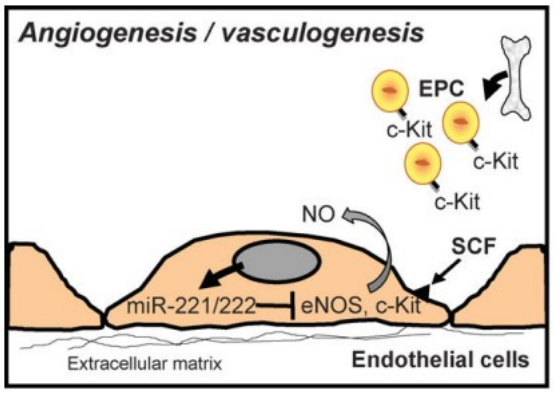

Figure 2.7-3 Role of miRNA-221 for vascular biology [121]

2.8. Relationship between miRNA-221 and Leptin

Table 2.8-1 The summary of miRNA-221 and metabolic relationship in literature [91]

Circulating miRNAs in human cardiometabolic disease				
miR-221, -130b, -423-5p	↓ Plasma	Obesity	Human	—
miR-221, let-7g	↑ Plasma	Metabolic syndrome, females	Human (♀)	—
Skeletal muscle: miRNAs in peripheral IR				
miR-221	↑	Genetic IR	Mouse (*ob/ob*)	AdipoR1/Adiponectin and PTB pathway
miRNAs involved in hepatic glucose homeostasis				
miR-221	↓ AdipoR1	Overexpressed miRNA	HumanHepG2 cells *in vitro*	AdipoR1/Adiponectin and PTB pathway

The miR-221 in humans and mice contains two associate, miR-222 and miR-221, both related with metabolic disease and existed in close intimacy on the X chromosome [91]. miR-221 with impact on adiponectin expression can arrange insulin resistance [91]. miR-221 imitates suppressed adiponectin expression, in human adipocytes which is cultured [91]. Newly, its found that miR-221 was electively higher in the livers of ob/ob mouse, but not in the muscle, spite of miR-221 suppressed adiponectin receptor 1 in muscle cells and liver in vitro by Lustig et al[122] Human adipocytes miR-221 concentrations (in vitro) of were unfavorably connected with body mass index of donors and tumor necrosis factor alpha mRNA concentrations [123].

Inversely, miR-221 expression of the subcutaneous adipose tissue was favorably connected with the body mass index in nondiabetic Pima Indians [14]. While a study [96] revealed higher miR-221 in the non-obese women who have metabolic syndrome, Ortega et al. [124] reveals lower plasma miR-221 concentration in obese patients. The inequality in these results might be because of the undefined variables in the cohorts or methodological differences. MiR-221 might potentially improve the IR by suppressing the adiponectin signaling and act as a plasma marker although the conflicting human findings, [91].

The studies reveled that adipose miR-221 expression is positively connected with high BMI and also let us know that adipose miR-221 is upregulator in obesity [12, 125] So far, the upregulation processes are unknown[14]. Tumor necrosis factor-α (TNF) and leptin, showed the effect of downregulation for miR-221 and they are known to be higher level in the obese state. It recommends a complicated mechanism in vivo, could be desensitization to impacts of TNF-α and leptin in the chronically obese state [14, 126-129].

Varied researches have revealed a disregulation of miRNAs expression when there was an inflammation in adipose tissue. The profile of the expression of miRNAs matches with the adipocytes from a leptin deficient ob/ob mouse and the TNF-α-treated adipocyte 3T3-L1 cell line [13] Leptin or TNF-α induce a lower miR-221 and an higher of miR-335 in human preadipocyte [130, 131]. TNF-α causes to a rise in the expression of miR-146a, miR-130, miR-150, miR-146b, miR-222, miR-221 and fall of miR-143 and miR-103 concentration in the murine adipocyte model [13, 132-134].

3.MATERIALS AND METHODS

3.1. Description of Patient and Control Groups

All peripheral blood samples and sera of study and controls were collected at Department of Cardiology of Yeditepe University. Inclusion criteria for study and controls were;

- Being over 18 years old,
- not pregnant,
- not bypassed before,
- have an indication for coronary angiography except acute coronary syndrome

78 patients with control group were included. The baseline and demographic findings that included in the study were demographics (age, gender), risk factors (hypertension, DM, family history of cardiac disease, hyperlipidemia, smoking). Patients were regarded as hypertensive if they were already on anti-hypertensive therapy. Smoking was defined as the inhaled use of cigars, pipes or cigarettes in any quantity. Diagnostic criteria of World Health Organization (WHO) was used for defining DM [135].

The participants were examined physically, and divided into two groups as patients and controls after coronary angiographic investigations.

Patient's group is described whose coronary lesion needs invasive treatment depended on stenosis of ≥70%. Since stenosis of the left main stem (stenosis ≥50%) this condition was evaluated as well. Control group was described who had normal coronary artery.

Coronary angiograms also were evaluated by quantification of the lesions according to the SYNTAX score, as described elsewhere. The SYNTAX score is a unique tool to score complexity of CAD. The SYNTAX scores were derived from the sum of the individual scores for every lesion that is defined as ≥50% luminal obstruction in vessels ≥1.5 mm. A dedicated software was used to calculate the SYNTAX score, for all patients (available at http://www.syntaxscore.com/calc/start.htm). Later, the SYNTAX score was divided into three groups; low (≤16), intermediate (16-22), and high (>22).

3.2. Sample Collection

After give decision to perform coronary angiography, gel-containing tubes were used for collecting 10 ml of peripheral blood for serum separation for ELISA analysis and miRNA isolation. Ob-R and leptin levels in serum samples were determined by an enzyme immunoassay method, a two-site sandwich ELISA to quantitate LR/Ob-R and Leptin in samples. Ethylene diamine tetra acetic acid (EDTA) containing tubes were stored at +4 °C for deoxyribonucleic acid (DNA) isolation.

Gel-containing tubes were rested 15 minutes for coagulation by gently turning around a few times in every 5 minutes. After coagulation period, tubes were centrifuged in 4500 rpm for 20 minutes, supernatant aliquot in eppendorf tubes, and stored in -80 °C. Blood samples collected within the scope of the Declaration of Helsinki and the study protocol was approved by the Yeditepe University Ethics Committee (no: 07.05.2020/1217)

3.3. Chemicals, Consumables and Instruments

3.3.1 Chemicals and Consumables

NanoDrop2000 (Thermoscientific, Waltham, Massachusetts, USA), Trizol (Quiazol, Qiagen), Chloroform (Sigma, Aldrich) %100 Ethanol (Sigma Aldrich), miRCURYLNA RT Kit (Qiagen, Exiqon, Hilden, Germany), miRCURY™ RNA Isolation Kit (Qiagen, Exiqon, Hilden, Germany), Qubit miRNA Assay Kit's standard protocol on the Qubit 3.0 Fluorometer (Thermoscientific, Waltham, Massachusetts, USA), Leptin receptor (LR/Ob-R) ELISA Kit (Abbkine Scientific, California/ USA) and Leptin ELISA Kit (LDN, Nordhorn/ Germany), 96 well plate (Applied Biosystems®, Thermo-Fisher Scientific Incorporated), 96 well plate optical seal (Applied Biosystems®, Thermo-Fisher Scientific Incorporated) PCR grade water (Invitrogen, Thermo-Fisher Scientific Incorporated).

3.3.2. Instruments

iPrep Pure Link Nucleic Acid Isolation Instrument (Invitrogen®, Thermo-Fisher Scientific Incorporated), ABI 7500 Fast Real Time Real Time PCR Instrument (Applied Biosystems®, Thermo-Fisher Scientific Inc.), T100 Thermalcycler (BioRad) Qubit 3.0 Flourometer (Applied Biosystems®, Thermo-Fisher Scientific Inc.), Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R, Beckman Coulter), Microplate Reader (WHYM201, Poweam Medical Co.), Microplate Autowasher (WHYM200, Poweam Medical Co.), NanoDrop 2000 Spectrophotometer (Thermo-Fisher Scientific Incorporated), +4 °C Fridge (Haier), −20 °C Deepfreeze (Haier), −80 0 C Ultra Deepfreeze (Haier) Micropipette Set (Denville Scientific), Ultra Pure Distilled Water Instrument (Purelab option Q, Elga)

3.4.1 miRNA Isolation

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

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

The miRCURY™ RNA isolation kit includes optimized protocols for a variety of sample types. These optimized protocols were consisting of four steps:

- Protein precipitation and Lysis
- Addition of the ethanol / isopropanol and loading to column
- Washing the column
- Washing the solution elution of the purified RNA from column

3.4.2 cDNA Synthesis

The isolated miRNA samples exposed to reverse transcription process from to first-strand cDNA synthesis reactions were performed by using the miRCURYLNA RT Kit (Exiqon, Qiagen, Hilden, Germany). The each template RNA sample diluted to 5 ng/µl using nuclease-free water. Then the samples prepared for the reverse transcription reactions on ice according to instructions shown in Table 3.4.2-1.

Table 3.4.2-1 cDNA mixture for PCR Reaction

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

After cDNA mixture for PCR Reaction prepared samples incubated due to manufacturer's instructions are shown in Table 3.4.2-2.

Table 3.4.2-2 Reverse transcription reaction temperature cycling protocol.

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

3.5.1 Measurement of miRNA Purity

The purity of the miRNA samples was measured with the NanoDrop 2000 instrument (Thermoscientific, Waltham, Massachusetts, USA).

The purity of miRNA samples was determined by the OD260 / OD280 ratio. The Sample's optical density range greater than 2 were accepted as pure between OD260/OD280 ratios.

3.5.2. Determination of miRNA Levels by Fluorometer

miRNA levels were determined flurometrically. miRNA concentration of transcripted samples determined and equalized by Qubit miRNA Assay Kit's standard protocol on the Qubit 3.0 Fluorometer (Thermoscientific, Waltham, Massachusetts, USA). In the first step, 200 µl working solution was prepared by mixing 199 mil miRNA Buffer and 1 mil miRNA Reagent (Qubit miRNA Assay, Invitrogen, Thermo Fisher Scientific Inc.). 10µl standard is added to 190µl working solution on standard 1 and standard 1 and 10µl is added to 190µl working solution on standard 2, standard number 2 is prepared and standards are introduced to the device in turn. For each sample measurement, 2µl sample was added to 198µl working solution and 200 mixture was obtained in total and miRNA levels were determined by reading in the device [136].

3.5.3 The miRNA Expression Levels Analyzed by Real-Time PCR

The target miRNA selected for this study were determined by using the "mirbase" and "targetscan" databases [111, 137]. miRNA and it targets were analyzed using these databases regarding molecular mechanisms in glioblastoma. miRNA221 was chosen as the target miRNA for this thesis. Primer sequence of the miRNA221 can be seen in Table 3.5.3-1 and reaction mix setup for PCR miRNA expression analysis was shown in Table 3.5.3-2.

Table 3.5.3-1. miRNA Primer Sequence

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

Table 3.5.3-2. Reaction mix setup for PCR miRNA Expression Analysis

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

As shown in Figure 3.5.3-1 two-part protocol of the miRCURY LNA™ Universal RT miRNA PCR performed as:

1. First-strand cDNA synthesis
2. Real-time PCR amplification

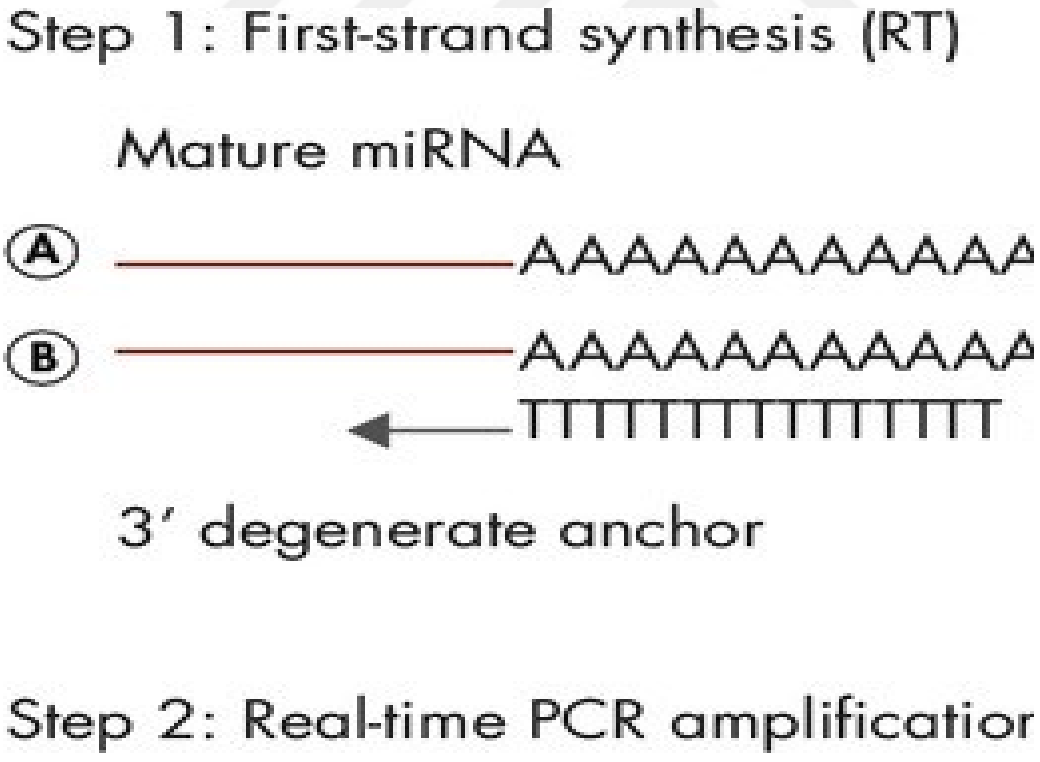

Figure 3.5.3-1 Schematic outline of miRCURY LNA miRNA PCR System

Comprehension of miRNA expression levels has been realized by delta CT (ΔCT), miRNA221 was chosen as the target miRNAs for thesis. Comprehension of miRNA expression levels has been determined by using Ct and delta ΔCT methods. The internal control (housekeeping assay, RNU6) has been used for calculation of ΔCT miRNA expression level determinations were accomplished using the ΔCT equations [138].

3.6. Measurement of Serum Leptin, Soluble Leptin Receptor

sOB-R levels were evaluated with ELISA method by using Abbkine Scientific (California/ USA) ELISA kits. Leptin levels were calculated with ELISA Kit Labor Diagnostica Nord (LDN, Nordhorn/Germany). All two kits are depending Sandwich ELISA principle, which is working with two antibodies (one for establishing the molecule on bottom of the well and one for enzymatic marking) binding from different epitopes on the target molecule. Reaction well plates are precoated with stabilization antibodies (specific for target molecule) and after stabilization marking antibodies are applied on target molecules in reaction wells.

Serum samples which are stored in -80 °C were thawed in room temperature and ELISA kits were heated up to room temperature, stock solutions (Wash Buffer, Sample Diluent, Assay Buffer, Standard Solutions, Leptin Antibody and Streptavidin-horseradish peroxidase (HRP) Conjugate) diluted and serum samples diluted ½ with Sample Diluent as 50µl Serum 50µl Sample Diluent with final volume of 100 µl before procedure. 100 µl of samples and standards added in wells which are precoated with and antibody for our target molecule, sealed with an adhesive film, and incubated in the room temperature for two hours on a microplate shaker in medium speed for the immobilization of our target molecule. After incubation, liquid in wells aspirated and all wells washed with 250 µl of wash buffer twice, after second wash buffer aspirated and microplate tapped gently on absorbent paper for avoiding any wash buffer remains. After washing, 100 µl of Leptin Antibody added and wells sealed with an adhesive film and incubated in the room temperature for one hour on a microplate shaker in medium speed. After incubation, liquid in wells aspirated and all wells washed with 250 µl of wash buffer twice, after second wash buffer aspirated and microplate tapped gently on absorbent paper for avoiding any wash buffer remains.

After washing, 100 µl of HRP 17 Substrate solution added in wells, sealed with an adhesive film, wrapped with tin foil for protecting from the light and incubated in room temperature for 15 minutes on microplate shaker in medium speed. After incubation, 50µl Stop solution added in wells, gently shacked with hand to mix the solution and read in microplate reader at 450 nm wavelength. Concentration calculations have done by standard curve method depending on absorbance of the standards.

3.7. Statistical Analysis

All results were evaluated with Statistical Package for the Social Sciences (SPSS) statistical analyze program. Pearson and Spearman correlation test were used for correlations. Differences between the study and controls were evaluated with student t-test or Mann-Whitney-U test. Relationships between variables were determined with Chi-square and Fischer Exact tests.

4. RESULTS

4.1. Demographic Results of Working Groups

We evaluated a total of 40 patients that 23 were male (57,5%) and 17 were female (42,5%). Control group included 38 individuals, of whom 17 were male (44,7%) and 21 were female (55,3%). Demographics of patients and controls were shown in Table 4.1-1 Hypertension, obesity is statistically high in CAD group and family history of CAD is significaly low in CAD patients. There was no significant difference between for other demographics.

Table 4.1-1 Demographics of patients and control group

		Non- Coronary Artery Disease (n=38)	Coronary Artery Disease (n=40)	p value
Age, years (mean±SD)		57,82±9,52	60,88±9,76	**P=0,17**
Gender	**Female**	%55,3	%42,5	x^2:1,271[a]
	Male	%44,7	%57,5	**p=0,260**
Height (cm) (mean±SD)		166,26±10,45	167,20±9,49	**P=0,68**
Weight (kg) (mean±SD)		81,11±13,42	83,75±13,32	**P=0,39**
BMI, kg/m² (mean±SD)		29,45±5,49	30,14±4,10	**P=0,53**
Hypertension	**Yes**	%52,6	%75	x^2: 4,237[a]
	No	%47,4	%25	**p=0,040***
Diabetes	**Yes**	%39,5	%50	x^2:0,873[a]
	No	%60,5	%50	**p=0,350**
Obesity	**Yes**	%34,2	%57,5	x^2:4,253[a]
	No	%65,8	%42,5	**p=0,039***
Hyperlipidemia	**Yes**	%52,6	%57,5	x^2:0,187[a]
	No	%47,4	%42,5	**p=0,666**
Family history of CAD	**Yes**	%50	%25	x^2:5,215[a]
	No	%50	%75	**p:0,022***

CAD: Coronary Artery Disease, BMI: Body Mass Index, n: number of individuals, SD: Standard Deviation, Statistically significant $p<0.05$.

The difference between groups was analyzed by using advanced Chi-square test (X2) and double independent sample student t-test.

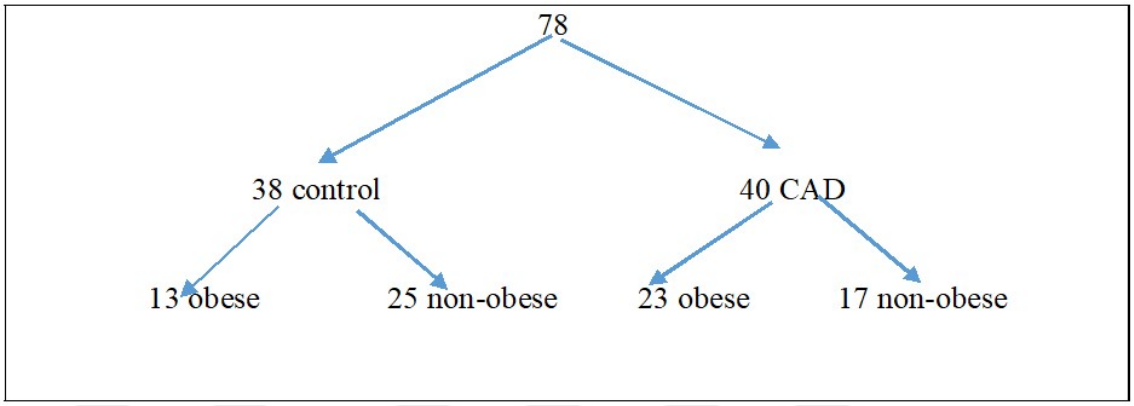

Figure 4.1-1 Distribution of samples

4.2. Blood tests

There was no significant difference between groups for the cholesterol levels. (Table 4.2-1)

Table 4.2-1 Cholesterol Levels

	Control (n= 38)	**Coronary Artery Disease (n= 40)**	**p value**
Total cholesterol (mean±SD) mg/dL	203,94±48,01	204,11±49,49	**P=0,99**
LDL-C (mean±SD) mg/dL	127,88±45,32	123,76±43,61	**P=0,70**
HDL-C (mean±SD) mg/dL	46,63±27,60	59,74±78,30	**P=0,35**
Triglyceride (mean±SD) mg/dL	175,62±79,34	174,71±93,96	**P=0,97**

SD: Standard Deviation, C: Cholesterol, n: number of individuals, significance level $p<0.05$.

4.3. Leptin, Soluble Leptin Receptor, FLI

4.3.1. Leptin, Soluble Leptin Receptor, FLI levels in CAD and Non-CAD groups

There was no statistically significant difference observed for leptin levels between groups (P=0,579). sOB-R levels were statistically high in patient group (p<0,001*). FLI was statistically low in patient group (p<0,001*).

Table 4.3.1-1 Leptin, Soluble Leptin Receptor, FLI levels in CAD and Non-CAD groups

	Non-CAD (n=38)	**Coronary Artery Disease (n=40)**	**p value**
Leptin (mean±SD) ng/ml	42,41±29,22	46,44±31,63	**p=0,579**
Soluble Leptin Receptor (sOB-R) (mean±SD) ng/ml	34,49±37,27	244,34±11,60	**p<0,001***
Soluble Leptin Receptor (sOB-R)/ **Leptin (FLI)**	1,74±1,29	0,19±0,13	**p<0,001***

SD: Standard Deviation, n: number of individuals, significance level p<0.05.

4.3.2. When re-administered patient and control groups as obese and non-obese groups,

No significant difference observed between two groups for sOB-R levels (p=0,227) and FLI (0,650). Leptin levels were statistically high in obese group (p=0,012).

Table 4.3.2-1 Leptin, Soluble Leptin Receptor, FLI levels in Obese and Non-Obese groups

	Obese (n=36)	**Non-Obese (n=42)**	**p value**
Leptin (mean±SD) ng/ml	53,97±29,21	36,20±29,32	**p=0,012***
Soluble Leptin Receptor (sOB-R) (mean±SD) ng/ml	164,58±106,34	133,47±109,82	**p=0,227**
Soluble Leptin Receptor (sOB-R)/ **Leptin (FLI)**	0,97±1,23	0,84±1,12	**p=0,650**

SD: Standard Deviation, n: number of individuals, significance level p<0.05.

4.3.3. When we divide the non-obese group into those with and without coronary artery disease,

There was no statistically significant difference in leptin levels between (P=0,709). sOB-R levels were statistically high in CAD group (p=0,027). FLI was statistically low in patient group (p=0,0001).

Table 4.3.3-1 Leptin, Soluble Leptin Receptor, FLI levels only in Non-Obese group when divided into CAD and Non-CAD groups,

NON-OBESE GROUP	**CAD (n=17)**	**Non-CAD (n=25)**	**p value**
Leptin (mean±SD) ng/ml	36,1375±29,86008	32,2639±29,75672	**p=0,709**
Soluble Leptin Receptor (sOB-R) (mean±SD) ng/ml	247,8620±11,39507	50,8935±74,75217	**p=0,027**
Soluble Leptin Receptor (sOB-R)/ **Leptin (FLI)**	0,1473 ±0,12485	1,1849 ±1,25445	**p<0,001***

SD: Standard Deviation, n: number of individuals, significance level p<0.05.

4.3.4. When we divide the obese group into two groups as CAD and non-CAD,

There was no statistically significant difference in leptin levels between two groups (p=0,410). sOB-R levels was statistically high in patient group (p=0,005). FLI was statistically low in patient group (p<0,001*).

Table 4.3.4-1 Leptin, Soluble Leptin Receptor, FLI levels only in Obese group when divided into CAD and Non-CAD groups

OBESE	**CAD (n=23)**	**Non-CAD (n=13)**	**p value**
Leptin (mean±SD) ng/ml	52,0024±32,65419	49,0495±29,23009	**p=0,410**
Soluble Leptin Receptor (sOB-R) (mean±SD) ng/ml	241,1701±11,20950	23,9832±6,56328	**p=0,005**
Soluble Leptin Receptor (sOB-R)/ **Leptin (FLI)**	0,2154±0,13514	2,1527 ±1,29500	**p<0,001***

SD: Standard Deviation, n: number of individuals, significance level p<0.05.

4.3.5. When we divide the non-CAD group into two groups as obese and non-obese,

Leptin levels were high in obese group, however it was not statistically significant (p=0,106). sOB-R levels were low in obese group, however it was not statistically significant (p=0,206). FLI was statistically low in non-obese group (p=0,032).

Table 4.3.5-1 Leptin, Soluble Leptin Receptor, FLI levels only in Non-CAD group when divided into Obese and Non-Obese groups

NON-CAD	**Obese (n=13)**	**Non-Obese (n= 25)**	**p value**
Leptin (mean±SD) ng/ml	49,0495±29,23009	32,2639±29,75672	**p=0,106**
Soluble Leptin Receptor (sOB-R) (mean±SD) ng/ml	23,9832±6,56328	50,8935±74,75217	**p=0,206**
Soluble Leptin Receptor (sOB-R)/ **Leptin (FLI)**	2,1527±1,29500	1,1849 ±1,25445	**p=0,032**

SD: Standard Deviation, n: number of individuals, significance level $p<0.05$.

4.3.6. Examining the relationship between SYNTAX scoring and leptin, soluble leptin receptor and FLI,

Pearson Correlation Analysis showed that is no correlation between SYNTAX Score and leptin. There was statistically significant positive correlation between SYNTAX Score and sOB-R (p<0,001*), negative correlation between SYNTAX Score and FLI (p<0,001*).

Table 4.3.6-1 Correlation analysis of Leptin, Soluble Leptin Receptor, FLI levels with SYNTAX score

	SYNTAX Score in allover study group	
CORRELATION PARAMETER	r^2	P value
LEPTIN	0,207	0,069
LEPTIN RECEPTOR	0,663	**<0,001***
FLI	-0,404	**<0,001***

Pearson Correlation Analysis was used for to show correlation between SYNTAX Score and serum levels of Leptin, sOB-R and FLI. The Values Marked with * are showing statistically significance.

4.4.1. microRNA Result

ΔCT values were calculated for analyzing miRNA221expression levels and these values are shown in Table 4.4.1-1. The levels of miRNA221 expression were determined by comparing the ΔCT values of miRNAs and the internal control (RNU6). When the expression levels of miRNA221 was compared according to the ΔCT factor, it was determined that the mean expression value of the patient group was 3,0313 ± 2,83618 while control group had 1 ± 0,94704, there was statistically significant difference between the groups (p:0,024) (Table 4.4.1-1). As it shown in the Figure 4.4.1-1 the expression levels of both miRNA221 were found to be significantly increased in the patient group than the control group.

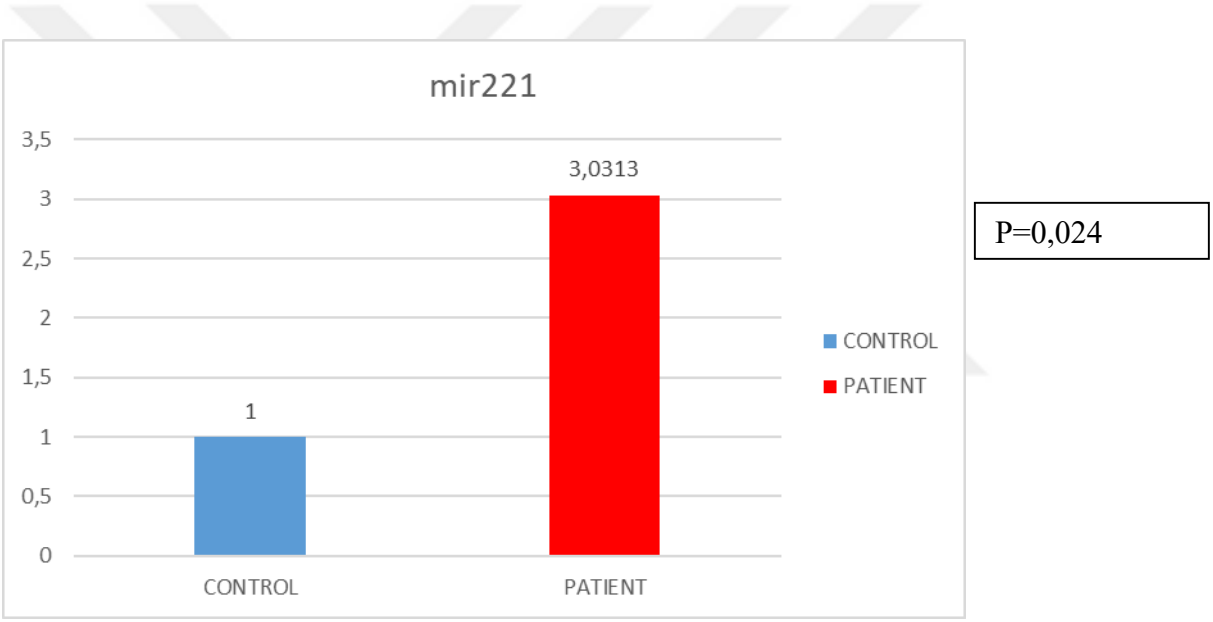

Figure 4.4.1-1 Comparison of miRNA221 expression levels between patient and control groups according to mean ΔCT value.

miRNA 221 expression level was 3 fold significantly higher in patient grup (P=0,024). miRNA 221 expression level was upregulated.

Table 4.4.1-1 Comparison of plasma miRNA221 expression levels as ΔC_T

MiRNA 221-3p	ΔC_T	P value
CAD group	3,0313 ± 2,83618	0,024
Control group	1 ± 0,94704	

X± SD (Mean ± Standard Deviation), statistically significance p<0,05.

Further, Pearson Correlation Analysis showed that was no correlation between miRNA-221 and leptin, miRNA-221 and FLI, miRNA-221 and syntax score. There was statistically significant correlation between miRNA-221 and sOB-R (p=0,029). (Table 4.4.1-2)

Table 4.4.1-2 Correlation analysis of Leptin, Soluble Leptin Receptor, FLI levels with miRNA -221 levels

	miRNA- 221 expression levels in allover study group	
CORRELATION PARAMETER	r^2	P value
LEPTIN	0,078	0,613
LEPTIN RECEPTOR	0,330	0,029*
FLI	-0,216	0,160
SYNTAX SCORE	0,120	0,437

Pearson Correlation Analysis was used for to show correlation between serum expression levels of miRNA-221 and serum levels of Leptin, Soluble Leptin Receptor and FLI. The Values Marked with * are showing statistically significance.

4.4.2. miRNA 221 ROC Analysis

ROC analysis was performed using the MedCalc Program to determine all plasma miRNA levels and the diagnostic value in the patient and control groups. As seen in Figure 4.4.2-1, it was determined that miRNA 221 expression levels could be evaluated as threshold values in patient groups (p=0.0290).

According to fold change values, the threshold value for miRNA221 was determined as >1.83, Area Under Curve (AUC)=0.681, sensitivity=60, specificity=92.9, and %95 Confidence Interval (CI) between 0.523-0.813.

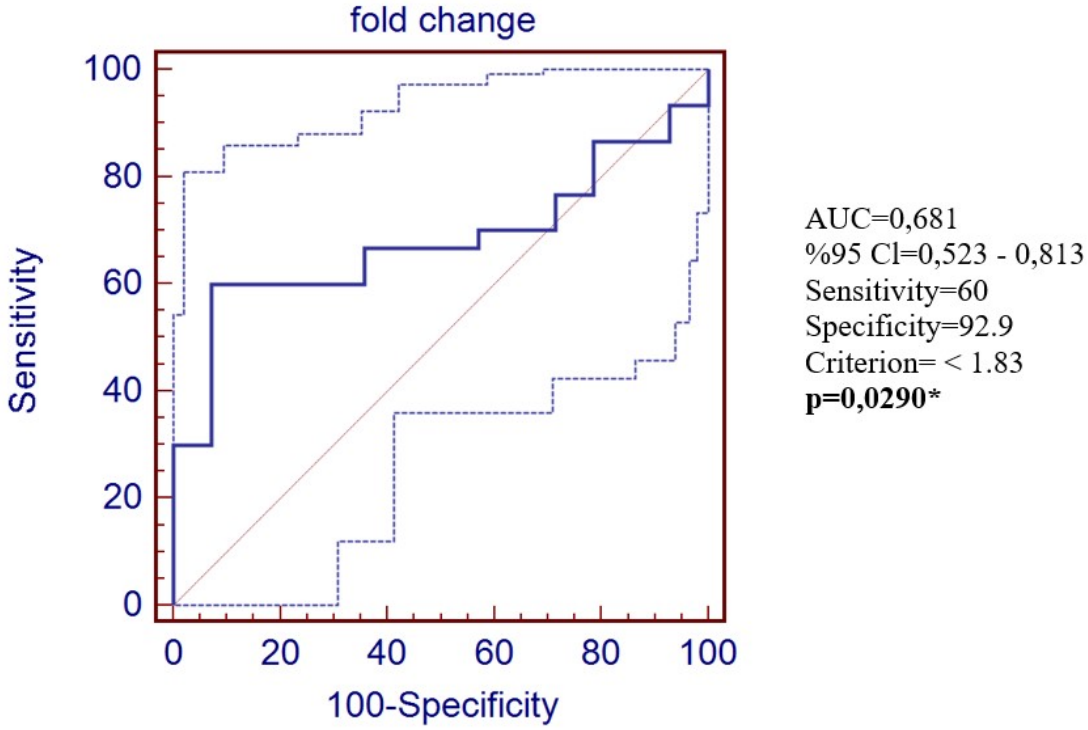

Figure 4.4.2-1. ROC analysis of miRNA221 expression in patients with CAD.

AUC: Area Under Curve, CI: Confidence Interval, * p<0.005.

5. DISCUSSION and CONCLUSION

The results of our study indicate that sOB-R levels were statistically higher and FLI results were statistically lower in patients who have CAD without acute coronary syndrome and need invasive treatment than the normal coronary artery group. In addition, leptin levels were not found significantly different between groups. As a result of the subgroup analyzes performed, the relationship between CAD and sOB-R levels and also with FLI results were independent from obesity. Also we used SYNTAX score for assessment severity of the CAD and we found there is statistically significant positive correlation between SYNTAX score and sOB-R and negative correlation between SYNTAX score and FLI. Beside these findings we found the expression levels miRNA-221 was found significantly 3 fold higher in the patient group than the controls. In addition, there was statistically significant correlation between miRNA-221 and sOB-R but no correlation with leptin, FLI and SYNTAX Score.

CAD is a major reason of mortality and morbidity, causes to 17.3 million deaths each year[139]. True pathogenesis of CAD still unknown. Dyslipidemia, smoking, being overweight, hypertension, obesity and DM are the risk factors [140]. One of the major risk factor for CAD is obesity. Leptin is good in decreasing appetite, arrangement of lipid metabolism and increasing the energy expenditure that all regulating obesity [141]. Serum or plasma leptin levels may show some physiological effects, like improve vascular smooth muscle cells growth, inducing the renin–angiotensin–aldosterone system, advance endothelial cell oxidative stress, induce formation of reactive oxygen species and causing arterial vascular wall injury [142, 143]. These features have an effect on evolution of CAD.

Leptin also has good impacts, like higher NO generation, taking part in the making of angiogenesis and enhancing coronary blood flow [144] [145]. According to the some studies, leptin have also different impacts, such as pro-thrombotic features in the cardiovascular system and obesity-related hypertension [146-148].

According to the possible angiogenic, thrombotic and atherogenic impacts of leptin, and being of its receptors in the endothelial cells, if the concentration becomes higher, it might cause restenosis after some vascular accidents [142, 149, 150].

Discussion for the impacts of leptin on CAD patients and some researches have revealed a moderate relationship between risk of CAD incident and level of plasma leptin [151] other have shown a positive impact for leptin [152, 153].

In the current study there was no statistically significant difference between CAD group and control group in terms of leptin levels.

A research revealed that leptin was related with higher atherogenic mechanisms[154]. Other research showed that serum levels of leptin were importantly increased in CAD individuals compared with healthy individuals also its connected to the extent of CAD, according to the including vessels [155]. Past results expose a negative impact of leptin in the pathogenesis of atherosclerosis and revealed that leptin was an independent risk factor for CAD [156]. Moreover the risk of CAD being higher with leptin than other risks factors such as race, sex, BMI and smoking [157]. Intercalary, a research also indicates that after excluding other risk factors, the odds ratio for higher disease intensity among people with increased leptin levels was protected[158].

A prospective cohort study in the USA found that leptin levels are not related with incident cardiovascular events [159].

A few researches have reported that this hormone may notice MI, independently of traditional risk factors [160], and it is a free predictive of MI in people with arterial hypertension [161]. Besides, leptin known for protective properties on the renal, cardiovascular and gastric systems in ischemia/reperfusion injury, thus indicating leptin as a definite deleterious hormone is not true[162].

Leptin is also a potential participant to cardiovascular risks related with obesity [162]. In current study, there was no statistically significant difference between two groups in terms of leptin levels but when we re-administered CAD and non-CAD groups as obese and non-obese groups, leptin levels are statistically high in obese group. Also when we divide the obese group into two groups as CAD and non-CAD, there was no statistically significant difference between two groups in terms of leptin levels. In summary leptin levels were related with obesity but were not related with CAD.

Though a research revealed that increased significant serum level of leptin among CAD patients than the other patients, but it's relation with CAD severity and Gensini Score was not significant [155].

In other study, leptin was negatively associated with SYNTAX score after adjustment for age, BMI, serum glucose and total cholesterol [163]. On the contrary, in another study, there was positive correlation between plasma leptin level and SYNTAX score [164].

In this study, the extent and severity of CAD was expressed as the anatomic SYNTAX score based on the coronary angiograms of recruited patients. Although it was originally developed to help decide the optimal revascularization strategy [27], since then the SYNTAX score was shown to have a prognostic value in predicting major cardiovascular and cerebrovascular events in patients undergoing PCI [165] and is still clinically relevant [166].

In our study, there was no correlation between SYNTAX Score and leptin. Variations between our results about connection between leptin and severity of CAD with other results is not understood but it might be related with a lower number of evaluated patients in the current study, the performance of coronary angiography and score estimation and its explication by interventional cardiologists.

The leptin-related damage to the cardiovascular system because of the leptin's inability to elicit its effects truly might be discussed due to the obese patients which are resistant to leptin [162] .

Leptin resistance's pathophysiological status is particularly characterized by lower leptin impacts on the adjustment of energy expenditure and food uptake [5] Obese people with no defined mutation of the leptin receptor, can suffer from this. Illogically, leptin resistance is unrelated with decreased leptin synthesis, cause the overweight and also obese people has higher amount of concentration [41, 42]. So the leptin resistance significantly affects individuals who are underweight and normal weight both, which is likely to be directed by the sOB-R. As a result, the sOB-R is directly included to the case of the formation of leptin-resistant conditions. Moreover, estimating the serum sOB-R may be a helpful scale to evaluate the extent of an present leptin resistance. [1].

The status of leptin action and leptin resistance has a biomarker called The FLI and it is the fraction of total leptin and sOB-R concentrations [8].

Base on that, in current study we evaluate the sOB-R and FLI, which are biomarkers of leptin resistance and the status of leptin action [8]. In current study, sOB-R levels were statistically high in CAD group and FLI was statistically low in CAD group. We re-administered CAD and non-CAD groups as obese and non-obese groups. Both non-obese and obese groups' sOB-R levels were statistically higher in CAD groups than non-CAD groups. For both groups' FLI findings were statistically low in CAD group than non- CAD group. Between obese and non-obese, there were no statistically significant difference in terms of sOB-R levels and FLI. It means, CAD is the reason for high sOB-R levels. Because of the high sOB-R levels, we found FLI lower in CAD than in non-CAD group, contrary to what is known about leptin resistance. And these findings are regardless of obesity. Examining the relationship between SYNTAX Scoring with sOB-R and FLI, Pearson Correlation Analysis showed there is a significantly positive correlation between SYNTAX Score and sOB-R, negative correlation between SYNTAX Score and FLI. These findings also showed that, sOB-R and FLI related with the severity of CAD. These findings might be important due to the lack of studies.

Mostly, both higher and lower levels of serum sOB-R seem to reflect the pathophysiological status of leptin resistance [1]. Leptin resistance recently stands for broken or unavailable leptin sensitivity spite of the higher level of endogenous leptin yet ignores alterations of serum sOB-R levels[1]. Leptin resistance might be limited to distinct organs like the hypothalamus systemic, skeletal muscle, liver or systemic[74, 75, 126]. Changes of sOB-R level have critical matter, if underweight and normal individuals affected by leptin resistance reversibly and temporarily. The higher the excess of sOB-R over leptin, the more likely leptin resistance emerges through inhibiting effects of sOB-R on the ligand. Clinical researches showed sOB-R levels up to a 100-fold molar excess over leptin for people onset of type-I DM and underweight people [64, 74, 75]. According to the results of another released study[7], it was requested an inverse u-shaped model for the evaluation of leptin sensitivity. Thus leptin and sOB-R assessment serve as diagnostic gauge for grading leptin sensitivity. Reduced sOB-R level could show an impaired leptin sensitivity and decreased OB-R membrane expression in obese individuals.

Higher levels of sOB-R followed by simultaneous reduction of leptin levels after a bariatric surgery, intervention programs or dietary change, can show healing leptin sensitivity. For underweight and normal children show increased sOB-R concentrations and reduced leptin, these measures parallely might develop clinical estimate for the course of puberty, as a functional leptin signal transduction like to be requested for its start [32, 76, 77]. The current literatures specify the significance of the sOB-R as predictor and modulator of leptin effects. Furthermore it gives a source for future exploration on the relationship between leptin resistance, leptin sensitivity and sOB-R [1].

Checking against the healthy control of age-matched subjects, sOB-R is importantly decreased and FLI and leptin hormone are importantly increased, according to the different data, in all polycystic ovary syndrome individuals [86]. Its revealed that abnormal expression of OB-R and leptin might be related with cancer growth and progression [167]. In a different research, high circulating levels of OB-R were shown in cancer patient and leptin concentrations were found reduced in cancer tissues [168].

In our study we did not study about leptin's tissue levels. We found sOB-R levels high and leptin levels nearly the same between groups. It may be different mechanisms going on for both cancer and CAD, which are independent from leptin resistance. Other option for high level of findings in our study is, OB-R has an inverse u-shaped model for the evaluation of leptin sensitivity. More researches should be made on leptin and OB-R to figure out leptin's action on CVDs.

miRNAs, manages gene expression at the posttranscriptional level[169, 170]. miRNAs works in various pathways like metabolism and they are transported into the circulation [93-95]. An irregular metabolic process because of abnormal work of miRNAs is entangled in the development of type-II DM, metabolic syndrome, and CVD [93]. Following miRNA in the circulation might ensure: evaluation of disease progression, treatment, susceptibility, risk and offers an approval of a preclinical diagnosis [91].

Leptin duty and expressions are also arranged by the instrumentation of different miRNA. Inducement of leptin may manage several types of miRNAs [171].

miRNA-221, from the data we select to examine in our community, shows reduced expression in obesity. [91, 101].Yet, a study showed that a population of Chinese women circulating levels of miRNA-221 were higher in non-obese with metabolic syndrome [96]. Expression of miR-221; known to be down regulated in the and circulation of obese individuals, rising with Bariatric surgery and Physical activity [99, 123].

Results of a study show that adipose miRNA-221 expression is favorably connected with BMI in the Pima Indian population. This connection is matches with former studies indicate that adipose miRNA-221 is upregulated in obesity [12, 13];nevertheless, processes for the upregulation in the research are unknown. TNF-α and leptin, showed the effect of downregulation for miR-221 and they are known to be higher level in the obese state. It recommends a complicated mechanism in vivo, could be desensitization to impacts of TNF-α and leptin in the chronically obese state [126-129].

So, clusters of miRNA-222 and miRNA-221 are good distributed and the most bountiful miRNAs among these cells, vascular smooth muscle cell (VSMC), endothelial, platelet. [172, 173]. miRNA-222 and miRNA-221 act as pro-inflammatory obstructer of angiotensin II and inverse leucocytes adhesion (in vitro and in vivo) [174], recommending a potential role of these miRNA clusters in CAD dysregulation.

According to a study using human vascular endothelial cells, miRNA-222 and miRNA-221 between other miRNAs were understood to be effective angiogenic features [10]. Different report indicates that an anti-angiogenic properties of miRNA-222 and miRNA-221 in human endothelial cells [11]. An miRNA-222 and miRNA-221 transfected endothelial cell model and its understood that they inhibit wound healing ,tube formation and cell migration in endothelial cells in vitro [10]. miRNA-222 and miRNA-221 is the member of the identical family and are situated very close intimacy to the Xp11 3 chromosome [172, 175, 176].a research proved that miRNA-222 ⁄ 221 levels in EPCs (circulating endothelial progenitor cells) found to be more in CAD individuals than in non-CAD patients[177]. On the top of it, concentrations of miR-222 and miR 221 were negatively connected to the counts of EPCs in CAD individuals[178].

miRNA-222 and miRNA221 decreases the expression of c-kit, the receptor for broken cell proliferation and stem cell factor in hematopoietic progenitor cells, figured out by Felli et al. [108]. A new research indicated that the overturn of Dicer rised eNOS expression, while transfection of miRNA-2222 and miRNA-221 imitates partially restored increased eNOS protein levels [11]. Withal its demonstrated that eNOS-derived NO production is very important in the functional activity and mobilization of EPCs [118, 120][22,23]. Investigations demonstrate that elevated miRNA-222 ⁄ 221 expression in EPCs downregulates the mobilization and differentiation of EPC via eNOS-dependent pathways and⁄or c-kit- in CAD individuals[177].

In the current study the expression levels of miRNA-221 were found to be significantly high (3 fold) in CAD group than the control group. Add to this finding, in our study we detected that miRNA-221 did not find correlated with severity of CAD.

Further, there was no correlation between miRNA-221 and leptin and also miRNA-221 and FLI. There was statistically significant correlation between miRNA-221 and sOB-R. sOB-R mediates leptin actions [43] and also is just as relevant for the formation of leptin resistant conditions [1].

A limitation of the present study is the small number of CAD patients. Further studies will need to determine the mechanisms by including acute coronary syndrome and showing the levels of markers on tissues.

Conclusion

In conclusion, the results showed that sOB-R level is statistically higher and FLI is statistically lower in CAD group than the normal coronary artery group. miRNA-221 levels statistically higher in CAD group than the normal coronary artery group. Also sOB-R levels and FLI are correlated with the severity of CAD. Further, if these results are confirmed in other studies, sOB-R levels, FLI and miRNA-221 may be useful to detect significant CAD before performing coronary angiography.

6.REFERENCES

1. Schaab, M. and J. Kratzsch, The soluble leptin receptor. Best practice & research Clinical endocrinology & metabolism, 2015. **29**(5): p. 661-670.
2. Piccolo, R., et al., Stable coronary artery disease: revascularisation and invasive strategies. Lancet, 2015. **386**(9994): p. 702-13.
3. Stankovic, I., et al., Myocardial mechanical and QTc dispersion for the detection of significant coronary artery disease. Eur Heart J Cardiovasc Imaging, 2015. **16**(9): p. 1015-22.
4. Poetsch, M.S., A. Strano, and K. Guan, Role of leptin in cardiovascular diseases. Frontiers in Endocrinology, 2020. **11**: p. 354.
5. Myers, M.G., M.A. Cowley, and H. Münzberg, Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol., 2008. **70**: p. 537-556.
6. Lammert, A., et al., Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun, 2001. **283**(4): p. 982-8.
7. Sun, Q., et al., Leptin and soluble leptin receptor levels in plasma and risk of type 2 interleukin in U.S. women: a prospective study. Diabetes, 2010. **59**(3): p. 611-8.
8. Herrick, J.E., G.S. Panza, and J.M. Gollie, Leptin, leptin soluble receptor, and the free leptin index following a diet and physical activity lifestyle intervention in obese males and females. Journal of obesity, 2016. **2016**.
9. !!! INVALID CITATION !!! (8).
10. Poliseno, L., et al., MicroRNAs modulate the angiogenic properties of HUVECs. Blood, 2006. **108**(9): p. 3068-71.
11. Suarez, Y., et al., Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res, 2007. **100**(8): p. 1164-73.
12. Ortega, F.J., et al., MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. PLoS One, 2010. **5**(2): p. e9022.
13. Xie, H., B. Lim, and H.F. Lodish, MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. Diabetes, 2009. **58**(5): p. 1050-7.
14. Meerson, A., et al., Human adipose microRNA-221 is upregulated in obesity and affects fat metabolism downstream of leptin and TNF-alpha. Diabetologia, 2013. **56**(9): p. 1971-9.
15. Saraste, A. and J. Knuuti, ESC 2019 guidelines for the diagnosis and management of chronic coronary syndromes. Herz, 2020. **45**(5): p. 409-420.
16. Weir, H.K., et al., Heart Disease and Cancer Deaths - Trends and Projections in the United States, 1969-2020. Prev Chronic Dis, 2016. **13**: p. E157.
17. Johnson, N.B., et al., CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors—United States, 2005–2013. 2014.
18. Murphy, S.L., et al., Mortality in the United States, 2014. NCHS Data Brief, 2015(229): p. 1-8.
19. Kannel, W.B., et al., Relation of body weight to development of coronary heart disease. The Framingham study. Circulation, 1967. **35**(4): p. 734-44.

20. Poirier, P., et al., Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation, 2006. **113**(6): p. 898-918.
21. Carreras-Badosa, G., et al., Dysregulation of Placental miRNA in Maternal Obesity Is Associated With Pre- and Postnatal Growth. J Clin Endocrinol Metab, 2017. **102**(7): p. 2584-2594.
22. RA, L.M., et al. Childhood obesity. Recommendations of the nutrition committee of the Spanish association of pediatrics. Part I. Prevention. Early detection. Role of the pediatrician. in Anales De Pediatria (Barcelona, Spain: 2003). 2006.
23. Force*, U.P.S.T., Screening for obesity in adults: recommendations and rationale. Annals of internal medicine, 2003. **139**(11): p. 930-932.
24. Sones, F., Cinecoronary arteriography. Circulation, 1959. **20**: p. 773-774.
25. Poon, K. and D. Walters, Indications for coronary angiography, in Advances in the Diagnosis of Coronary Atherosclerosis. 2011, IntechOpen.
26. Members, W.C., et al., 2021 ACC/AHA/SCAI guideline for coronary artery revascularization: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Journal of the American College of Cardiology, 2022. **79**(2): p. e21-e129.
27. Sianos, G., et al., The SYNTAX Score: an angiographic tool grading the complexity of coronary artery disease. EuroIntervention, 2005. **1**(2): p. 219-27.
28. Essam, F., A. Jumaily, and S. Zgaer, A review: Leptin structure and mechanism actions. BEPLS, 2014. **4**: p. 185-92.
29. Minokoshi, Y., et al., Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature, 2002. **415**(6869): p. 339-343.
30. Shimomura, I., et al., Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature, 1999. **401**(6748): p. 73-76.
31. Sweeney, G., et al., High leptin levels acutely inhibit insulin-stimulated glucose uptake without affecting glucose transporter 4 translocation in l6 rat skeletal muscle cells. Endocrinology, 2001. **142**(11): p. 4806-4812.
32. Donoso, M., et al., Increased circulating adiponectin levels and decreased leptin/soluble leptin receptor ratio throughout puberty in female ballet dancers: association with body composition and delay in puberty. European Journal of Endocrinology, 2010. **162**(5): p. 905-911.
33. Agarwal, S.K., et al., Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. The journal of clinical endocrinology & metabolism, 1999. **84**(3): p. 1072-1076.
34. Barkan, D., et al., Leptin modulates the glucocorticoid-induced ovarian steroidogenesis. Endocrinology, 1999. **140**(4): p. 1731-1738.
35. Forbes, S., et al., Integrated control of appetite and fat metabolism by the leptin-proopiomelanocortin pathway. Proceedings of the National Academy of Sciences, 2001. **98**(7): p. 4233-4237.
36. Prieur, X., et al., Leptin regulates peripheral lipid metabolism primarily through central effects on food intake. Endocrinology, 2008. **149**(11): p. 5432-5439.
37. Matarese, G., et al., Regulatory T cells in obesity: the leptin connection. Trends in molecular medicine, 2010. **16**(6): p. 247-256.

38. Faggioni, R., et al., Leptin-deficient (ob/ob) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor α and IL-18. Proceedings of the National Academy of Sciences, 2000. **97**(5): p. 2367-2372.
39. Shapiro, N.I., et al., Leptin exacerbates sepsis-mediated morbidity and mortality. The Journal of Immunology, 2010. **185**(1): p. 517-524.
40. Reis, B.S., et al., Leptin receptor signaling in T cells is required for Th17 differentiation. The Journal of Immunology, 2015. **194**(11): p. 5253-5260.
41. Reinehr, T., et al., Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children. International journal of obesity, 2005. **29**(10): p. 1230-1235.
42. Lönnqvist, F., et al., Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nature medicine, 1995. **1**(9): p. 950-953.
43. Tartaglia, L.A., et al., Identification and expression cloning of a leptin receptor, OB-R. Cell, 1995. **83**(7): p. 1263-1271.
44. Akasaka, Y., et al., Direct evidence for leptin-induced lipid oxidation independent of long-form leptin receptor. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 2010. **1801**(10): p. 1115-1122.
45. Moharana, K., et al., Structural and mechanistic paradigm of leptin receptor activation revealed by complexes with wild-type and antagonist leptins. Structure, 2014. **22**(6): p. 866-877.
46. Clement, K., et al., A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature, 1998. **392**(6674): p. 398-401.
47. Yang, G., et al., Modulation of direct leptin signaling by soluble leptin receptor. Molecular Endocrinology, 2004. **18**(6): p. 1354-1362.
48. Ge, H., et al., Generation of soluble leptin receptor by ectodomain shedding of membrane-spanning receptors in vitro and in vivo. Journal of Biological Chemistry, 2002. **277**(48): p. 45898-45903.
49. Maamra, M., et al., Generation of human soluble leptin receptor by proteolytic cleavage of membrane-anchored receptors. Endocrinology, 2001. **142**(10): p. 4389-4393.
50. Bjørbæk, C., et al., Identification of SOCS-3 as a potential mediator of central leptin resistance. Molecular cell, 1998. **1**(4): p. 619-625.
51. Morrison, C.D., et al., Increased hypothalamic protein tyrosine phosphatase 1B contributes to leptin resistance with age. Endocrinology, 2007. **148**(1): p. 433-440.
52. Enriori, P.J., et al., Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell metabolism, 2007. **5**(3): p. 181-194.
53. Bence, K.K., et al., Neuronal PTP1B regulates body weight, adiposity and leptin action. Nature medicine, 2006. **12**(8): p. 917-924.
54. Howard, J.K., et al., Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. Nature medicine, 2004. **10**(7): p. 734-738.
55. Kievit, P., et al., Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell metabolism, 2006. **4**(2): p. 123-132.
56. Mori, H., et al., Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. Nature medicine, 2004. **10**(7): p. 739-743.

57. Ozcan, U., et al., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science, 2004. **306**(5695): p. 457-61.
58. Ozcan, L., et al., Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab, 2009. **9**(1): p. 35-51.
59. Caro, J.F., et al., Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet, 1996. **348**(9021): p. 159-61.
60. Hosoi, T., et al., Endoplasmic reticulum stress induces leptin resistance. Mol Pharmacol, 2008. **74**(6): p. 1610-9.
61. Zhang, X., et al., Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell, 2008. **135**(1): p. 61-73.
62. El-Haschimi, K., et al., Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest, 2000. **105**(12): p. 1827-32.
63. Chan, J.L., et al., Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes, 2002. **51**(7): p. 2105-2112.
64. Stein, K., et al., Influence of nutritional recovery on the leptin axis in severely malnourished children. J Clin Endocrinol Metab, 2006. **91**(3): p. 1021-6.
65. Zepf, F.D., et al., Differences in zinc status and the leptin axis in anorexic and recovered adolescents and young adults: a pilot study. Food Nutr Res, 2012. **56**.
66. Rose-John, S. and P.C. Heinrich, Soluble receptors for cytokines and growth factors: generation and biological function. Biochemical Journal, 1994. **300**(Pt 2): p. 281.
67. Yamagishi, S.-i., T. Matsui, and K. Nakamura, Kinetics, role and therapeutic implications of endogenous soluble form of receptor for advanced glycation end products (sRAGE) in diabetes. Current drug targets, 2007. **8**(10): p. 1138-1143.
68. Cook, J.D., R.D. Baynes, and B.S. Skikne, The physiological significance of circulating transferrin receptors. Nutrient regulation during pregnancy, lactation, and infant growth, 1994: p. 119-126.
69. Schaab, M., et al., Novel regulatory mechanisms for generation of the soluble leptin receptor: implications for leptin action. PLoS One, 2012. **7**(4): p. e34787.
70. Zhang, J. and P.J. Scarpace, The soluble leptin receptor neutralizes leptin-mediated STAT3 signalling and anorexic responses in vivo. Br J Pharmacol, 2009. **158**(2): p. 475-82.
71. Tu, H., et al., Soluble receptor inhibits leptin transport. J Cell Physiol, 2008. **214**(2): p. 301-5.
72. Tu, J.L., et al., Unique GTP-binding pocket and allostery of uridylate kinase from a gram-negative phytopathogenic bacterium. J Mol Biol, 2009. **385**(4): p. 1113-26.
73. Monteleone, P., et al., Opposite modifications in circulating leptin and soluble leptin receptor across the eating disorder spectrum. Mol Psychiatry, 2002. **7**(6): p. 641-6.
74. Kratzsch, J., et al., Increased serum soluble leptin receptor levels in children and adolescents with type 1 diabetes mellitus. Eur J Endocrinol, 2004. **151**(4): p. 475-81.
75. Kratzsch, J., et al., Metabolic decompensation in children with type 1 diabetes mellitus associated with increased serum levels of the soluble leptin receptor. Eur J Endocrinol, 2006. **155**(4): p. 609-14.
76. Kratzsch, J., et al., Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence. J Clin Endocrinol Metab, 2002. **87**(10): p. 4587-94.

77. Mantzoros, C.S., J.S. Flier, and A.D. Rogol, A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab, 1997. **82**(4): p. 1066-70.
78. Elefteriou, F., et al., Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci U S A, 2004. **101**(9): p. 3258-63.
79. Elefteriou, F., et al., Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature, 2005. **434**(7032): p. 514-20.
80. Bandeira, F.F., et al., High bone mass associated with berardinelli lipodystrophy. Endocr Pract, 2007. **13**(7): p. 764-9.
81. Lou, P.H., et al., Reduced body weight and increased energy expenditure in transgenic mice over-expressing soluble leptin receptor. PLoS One, 2010. **5**(7): p. e11669.
82. Cohen, S.E., et al., High circulating leptin receptors with normal leptin sensitivity in liver-specific insulin receptor knock-out (LIRKO) mice. J Biol Chem, 2007. **282**(32): p. 23672-8.
83. Sinha, M.K., et al., Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. J Clin Invest, 1996. **98**(6): p. 1277-82.
84. Owecki, M., et al., Leptin, soluble leptin receptors, free leptin index, and their relationship with insulin resistance and BMI: high normal BMI is the threshold for serum leptin increase in humans. Horm Metab Res, 2010. **42**(8): p. 585-9.
85. Baranowska-Bik, A., et al., Plasma leptin levels and free leptin index in women with Alzheimer's disease. Neuropeptides, 2015. **52**: p. 73-8.
86. Rizk, N.M. and E. Sharif, Leptin as well as Free Leptin Receptor Is Associated with Polycystic Ovary Syndrome in Young Women. Int J Endocrinol, 2015. **2015**: p. 927805.
87. Wahid, F., et al., MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2010. **1803**(11): p. 1231-1243.
88. Sheinerman, K.S. and S.R. Umansky, Circulating cell-free microRNA as biomarkers for screening, diagnosis and monitoring of neurodegenerative diseases and other neurologic pathologies. Frontiers in cellular neuroscience, 2013. **7**: p. 150.
89. Zhang, W., et al., MicroRNAs in Serum Exosomes as Potential Biomarkers in Clear-cell Renal Cell Carcinoma. Eur Urol Focus, 2018. **4**(3): p. 412-419.
90. Larrea, E., et al., New Concepts in Cancer Biomarkers: Circulating miRNAs in Liquid Biopsies. Int J Mol Sci, 2016. **17**(5).
91. Deiuliis, J.A., MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. Int J Obes (Lond), 2016. **40**(1): p. 88-101.
92. Yang, Z., et al., Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes. Acta Diabetol, 2014. **51**(5): p. 823-31.
93. Chen, W.M., et al., Modulation of microRNA Expression in Subjects with Metabolic Syndrome and Decrease of Cholesterol Efflux from Macrophages via microRNA-33-Mediated Attenuation of ATP-Binding Cassette Transporter A1 Expression by Statins. PLoS One, 2016. **11**(5): p. e0154672.
94. Karolina, D.S., et al., Circulating miRNA profiles in patients with metabolic syndrome. J Clin Endocrinol Metab, 2012. **97**(12): p. E2271-6.

95. Kimura, Y., et al., Clinical Significance of Determining Plasma MicroRNA33b in Type 2 Diabetic Patients with Dyslipidemia. J Atheroscler Thromb, 2016. **23**(11): p. 1276-1285.
96. Wang, Y.-T., et al., Circulating microRNAs have a sex-specific association with metabolic syndrome. Journal of biomedical science, 2013. **20**(1): p. 1-10.
97. Wang, X., et al., Determination of 14 circulating microRNAs in Swedes and Iraqis with and without diabetes mellitus type 2. PLoS One, 2014. **9**(1): p. e86792.
98. Flowers, E., et al., Circulating micrornas associated with glycemic impairment and progression in Asian Indians. Biomark Res, 2015. **3**: p. 22.
99. Villard, A., et al., Diagnostic Value of Cell-free Circulating MicroRNAs for Obesity and Type 2 Diabetes: A Meta-analysis. J Mol Biomark Diagn, 2015. **6**(6).
100. Zampetaki, A., et al., Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res, 2010. **107**(6): p. 810-7.
101. Alexander, R., H. Lodish, and L. Sun, MicroRNAs in adipogenesis and as therapeutic targets for obesity. Expert Opin Ther Targets, 2011. **15**(5): p. 623-36.
102. Navickas, R., et al., Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review. Cardiovasc Res, 2016. **111**(4): p. 322-37.
103. Ahlin, F., et al., MicroRNAs as circulating biomarkers in acute coronary syndromes: A review. Vascul Pharmacol, 2016. **81**: p. 15-21.
104. Schulte, C., M. Karakas, and T. Zeller, microRNAs in cardiovascular disease - clinical application. Clin Chem Lab Med, 2017. **55**(5): p. 687-704.
105. Small, E.M. and E.N. Olson, Pervasive roles of microRNAs in cardiovascular biology. Nature, 2011. **469**(7330): p. 336-42.
106. Florijn, B.W., et al., Gender and cardiovascular disease: are sex-biased microRNA networks a driving force behind heart failure with preserved ejection fraction in women? Cardiovasc Res, 2018. **114**(2): p. 210-225.
107. Kaur, A., et al., Systematic review of microRNA biomarkers in acute coronary syndrome and stable coronary artery disease. Cardiovasc Res, 2020. **116**(6): p. 1113-1124.
108. Felli, N., et al., MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci U S A, 2005. **102**(50): p. 18081-6.
109. ; Available from: http://www.genecards.org/.
110. miRDB. Available from: http://www.mirdb.org/.
111. mirBASE. Available from: http://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000764.
112. Orlic, D., et al., Bone marrow cells regenerate infarcted myocardium. Nature, 2001. **410**(6829): p. 701-5.
113. Beltrami, A.P., et al., Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell, 2003. **114**(6): p. 763-76.
114. Murohara, T., et al., Role of endothelial nitric oxide synthase in endothelial cell migration. Arterioscler Thromb Vasc Biol, 1999. **19**(5): p. 1156-61.
115. Rudic, R.D., et al., Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. J Clin Invest, 1998. **101**(4): p. 731-6.

116. Murohara, T., et al., Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest, 1998. **101**(11): p. 2567-78.
117. Zeiher, A.M., Endothelial vasodilator dysfunction: pathogenetic link to myocardial ischaemia or epiphenomenon? Lancet, 1996. **348 Suppl 1**: p. s10-2.
118. Aicher, A., et al., Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med, 2003. **9**(11): p. 1370-6.
119. Iwakura, A., et al., Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. Circulation, 2003. **108**(25): p. 3115-21.
120. Landmesser, U., et al., Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. Circulation, 2004. **110**(14): p. 1933-9.
121. Urbich, C., A. Kuehbacher, and S. Dimmeler, Role of microRNAs in vascular diseases, inflammation, and angiogenesis. Cardiovascular research, 2008. **79**(4): p. 581-588.
122. Lustig, Y., et al., RNA-binding protein PTB and microRNA-221 coregulate AdipoR1 translation and adiponectin signaling. Diabetes, 2014. **63**(2): p. 433-445.
123. Chou, W.-W., et al., Decreased microRNA-221 is associated with high levels of TNF-α in human adipose tissue-derived mesenchymal stem cells from obese woman. Cellular Physiology and Biochemistry, 2013. **32**(1): p. 127-137.
124. Ortega, F.J., et al., Targeting the circulating microRNA signature of obesity. Clin Chem, 2013. **59**(5): p. 781-92.
125. Denvir, M.A., et al., Phase 2 Randomised Controlled Trial and Feasibility Study of Future Care Planning in Patients with Advanced Heart Disease. Sci Rep, 2016. **6**: p. 24619.
126. Konner, A.C. and J.C. Bruning, Selective insulin and leptin resistance in metabolic disorders. Cell Metab, 2012. **16**(2): p. 144-52.
127. Wauman, J. and J. Tavernier, Leptin receptor signaling: pathways to leptin resistance. Front Biosci (Landmark Ed), 2011. **16**(7): p. 2771-93.
128. Cseh, K., et al., The role of tumour necrosis factor (TNF)-alpha resistance in obesity and insulin resistance. Diabetologia, 2000. **43**(4): p. 525.
129. Loh, K., et al., Elevated hypothalamic TCPTP in obesity contributes to cellular leptin resistance. Cell Metab, 2011. **14**(5): p. 684-99.
130. Lorente-Cebrian, S., et al., MicroRNAs regulate human adipocyte lipolysis: effects of miR-145 are linked to TNF-α. PloS one, 2014. **9**(1): p. e86800.
131. Zhu, L., et al., MiR-335, an adipogenesis-related microRNA, is involved in adipose tissue inflammation. Cell biochemistry and biophysics, 2014. **68**(2): p. 283-290.
132. Kim, C., et al., TNFα-induced miR-130 resulted in adipocyte dysfunction during obesity-related inflammation. FEBS letters, 2013. **587**(23): p. 3853-3858.
133. Shi, C., et al., IL-6 and TNF-α induced obesity-related inflammatory response through transcriptional regulation of miR-146b. Journal of interferon & cytokine research, 2014. **34**(5): p. 342-348.
134. Karkeni, E., et al., Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: A new mechanism for the regulation of inflammation by vitamin D. Epigenetics, 2018. **13**(2): p. 156-162.

135. Kistorp, C., et al., Prevalence and characteristics of diabetic patients in a chronic heart failure population. International journal of cardiology, 2005. **100**(2): p. 281-287.
136. Li, X., et al., Lowering the quantification limit of the QubitTM RNA HS assay using RNA spike-in. BMC molecular biology, 2015. **16**(1): p. 1-7.
137. targetscan. Available from: http://www.targetscan.org/.
138. Livak, K.J. and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 2001. **25**(4): p. 402-8.
139. Benjamin, E.J., et al., Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation, 2018. **137**(12): p. e67-e492.
140. the risk factors for cardiovascular disease. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
141. Yang, H., et al., Leptin concentration and risk of coronary heart disease and stroke: A systematic review and meta-analysis. PLoS One, 2017. **12**(3): p. e0166360.
142. Koh, K.K., S.M. Park, and M.J. Quon, Leptin and cardiovascular disease: response to therapeutic interventions. Circulation, 2008. **117**(25): p. 3238-49.
143. Bouloumie, A., et al., Leptin induces oxidative stress in human endothelial cells. FASEB J, 1999. **13**(10): p. 1231-8.
144. Zhang, T., et al., Leptin Expression in Human Epicardial Adipose Tissue Is Associated with Local Coronary Atherosclerosis. Med Sci Monit, 2019. **25**: p. 9913-9922.
145. Fruhbeck, G. and J. Gomez-Ambrosi, Modulation of the leptin-induced white adipose tissue lipolysis by nitric oxide. Cell Signal, 2001. **13**(11): p. 827-33.
146. Eriksson, M.A., et al., Sex-related differences in the associations between hyperleptinemia, insulin resistance and dysfibrinolysis. Blood Coagul Fibrinolysis, 2008. **19**(7): p. 625-32.
147. Kshatriya, S., et al., Obesity hypertension: the emerging role of leptin in renal and cardiovascular dyshomeostasis. Curr Opin Nephrol Hypertens, 2010. **19**(1): p. 72-8.
148. Schafer, K., et al., Leptin promotes vascular remodeling and neointimal growth in mice. Arterioscler Thromb Vasc Biol, 2004. **24**(1): p. 112-7.
149. Beltowski, J., Leptin and atherosclerosis. Atherosclerosis, 2006. **189**(1): p. 47-60.
150. Beltowski, J., G. Wojcicka, and A. Jamroz-Wisniewska, Role of nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) in the regulation of blood pressure by leptin in lean and obese rats. Life Sci, 2006. **79**(1): p. 63-71.
151. Sattar, N., et al., Leptin and coronary heart disease: prospective study and systematic review. J Am Coll Cardiol, 2009. **53**(2): p. 167-75.
152. Simiti, L.A., et al., Better prognosis in overweight/obese coronary heart disease patients with high plasma levels of leptin. Clujul Med, 2016. **89**(1): p. 65-71.

153. Ku, I.A., et al., Association of low leptin with cardiovascular events and mortality in patients with stable coronary artery disease: the Heart and Soul Study. Atherosclerosis, 2011. **217**(2): p. 503-8.
154. Khafaji, H.A., et al., Elevated serum leptin levels in patients with acute myocardial infarction; correlation with coronary angiographic and echocardiographic findings. BMC Res Notes, 2012. **5**: p. 262.
155. Rahmani, A., et al., Association between plasma leptin/adiponectin ratios with the extent and severity of coronary artery disease. BMC Cardiovascular Disorders, 2020. **20**(1): p. 1-9.
156. Katsiki, N., D.P. Mikhailidis, and M. Banach, Leptin, cardiovascular diseases and type 2 diabetes mellitus. Acta Pharmacol Sin, 2018. **39**(7): p. 1176-1188.
157. Chai, S.B., et al., Leptin and coronary heart disease: a systematic review and meta-analysis. Atherosclerosis, 2014. **233**(1): p. 3-10.
158. Fukuta, H., et al., Relation of plasma levels of adiponectin to left ventricular diastolic dysfunction in patients undergoing cardiac catheterization for coronary artery disease. Am J Cardiol, 2011. **108**(8): p. 1081-5.
159. Martin, S.S., et al., Leptin and incident cardiovascular disease: the Multi-ethnic Study of Atherosclerosis (MESA). Atherosclerosis, 2015. **239**(1): p. 67-72.
160. Thogersen, A.M., et al., Interactions between fibrinolysis, lipoproteins and leptin related to a first myocardial infarction. Eur J Cardiovasc Prev Rehabil, 2004. **11**(1): p. 33-40.
161. Wallerstedt, S.M., et al., Serum leptin and myocardial infarction in hypertension. Blood Press, 2004. **13**(4): p. 243-6.
162. Ghantous, C.M., et al., Differential Role of Leptin and Adiponectin in Cardiovascular System. Int J Endocrinol, 2015. **2015**: p. 534320.
163. Runjic, F., et al., Association of anticardiolipin antibodies, complement and leptin with the severity of coronary artery disease expressed as syntax score. J Physiol Pharmacol, 2020. **71**(3).
164. Emara, A.A., M.A. Moharam, and A.E. Reyad, Correlation between plasma leptin hormone in diabetic patients and the extent of coronary artery disease. Menoufia Medical Journal, 2018. **31**(2): p. 607.
165. Serruys, P., et al., Assessment of the SYNTAX score in the Syntax study (EuroIntervention (2009) 5,(50-56)). EuroIntervention, 2009. **5**(3): p. 401.
166. Guedeney, P., et al., Prognostic value of SYNTAX score in patients with infarct-related cardiogenic shock: insights from the CULPRIT-SHOCK trial. Cardiovascular Interventions, 2020. **13**(10): p. 1198-1206.
167. Kim, E.Y., et al., Susceptibility of gastric cancer according to leptin and leptin receptor gene polymorphisms in Korea. J Korean Surg Soc, 2012. **83**(1): p. 7-13.
168. Çakır, I., et al., Correlation between the expressions of leptin and its receptors (ObR, sObR) in gastric cancer/Mide kanserinde leptin ve reseptörlerinin (ObR, sObR) ekspresyonları arasındaki korelasyon. Turkish Journal of Biochemistry, 2016. **41**(4): p. 243-249.
169. Bartel, D.P., MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004. **116**(2): p. 281-97.
170. Kim, V.N., Small RNAs: classification, biogenesis, and function. Mol Cells, 2005. **19**(1): p. 1-15.
171. Jasinski-Bergner, S. and H. Kielstein, Adipokines regulate the expression of tumor-relevant MicroRNAs. Obesity Facts, 2019. **12**(2): p. 211-225.

172. Altuvia, Y., Landgraf P, Lithwick G, Elefant N, Pfeffer S, Aravin A, Brownstein MJ, Tuschl T, Margalit H. Clustering and conservation patterns of human microRNAs. Nucleic Acids Res, 2005. **33**: p. 2697-2706.
173. Zhu, N., et al., Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. Atherosclerosis, 2011. **215**(2): p. 286-293.
174. Hartmann, D. and T. Thum, MicroRNAs and vascular (dys) function. Vascular pharmacology, 2011. **55**(4): p. 92-105.
175. Lewis, B.P., C.B. Burge, and D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. cell, 2005. **120**(1): p. 15-20.
176. Calin, G.A., et al., Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences, 2004. **101**(9): p. 2999-3004.
177. Minami, Y., et al., Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. European journal of clinical investigation, 2009. **39**(5): p. 359-367.
178. Hosoi, T., et al., Effect of pranoprofen on endoplasmic reticulum stress in the primary cultured glial cells. Neurochem Int, 2009. **54**(1): p. 1-6.

7.APPENDICES

7.1. ROW DATA

```
CROSSTABS
  /TABLES=hasta_kontrol BY OBEZ sex HL DM HT aile
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ KAPPA RISK MCNEMAR
  /CELLS=COUNT EXPECTED ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

Output Created		05-APR-2022 08:32:25
Comments		
Input	Data	C:\Users\selvi.duman\Desktop\AYÇA TEZ\Yeni klasör\ayça tez (1).sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	78
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=hasta_kontrol BY OBEZ sex HL DM HT aile /FORMAT=AVALUE TABLES /STATISTICS=CHISQ KAPPA RISK MCNEMAR /CELLS=COUNT EXPECTED ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,03
	Elapsed Time	00:00:00,11
	Dimensions Requested	2
	Cells Available	131029

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
hasta_kontrol * OBEZ	78	100.0%	0	0.0%	78	100.0%
hasta_kontrol * sex	78	100.0%	0	0.0%	78	100.0%
hasta_kontrol * HL	78	100.0%	0	0.0%	78	100.0%
hasta_kontrol * DM	78	100.0%	0	0.0%	78	100.0%
hasta_kontrol * HT	78	100.0%	0	0.0%	78	100.0%
hasta_kontrol * aile	78	100.0%	0	0.0%	78	100.0%

hasta_kontrol * OBEZ

Crosstab

			OBEZ		Total
			obez	değil	
hasta_kontrol	kontrol	Count	13	25	38
		Expected Count	17.5	20.5	38.0
		% within hasta_kontrol	34.2%	65.8%	100.0%
		% within OBEZ	36.1%	59.5%	48.7%
		% of Total	16.7%	32.1%	48.7%
	hasta	Count	23	17	40
		Expected Count	18.5	21.5	40.0
		% within hasta_kontrol	57.5%	42.5%	100.0%
		% within OBEZ	63.9%	40.5%	51.3%
		% of Total	29.5%	21.8%	51.3%
Total		Count	36	42	78
		Expected Count	36.0	42.0	78.0
		% within hasta_kontrol	46.2%	53.8%	100.0%
		% within OBEZ	100.0%	100.0%	100.0%
		% of Total	46.2%	53.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.253[a]	1	.039		
Continuity Correction[b]	3.368	1	.066		
Likelihood Ratio	4.297	1	.038		
Fisher's Exact Test				.045	.033
Linear-by-Linear Association	4.199	1	.040		
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 17,54.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.076	.036	2.062	.039
N of Valid Cases		78			

a. Not assuming the null hypothesis.

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

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	.384	.154	.962
For cohort OBEZ = obez	.595	.355	.996
For cohort OBEZ = değil	1.548	1.010	2.373
N of Valid Cases	78		

hasta_kontrol * sex

Crosstab

			sex		Total
			KADIN	ERKEK	
hasta_kontrol	kontrol	Count	21	17	38
		Expected Count	18.5	19.5	38.0
		% within hasta_kontrol	55.3%	44.7%	100.0%
		% within sex	55.3%	42.5%	48.7%
		% of Total	26.9%	21.8%	48.7%
	hasta	Count	17	23	40
		Expected Count	19.5	20.5	40.0
		% within hasta_kontrol	42.5%	57.5%	100.0%
		% within sex	44.7%	57.5%	51.3%
		% of Total	21.8%	29.5%	51.3%
Total		Count	38	40	78
		Expected Count	38.0	40.0	78.0
		% within hasta_kontrol	48.7%	51.3%	100.0%
		% within sex	100.0%	100.0%	100.0%
		% of Total	48.7%	51.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.271[a]	1	.260		
Continuity Correction[b]	.811	1	.368		
Likelihood Ratio	1.274	1	.259		
Fisher's Exact Test				.365	.184
Linear-by-Linear Association	1.254	1	.263		
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 18,51.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	-.043	.037	-1.127	.260
N of Valid Cases		78			

a. Not assuming the null hypothesis.

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

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	1.671	.683	4.092
For cohort sex = KADIN	1.300	.821	2.060
For cohort sex = ERKEK	.778	.500	1.211
N of Valid Cases	78		

hasta_kontrol * HL

Crosstab

			HL		Total
			VAR	YOK	
hasta_kontrol	kontrol	Count	20	18	38
		Expected Count	20.9	17.1	38.0
		% within hasta_kontrol	52.6%	47.4%	100.0%
		% within HL	46.5%	51.4%	48.7%
		% of Total	25.6%	23.1%	48.7%
	hasta	Count	23	17	40
		Expected Count	22.1	17.9	40.0
		% within hasta_kontrol	57.5%	42.5%	100.0%
		% within HL	53.5%	48.6%	51.3%
		% of Total	29.5%	21.8%	51.3%
Total		Count	43	35	78
		Expected Count	43.0	35.0	78.0
		% within hasta_kontrol	55.1%	44.9%	100.0%
		% within HL	100.0%	100.0%	100.0%
		% of Total	55.1%	44.9%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.187[a]	1	.666		
Continuity Correction[b]	.042	1	.838		
Likelihood Ratio	.187	1	.666		
Fisher's Exact Test				.820	.419
Linear-by-Linear Association	.184	1	.668		
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 17,05.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.017	.039	.432	.666
N of Valid Cases		78			

a. Not assuming the null hypothesis.

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

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	.821	.336	2.007
For cohort HL = VAR	.915	.612	1.369
For cohort HL = YOK	1.115	.681	1.823
N of Valid Cases	78		

hasta_kontrol * DM

Crosstab

			DM		Total
			VAR	YOK	
hasta_kontrol	kontrol	Count	15	23	38
		Expected Count	17.1	20.9	38.0
		% within hasta_kontrol	39.5%	60.5%	100.0%
		% within DM	42.9%	53.5%	48.7%
		% of Total	19.2%	29.5%	48.7%
	hasta	Count	20	20	40
		Expected Count	17.9	22.1	40.0
		% within hasta_kontrol	50.0%	50.0%	100.0%
		% within DM	57.1%	46.5%	51.3%
		% of Total	25.6%	25.6%	51.3%
Total		Count	35	43	78
		Expected Count	35.0	43.0	78.0
		% within hasta_kontrol	44.9%	55.1%	100.0%
		% within DM	100.0%	100.0%	100.0%
		% of Total	44.9%	55.1%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.873[a]	1	.350		
Continuity Correction[b]	.499	1	.480		
Likelihood Ratio	.875	1	.350		
Fisher's Exact Test				.372	.240
Linear-by-Linear Association	.862	1	.353		
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 17,05.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.034	.036	.934	.350

N of Valid Cases	78			

a. Not assuming the null hypothesis.

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

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	.652	.266	1.602
For cohort DM = VAR	.789	.478	1.303
For cohort DM = YOK	1.211	.809	1.810
N of Valid Cases	78		

hasta_kontrol * HT

Crosstab

			HT		Total
			var	yok	
hasta_kontrol	kontrol	Count	20	18	38
		Expected Count	24.4	13.6	38.0
		% within hasta_kontrol	52.6%	47.4%	100.0%
		% within HT	40.0%	64.3%	48.7%
		% of Total	25.6%	23.1%	48.7%
	hasta	Count	30	10	40
		Expected Count	25.6	14.4	40.0
		% within hasta_kontrol	75.0%	25.0%	100.0%
		% within HT	60.0%	35.7%	51.3%
		% of Total	38.5%	12.8%	51.3%
Total		Count	50	28	78
		Expected Count	50.0	28.0	78.0
		% within hasta_kontrol	64.1%	35.9%	100.0%
		% within HT	100.0%	100.0%	100.0%
		% of Total	64.1%	35.9%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.237[a]	1	.040		
Continuity Correction[b]	3.321	1	.068		
Likelihood Ratio	4.280	1	.039		
Fisher's Exact Test				.059	.034
Linear-by-Linear Association	4.183	1	.041		
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 13,64.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.083	.039	2.058	.040
N of Valid Cases		78			

a. Not assuming the null hypothesis.

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

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	.370	.142	.965
For cohort HT = var	.702	.494	.997
For cohort HT = yok	1.895	1.006	3.568
N of Valid Cases	78		

hasta_kontrol * aile

Crosstab

			aile		Total
			1,00	2,00	
hasta_kontrol	kontrol	Count	19	19	38
		Expected Count	14.1	23.9	38.0
		% within hasta_kontrol	50.0%	50.0%	100.0%
		% within aile	65.5%	38.8%	48.7%
		% of Total	24.4%	24.4%	48.7%
	hasta	Count	10	30	40
		Expected Count	14.9	25.1	40.0
		% within hasta_kontrol	25.0%	75.0%	100.0%
		% within aile	34.5%	61.2%	51.3%
		% of Total	12.8%	38.5%	51.3%
Total		Count	29	49	78
		Expected Count	29.0	49.0	78.0
		% within hasta_kontrol	37.2%	62.8%	100.0%
		% within aile	100.0%	100.0%	100.0%
		% of Total	37.2%	62.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.215[a]	1	.022		
Continuity Correction[b]	4.199	1	.040		
Likelihood Ratio	5.279	1	.022		
Fisher's Exact Test				.034	.020
McNemar Test				.[c]	
N of Valid Cases	78				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 14,13.

b. Computed only for a 2x2 table

c. Both variables must have identical values of categories.

Symmetric Measures

		Value
Measure of Agreement	Kappa	.[a]

N of Valid Cases	78

a. Kappa statistic cannot be computed. It requires a two-way table in which the variables are of the same type.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	3.000	1.152	7.815
For cohort aile = 1,00	2.000	1.072	3.732
For cohort aile = 2,00	.667	.463	.960
N of Valid Cases	78		

```
T-TEST GROUPS=hasta_kontrol(0 1)
  /MISSING=ANALYSIS
  /VARIABLES=yas kilo boy TOTAL LDL HDL TG VKİ
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		05-APR-2022 08:34:27
Comments		
Input	Data	C:\Users\selvi.duman\Desktop\AYÇA TEZ\Yeni klasör\ayça tez (1).sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	78
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=hasta_kontrol(0 1) /MISSING=ANALYSIS /VARIABLES=yas kilo boy TOTAL LDL HDL TG VKİ /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,00

Group Statistics

	hasta_kontrol	N	Mean	Std. Deviation	Std. Error Mean
yas	kontrol	38	57.8158	9.51771	1.54398
	hasta	40	60.8750	9.76175	1.54347
kilo	kontrol	38	81.1053	13.41598	2.17636
	hasta	40	83.7500	13.32003	2.10608
boy	kontrol	38	166.2632	10.45113	1.69540
	hasta	40	167.2000	9.49278	1.50094
TOTAL	kontrol	34	203.9412	48.01448	8.23442
	hasta	35	204.1143	49.49199	8.36567
LDL	kontrol	34	127.8824	45.31824	7.77201
	hasta	34	123.7647	43.60919	7.47891
HDL	kontrol	35	46.6286	27.60124	4.66546
	hasta	35	59.7429	78.30271	13.23557
TG	kontrol	34	175.6176	79.33665	13.60612
	hasta	35	174.7143	93.95887	15.88195
VKİ	kontrol	38	29.45	5.487	.890
	hasta	40	30.14	4.099	.648

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
yas	Equal variances assumed	.137	.713	-1.400	76
	Equal variances not assumed			-1.401	75.946
kilo	Equal variances assumed	.002	.968	-.873	76

	Equal variances not assumed			-.873	75.735
boy	Equal variances assumed	.648	.423	-.415	76
	Equal variances not assumed			-.414	74.380
TOTAL	Equal variances assumed	.014	.907	-.015	67
	Equal variances not assumed			-.015	67.000
LDL	Equal variances assumed	.138	.712	.382	66
	Equal variances not assumed			.382	65.903
HDL	Equal variances assumed	1.213	.275	-.934	68
	Equal variances not assumed			-.934	42.321
TG	Equal variances assumed	.414	.522	.043	67
	Equal variances not assumed			.043	65.738
VKİ	Equal variances assumed	1.784	.186	-.635	76
	Equal variances not assumed			-.631	68.394

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
yas	Equal variances assumed	.165	-3.05921	2.18459	-7.41020
	Equal variances not assumed	.165	-3.05921	2.18315	-7.40739
kilo	Equal variances assumed	.385	-2.64474	3.02799	-8.67549
	Equal variances not assumed	.385	-2.64474	3.02855	-8.67696
boy	Equal variances assumed	.679	-.93684	2.25870	-5.43543
	Equal variances not assumed	.680	-.93684	2.26433	-5.44824
TOTAL	Equal variances assumed	.988	-.17311	11.74363	-23.61350
	Equal variances not assumed	.988	-.17311	11.73840	-23.60306
LDL	Equal variances assumed	.704	4.11765	10.78603	-17.41735
	Equal variances not assumed	.704	4.11765	10.78603	-17.41794
HDL	Equal variances assumed	.353	-13.11429	14.03378	-41.11825
	Equal variances not assumed	.355	-13.11429	14.03378	-41.42924
TG	Equal variances assumed	.966	.90336	20.96483	-40.94261
	Equal variances not assumed	.966	.90336	20.91322	-40.85433
VKİ	Equal variances assumed	.527	-.694	1.093	-2.871

Equal variances not assumed	.530	-.694	1.101	-2.891

Independent Samples Test

		t-test for Equality of Means
		95% Confidence Interval of the Difference
		Upper
yas	Equal variances assumed	1.29177
	Equal variances not assumed	1.28896
kilo	Equal variances assumed	3.38602
	Equal variances not assumed	3.38749
boy	Equal variances assumed	3.56174
	Equal variances not assumed	3.57455
TOTAL	Equal variances assumed	23.26728
	Equal variances not assumed	23.25684
LDL	Equal variances assumed	25.65264
	Equal variances not assumed	25.65324
HDL	Equal variances assumed	14.88968
	Equal variances not assumed	15.20067
TG	Equal variances assumed	42.74933
	Equal variances not assumed	42.66106
VKİ	Equal variances assumed	1.483
	Equal variances not assumed	1.503

```
T-TEST GROUPS=hasta_kontrol(0 1)
  /MISSING=ANALYSIS
  /VARIABLES=LEPTIN LEPTIN_RESEPTR ORAN_LEPTIN_RECEPTOR
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		05-APR-2022 08:39:39
Comments		
Input	Data	C:\Users\selvi.duman\Desktop\AYÇA TEZ\Yeni klasör\ayça tez (1).sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>

	Split File	<none>
	N of Rows in Working Data File	72
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=hasta_kontrol(0 1) /MISSING=ANALYSIS /VARIABLES=LEPTIN LEPTIN_RESEPTR ORAN_LEPTIN_RECEPTOR /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,03

Group Statistics

	hasta_kontrol	N	Mean	Std. Deviation	Std. Error Mean
LEPTIN	kontrol	33	42.4116	29.21720	5.08606
	hasta	39	46.4371	31.63234	5.06523
LEPTIN_RESEPTR	kontrol	33	34.4920	37.26985	6.48785
	hasta	39	244.3439	11.59635	1.85690
ORAN_LEPTIN_RECEPTOR	kontrol	33	1.7435	1.28579	.22383
	hasta	39	.1913	.13176	.02110

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means
		F	Sig.	t
LEPTIN	Equal variances assumed	.257	.614	-.557
	Equal variances not assumed			-.561
LEPTIN_RESEPTR	Equal variances assumed	3.322	.073	-33.344
	Equal variances not assumed			-31.097
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	105.916	.000	7.502
	Equal variances not assumed			6.904

Independent Samples Test

		t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
LEPTIN	Equal variances assumed	70	.579	-4.02541
	Equal variances not assumed	69.436	.577	-4.02541
LEPTIN_RESEPTR	Equal variances assumed	70	.000	-209.85190
	Equal variances not assumed	37.247	.000	-209.85190
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	70	.000	1.55217
	Equal variances not assumed	32.569	.000	1.55217

Independent Samples Test

		t-test for Equality of Means	
		Std. Error Difference	95% Confidence Interval of the Difference
			Lower
LEPTIN	Equal variances assumed	7.22630	-18.43782
	Equal variances not assumed	7.17806	-18.34365
LEPTIN_RESEPTR	Equal variances assumed	6.29348	-222.40385
	Equal variances not assumed	6.74835	-223.52230
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	.20690	1.13952
	Equal variances not assumed	.22482	1.09454

Independent Samples Test

		t-test for Equality of Means
		95% Confidence Interval of the Difference
		Upper
LEPTIN	Equal variances assumed	10.38699
	Equal variances not assumed	10.29282
LEPTIN_RESEPTR	Equal variances assumed	-197.29994
	Equal variances not assumed	-196.18150
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	1.96483
	Equal variances not assumed	2.00980

```
T-TEST GROUPS=OBEZ(1 2)
  /MISSING=ANALYSIS
  /VARIABLES=LEPTIN LEPTIN_RESEPTR ORAN_LEPTIN_RECEPTOR
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		05-APR-2022 08:40:57
Comments		
Input	Data	C:\Users\selvi.duman\Desktop\AYÇA TEZ\Yeni klasör\ayça tez (1).sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	72
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=OBEZ(1 2) /MISSING=ANALYSIS /VARIABLES=LEPTIN LEPTIN_RESEPTR ORAN_LEPTIN_RECEPTOR /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,00

Group Statistics

	OBEZ	N	Mean	Std. Deviation	Std. Error Mean
LEPTIN	obez	34	53.9661	29.21445	5.01024
	değil	38	36.2048	29.31812	4.75603
LEPTIN_RESEPTR	obez	34	164.5766	106.33713	18.23667
	değil	38	133.4748	109.81889	17.81498
ORAN_LEPTIN_RECEPTOR	obez	34	.9694	1.22957	.21087
	değil	38	.8431	1.12048	.18177

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means
		F	Sig.	t
LEPTIN	Equal variances assumed	.000	.987	2.571
	Equal variances not assumed			2.571
LEPTIN_RESEPTR	Equal variances assumed	1.071	.304	1.218
	Equal variances not assumed			1.220
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	.312	.578	.456
	Equal variances not assumed			.453

Independent Samples Test

		t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
LEPTIN	Equal variances assumed	70	.012	17.76135
	Equal variances not assumed	69.173	.012	17.76135
LEPTIN_RESEPTR	Equal variances assumed	70	.227	31.10180
	Equal variances not assumed	69.547	.227	31.10180
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	70	.650	.12624
	Equal variances not assumed	67.179	.652	.12624

Independent Samples Test

		t-test for Equality of Means	
		Std. Error Difference	95% Confidence Interval of the Difference
			Lower
LEPTIN	Equal variances assumed	6.90951	3.98077
	Equal variances not assumed	6.90813	3.98063
LEPTIN_RESEPTR	Equal variances assumed	25.54041	-19.83695
	Equal variances not assumed	25.49411	-19.75041
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	.27695	-.42612
	Equal variances not assumed	.27840	-.42942

Independent Samples Test

	t-test for Equality of Means
	95% Confidence Interval of the Difference

		Upper
LEPTIN	Equal variances assumed	31.54194
	Equal variances not assumed	31.54208
LEPTIN_RESEPTR	Equal variances assumed	82.04055
	Equal variances not assumed	81.95401
ORAN_LEPTIN_RECEPTOR	Equal variances assumed	.67859
	Equal variances not assumed	.68189

7.2. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1876 **08/05/2020**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Ayça Türer Cabbar)

Yeditepe Üniversitesi Sağlık Bilimleri Fakültesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü , Kardiyoloji AD. Dr. Öğr. Üyesi Ayça Türer Cabbar'ın sorumlu araştırmacı olduğu **''Leptin ve miRNA'nın Koroner Arter Hastalığı ve Yaygınlığı İle İlişkisinin İncelenmesi''** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1849)** kayıtlı Numaralı KAEK Başvuru Dosyası , Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **07.05.2020** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu,yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No:1217)**

Prof. Dr. Turgay Çelik

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

7.3. CURRICULUM VITAE

Personal Informations

Name	Ayça	**Surname**	Türer Cabbar

Education

		Name of Institution	**date of graduation**
PhD			
Master	Kardiyoloji	**Dr. Siyami Ersek Göğüs Kalp Damar Cerrahisi Eğitim ve Araştırma Hastanesi**	2011
University	Tıp	**Ankara Üniversitesi Tıp Fakültesi**	2005
High school	-	**Tarsus Abdul Kerim Bengi Anadolu Lisesi**	1999

Languages	**foreign language (#)**
English	80

Başarılmış birden fazla sınav varsa(KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Work Experience		**Year**
Kardiyoloji Uzman Öğretim Üyesi	Yeditepe Üniversite Hastanesi	2014-
Kardiyoloji Uzman Doktor	Kocaeli Seka Devlet Hastanesi	2012-2014

Computer skills

Program	
Microsoft Office	Good

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

Articles published in journals included in SCI, SSCI, AHCI indexes

1.Turer Cabbar, A., Değertekin, M. M., Şimşek, M. A., Özveren, O., Güleç, S., Yanartaş, M., Gezer Tas, S., Olgun Yıldızeli, Ş., Mutlu, B., İşbir, T., & Yıldızeli, B. (2021). Evaluation of Asymmetric Dimethylarginine Levels in Patients With Chronic Thromboembolic Pulmonary Hypertension Undergoing Pulmonary Endarterectomy. Heart, Lung and Circulation. 2021 Jun 12;S1443-9506(21)00552-7. doi: 10.1016/j.hlc.2021.05.090.
2.Turer Cabbar, A. (2021). Left Ventricular Systolic Dysfunction due to Hydroxychloroquine Toxicity. American Journal of Therapeutics. doi: 10.1097/MJT.0000000000001194
3.Turer Cabbar, A., (2020). The electromechanical association artifact with limb leads electrodes placed upon the torso. Acta Cardiologica Sinica, 36(6), 681.
4. Turer Cabbar, A., Hünük, B., & Ünverengil, G. (2020). Early Detection of Subclinical Cardiac Involvement with Novel Cardiac Imaging Modalities in a Patient with Becker Muscular Dystrophy. Acta Cardiologica Sinica, 36(5), 528.
5.Aslanger, E., Yıldırımtürk, Ö., Şimşek, B., Sungur, A., Turer Cabbar, A., Bozbeyoğlu, E., Karabay, C. Y., Smith, S. W., & Değertekin, M. (2020). A new electrocardiographic pattern indicating inferior myocardial infarction. Journal of electrocardiology, 61, 41-46.
6.Yildirim, E., & Turer Cabbar, A. (2019). Association between copeptin and contrast-induced nephropathy in patients with ST-elevation myocardial infarction. Revista Portuguesa de Cardiologia, 38(12), 873-879.

National Articles

1.Türer Cabbar, A., Şimşek, M. A., Hünük, B., Özveren, O., Güzelburç, Ö., Değertekin, M. M. (2021). Akut koroner sendrom olmayan tïp 2 dïyabetïk hastalarda koroner arter hastaliği yayginliği ile mpv ilişkisinin araştirilmasi. Evaluation of the relationship between mpv and severity of coronary artery disease in type 2 diabetic patients without acute coronary syndrome. Medical Journal of Suleyman Demirel University, 28(3), 411-417
2.Türer Cabbar, A., & Eren, M. (2020). ST Segment yükselmeli miyokardial enfarktüs ile başvuran hastalarda asimetrik dimetilarjinin düzeyinin kisa ve uzun dönem prognoz ile ilişkisi. Namık Kemal Tıp Dergisi, 8(2), 225-233.

Internatioanal Oral Presentations

1.Turer Cabbar A, Değertekin MM. Is there a relation between corrected QT and slow coronary flow phenomenon. 16th International Congress of Update in Cardiology and Cardiovascular Surgery, 30 October-1 November, Turkey, 2020.
2.Özveren O, Turer Cabbar A, Güzelbey T. Changes in left atrium with cardiac iron overload in beta-thalassemia major patients without ventricular systolic dysfunction. 17th International Congress of Update in Cardiology and Cardiovascular Surgery, 5-7 November, Turkey, 2021.
3.Akdeniz FT, Türer Cabbar A, Güleç Yılmaz GS, Fatlawi M, Çapar B, Dalan AB, İsbir T. Association of tumor necrosis factor (TNF) alpha and beta polymorphism in coronary artery disease. 7th International Multidisciplinary Congress on Good Medical Research, 24-31 October, 2020. (Özet Bildiri/Sözlü Sunum)(Yayın No:6401457)
4.Güleç yılmaz GS, Türer Cabbar A, Marsomy OA, Akdeniz FT, Sümerkent K, Barut Z, Isbir T. Effects of interleukin 1 beta gene polymorphisim in patients with atherosclerosis. 7th International Multidisciplinary Congress on Good Medical Research, 24-31 October, 2020. (Özet Bildiri/Sözlü Sunum)(Yayın No:6401543)

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)
