جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية الطب

الأهمية التشخيصية (MiR-1,MiR-145)في الدم و أهمية التباين النمطي الجيني SIRT3 في تقبل المرض في مرضى احتشاء العضلة القلبية

اطروحة مقدمة
الى مجلس كليه الطب / جامعة القادسية
وهي جزء من متطلبات نيل درجة دكتوراة فلسفة في علم الاحياء المجهرية الطبية

من قبل
علاوي جواد حسن ألعبيدي
بكالوريوس علوم –علوم حياة/جامعة بابل 1997
ماجستير أحياء مجهرية طبية/جامعة القادسية 2012

بأشراف
ا.م.د. إبراهيم عبد المجيد التميمي
ا.د. عقيل رحيم البرقعاوي

٢٠١٨ م
١٤٤٠هـ

Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Al-Qadisiyah
College of Medicine

Diagnostic Importance of Circulating (miR-1, miR-145) and Disease Susceptibility Value of SIRT3 Gene Polymorphism in Acute Myocardial Infarction

A thesis
Submitted to the Council of the College of Medicine / University of Al-Qadisiyah in Partial Fulfillment of the Requirements for the Degree of Philosophy Doctorate in Medical Microbiology

By
Allawi Jawad Hassan Al-Obaidi
B.Sc. Biology. Babylon University (1997)
M.Sc. Medical Microbiology Al-Qadisiyah University (2012)

Supervised by

Assist. Prof. Dr. Ibrahim A. Altamemi

Prof. Dr .Aqeel R. Al-brakawi

2018 A.D. **1440 A.H**

Supervisor's Certificate

I certify that this thesis (**Diagnostic importance of circulating (miR-1, miR-145), and disease susceptibility value of the SIRT3 Gene in Acute myocardial infarction**) has been prepared under my supervision at the Department of Microbiology, College of Medicine/ University of Al-Qadisiyah, as a partial fulfillment of the requirements of the degree of Doctorate of Philosophy of science in Medical Microbiology.

Signature
Assistant Professor
Dr. Ibrahim Abdul-majeed Altamemi
Supervisor
Department of Medical Microbiology

Signature
Professor
Dr .Aqeel R. Al-brakawi
Supervisor
Department of Internal medicine

College of Medicine/ University of Al-Qadisiyah
Date: / / 2018

Recommendation of Head of Medical Microbiology Department

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature

Professor

Dr. Adnan H. Al-Hamadany

Head of Medical Microbiology Department

College of Medicine/ University of Al-Qadisiyah

Date: / / 2018

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ)

سورة فصلت الاية(٥٣)

صدق الله العلي العظيم

DEDICATION

TO MY PARENTS

TO MY WIFE

TO MY FLOWERS, MY DAUGHTER ASEEL AND MY BOY MUJTABA

Acknowledgements

First of all, I would like to thank the grace of God that has seen me during the completing this work .

I would like to thank my supervisor Assist. Prof. Dr. Ibrahim Abdul-majeed Altamemi , for his ongoing support, advice and encouragement throughout this project. I was privileged to have Prof. Dr .Aqeel R. Al-brakawi, (Internal medicine consultant / College of Medicine) to guide me through this thesis and give me the confidence to believe in my ideas.

I would like to express my special thanks to the dean of college of medicine /Al-Qadisyia University and special thanks to Professor Dr. Adnan Alhamadani , Head of Medical Microbiology & Parasitology Dept. / College of Medicine .

My great gratitude should go to my colleagues and all of the staff in the laboratories and coronary care unit staffs of Al-Diwaniyha Teaching Hospital. Thanks for all those who participated in this thesis, by their support and assistance.

My deepest appreciation to the patients who expressed their assistance and made this work possible.

I am grateful to my Wife, my family , my friends and colleagues who encouraged and supported me at different times during this process.

Summary

Myocardial infarction (MI) is the most common cause of cardiac injury, and subsequent reperfusion further enhances the activation of innate and adaptive immune responses and cell death programs.

There are many genetic factors which are makes an individual more susceptible to Myocardial infarction occurrence such as SIRT3 gene.

Recent studies were included SIRT3 gene polymorphism because it has important function in MI , SIRT3 regulates the enzymatic activity of the key enzymes of oxidative phosphorylation via deacetylation, there by regulating mitochondrial energy metabolism.

Myocardial infarction managements must be quickly and the time of MI diagnosis is necessary for treatment .there are many biomarkers used in MI diagnosis but not MI specific biomarker as troponine which is a good biomarker but after myocytes necrosis occur therefore other the recent studies are suggest using miRNAs as MI biomarker. MiRNAs are endogenous, noncoding, single-stranded RNAs of ~22 nucleotides and constitute a novel class of gene regulators.

The aim of present study is to find out a non invasive diagnostic miRNA biomarker (miRNA-1,miRNA-145) for myocardial infarction with high sensitivity and specificity and to study if there is a role for SIRT3 gene polymorphism in Myocardial infarction susceptibility. To achieve this goal ,a blood sample were collected from three groups. The first group was 24 patients with myocardial infarction which include (16male and 8female) ,who were observation in coronary Care Unit of Al-Diwaniyha teaching hospital , Second group was include 24 patients who have risk factor for MI (Hypertension, Hyperlipidemia, Diabetes mellitus), Third group was include 19 healthy volunteers(non coronary

artery diseases). Blood sample were collected by venipuncture from these groups (five millimeter of venous blood). Each blood sample of three groups were collected to 2 ml of blood collected directly in a sterile tube containing EDTA for DNA extraction ,(Amplification refractory mutation system)ARMS-method application to studySIRT3 gene polymorphism (**rs56312618**; **rs12293349** ;**rs11246029**), 3 ml were collected in EDTA free plane tube and allowed to clot then serum has been separated in Eppendrof tube then use for miRNA-1and miRNA-145 qPCR

Regarding **to** gender distribution about (66.7%)of MI patients were male, and (33.3%)MI patients were female, while the risk group included (41.7%) male and (58.3%) female and the control group included (68.4%) male and (31.6%) women. There was no significant difference in mean age among the three groups regarding distribution of patients according to gender (P=0.121), which ensures gender match that is mandatory for such a study According to result of present work.

There were only significant correlation concerning **rs11246029** genotype was seen in **CC** variant between MI and control group with an EF= 0.56. While in case of **rs12293349** genotype the significant difference was seen in **CT** variant, between MI and control (p=0.05) group with an EF= **0.55**.The **rs56312618** genotype show significant difference in **AA** variant, between MI and control group(p=0.027), also with EF=0.59. while of miRNA-145 fold change was significantly lowest in the Myocardial infarction group followed by risk group and then by control group (P<0.05).

Receiver operator characteristic (ROC) analysis was done in order to find sensitivity and specifity ; the cut off value was identified at miRNA-1 of >5.28 fold change with a sensitivity of 91.67 % and a specificity of

90.7%,while the cut off value of miRNA-145 has cut off ≤0.7 fold change with a sensitivity of 95.83 % and a specificity of 89.47% .Concerning the prognostic value of above miRNA (ROC) analysis , the miNAR-1 have predictive value of MI recurrence with sensitivity and specificity(40%,89.47%) respectively. while, miRNA-145 have a predictor of recurrence of MI with sensitivity and specificity (40% , 94.74 %) respectively.

Conclusion: The individuals with SIRT3 gene promoter; rs56312618 genotype(AA variant) ;rs12293349 genotype (CT variant);rs11246029 (CC variant) were seem at risk for myocardial infarction.

The characteristic of (miR-1,miR145) and theirs high sensitivity and Specificity in this study bush to use them as alone biomarker or supported for Other biomarker in AMI diagnosis .

In addition miRNA-1, miRNA-145 can be using as biomarkers in Myocardial infarction prognosis interpretation but with a caution.

Present study didn't find any significant associated between (miRNA-1 or miRNA-145) and SIRT3 gene polymorphism.

There was negative correlation between miRNA-1 and miRNA-145 ($r = -0.501$, $P<0.001$).

List of Abbreviation

ACS	Acute coronary syndrome
AUC	Accuracy
AP1	Activator protein 1
AMI	Acute Myocardial infarction
ATP	Adinine triphosphate
ARMS	Amplification refractory mutation system
BNP	B-type natriuretic peptide
CCU	Central care unite
cDNA	Complementary DNA
CAD	Coronary artery diseases
CRP	C-reactive protein
CK	Creatine phosphokinase
CypD	Cyclophilin D
dNTP	Deoxyribonucleotide triphosphate
DM	Diabetes mellitus
DEPC	Diethyl pyrocarbonate
EF	Effect fraction
E F	Effect fraction
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
F	Forward
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDF-15	Growth-differentiation factor-15
H-FABP	Heart fatty acid-binding protein
HSP60	Heat shock protein 60
hnRNPA2B1	heterogeneous nuclear ribonucleoprotein
HDL	High density lipid
HDL	high-density lipoprotein
IL	Interleukin
KCNJ2	Inward-rectifier potassium ion channel
IMA	Ischemia-modified albumin
IHD	Ischemic heart diseases
IDH2	Isocitrate dehydrogenase 2

MnSOD	The mitochondrial antioxidant manganese superoxide dismutase
miRNA (miR)	Micro Ribose Nucleic acid
MGB	Minor groove binder
MPO	Myeloperoxidase
MI	Myocardial infarction
MYO	Myoglobin
NCBI	National center of biotechnology information
NAD	Nicotinamide adenine dinucleotide
NF-κB	Nuclear Factor κB
OD	Odd Ratio
OpA1	Optic atrophy 1
PCV	packed cell volume
PTX-3	Pentraxin 3
mPTP	Permeability transition pore
PAPPA	Pregnancy-associated plasma protein A
P F	Probability fraction
PDCD4	Programmed cell death protein 4
PKCe	Protein kinase C epsilon type
ROS	Reactive oxygen species
RT-qPCR	Real time- quantitative polymerase chain reaction
ROC	Receiver operating characteristic curves
rs	Reference sequence
FAM	Reporter dye
R	Reverse
SNPs	Single nucleotide polymorphisms
sCD40L	Soluble cluster of differentiation 40 ligand
STEMI	ST elevation MI
SOD2	Superoxide dismutase 2
cTns	Troponin c
TNF- α	Tumor necrosis factor-α
VSMCs	Vascular smooth muscle cells
WBC	White blood cells
WHO	World health organization

contents

List of Tables

List of Figures

List of appendices

Chapter One

Introduction

1 Introduction

Acute myocardial infarction resulting from the rupture or erosion of an atherosclerotic plaque with thrombotic occlusion of an epicardial coronary artery(Ai, Zhang et al. 2010) Myocardial It is the most prevalent cause of death(Nymark 2015). The European Society of Cardiology guidelines suggest that every sixth male and every seventh woman in Europe will die due to an AMI (Cardiology 2012). The Known MI risk factors include hypertension, smoking, diabetes, hyperlipoproteinemia , hypercholesterolemia and genetic factors as PCSK9, SORT1, MIA3, WDR12, MRAS,SIRT3 gene and others genes . SIRT3 gene controls the flow of mitochondrial oxidative pathways and reactive oxygen species (ROS) production, ROS generation and mitochondrial functions, leading to death of cardiomyocytes(Sun, Liu et al. 2018). Though genome-wide association studies have identified more than 50 genetic loci to CAD, these genetic variants account for only 10% of cases(Roberts 2014, Ozaki and Tanaka 2016). To date, genetic causes and underlying molecular mechanisms for CAD remain largely unclear. It has been hypothesized that low frequency and rare variants with large effects may account for some of the missing heritability for CAD(Yin, Pang et al. 2016). There are twenty-three DNA sequence variants (DSVs) are identified with SIRT3 gene promoter ,some this variant was significantly associated with an increased susceptibility for coronary artery diseases (Yin, Pang et al. 2016).SIRT3 gene promoter Polymorphisms can be used to Prediction of the risk of Myocardial Infarction (Matsushima and Sadoshima 2015).

Myocardial Infarction diagnosis is based on clinical history, electrocardiogram (ECG), and enzyme quantifications in blood and postmortem outcomes(Mendis, Thygesen et al. 2010). Creatine kinase–MB isoenzymes, cardiac myoglobin, and troponins are current AMI biomarkers that have been commonly used in clinical diagnosis(Wang, Zhu et al. 2010). The gold standard for diagnosing AMI is still cardiac troponin I (cTnI), but its plasma concentrations are not very sensitive and accurate. A false positive result might appear in non-cardiac diseases such as chronic kidney disease, severe sepsis and septic shocks(Jaffe and Apple 2000). Thus detecting specific and accurate biomarkers is a necessity for an optimal diagnosis, prognosis, prevention and treatment in MI.

Many recent studies have reported the existence and the prominence of circulating miRNAs in the plasma. Those miRNAs are stable since they are protected in microparticles (including exosomes, microvesicles, and apoptotic bodies) and can form miRNA-protein complexes that are resistant to degradation (Arroyo, Chevillet et al. 2011, Creemers, Tijsen et al. 2012). In addition, circulating miRNAs are highly specific and selective with prolonged half-life, and are able to differentiate between pathologies. Thus, circulating miRNAs fulfill a number of criteria that allow them to be considered as ideal clinical biomarkers that can be quantified by real-time- PCR or microarrays(Creemers, Tijsen et al. 2012).thus, miRNAs were included in this study.

Some studies have shown that the expression of miR-1 is down-regulated in myocardial infarction (Sayed, Hong et al. 2007, Li, Song et

al. 2010). However, in other cardiovascular experimental models, reports a expression of miR-1 is up-regulated(Bostjancic, Zidar et al. 2010, Ai, Zhang et al. 2012). Other study suggests that miR-145 could be a valuable biomarker for cardiovascular diseases(Weber, Baker et al. 2011, Samples 2012, Wei, Nazari-Jahantigh et al. 2013).

Thus ,according to such above ,the aim of present study is to find out a non invasive diagnostic miRNA biomarker for myocardial infarction with high sensitivity and specificity and to study if there is a role for SIRT3 gene polymorphism in in MI susceptibility this aim achieve through following objectives.

- Study (**rs56312618** ; **rs12293349** ;**rs11246029** SIRT3 gene promoter variant among MI patients by ARMC-PCR technique to find out which of them have valuable role in disease susceptible .
- Measuring fold change of circulating miRNAs (miRNA-, miRNA-145)in MI patient by RT-PCR and find out its sensitivity and specificity by ROC analysis .

Chapter Two

Literatures Review

2.1 Myocardial infarction definition

Myocardial infarction (MI) is multifactorial, progressive, and complex disease, means that part of the heart muscle suddenly loses its blood supply, Without prompt treatment, this can lead to damage to the affected part of the heart. It sometimes called a heart attack or coronary thrombosis(Mythili and Malathi 2015). state that a myocardial infarction has occurred when two of the following three criteria are met: symptoms consistent with ischemia, or decreased blood flow to the heart; changes in an Electrocardiogram; and elevated enzymes—most commonly, one called CK-MB(Smith, Saunders et al. 2013).

September 2000, widely accepted definition of myocardial infarction was introduced—a definition developed by a joint committee of the European Heart Society and the American College of Cardiology. The most significant change is that the definition adds cardiac troponin, a protein found only in heart muscle tissue, to the measures already used to determine whether or not a myocardial infarction has occurred(Mythili and Malathi 2015).

2.2 Clinical Classification of Myocardial Infarction

Clinical Classification of Myocardial Infarction For the sake of immediate treatment strategies, such as reperfusion therapy, it is usual practice to designate MI in patients with chest discomfort, or other ischaemic symptoms that develop ST elevation in two contiguous leads, as an 'ST elevation MI' (STEMI). In contrast, patients without ST elevation at presentation are usually designated as having a 'non-ST elevation MI' (NSTEMI). Many patients with MI develop Q waves (Q wave MI), but others do not (non-Q MI). Patients without elevated biomarker values can be diagnosed as having unstable angina. In addition

to these categories, MI is classified into various types, based on pathological, clinical and prognostic differences, along with different treatment strategies(Thygesen, Alpert et al. 2013).

Asymptomatic patients who develop new pathological Q wave criteria for MI detected during routine ECG follow-up, or reveal evidence of MI by cardiac imaging, that cannot be directly attributed to a coronary revascularization procedure, should be termed 'silent MI' (Toma, Fu et al. 2010). In studies, silent Q wave MI accounted for 9–37% of all nonfatal MI events and were associated with a significantly increased mortality risk (Burgess, Hunt et al. 2009). Improper lead placement or QRS confounders may result in what appear to be new Q waves or QS complexes, as compared to a prior tracing. Thus, the diagnosis of a new silent Q wave MI should be confirmed by a repeat ECG with correct lead placement, or by an imaging study, and by focused questioning about potential interim ischemic symptoms(Thygesen, Alpert et al. 2012).

2.3 Epidemiology

The true natural history of myocardial infarction is hard to establish for a number of reasons: the common occurrence of silent infarction, the frequency of acute coronary death outside hospital and the varying methods used in the diagnosis of the condition(Bueno, Caforio et al. 2017). Community studies(Roger 2015) have consistently shown that the overall fatality of acute heart attacks in the first month is between 30% and 50%, and of these deaths about one-half occur within the first 2 h(Roger 2015). This high initial mortality seems to have altered little over the last 30 years, By contrast with community mortality, there has been a profound fall in the fatality of those treated in hospital. Prior to the introduction of coronary care units in the 1960s, the in-hospital mortality

seems to have averaged some 25–30%. A systematic review of mortality studies in the pre-thrombolytic era of the mid-1980s showed an average fatality of 18%(Members, Steg et al. 2012). With the widespread use of fibrinolytic drugs, aspirin and coronary interventions the overall 1-month mortality has since been reduced to 6–7%, at least in those who participate in large-scale trials and qualify for fibrinolysis, aspirin and/or coronary interventions. In European Heart Survey, mortality in patients presenting with ST-segment elevation acute coronary syndromes was 8.4% at 1 month. The WHO-MONICA investigators convincingly demonstrated that, also at the population level, the introduction of new treatments for coronary care was strongly linked with declining coronary event rates and 28-day case fatality(Members, Steg et al. 2012).

Worldwide, Myocardial infarction is a common presentation of coronary artery disease, The World Health Organization estimated in 2017, that 12.2% of worldwide deaths were from ischemic heart disease(Mestl and Edwards 2011). AMI is the most prevalent cause of death(Nymark 2015). with it being the leading cause of death in high- or middle-income countries and second only to lower respiratory infections in lower-income countries(Mestl and Edwards 2011). Ischemic heart diseases IHD is becoming a more common cause of death in the developing world. In Iraq WHO2016 reports 33% death because of cardiovascular diseases(Organization 2016).

2.4Etiology and risk factors

There are some etiology and risk factors which can make a person to be more susceptible to heart attack.

1. High blood pressure (normal blood pressure is 120/80 mm Hg and if the number is increasing, it can create a risk for heart problems).

Controlling hypertension by using appropriate medication (Lanas, Avezum et al. 2007).

2. Dyslipidemia as High triglyceride and hyperlipidemia (high cholesterol levels, LDL or triglyceride puts you at risk for AMI). The triglyceride is a fatty substance that is stored in some foods and after you eat it, can stay in your fat cells or arteries and cause heart problems (Smith, Allen et al. 2006).

3. Diabetes or high blood sugar .people with diabetes have a bigger risk of acute myocardial infarction (AMI) because it increase the rate of atherosclerotic progression which has a bad impact on lipid profile, and high blood sugar may damage blood vessels and nerves which contribute to risk of coronary heart disease and heart attacks.(Chowdhury, Warnakula et al. 2014).

4. Obesity (according to many studies it enriches a possibility of heart attack)(Lanas, Avezum et al. 2007).

5. Tobacco use tobacco is known as one of the damagers of blood vessel walls and the body's response to it is the formation of atherosclerosis and trigger risk of AMI . It may also cause other cardiovascular diseases (Kivimäki, Nyberg et al. 2012).

6. Male gender the studies showed that AMI risk is higher in men than women in all age groups (Srivastava, Tiwari et al. 2014).

7. Genetic susceptibility of myocardial infarction.

However, there are other risk factors for AMI as lack of exercises, unhealthy diet, excessive alcohol, and stress (Rosengren, Hawken et al. 2004, Yusuf, Hawken et al. 2004, Steptoe and Kivimäki 2012) .

2.5 Myocardial infarction Pathogenesis

An acute coronary syndrome is nearly always caused by a sudden reduction in coronary blood flow caused by atherosclerosis with thrombosis superimposed, with or without concomitant vasoconstriction (Davies 2000). The clinical presentation and outcome depend on the location of the obstruction and the severity and duration of myocardial ischaemia (Galaup and Germain 2012). In myocardial infarction with ST segment elevation, occlusive and persistent thrombosis prevails(Skyschally, Schulz et al. 2008). About 2/3 to 3/4 of fatal coronary thrombi are precipitated by sudden rupture of a vulnerable plaque (inflamed, lipid-rich plaque covered by a thin fibrous cap) (Falk, Shah et al. 1995, Skyschally, Schulz et al. 2008). Other poorly defined mechanisms such as plaque erosion account for the rest. As many as 3/4 of all infarctrelated thrombi appear to evolve over plaques causing only mild-to-moderate stenosis prior to infarction and after thrombolysis(Falk, Shah et al. 1995, Jennings 2013).

However, severe stenoses are more likely to undergo plaque events leading to infarction than mild ones(Alderman, Corley et al. 1993). Myocardial infarction caused by complete coronary artery occlusion begins to develop after 15–30 min of severe ischaemia (no forward or collateral flow) and progresses from the subendocardium to the subepicardium in a time-dependent fashion the wave-front phenomenon)(Jennings 2013). Reperfusion, including recruitment of collaterals, may save myocardium at risk from undergoing necrosis, and subcritical but persistent flow may extend the time-window for achieving myocardial salvage by complete reperfusion(Schömig, Mehilli et al.

2005). The thrombotic response to plaque disruption is dynamic: thrombosis and thrombolysis, often associated with vasospasm, occur simultaneously, causing intermittent flow obstruction and distal embolization (Erbel and Heusch 2000). The latter leads to microvascular obstruction which may prevent successful myocardial reperfusion despite a patent epicardial infarctrelated artery (Topol and Yadav 2000). In coronary thrombosis, the initial flow obstruction is usually due to platelet aggregation, but fibrin is important for the subsequent stabilization of the early and fragile platelet thrombus (Van de Werf, Ardissino et al. 2003). Therefore, both platelets and fibrin are involved in the evolution of a persisting coronary thrombus(Van de Werf, Ardissino et al. 2003).

2.6 Clinical Features

The term myocardial infarction reflects cell death of cardiac myocytes caused by ischaemia, which is the result of a perfusion imbalance between supply and demand(Mendis, Thygesen et al. 2010). Ischaemia in a clinical setting most often can be identified from the patient's history and from the ECG. Possible ischaemic symptoms include various combinations of chest, upper extremity, jaw, or epigastric discomfort with exertion or at rest(Cutlip, Windecker et al. 2007). The discomfort associated with acute myocardial infarction usually lasts at least 20 min. Often, the discomfort is diffuse, not localized, not positional, not affected by movement of the region, and it may be accompanied by dyspnoea, diaphoresis, nausea, or syncope. These symptoms are not specific to myocardial ischaemia and can be misdiagnosed and thus attributed to gastrointestinal, neurological, pulmonary, or musculoskeletal disorders. Myocardial infarction may occur with atypical symptoms, or even

without symptoms, being detected only by ECG, biomarker elevations, or cardiac imaging (Cutlip, Windecker et al. 2007).

2.7 Genetic susceptibility of myocardial infarction:

Beyond the well-recognized clinical and environmental risk factors, studies point to a substantial heritability for ischemic stroke (Lanktree, Dichgans et al. 2010) . However, elucidation of genetic risk factors for stroke remains a challenge. genome-wide association studies (GWAS) have begun to identify several promising susceptibility loci (Bellenguez, Bevan et al. 2012, Malovini, Bellazzi et al. 2016) . In addition, a large number of common susceptibility variants have been identified by GWAS targeting epidemiologically proven stroke risk factors. Though genome-wide association studies have identified more than 50 genetic loci to coronary artery diseases(CAD) , these genetic variants account for only 10% of cases (Malovini, Bellazzi et al. 2016, Ozaki and Tanaka 2016). To date, genetic causes and underlying molecular mechanisms for CAD remain largely unclear. It has been hypothesized that low frequency and rare variants with large effects may account for some of the missing heritability for CAD. Recently, epigenetic factors have been suggested to contribute to aging and age-associated diseases (Perridon, Leuvenink et al. 2016).

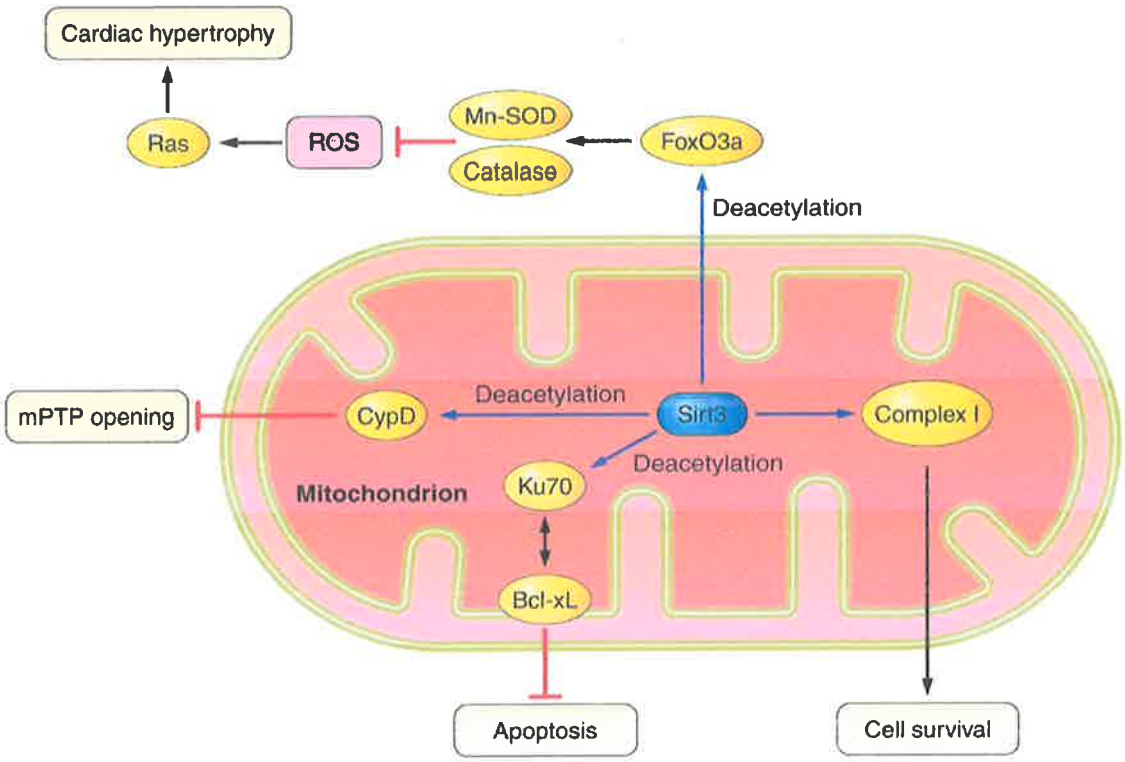
2.7.1 SIRT3 gene, function and Signaling pathways

More than 90% of ATP in the normal myocardium is derived from mitochondrial oxidative phosphorylation, followed by anaerobic glycolysis of glucose. Fatty acid beta oxidation is the main source of mitochondrial oxidative phosphorylation, followed by glucose, lactic acid, and ketone body aerobic oxidation(Stanley, Recchia et al. 2005). SIRT3 regulates the enzymatic activity of the key enzymes of oxidative phosphorylation via deacetylation, thereby regulating mitochondrial

energy metabolism . SIRT3 is a NAD+-dependent protein deacetylase that is a member of the silent information regulator 2 (SIR2) family(Giralt and Villarroya 2012). SIRT3 can exert controls on a wide range of important biological activities including regulation of nuclear gene expression , metabolic control(Shi, Wang et al. 2005), neuro protection(Kong, Wang et al. 2010), cardiovascular disease, cancer(Alhazzazi, Kamarajan et al. 2011), and aging(Bellizzi, Rose et al. 2005). SIRT3 is the only sirtuin protein reported to affect human lifespan(Kong, Wang et al. 2010);(Brown, Xie et al. 2013). As it is localized exclusively in mitochondria. SIRT3 influences energy metabolism processes (e.g., tricarboxylic acid cycle, respiratory chain, fatty acid b-oxidation, and ketogenesis) by targeting the responsible enzymes (Giralt and Villarroya, 2012). It also controls the flow of mitochondrial oxidative pathways and, ultimately, the rate of reactive oxygen species (ROS) production.

the relationship between SIRT3 and apoptosis is not clear, and whether SIRT3 promotes or inhibits apoptosis is still controversial(Samant, Zhang et al. 2014). However, SIRT3 was shown to mainly inhibit cardiomyocyte apoptosis in most studies. As mentioned previously, SIRT3 can inhibit apoptosis by regulating oxidative stress(Samant, Zhang et al. 2014). In addition, SIRT3 also inhibits apoptosis by the following route: first, SIRT3 deacetylates and activates optic atrophy 1 (OPA1). Loss of OPA1 impairs mitochondrial fusion, perturbs cristae structure, and increases the susceptibility of cells toward apoptosis (Signorile, Santeramo et al. 2017)Second, SIRT3 activates Ku70 by deacetylation. The activated Ku70 binds to Bax, which inhibits Bax-induced cardiomyocyte apoptosis(Sundaresan, Samant et al. 2008). Third, SIRT3 deacetylates cyclophilin D and closes the mitochondrial

permeability transition pore (mPTP) to maintain the normal morphology of mitochondria, thereby inhibiting apoptosis(Cheng, Yang et al. 2016). Permeability transition pore (mPTP) can lead to mitochondrial dysfunction. Note that mPTP is regulated by cyclophilin D (CypD) which is deacetylated by SIRT3, The absence of SIRT3 in cardiac muscle becomes a stimulus to increase the opening of mPTP (Hafner, Dai et al. 2010). Signaling pathways of Sirt3 in the heart is regulates Mitochondrial permeability transition pore (mPTP) opening, apoptosis, and cell survival, mainly through deacetylation of mitochondrial proteins, including cyclophilin D (CypD), Ku70, and complex I, independently of transcription. Sirt3 also deacetylates FoxO, leading to transcriptional upregulation of MnSOD and catalase and inhibition of cardiac hypertrophy(Pillai, Sundaresan et al. 2010).



Figure(2- 1)Signaling pathways of Sirt3 in the heart (Pillai, Sundaresan et al. 2010)

In human endothelial cells, SIRT3 mediates the cellular response to hypoxia and protects the cells from high glucose-induced cytotoxicity (Tseng, Wu et al. 2014, Liu, Cao et al. 2015). SIRT3 has recently been reported to be involved in inflammation as well as platelet aging and thrombosis(Barger, Anderson et al. 2015, Kumari, Chaurasia et al. 2015). In addition, overexpressed SIRT3 enhances autophagy, which plays important role in myocardial infarction (Liang, Benavides et al. 2013, Mei, Thompson et al. 2015).

2.7.2 SIRT3 Gene Polymorphism and MI Susceptibility

The human SIRT3 gene is localized to the chromosome 11p15.5, and encodes an NAD-dependent mitochondrial deacetylase of 399-amino acids containing an N-terminal mitochondrial targeting signal and a central catalytic domain(Schwer, North et al. 2002). SIRT3 gene is expressed in a variety of tissues with higher expression in adipose tissue, brain and heart in embryos and adults (NCBI Unigene EST Profile Viewer), indicating the tissue-specific regulation of the SIRT3 gene expression. The human SIRT3 gene promoter contains high GC contents and lacks the TATA box sequence and there are binding sites for activator protein 1 (AP1), GATA-binding factor, nuclear Factor κB (NF-κB) and transcription factor ZF5, as well as multiple specificity protein 1 (SP1) binding sites(Bellizzi, Dato et al. 2007). Nuclear respiratory factor 2, a transcription factor that regulates mitochondrial genes, binds to the promoter of SIRT3 gene and induces its expression (Satterstrom, Swindell et al. 2015).

There are twenty-three DNA sequence variants (DSVs) are identified with SIRT3 gene promoter , rs71019893 , rs3817629, rs56312618,

rs1045288, rs12293349, rs369344513, rs2272563, rs369178836 rs185277566 and rs71019893(Yin, Pang et al. 2016).

However, molecular mechanisms by which SIRT3 gene expression is changed have not been reported. speculated that the DNA sequence variants within the regulatory regions of the SIRT3 gene may account for the changed SIRT3 gene expression. The DNA sequence variants and SNPs of the SIRT3 gene promoter identified in MI patients may alter transcriptional activity of SIRT3 gene promoter and change SIRT3 level, contributing to the MI development as a risk factor. The investigation into the molecular mechanisms by which SIRT3 gene promoter activity are affected. (Yin, Pang et al. 2016).

A series of downstream substrates of SIRT3, as well as histone, have been identified. SIRT3 deacetylates and activates several enzymes that are critical in maintaining cellular ROS levels and promote resistance to oxidative stress, including superoxide dismutase 2 (SOD2) and isocitrate dehydrogenase 2 (IDH2)(Merksamer, Liu et al. 2013). In experimental animals, SIRT3 deacetylates FOXO3a, a transcription factor that upregulates SOD2 and catalase, and decreases ROS levels(Sundaresan, Gupta et al. 2009). Increased ROS levels and oxidative stress have been demonstrated to contribute to the atherogenesis(V Goncharov, V Avdonin et al. 2015). In human endothelial cells, SIRT3 deacetylates and stabilizes FOXO3 to protect mitochondria against oxidative stress, and activates mitochondrial aldehyde dehydrogenase 2, a key enzyme to remove reactive aldehydes(Samant, Zhang et al. 2014). acyl-CoA dehydrogenase, a key mitochondrial fatty acid oxidation enzyme(Alrob, Sankaralingam et al. 2014). SIRT3 regulates fatty acid oxidation through heat shock protein 10(Lu, Chen et al. 2015). In human diploid fibroblasts, SIRT3 overexpression antagonizes high glucose-induced cellular senescence via

the SIRT3-FOXO1 signaling pathway(Zhang, Cui et al. 2013). A recent study has shown that SIRT3 targets human very long-chain acyl-CoA dehydrogenase, a key fatty acid oxidation enzyme(Verdin, Zhang et al. 2015). Therefore, decreased SIRT3 levels may contribute to MI development by: 1) affecting lipid metabolism, inflammation and other pathways, initiating the atherosclerosis; and 2) interfering with fatty acid oxidation, ROS generation and mitochondrial functions, leading to death of cardiomyocytes. Exact molecular mechanisms need further investigated and elucidated(Verdin, Zhang et al. 2015).

2.8 MI Diagnostic criteria

2.8.1 Clinical presentation

The general appearance of patients may vary according to the experienced symptoms; the patient may be comfortable, or restless and in severe distress with an increased respiratory rate. A cool and pale skin is common and points to vasoconstriction. Some patients have low-grade fever (38–39 °C). Blood pressure may be elevated or decreased, and the pulse can become irregular(Joshi 2009)

2.8.2 Electrocardiogram (ECG):

The primary purpose of the electrocardiogram is to detect ischemia or acute coronary injury in broad, symptomatic emergency department populations. A serial ECG may be used to follow rapid changes in time. The standard 12 lead ECG does not directly examine the right ventricle, and is relatively poor at examining the posterior basal and lateral walls of the left ventricle. In particular, acute myocardial infarction in the distribution of the circumflex artery is likely to produce a nondiagnostic ECG(Cannon 1998).

2.8.3 Routin Labortory diagnosis

2.8.3.1 Serum Biomarker

The early recognition of cardiac ischemia and accurate placement of the patient in the risk spectrum of the acute coronary syndrome are critical to the effective management of patients with acute myocardial infarction (AMI). Apart from clinical history, physical examination and accurate ECG interpretations, cardiac biomarkers are equally valuable in the initial evaluation of patients with nontraumatic chest pain. Previously the diagnosis of an AMI was based on World Health Organisation (WHO) criteria which defined MI as the presence of two out of three characteristics comprising: symptoms of acute ischemia (chest pain), development of Q waves in ECG and elevated activities of traditional serum enzymes comprising: total CK, CK-MB, ASAT and LDH.there are many serum biomarkers using with routine myocardial infarction diagnosis which are following :

Troponins are a complex of three protein subunits, namely troponin C, troponin T and troponin I, located on the thin filaments of the skeletal and cardiac muscle fibers. Troponin C is the calcium-binding component, troponin T is the tropomyosin-binding component and troponin I is the inhibitory component. As the isoforms of troponin C is identical in the skeletal and cardiac muscle, troponin C is not extremely specific for myocardial injury (Mythili and Malathi 2015). The isoforms of troponin T and troponin I differ in the skeletal and the cardiac muscle, and thus are extremely specific for cardiac tissue necrosis(Apple 1999). Troponin T is present chiefly in the bound form to the contractile elements of the myocardial cells; however, it is also present free in the cytoplasm. Troponin T exhibits a dual release initially of the cytoplasmic component and later of the bound component (Katus, Remppis et al. 1991). Troponin

I is extremely specific for the cardiac muscle and has not been isolated from the skeletal muscle. This absolute specificity makes it an ideal marker of myocardial injury (Higgins and Higgins 2003). They are released into the circulation 6-8 h after myocardial injury, peak at 12-24 h and remain elevated for 7-10 days(Tucker, Collins et al. 1997). The only disadvantage of cTn is the late clearance that makes it difficult to identify a recurrent myocardial infarction.

Myoglobin (MYO) is a small cytoplasmic oxygen-binding protein found in the skeletal as well as the cardiac muscle. It is released extremely early into the serum, 1 h after the onset of myocardial injury, peaks at 4-12 h and returns to baseline values immediately(Vaidya 1994). The major disadvantage of MYO is the lack of specificity to the cardiac tissue due to the presence of large amounts of MYO in the skeletal muscle(Mair, Morandell et al. 1995). The levels of MYO can therefore not be used as a single diagnostic marker, but in conjunction with the troponins or CK-MB. Thus, serum levels of MYO can be used to rule out, rather than diagnose, myocardial infarction(Gibler, Gibler et al. 1987, Wang, Lee et al. 2013).

Creatine phosphokinase (CK) and Creatine phosphokinase -MB . CK is an enzyme that is found primarily in the cardiac muscle and skeletal muscle. This enzyme has 3 isoenzymes: MM, MB and BB. CK-MM is the skeletal muscle fraction, CK-MB is the cardiac muscle fraction and CK-BB is the brain fraction of the total CK(Blomberg, Kimber et al. 1975). Previously, the total CK was assessed for myocardial infarction. However, as the total CK contains 95% of the CK-MM fraction, The CK-MB rises in the serum at 4-9 h after the onset of chest pain, peaks ~24 h and returns to baseline values at 48-72 h figure (2-2). The one advantage of CK-MB over the troponins is the early clearance that helps in the

detection of reinfarction. Thus, the serum level of troponin along with the level of the CK-MB fraction is assessed for the diagnosis of myocardial infarction(Gerhardt, Katus et al. 1991, Wang, Lee et al. 2013).

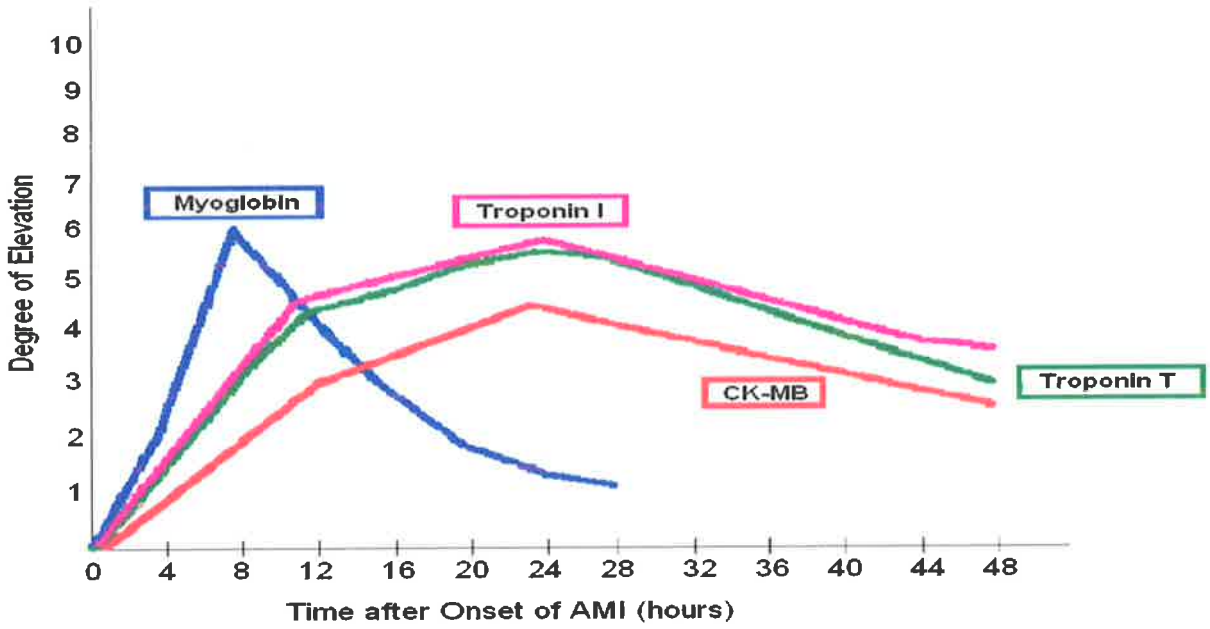


Figure (2 -2): cardiac markers levels (Ruseva 2005)

2.8.4Clinical Importance of Biomarker

An ideal biomarker should have the following characteristics: relatively high concentration within cardiac tissue, have no significant tissue sources other than the heart, have high clinical sensitivity and specificity, be detectable in the blood early after the onset of chest pain, have elevated blood levels for several days after the onset of symptoms, and have an assay with a quick turnaround time. Since no single biomarker fulfils all of these criteria, the NACB proposes the use of two biomarkers for the diagnosis of AMI: an early marker – myoglobin and a definitive marker- cardiac troponins. When cardiac troponin is not available, the next best alternative is CK-MB (measured by mass assay) (Ruseva 2005).

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." (Colburn, DeGruttola et al. 2001) A joint venture on chemical safety, the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labor Organization, has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease"(Strimbu and Tavel 2010). An even broader definition takes into account not just incidence and outcome of disease, but also the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients. In their report on the validity of biomarkers in environment risk assessment, the WHO has stated that a true definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological(Cortes, García-Cañaveras et al. 2017) .

The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction."(Cortes, García-Cañaveras et al. 2017). Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues. Medical signs have a long history of use in clinical practice—as old as medical practice itself—and biomarkers are merely the most objective, quantifiable medical signs modern laboratory science allows us to measure reproducibly(Galaup and Germain 2012). The use of biomarkers, and in

particular laboratory-measured biomarkers, in clinical research is somewhat newer, and the best approaches to this practice are still being developed and refined. The key issue at hand is determining the relationship between any given measurable biomarker and relevant clinical endpoints(Strimbu and Tavel 2010).

There are many serum cardiac biomarker following

C-reactive protein (CRP) is an acute phase protein secreted by the hepatocytes during an inflammatory stimulus(Stefano De Servi, Mariani et al. 2005). It has been shown that CRP is increased in patients with unstable angina; however, owing to lack of sensitivity and specificity, it cannot be used as a diagnostic marker (Duffy and Salerno 2004).

Pentraxin 3 (PTX-3) is a specific marker of vascular inflammation produced by the vascular endothelial cells, vascular smooth muscle cells, macrophages, and neutrophils in response to an inflammatory stimulus(Inoue, Sugiyama et al. 2007). The PTX-3 level has been proposed as a prognostic biomarker of adverse outcome in patients with unstable angina pectoris, myocardial infarction and heart failure(Suzuki, Takeishi et al. 2008).

Interleukin (IL)-6. Another marker of early atherosclerosis is IL-6, which has a major role in the recruitment and activation of inflammatory cells in response to ischemia and further during the reperfusion of the infarcted myocardium(Karpinski, Plaksej et al. 2009). .

Myeloperoxidase (MPO) is a metalloproteinase produced by the polymorphonuclear leukocytes and macrophages. It initiates the production of reactive oxygen species that are important for the development of atheroma and plaque rupture (Khan, Kelly et al. 2007). Thus, an increased level of MPO is a marker of plaque instability(Wang,

Zhang et al. 2007). Furthermore, it serves as a predictive marker for future cardiovascular adverse events(Cavusoglu, Ruwende et al. 2007).

Pregnancy-associated plasma protein A (PAPPA) is also a metalloproteinase that has an active role during the rupture of an atherosclerotic plaque(Bayes-Genis, Conover et al. 2001). It is primarily produced by the syncytiotrophoblasts of the placenta, as well as by the fibroblasts, vascular endothelial cells and vascular smooth muscle cells. In atherosclerosis, it has been associated with plaque progression and instability(Lund, Qin et al. 2006).

Soluble cluster of differentiation 40 ligand (sCD40L). sCD40L of the tumor necrosis factor-α (TNF-α) family is upregulated on the platelets located in the intraluminal thrombus. The activation of the inflammatory and coagulant pathways during thrombogenesis causes the release of CD40L into the circulation, thus indicating plaque rupture and subsequent myocardial infarction(Lund, Qin et al. 2006).

TNF-α is a pleiotropic cytokine produced by the endothelial cells, smooth muscle cells and macrophages. TNF-α levels are markedly elevated in advanced heart failure(Levine, Kalman et al. 1990). The role of TNF-α in atherosclerosis is the production of tissue inhibitors of metalloproteinases by the fibroblasts. Thus, the production of excess amounts of metalloproteinases causes rupture of the atheromatous plaque (Shingu, Nagai et al. 1993). This confirms the role of TNF-α in the regulation of the inflammatory cascade. Thus, elevated levels of TNF-α are indicative of recurrent non-fatal myocardial infarction or a fatal cardiovascular event (Ridker, Rifai et al. 2000).

Heart fatty acid-binding protein (H-FABP) is a small cytosolic low molecular weight protein found in the cardiac tissues that are responsible for the transport of fatty acids from the plasma membrane to sites of β-

oxidation in mitochondria and peroxisomes, and to the endoplasmic reticulum for lipid synthesis(Chan and Ng 2010). It is chiefly present in the myocardium and, to a lesser extent, in the brain, kidney and skeletal muscle. H-FABP is released extremely early into the serum following myocyte rupture(Haltern, Peiniger et al. 2010). An increased concentration of H-FABP appears as early as 30 min after myocardial injury, peaks at 6-8 h and returns to baseline levels at ~24 h (Mad, Domanovits et al. 2007). Additionally, H-FABP can be used as a predictive biomarker of mortality following acute coronary syndrome (ACS) (Ruzgar, Bilge et al. 2006). B-type natriuretic peptide (BNP). BNP is a neurohormone released from the cardiac cells. Studies have shown that elevated BNP is a predictive marker of death and heart failure. However, they are not useful for the diagnosis of AMI (Morrow, de Lemos et al. 2003).

Ischemia-modified albumin (IMA) Under ischemic conditions, the level of IMA in the blood is significantly increased, thus aiding in the diagnosis of acute ischemia prior to the onset of myocardial necrosis (Mastella, Moresco et al. 2009). The measurement of IMA is enabled by the binding of the cobalt to the damaged N-terminus of the albumin. The increase in IMA levels occurs immediately after the onset of ischemia and returns to baseline values within 6-12 h, thus enabling early identification of ischemia (Hjortshøj, Dethlefsen et al. 2009).Growth-differentiation factor-15 (GDF-15).

GDF-15 is a member of the transforming growth factor-β family of cytokines that is primarily expressed by the placenta; however, under abnormal conditions, it can be expressed by various tissues (Xu, Kimball et al. 2006).

During cardiac ischemia, the level of GDF-15 is increased favoring diagnosis of ACS (Kempf, Sinning et al. 2009). However, owing to its lack of specificity, it can be used as a predictive marker of mortality rather than a diagnostic marker following ACS (Kempf, Björklund et al. 2007).

Copeptin, the C-terminal portion of provasopressin is cosecreted with vasopressin. Copeptin is secreted extremely early in the course of an AMI from the pituitary (Voors, von Haehling et al. 2009). Copeptin levels are significantly increased within minutes in patients with AMI. Thus, copeptin can be used as a diagnostic and prognostic marker of myocardial injury (Reichlin, Hochholzer et al. 2009).

F2 isoprostanes are products of arachidonic acid metabolism. During atherosclerosis, the F2 isoprostanes are secreted by a variety of cells including the monocytes. Studies have shown that the F2 isoprostane level is increased in the urine of patients with unstable angina. Additionally, it can be used as a predictive marker of complications in non-fatal myocardial infarction, development of heart failure and fatality (LeLeiko, Vaccari et al. 2009).

2.8.5 MiRNAs structure and function

MicroRNAs (miRNAs) are endogenous, noncoding, single-stranded RNAs of ~22 nucleotides and constitute a novel class of gene regulators (Chua, Armugam et al. 2009). Analogous to the first RNA revolution in the 1980s, when Cech's group discovered the enzymatic activity of RNA, the more recent discoveries of RNAi and miRNA may represent the second RNA revolution(Kong and Han 2005). Although the first miRNA, lin-4, was discovered in 1993(Kong and Han 2005). about 800 miRNAs have been cloned and sequenced in humans, and the estimated number of

miRNA genes is as high as 1,000 in the human genome(Bentwich, Avniel et al. 2005).

The mature miRNAs bind to the 3′-UTR of their mRNA targets and negatively regulate gene expression via degradation or translational inhibition. Functionally, an individual miRNA is important as a transcription factor because it is able to regulate the expression of its multiple target genes(Chen and Rajewsky 2007). As a group, miRNAs are estimated to regulate over 30% of the genes in a cell(Lewis, Burge et al. 2005). It is thus not surprising that miRNAs are involved in the regulation of almost all major cellular functions including apoptosis and necrosis. Accordingly, miRNAs may precipitate in many diseases including cardiovascular disease (Zhang 2008, Zhang 2008, Cheng, Tan et al. 2010).

others studies have revealed that miRNAs exist in circulating blood. In contrast to our original thought, the cell-free miRNAs are relatively stable due to binding with other materials such as exosomes in circulating blood(Cortez and Calin 2009). Moreover, cancer tissue miRNAs are able to be released into circulating blood and the serum or plasma cell-free miRNAs can be used as novel biomarkers for diverse cancers(Cortez and Calin 2009). However, the quantitative method to measure the absolute amount of a miRNA in blood has not been well-established due to lack of stable control RNAs in blood especially under disease conditions. More importantly, the roles of the circulating-cell free miRNAs in patient with cardiovascular diseases are currently unclear(Cheng, Tan et al. 2010).

2.8.6 important criterion that make miRNA to be considered as a biomarker for MI:

Acute myocardial infarction diagnosis is based on ECG findings and measurements of blood biomarkers of myocardial damage, among which

cardiac troponins (cTns) are the most widely used. High-sensitivity troponin assays have been developed, but they suffer from a lack of specificity since elevation of cTn levels can be due to non-cardiac causes. Therefore, there is an unmet need for novel, early and specific biomarkers of AMI(Vella, Vella et al. 2015).

1- Early detection of acute myocardial infarction (AMI) is crucial for deciding the course of treatment to preserve and prevent further damage to the myocardial tissue(Bryant, Pawlowski et al. 2012, Vella, Vella et al. 2015).

2- Animal and clinical studies have demonstrated that miRNAs increase in the plasma shortly after the onset of a coronary event(Bryant, Pawlowski et al. 2012).

3- Certain miRNA display faster release kinetics than seen for troponins detected with high-sensitivity assays(Bryant, Pawlowski et al. 2012).

4- biomarker for MI is that this miRNA is not found in the plasma/extracellular space as a result of any other pathology. That is, the miRNA used to diagnose a myocardial event must not be detectable in patients with other complications, such as liver disease, kidney dysfunction, or diabetes.

5- The best miRNA to use as a marker will be the one with exclusive expression in myocardial tissue;

6- In the heart, miRNAs are widely expressed and regulate multiple physiological and pathological pathways such as apoptosis, fibrosis or angiogenesis (Goretti, Wagner et al. 2014).

7- miRNAs are present and stable in the bloodstream(Mitchell, Parkin et al. 2008).

8- circulating miRNAs can be either released by dying cells or be actively secreted by living cells, acting as paracrine factors(Goretti and Devaux 2016).

9- circulating levels of muscle-enriched miR-1 and miR-133a were significantly increased 15 mins after transcoronary ablation of septal hypertrophy(Liebetrau, Möllmann et al. 2013), supporting the hypothesis that heart-derived miRNAs may constitute early diagnostic biomarkers of AMI.

10- Stability of extracellular miRNA. There are multiple factors that account for the protection of circulating miRNAs against RNase-dependent degradation. For example, circulating miRNAs are not free in blood, which are normally included in microvesicles or exosomes. Furthermore, the circulating miRNAs can form the protein-miRNA complexes with some special proteins (such as nucleolar RNA-binding protein, nucleophosmin to resist the degradation (Wang, Zhang et al. 2010).

2.8.7Stability of miRNAs

The high stability of miRNAs in the body fluids makes miRNA a good candidate for a biomarker. As discussed above, miRNAs in the blood are protected from nuclease-mediated cleavage. Other typical longer RNAs such as messenger RNAs are too long to be protected inside protein complexes or small vesicles. In summary, the diversity and stability of miRNAs in combination with technical availabilities establish miRNAs as novel and promising biomarkers for the diagnosis of human diseases.(Kim 2015).

Another interesting discovery was that extracellular miRNAs are embedded in high-density lipoprotein (HDL)(Vickers, Palmisano et al.

2011). The expression profile of miRNAs in HDL showed that the miRNA population clearly differs between samples from healthy persons and samples from persons with atherosclerosis. Moreover, the nSMase2-dependent pathway was noted to be involved in the release of miRNA-containing HDL from the cells(Vickers, Palmisano et al. 2011) More studies are required to determine whether the alteration of the miRNA level in HDL is linked to disease progression.(Kim 2015).

Although most studies to identify miRNA biomarkers use blood, plasma, or serum, miRNAs are observed in most body fluids (Weber, Baxter et al. 2010, Russo, Di Bella et al. 2012).Because different miRNAs are profiled from each body fluid, researchers should select the proper fluid sample for their purpose. Widespread distribution of miRNAs in the body fluids suggests that miRNA biomarkers could be applicable for diverse diseases(Kim 2015).

2.8.8 Origin and distribution of extracellular miRNAs

One simple explanation for the origin of extracellular miRNAs is that the death of cells as a consequence of disease or other necrotic events results in the passive release of miRNAs in the cytoplasm, and that the released miRNAs are detected in the blood. Although this kind of miRNA release is feasible, it is likely that a regulatory pathway is also involved in the cellular release of miRNAs (Valadi, Ekström et al. 2007).

Initial study found that the miRNA profile inside cells differs from the miRNA profile in the culture media, which suggests the existence of a selective pathway for the release of miRNAs.(Valadi, Ekström et al. 2007). miRNAs are found in multivesicular bodies (MVBs), the cellular structure containing vesicles that are released into the extracellular space

as exosomes(Gibbings, Ciaudo et al. 2009). Later, it was shown that neutral sphingomyelinase 2 (nSMase2) is involved in the secretion of miRNA-containing exosomes into the extracellular space(Kosaka, Iguchi et al. 2010). The secretion of exosomes is triggered by ceramides, whose synthesis is regulated by neutral sphyngomyelinase 2(nSMase2)(Kosaka, Iguchi et al. 2013). Also, heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) was identified to recognize specific sequence motifs in a subset of miRNAs and to regulate their sorting into exosomes(Villarroya-Beltri, Gutiérrez-Vázquez et al. 2013). Although these studies identified the factors involved in the release of cellular miRNAs into the extracellular space, we still do not understand the detailed mechanism governing this process. The elucidation of the mechanism by which miRNAs are selected to be released from donor cells, how those specific groups of miRNAs are embedded inside the exosomes, and when the release of miRNAs is triggered would be helpful for studies attempting to use extracellular miRNAs as biomarkers in diseases(Wang, Zhang et al. 2010).

Extracellular miRNAs have been discovered in diverse macromolecules in the blood and other body fluids. Because of their small size, miRNAs can be incorporated into some proteins and protected from nuclease attack, Ago protein is the most prevalent such protein, because normal biogenesis results in the incorporation of miRNA into the Ago protein. The majority of extracellular miRNAs cofractionate with Ago2 protein complexes, whereas only a small portion of miRNAs are encapsulated in the extracellular vesicles or exosomes in the human plasma.(Arroyo, Chevillet et al. 2011, Turchinovich, Weiz et al. 2011). In the bloodstream, various extracellular vesicles or exosomes circulate throughout the body. The extracellular vesicles are made by direct

budding of the cellular membrane, whereas the exosomes are released from the cells as a result of the fusion of Multivesicular bodies (MVBs) into the plasma membrane.(Colombo, Raposo et al. 2014). Early studies identified diverse miRNAs in these extracellular structures (Hunter, Ismail et al. 2008, Taylor and Gercel-Taylor 2008). In addition, many following studies determined the level of those miRNAs and showed that the miRNA level could be used to predict whether a person has a disease or not(Schwarzenbach, Nishida et al. 2014).

2.8.9MiRNA -1

MiRNA -1(MiR-1) is primarily expressed in cardiac and skeletal muscles and consists of two subfamilies, miR1-1 and miR1-2. The two subfamilies have identical sequences, but are encoded by two distinct genes in chromosomes 18 and 20, respectively. MiR-1, involved in cardiogenesis and muscle differentiation, may regulate cardiac arrhythmogenicity by repressing the expression of the gap junction protein, connexin (GJA1) and the inward-rectifier potassium ion channel (KCNJ2)(Xu, Lu et al. 2007, Yang, Lin et al. 2007, Khalil, Kamar et al. 2017).

In the rat, the circulating miR-1 level is rapidly increased 1 hr after coronary artery ligation, and peaked at 200-fold higher than baseline 6 hrs after AMI. The elevated miR-1 level returned to basal levels 3 days after AMI, a time course earlier than traditional AMI biomarkers, such as troponin (Cheng, Tan et al. 2010, Wang, Zhu et al. 2010, Fang and Yeh 2015).

It is closely related to the physical and pathological processes of vascular modeling, including heart development, arrhythmias, ischemia,

myocardial infarction and cardiac hyper-trophy(Townley-Tilson, Callis et al. 2010). Some studies have shown that the expression of miR-1 is down-regulated in cardiac hypertrophy and chronic myocardial infarction and speculate that miR-1 may inhibit cell proliferation and hypertrophy(Sayed, Hong et al. 2007, Li, Song et al. 2010). However, in other cardiovascular experimental models, such as ischemic precondition-ing, ischemia/reperfusion, acute myocardial infarction, oxidative stress, and hyperglycemia, the expression of miR-1 is up-regulated(Bostjancic, Zidar et al. 2010, Ai, Zhang et al. 2012). Therefore, miR-1 could be an activator of these diseases. Some researchers have shown the mechanisms of action of miR-1 on vascular diseases to further clarify the function of miR-1 according to the size of heart or hypertrophy, the contractile function, and the conductance of heart (Wang, Zhang et al. 2010, Khalil, Kamar et al. 2017).

Zao *et al.* reported that miR-1 targets the transcription factor Hand2, which increases the proliferation of ventricular cardiomyocytes, miR-1 was suggested to prevent vascular injuries by decreasing the thickness of cell walls (Zhao, Samal et al. 2005). In other animal models, miR-1 also plays a protective role and has been linked to hypertrophy. In the model of functional overload and trans-aortic constriction (TAC) induced hypertrophy, the expression of miR-1 is down-regulated to half through binding of the 3'UTR of target genes, cyclin dependent kinase 9, Ras GTPase-activating protein, fibronectin, and Ras-homolog enriched in the brain(Sayed, Hong et al. 2007), which was closely linked to the regulation of genes related to calcium. Some studies reported miR-1-induced down regulation of the expression of the transcription factors, mef2a and gata4, which are related to calmodulin and cardiac hypertrophy along with myocardial infarction. Inhibition of these target

genes is an ideal way to treat cardiac hypertrophy. Furthermore, miR-1 inhibits cardiac hypertrophy thus protecting the heart from hypertrophy-related injury (Ikeda, He et al. 2009)and preventing the expression of calmodulin. Elia *et al.* report IGF-1 as the target gene of miR-1(Elia, Contu et al. 2009).

miR-1 is overexpressed in individuals with coronary artery disease, and that when overexpressed in normal or infarcted rat hearts, it exacerbates arrhythmogenesis. Elimination of miR-1 by an antisense inhibitor in infarcted rat hearts relieved arrhythmogenesis(Yang, Lin et al. 2007). miR-1 overexpression slowed conduction and depolarized the cytoplasmic membrane by post-transcriptionally repressing KCNJ2 (which encodes the K(+) channel subunit Kir2.1) and GJA1 (which encodes connexin 43), and this likely accounts at least in part for its arrhythmogenic potential. Thus, miR-1 may have important pathophysiological functions in the heart(Yang, Lin et al. 2007).

2.8.10 MiRNA-145

The microRNA-143/-145 (miR-143 and miR-145) encoding genes are located in close proximity with each other on human chromosome 5 and are believed to be cotranscribed in the same bicistronic transcript(Weber, Schober et al. 2010). The miR-143/-145 gene cluster is expressed in the heart and in VSMCs(Boettger, Beetz et al. 2009, Deacon, Nevis et al. 2010). MiR-143 is believed to play an essential role in the function and formation of the cardiac chamber via regulation of myocardial cell morphology (Deacon, Nevis et al. 2010).MiR-143 and miR-145 are essential for VSMC differentiation (Cordes, Sheehy et al. 2009) and are molecular keys to determine VSMC phenotypic switching(Cheng, Liu et al. 2009, Rangrez, Massy et al. 2011). It is

interesting that miR-143 and miR-145 can be upregulated in endothelial cells in response to shear stress and subsequently are exported in exosome-like vesicles that regulate VSMC phenotype(Hergenreider, Heydt et al. 2012). In addition, it has been reported that circulating miR-145 levels differ in patients with coronary artery disease(Weber, Baker et al. 2011, D'Alessandra, Carena et al. 2013); in patients with acute myocardial infarction (AMI), the level of miR-145 in total peripheral blood correlates with infarct size(Meder, Keller et al. 2011). This suggests that miR-145 could be a valuable biomarker for cardiovascular diseases. Furthermore, several clinical studies have shown that miR-143/-145 dysregulation is associated with many cardiovascular diseases, including essential hypertension, atherosclerosis, pulmonary arterial hypertension, and coronary artery disease(Weber, Baker et al. 2011, Samples 2012, Wei, Nazari-Jahantigh et al. 2013).

MiRNA-145 is a mediator in the regulation of the proliferation and differentiation of vascular smooth muscle cells (VSMCs), in which it is highly expressed, and is the most abundant miRNA in healthy arterial walls(Cordes, Sheehy et al. 2009). Although the expression of different circulating miRNAs among CAD patients has been reported(Pleister, Selemon et al. 2013).the functions of miRNA-145 in cardiovascular disease remain unknown (Xu, Cao et al. 2017).

Furthermore, Programmed cell death protein 4(PDCD4) was identified as a novel target of miR-145 in cardiomyocytes, and overexpression of PDCD4 could remarkably restore the miR-145-inhibited cardiomyocytes apoptosis and mitochondrial dysfunction after hypoxia injury(Xu, Cao et al. 2017).

Chapter Three

Materials and Methods

3.1 Subjects

3.1.1 patient group and sample collection

A case control study based on three group. as shown in figure (3 -1) , first group was 24 patients with MI which include (16male and 8female) ,who were observation in cornary Care Unite of Al-Diwaniyha teaching hospital in the period from January 2017 to February 2018 under the supervision of internal medicine specialists were included in this study and the information about each case collected from the patient as well as the tests that have been done in hospital labortories. such as (complete blood picture, lipid profile and cardiac markers) and in addation to parameters that fond in questionnaire in appendix. Second group was 24 patients who have risk factor for MI (Hypertension, Hyperlipidemia, Diabetes mellitus (DM),smoking , obesity) this group has been collected from internal medicine consultation department .

Third group was include 19 healthy volunteers(non coronary artery diseases). Blood sample were collected by venipuncture from these groups (five millimeter of venous blood)were drawing by disposable syringe under aseptic technique . Each blood sample of three groups were collected into two parts:

1- Two milliliter of blood collected directly in a sterile tube containing EDTA for DNA extraction ,then uses Amplification refractory mutation-PCR system (ARMS-PCR) technique application to studySIRT3 gene polymorphism, these samples should be frozen immediately at -20C.

2- Three milliliter were collected in EDTA free plane tube and allowed to clot then serm was separated by centrifugation 13000pm for 5 minute

.The serum has been collected in Eppendrof tube then stored at -20 to be use for miRNA-1and miRNA-145 qPCR as in study deign figure (3-1) .

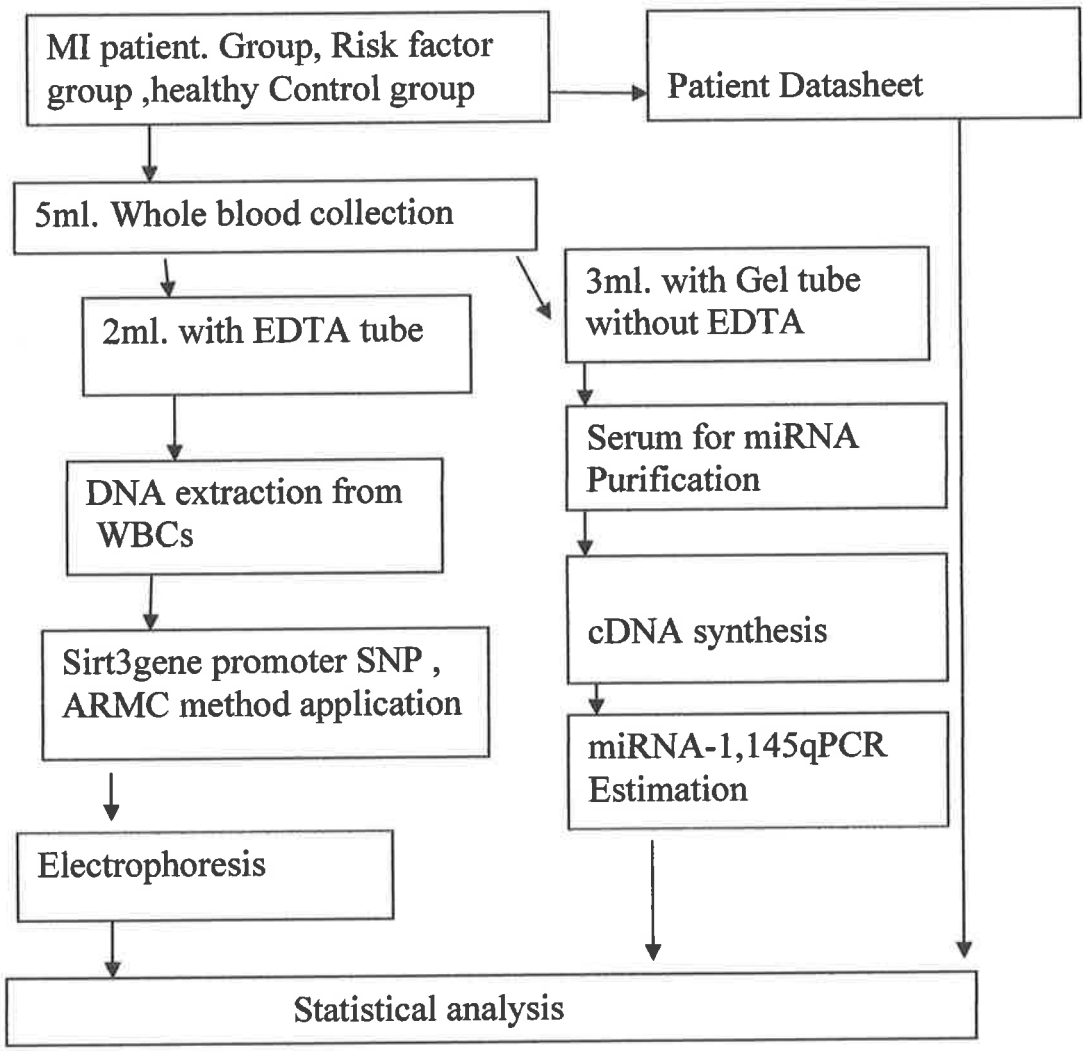


Figure (3 - 1) Shows the study design

3.1.2 Data collection

Data were collected by direct interview (including demographic data, social status, period of symptoms, familial history with myocardial diseases , MI recurrent occur and other diseases as hypertension, DM, dyshyperlipidemia , (appendix 1).

3.2 Materials

3.2.1. Instruments and Equipments:

Table (3-1): Instruments and equipments that used in this study:

No.	**Instrument / equipment**	**Company / Country**
1	Adjustable Micro Pipette 0.5-10 µl,10-100µl, 20- 200µl, 100-1000µl	Transferpette / Germany
2	Eppendorf tubes	Sigma/England
3	Exispin vortex centrifuge	Bioneer/ Korea
4	Gel and Clot/activator tubes	Xinle /china
5	High speed Cold Centrifuge	Eppendorf/ Germany
6	Incubator	Memmert /Germany
7	Micro AMP Optical 8- cap strip	Biosystems /USA
8	Micro AMP Optical 8- tube strip	Biosystems /USA
9	Micro-centrifuge tubes (1.5 and 2ml)	Eppendorf / German
10	Miniopticon Real Time PCR	Bio-Rad/ USA
11	Nanodrop	Thermo Scientific/ USA
12	Pipette tips crystal (0.5-10 µl)	Human/Germany
13	Racks for plain tubes	Deans gate /china

14	Refrigerator	Concord/ lebanon
15	Sterilized Latex Surgical Gloves	Deans gate /china
16	Thermocycler apparatus	Bio-Rad/ USA
17	tips(RNase/DNase free) (100,1000 µl)	Promega/ USA
18	Vortex	CYAN/ Belgium
19	Water bath	PolyScience /USA

3.2.2 Chemicals

The chemical and biological materials used in this work are listed in Table(3-2) below:

Table (3-2):List of Chemical materials with their remarks

No.	Chemicals	Company	Country
1	Chloroform	Labort	India
2	DEPC water	Bioneer	Korea
3	Isopropanol	Labort	India
4	Ethanol 100%	Labort	India
5	Ethanol 70%	Labort	India
6	RNase free water	Bioneer	Korea

3.2.3 Kits

Table (3-3): The kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	DNA mini kit extraction kit (Frozen Blood).	Geneaid	USA
2	**Total RNA Extraction Kit AccuZol™**	Bioneer	Korea
	Trizol reagent 100ml		
3	**DNase I enzyme kit**	Promega	USA
	Dnase I enzyme		
	10x buffer		
	Free nuclease water		
	Stop reaction		
4	**M-MLV Reverse Transcriptase kit**	Bioneer	Korea
	M-MLV Reverse Transcriptase (10,000U)		
	5X M-MLV RTase reaction buffer		
	dNTP		
	100mM DTT		
	RNase Inhibitor		
5	**NEXpro™ Master Mix (Probe)**	Geneslabs	Korea
	qPCR PreMix for TaqMan probe: Taq DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) and 10X buffer		

3.3.4 SIRT3 gene promoter Primers

Tables of SIRT3 gene promoter Primers

Primer	Sequence	
rs11246029	T allele	CCCGGTCCCGCCTCCGAGT
	C allele	AAGGAGGCGGGGGCGTGG
	F	CGACCCGTTCAACTACCCGGCC
	R	TCACCGCCATCCGGGTTGAAAA

NCBI-SNP: **rs11246029**

Primer	Sequence	
rs12293349	T allele	CATCCCGGTTGTTCTTCTGGGT
	C allele	ATGACAGCAGGAAGACCCCAGG
	F	AGAGACGCGCTGTAACCGAGC
	R	CGGCGCTCACTTCTTCGTGTAG

NCBI-SNP: **rs12293349**

Primer	Sequence	
rs56312618	G allele	ATCCCGGTTGTTCTTCTGGGG
	A allele	CATGACAGCAGGAAGACCCCAGT
	F	AGAGACGCGCTGTAACCGAGC
	R	GCCCCCTCCGTCTCCCTCTAT

NCBI-SNP: **rs56312618**

3.3.5 microRNA Primers and probes

The Primers and probes for microRNA(1, 145) were design in this study by using **(The Sanger Center miRNA database Registry)** to selected miRNA sequence **and using miRNA Primer Design Tool.** These primers and probe were provided by (Bioneer company, Korea) as following table:

Primer		Sequence
miR-1RT primer		GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGA GCCAACATACAT
miR-1 primers	F	GTGCAGGGTCCGAGGT
	R	GTTGGGTGGAATGTAAAGAAGT
miR-1probe		FAM-CAGAGCCAACATACAT-MGB

>hsa-miR-1-3p MIMAT0000416

Primer		Sequence
miR-145 RT primer		GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGA GCCAACAGGGAT
miR-145 primers	F	GTGTCCAGTTTTCCCAGGA
	R	GTGCAGGGTCCGAGGT
miR-145 probe		FAM-CAGAGCCAACAGGGAT-MGB

>hsa-miR-145-5p MIMAT0000437

Primer	Sequence	
GAPDH	F	AATTCCATGGCACCGTCAAG
	R	ATCGCCCCACTTGATTTTGG
GAPDH probe	CCCATCACCATCTTCCAGGAGCG	

NM_001256799.2 Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transcript variant 2, mRNA

3.4.1 Genomic DNA Extraction

Genomic DNA from blood samples were extracted by using Genomic DNA mini kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions as following steps:

1. A 200µl of frozen blood was transferred to sterile 1.5ml microcentrifuge tube, and then about 30µl of proteinase K was added and mixed by vortex. And incubated at 60℃ for 15 minutes.

2. After that, 200µl of lysis buffer was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70℃ for 15 minutes, and inverted every 3 minutes through incubation periods.

3. 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously.

4. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow.through were discarded and placed the column in a new 2 ml collection tube.

5. 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow. through was discarded and placed the column back in the 2 ml collection tube.

6. 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow. through was discarded and placed the column back in the 2 ml collection tube.

7. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.

8. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre.heated elution buffer were added to the center of the column matrix.

9. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elude the purified DNA.

3.4.2 Estimation of Genomic DNA purity &cocentration

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).

2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.

3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of blood genomic DNA was added to measurement.

3.4.5 ARMS-PCR Method

ARMS-PCR assay was performed for detection and SIRT3gene (rs11246029,rs12293349,rs56312618) polymorphism in 24 MI patients,24 risk factor individual and in 19healthy control blood samples. This method was carried out according to described method by(Medrano and de Oliveira 2014). as following steps:

3.4.5.1 ARMS-PCR master mix preparation

ARMS-PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done two reactions for each sample according to company instructions as following tables:

1- A-allele ARMS PCR reaction Mix:

PCR Master mix		Volume
DNA template		2µl
A-allele primers (10pmol)		1µl
Internal control primers	Forward primer (10pmol)	1µl
	Reveres primer (10pmol)	1µl
PCR water		15µl
Total volume		20µl

2- T-allele ARMS PCR reaction Mix:

PCR Master mix		Volume
DNA template		2µl
T-allele primers (10pmol)		1µl
Internal control primers	Forward primer (10pmol)	1µl
	Reveres primer (10pmol)	1µl
PCR water		15µl
Total volume		20µl

After that, these PCR master mix component that mentioned in table above placed in standard AccuPower PCR PreMix Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and loading dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea).

3.4.5.2 ARMS-PCR Thermocycler Conditions

PCR thermocycler conditions were done for each gene independent as following tables:

PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	35cycle
Annealing	55°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

3.4.5.3 ARMS-PCR product analysis

The ARMS-PCR products were analyzed by agarose gel electrophoresis following steps:

1- 1% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 10ul of (100bp Ladder) in First well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- The ARMS-PCR products were visualized by using UV transilluminator.

3.4.6.1 Total RNA extraction

Total RNA were extracted from serum samples by using (TRIzol® reagent kit. Bioneer. Korea) and done according to company instructions as following steps:

1- 250µl serum samples was placed in 1.5 microcentrifuge tube then 750µl TRIzol® reagent was added to each tubes.

2- Then, 200µl chloroform was added to each tube and shaken vigorously for 60 seconds.

3- The mixture was incubated on ice for 5 minutes. Then centrifuged at 12000 rpm, 4C°, for 15 minutes.

5- Supernatant was transferred into a new Eppendorf tube, and 500µl isopropanol was added. Then,The mixture was mixed by inverting the tube 4-5 times and

incubated at 4C° for 10 minutes. Then, centrifuged at 12,000 rpm , 4C° for 10 minutes.

8- Supernatant was discarded, and 1ml 80% Ethanol was added and mixed by vortex again. Then, centrifuge at 12000 rpm, 4C° for 5 minutes.

9- The supernatant was discarded and the RNA pellet was left to air to dry.

12- 50µl DEPC water was added to each sample to dissolve the RNA pellet, Then, the extracted RNA sample was kept at -20.

3.4.6.2 DNase I Treatment

The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by Promega company, USA instructions as follow:

Mix	Volume
Total RNA 100ng/ul	10ul
DNase I enzyme	1ul
10X buffer	4ul
DEPC water	5ul
Total	20ul

After that, The mixture was incubated at 37C° for 30 minutes. Then, 1µl stop reaction was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action.

3.4.6.3 Assessment of the extracted RNA for miRNA

The concentration of the extracted total RNA was quantified by the use of Quantus fluorometer system following the manufacturer's instructions

(Promega, USA). This assay was performed by adding 99 to l of Tris EDTA to 1 to l of extracted RNA in eppendorf tube and then 100 to l of Quanti Fluor RNA dye was added. The mixer was kept for 5 minutes at dark place. Then the quantity of RNA (ng/ml) was measured, the appropriate application was chosen (Nucleic acid, RNA).

3.4.6.4 Assessment of the extracted RNA

The Taq Man MicroRNA assays were used looped-primer RT-PCR, a new real-time quantification method, for detection mature miRNAs. Total RNA containing miRNA was the starting material in RT-PCR reaction which was performed in two-steps as in figure (3-1):

1. In the reverse transcription (RT) step, cDNA was reversed transcribed from total RNA samples using specific miRNA primers from the TaqMan MicroRNA Assays and reagents from the TaqMan□MicroRNA Reverse Transcription Kit.
2. In the PCR step, PCR products were amplified from cDNA samples using the TaqMan MicroRNA Assay together with the TaqMan® Universal PCR Master Mix. As shown in figure (3-2)

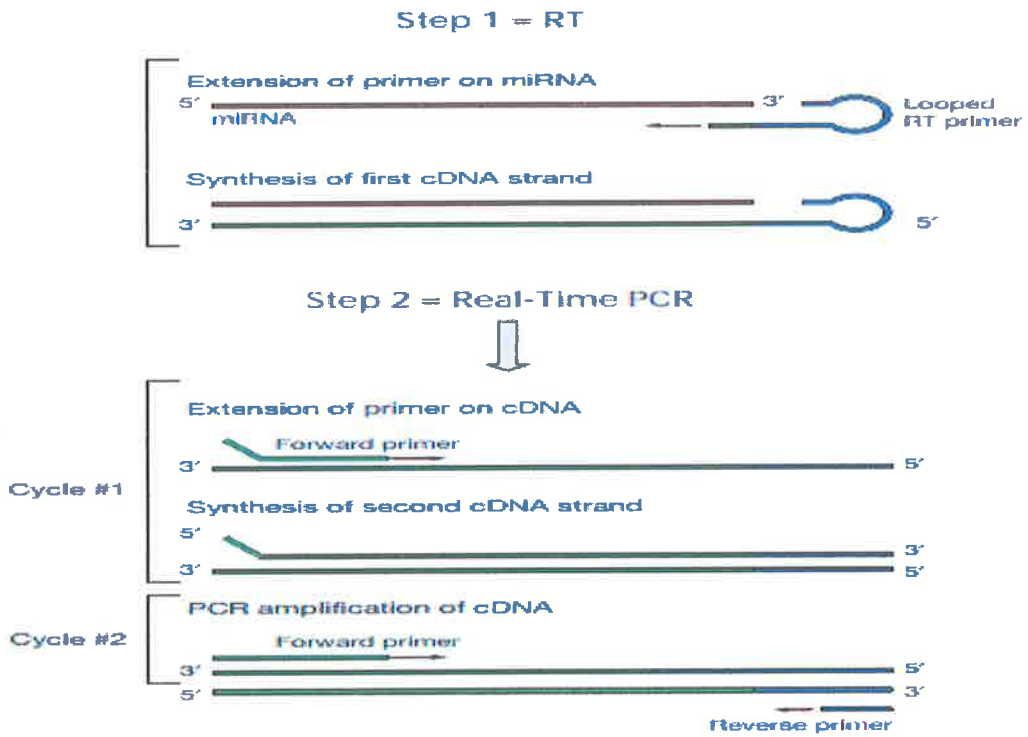


Figure (3-2): Two - step RT- PCR reaction (TaqMan small RNA assays protocol, 2011)

3.4.6.5 cDNA synthesis

3.3.10.1 cDNA synthesis for miR1 and miR145

DNase-I treated RNA samples were used in miRNA cDNA synthesis step for (1,145)miRNA by using **M-MLV Reverse Transcriptase kit** and done according to company instructions as following tables:

Step 1

RT master mix	Volume
Total RNA 100ng/ul	8ul
hsa-miR-21RT primer	1ul
DEPC water	1ul
Total	10ul

Than RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice.

Step 2

RT master mix	Volume
Step 1 RT master mix	10ul
M-MLV RTase (200u)	1ul
5X M-MLV RTase reaction buffer	4ul
100mM DTT	2ul
dNTP	2ul
RNase inhibitor	1ul
Total	20ul

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions:

Step	Temperature	Time
cDNA synthesis (RT step)	42 °C	1 hour
Heat inactivation	95 °C	5 minutes

3.4.6.7 cDNA synthesis for GAPDH gene

DNase-I treated RNA samples were also used in cDNA synthesis step for GAPDH gene by using **M-MLV Reverse Transcriptase kit** and done according to company instructions as following tables:

Step 1

RT master mix	Volume
Total RNA 100ng/ul	8ul
Random Hexamer primer	1ul
DEPC water	1ul
Total	10ul

Than RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice.

Step 2

RT master mix	Volume
Step 1 RT master mix	10ul
M-MLV RTase (200u)	1ul
5X M-MLV RTase reaction buffer	4ul
100mM DTT	2ul
dNTP	2ul
RNase inhibitor	1ul
Total	20ul

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions:

Step	Temperature	Time
cDNA synthesis (RT step)	42 °C	1 hour
Heat inactivation	95 °C	5 minutes

3.4.6.8 STEM-LOOP RT-qPCR

The stem loop RT-qPCR was used in quantification of miRNA-1 and miRNA-145 expression analysis that normalized by housekeeping gene

(GAPDH) in serum MI patients , risk and control group by using Real-Time PCR technique and this method was carried out according to method described by (Varkonyi-Gasic and Hellens 2011) and include the following steps:

1- qPCR master mix preparation

qPCR master mix was prepared by using **NEXpro™ Master Mix (Probe)**that dependant on TaqMan probe FAM dye detection of gene amplification in Real-Time PCR system and include the follow:

qPCR master mix	volume
21 miRNA cDNA template (100ng)	5µL
Forward primer(10pmol)	2.5 µL
Reverse primer (10pmol)	2.5 µL
TaqMan probe (20pmol)	2.5 µL
DEPC water	25 µL
Total	50 µL

After that, these qPCR master mix component that mentioned above placed in qPCR premix standard plate tubes that contain the other PCR TaqMan probe amplification components, then the plate mixed by Exispin vortex centrifuge for 3 minutes, than placed in Miniopticon Real-Time PCR system.

2- miRNA qPCR Thermocycler conditions

After that, the qPCR plate was loaded and the following thermocycler protocol in the following table:

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	5min	1
Denaturation	95 °C	20 sec	45
Annealing\Extention Detection(scan)	55 °C	30 sec	

3.4.6.9 Data analysis of qRT-PCR

The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) by using The ΔCT Method Using a Reference that described by(Livak and Schmittgen 2001). as following equations:

ΔΔCT = ΔCT, target in treated sample- ΔCT, target in calibrator sample

Gene	Test (Treatment group)	Cal. (Control group)
Target gene	CT (target, test)	CT (target, cal)
Reference gene	CT (ref, test)	CT (ref, cal)

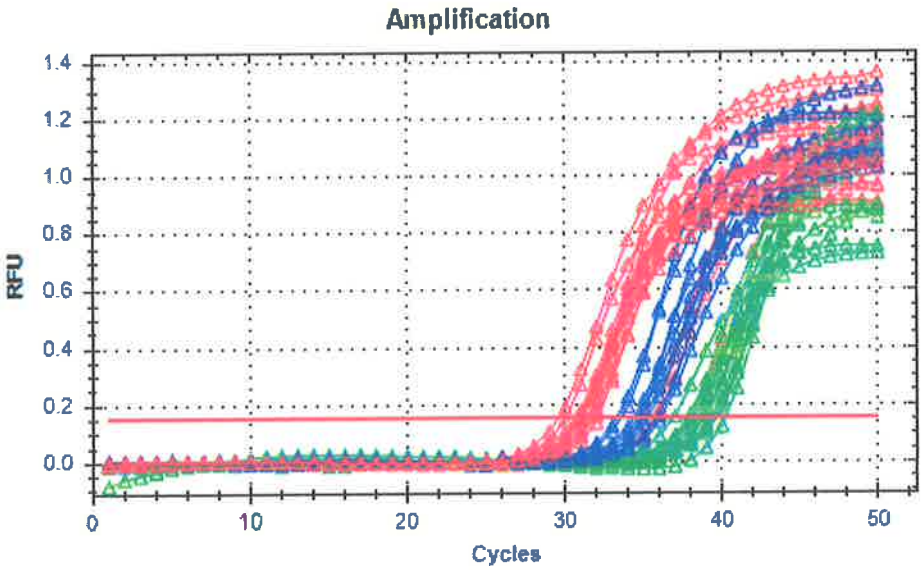


Figure (3-3): Real time PCR amplification plot for miRNA-1 in blood samples that show difference in threshold cycle numbers (Ct value) between Patients and Healthy control groups.

Red plot : Patient MI group, Blue plot: Patient Risk group, and Green plot: healthy control group.

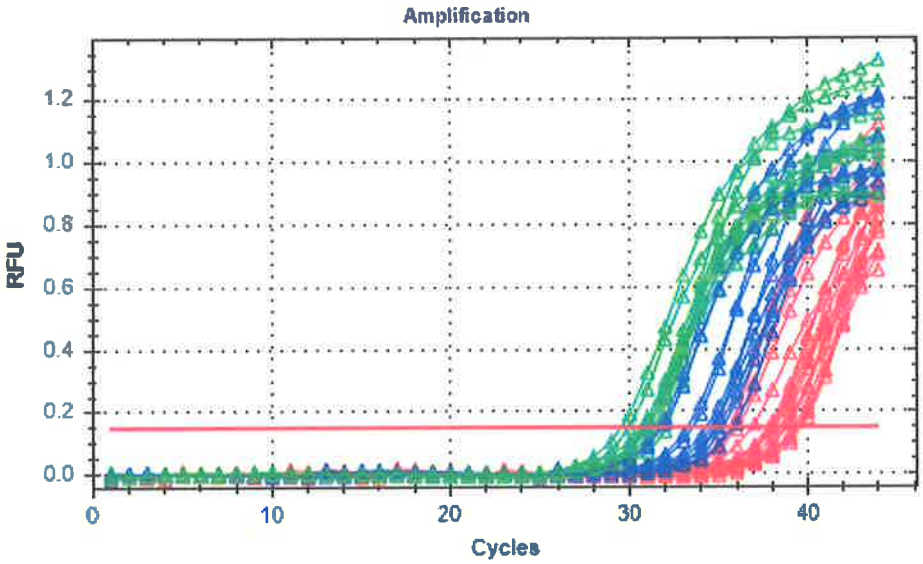


Figure (3-4): Real time PCR amplification plot for miR145 in blood samples that show difference in threshold cycle numbers (Ct value) between Patients and Healthy control groups.

Red plot : Patient MI group, Blue plot: Patient Risk group, and Green plot: healthy control group.

3.4.6.10 Relative quantification (the ΔΔCT method)

The ΔΔCT method, also referred to as the Comparative CT method, is a means of measuring relative quantification and was described by Livak and Schmittgen 2001(Livak and Schmittgen 2001). It determines the relative change in gene expression between a target gene under investigation and that of calibrator (control) gene. Most frequently, the untreated control is used as the calibrator.

The difference between the CT of the target gene (CT, target) and the CT of the endogenous control (CT, ec) is the ΔCT of the sample:

ΔCT = CT, target - CT, ec

The term ΔΔCT is calculated as the ΔCT of the target gene in the treated sample minus the ΔCT of the target in the untreated, calibrator sample:

ΔΔCT = ΔCT, target in treated sample- ΔCT, target in calibrator sample

The calibrator, since it is untreated, should have no change in its ΔΔCT value during the course of the experiment. Its change, therefore, is equivalent to zero. Since 20 equal one, the calibrator gene's expression is unity.

When the ΔΔCT method is used to measure gene expression, therefore, the results are expressed as a " fold" change in the expression level of the target gene normalized to the endogenous control and relative to the calibrator. It is given by the equation: Relative Fold Change = 2 ΔΔCT

3.5 Statistical analysis

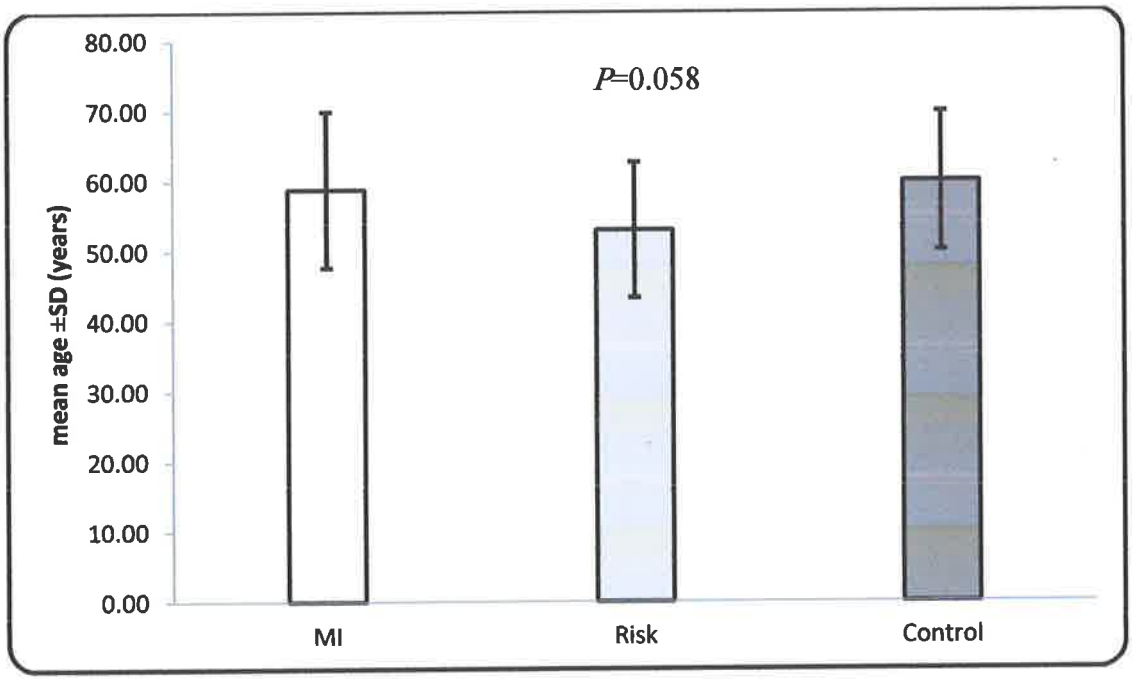
Data were summarized , presented and analyzed using statistical package for social science (SPSS version 23) and Microsoft office Excel 2016.Numeric data were presented as mean ,standard deviation, range, median and interquartile range (IQR) after performance of KolmogorovS normality test and making decision about normally and non normally distributed variable . kruskall walis test was used to study difference in mean rank among more than two groups . Chi-square test was used to study associated between any two categorical variables .Odd ratio and 95% confidence interval was estimated to measure risk. Receive operator characteristic curve analysis was used to find out the proper cutoff values and further analysis of sensitivity and specificity was carried out accordingly . the level of significance was considered at P-value of 0.05 or less and highly significant level at 0.01 or less(Cool 2007).

Chapter four

Results and Discussion

4.1 Demographic characteristics of the control and study groups

The current study included 24 patients with ST elevation myocardial infarction (MI), 24 persons who have risk factors of myocardial infarction and 19 healthy control subjects.



Figure(4-1): Bar chart showing mean age in control and study groups

The mean age of patients with MI was 58.83±11.04 years, the mean age of risk group was 53.04± 9.65 years and that of control group was 60.00±9.87 years. There was no significant difference in mean age among study and control groups enrolled in the present study (P=0.058), which ensures age matching that is mandatory for such a study, as shown in figure (4-1).

Table (4-1): Distribution according to gender of control and study groups

Gender	MI n=24	Risk n=24	Control n=19	χ^2	*P*
Male	16 (66.7)	10 (41.7)	13 (68.4)	4.22	0.121
Female	8 (33.3)	14 (58.3)	6 (31.6)		
M:F ratio	2:1	1:1.4	2.2:1		

Regarding to gender distribution about 16 patients (66.7%) were male, and 8 patients(33.3%) were female, while the the risk group included 10 (41.7%) male and 14 (58.3%) female and the control group included 13 (68.4%) male and 6 (31.6%) women. There was no significant difference in mean gender among the three groups regarding distribution of patients according to gender (P=0.121), which ensures gender match that is mandatory for such a study, as shown in table(4-1).

This result came in agreement with other Iraqi study done by(Al-Asadi and Kadhim 2014, Sanchis-Gomar, Perez-Quilis et al. 2016) which is reports MI prevalence was middle-aged individuals (35–54 years) , The prevalence of MI was higher in male compared with female, M I Risk factors in the present were patients with hypertensive , Diabetes mellitus patients and dyshyperlipidemia patients this factor incidence occurs within age group range between 35–74 years, which came compatible with previously conducted study (Sanchis-Gomar, Perez-Quilis et al. 2016) .

Mean weight of patients belonging to MI group was 90.38 ±11.99 kg; mean weight of risk group was 91.13±10.93 kg and the mean weight of control group was 94.11± 9.92 kg. There was no significant difference in mean weight among groups (P=0.358), as shown in figure 4-2.

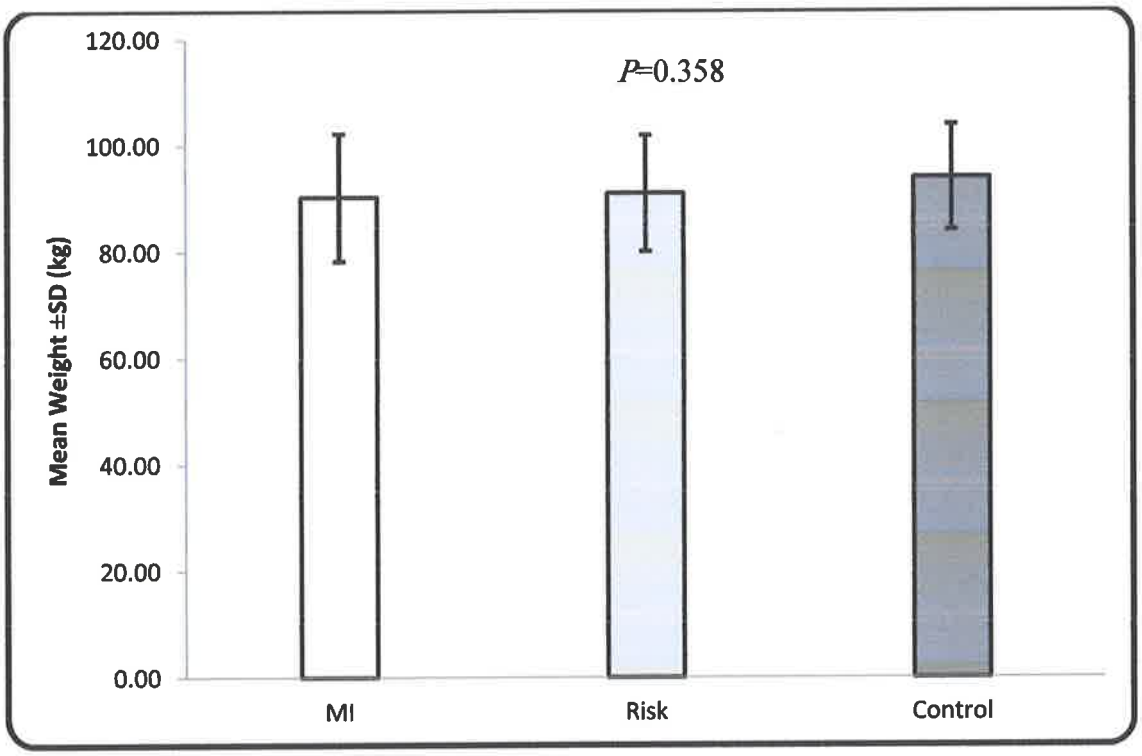


Figure 4-2: Bar chart showing mean weight in control and study groups

4.2 Hematological parameters of control and study groups

Hematological parameters included in the present study considered as non specific or indirect diagnostic markers of AMI, and it's done

Table (4-2): Mean hematological parameters among control and study groups

Characteristic	MI	Risk	Control
PCV %	43.96 ±12.04 A	39.79 ±13.64 A	46.63 ±3.35 A
Platelet $X10^3$/cc	271.65 ±68.25 A	236.58 ±54.07 B	235.63 ±44.10 B
WBC $X10^3$/cc	9.98 ±1.98 A	7.14 ±1.14 B	7.50 ±1.19 B
ESR mm/hr	24.63 ±19.23	---	---

Capital letters to indicate differences in mean; different letters indicate significant difference at P≤0.05; similar letters indicate insignificant difference at P≤0.05; A indicates the highest value.

according to physician's request as a routine which include (PCV, WBC, and platelets , ESR).Mean packed cell volume (PCV) % of patient group was 43.96 ±12.04; mean PCV% of risk group was 39.79 ±13.64 and that of control group was 46.63 ±3.35. There was no significant difference in mean PCV among the three groups (P>0.05), This finding dose not agrees with Al-Oqaily et al (AL-Oqaily 2014)who found that PCV can be manifested as myocardial infarction due to decreased oxygen carrying capacity to the heart, as shown in table 4-2. Mean platelet count of patient group was 271.65 ±68.25; mean platelet count of risk group was 236.58 ±54.07 and that of control group was 235.63 ±44.10.

Mean platelet count of MI group was significantly highest (P<0.05), whereas, there was no significant difference in mean platelet count between risk and control groups (P>0.05), as shown in table (4-2) This finding agree with Khandekar et al (Khandekar, Khurana et al. 2006) since they found that platelet distribution width and mean platelet volume are significantly raised in patients with acute myocardial infarction and unstable angina.

Mean white blood cells (WBC) count of patient group was 9.98 ±1.98; mean white blood cell (WBC) of risk group was 7.14 ±1.14 and that of control group was 7.50 ±1.19. Mean WBC count of MI group was significantly highest (P<0.05), whereas, there was no significant difference in mean WBC count between risk and control groups (P>0.05), as shown in table(4-2) this finding came in agreement with Madjid et al(Madjid, Awan et al. 2004), Hoffman et al(Hoffman, Blum et al. 2004), and Lee et al (Lee, Folsom et al. 2001) since they found that patients with elevated white blood cell counts are at higher risk of developing acute

myocardial infarction and acute coronary events. Mean erythrocyte sedimentation rate (ESR) of patients group was 24.63±19.23 mm/hr.

4.3 Serum lipid profile of control and study groups

Table (4-3): Mean serum lipids among control and study groups

Serum lipid	MI	Risk	Control
Triglyceride (mg/dl)	202.12 ±47.41 A	209.42 ±162.56 A	115.89 ±41.98 B
Total cholesterol (mg/dl)	222.42 ±35.92 A	202.92 ±44.44 B	186.21 ±37.79 C
HDL (mg/dl)	41.2±7.20 A	40.83 ±8.20 A	32.89 ±4.51 B

Capital letters to indicate differences in mean; different letters indicate significant difference at P≤0.05; similar letters indicate insignificant difference at P≤0.05; A indicates the highest value

The Mean serum triglyceride of patients with MI was 202.12±47.41 mg/dl; mean serum triglyceride of risk group was 209.42±162.56 mg/dl and that of control group was 186.21±37.79 mg/dl. It was significantly lowest in the control group (P<0.05); however, no significant difference was encountered in its level between MI and risk groups (P>0.05, as shown in table (4-3).

Moreover, mean serum Total cholesterol in patients with MI was 202.12 ±47.41 mg/dl; mean serum Total cholesterol of risk group was 209.42 ±162.56 mg/dl and that of control group was 186.21 ±37.79 mg/dl. It was significantly highest in the MI group followed by risk group and then by control group (P<0.05), as shown in table (3-4).

According to such data, The most MI patients and risk group patients were considered as hypertensive when they were already on antihypertensive treatments and antihyperlipidemia treatments therefore some time no significant difference in triglyceride , cholesterol and High density lipid (HDL) of plasma level when compared with healthy control.

This result agreement with Al-Gazally and Al-Saadi results (Al-Gazally and Al-Saadi 2016) and Holmes, Millwood et al (Holmes, Millwood et al. 2018) since they found Lipoproteins and lipids showed similar associations with MI . HDL particles, cholesterol concentrations are inversely associated, whereas triglyceride concentrations is positively associated with MI .

4.4 Distribution of patients groups according to frequency of attack

Patients belong to MI group were classified into those having first attack and those experience recurrence. Patients with first attack account for 19 out of 24 (79.2%), while patients with recurrence accoun for 5 out of 24 (20.8%), as shown in figure (4-3).

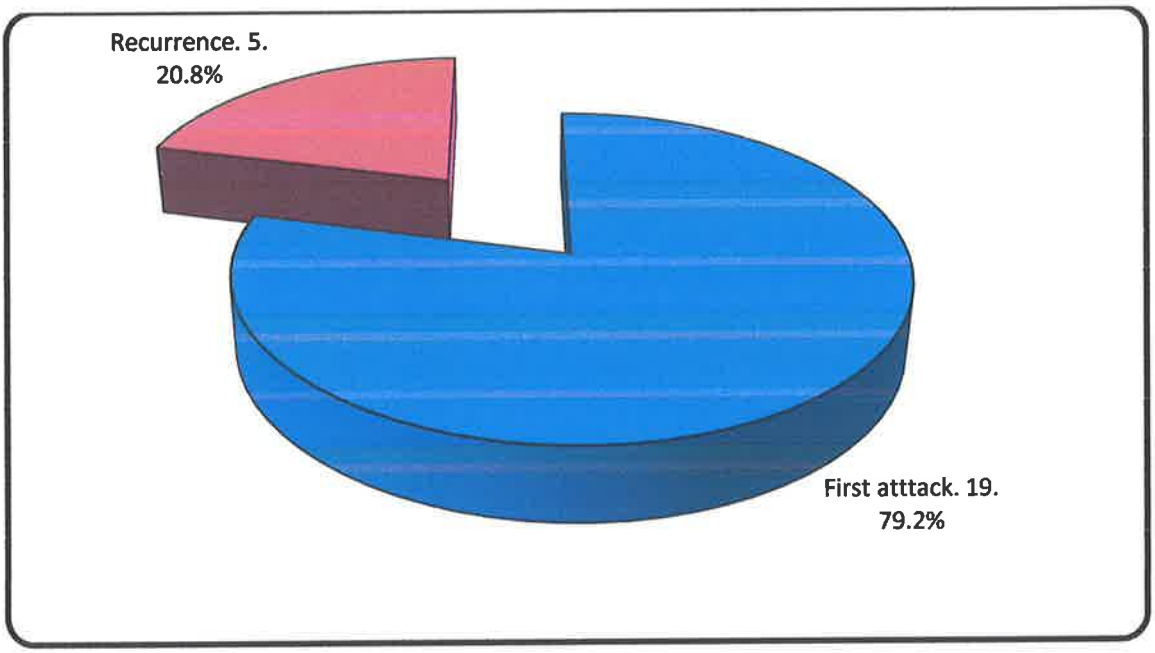


Figure 4-3: Pie chart showing the distribution of patients with MI according to frequency of attacks

4.5 Marker of cardiac muscle injury in groups enrolled in the present study

The serum level of cardiac muscle injury markers in MI patients are shown in table (4-4). Patients who were positive for Troponine accounted for 9 out of 24 (37.5%), those who were positive for creatinine kinase (CK) accounted for 9 out of 24 (37.5%), those who were positive for

Table (4-4): Markers of cardiac muscle injury

Marker	n	%
Troponine I	9	37.5
CK-MB	9	37.5
Myoglobin	9	37.5
Any one of them	13	54.2

Myoglobin accounted for 9 out of 24 (37.5%) and those who were positive for any one of the three markers accounted for 13 out of 24 (54.2%), as shown in table (4-4).

There are different results of cardiac markers in MI patients might be related to time of sample collection, therefore some patients with early MI stage, CK-MB test only was positive, others were negative , because The CK-MB rises in the serum at 4-9 h after the onset of chest pain, peaks ~24 h and returns to baseline
values at 48-72 h(Gerhardt, Katus et al. 1991, Wang, Lee et al. 2013). Myoglobin is released extremely early into the serum, 1 h after the onset of myocardial injury, peaks at 4-12 h and returns to baseline values immediately(Vaidya 1994). TroponineI are released into the circulation

6-8 h after myocardial injury, peak at 12-24 h and remain elevated for 7-10 days(Tucker, Collins et al. 1997). Therefore differents in this result each patient depent on MI progress which asociated with blood collection time .

4.6 SIRT3 Frequency distribution of genotype and allele belonging to rs11246029

The frequency distribution of genotype and allele belonging to rs11246029 figure (4-4); according to groups is shown in table (4-6). Genotype CC was seen in 16 (66.6%), and 6 (31.6%) of MI, and control groups respectively. Genotype CT was seen in 4 (16.7%), and 8 (42.1%) of MI, and control groups respectively. Genotype TT was seen in 4 (16.7%), and 5 (26.3%) of MI, and control groups respectively. There were highly significant difference in frequency distribution of CC genotype between MI and control groups (p=0.022) with an EF (0.56).

On the other hand, both of CT, and TT genotype show no significant differences between MI and control group (p=0.065, and P=0.065) respectively. As shown in Table (4-6).

Concerning the allelic distribution, allele C was seen in 36 (75.0%), 29 (60.4%) of MI, and control groups, respectively. Allele T was seen in 12 (25.0%), and 18 (47.4%) of MI, and control groups, respectively. Allele C was more frequently seen in patients with MI than in control group (P = 0.031), with EF (0.40). Patients are more likely to have allele C by an Odds ratio of 2.7, as shown in table (4-6). While allele T was more frequently seen in control than in MI group

(*P*=0.031),with PF (0.40). Patients are less likely to have allele T, as shown in table (4-6).

Table (4-6): The frequency distribution of genotype and allele belonging to rs11246029 between MI, Risk and control group.

Genotype	MI	Control	P	OR (95% CI)	EF	PF
CC	16 (66.6)	6 (31.6)	**0.022**	4.33 (1.2-15.69)	0.56	----
CT	4 (16.7)	8 (42.1)	0.065	0.28 (0.07-1.12)	----	0.47
TT	4 (16.7)	5 (26.3)	1.000	0.99 (0.22-4.33)	----	0.26
Allele	**MI**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	36 (75.0)	20 (52.6)	**0.031**	2.70 (1.08-6.72)	0.40	----
T	12 (25.0)	18 (47.4)	**0.031**	0.37 (0.15-0.92)	----	0.40
Genotype	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
CC	10 (41.7)	6 (31.6)	0.497	1.55 (0.44-5.47)	0.22	----
CT	9 (37.5)	8 (42.1)	0.759	0.83 (0.24-2.82)	0.10	----
TT	5 (20.8)	5 (26.3)	0.637	0.74 (0.18-3.05)	----	0.15
Allele	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	29 (60.4)	20 (52.6)	0.469	1.37 (0.58-3.25)	0.16	----
T	19 (39.6)	18 (47.4)	0.469	0.73 (0.31-1.72)	----	0.16

Regarding to Risk and control group, Genotype CC was seen in 10 (41.7%)and, 6 (31.6%) of Risk, and control groups respectively. Genotype CT was seen in 9 (37.5%) and 8 (42.1%) of Risk, and control groups respectively. While, Genotype TT was seen in 5 (20.8%) and 5 (26.3%) of Risk, and control groups respectively. There were no significant difference in frequency distribution of theses genotype between Risk and control groups (p=5 (20.8, p= 0.759, and p= 0.637)

respectively. However, both CC, and CT genotype were more frequently seen in risk group than in control group, with EF (0.22, 0.10 respectively). Risk group are more likely to have genotype CC, by an Odds ratio of 1.55, as shown in table (4-6).Regarding to allelic distribution, allele C was seen in 29 (60.4%), 20 (52.6%) of risk, and control groups, respectively. Allele T was seen in 19 (39.6%), and 18 (47.4%) of risk, and control groups, respectively. Allele C was more frequently seen in risk group than in control group, but statistically it seem not significant (P = 0.469), with EF (0.16). thus, Patients are more likely to have allele C by an Odds ratio of 1.37. as shown in table (4-6).

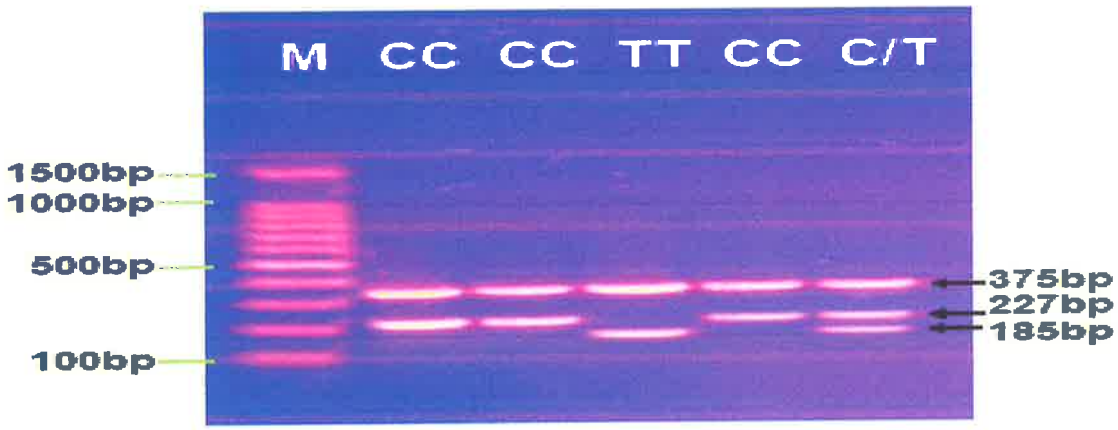


Figure (4-4): Agarose gel electrophoresis image that show the ARMS-PCR product analysis of SIRT3 gene (rs11246029) blood patients and healthy control samples. ARMS-PCR product was analysis by 1% agarose gel. Where M: marker (150-100bp), lane (CC) wild type homozygote was show at two bands (375bp internal control band and 227bp C allele bands), lane (TT) mutant type homozygote was show at two bands (375bp internal control band and 185bp T allele bands), and lane (C/T) heterozygote was show at three band digested at (375bp internal control band, 227bp C allele and 185bp T allele bands)

4.7 Frequency distribution of genotype and allele belonging to rs12293349.

The frequency distribution of genotype and allele belonging to rs12293349 figure (4-5) according to groups is shown in table (4-7). Genotype CC was seen in 7 (29.2%), and 10 (52.6%) of MI, and control groups respectively. Genotype CT was seen in 12 (50.0%), and 4 (21.1%) of MI, and control groups respectively. Genotype TT was seen in 5 (20.8%), and 5 (26.3%) of MI, and control groups respectively. The only significant difference was seen in frequency distribution of CT genotypes

Table (4-7): The frequency distribution of genotype and allele belonging to rs12293349 between MI, Risk, and control group.

Genotype	MI	Control	P	OR (95% CI)	EF	PF
CC	7 (29.2)	10 (52.6)	0.118	0.37 (0.11-1.131	----	0.41
CT	12 (50.0)	4 (21.1)	**0.051**	3.75 (0.96-14.65)	**0.55**	----
TT	5 (20.8)	5 (26.3)	0.953	0.74 (0.44-1.72)	----	0.15
Allele	**MI**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	26 (54.2)	24 (63.2)	0.401	0.69 (0.29-1.65)	----	0.19
T	22 (45.8)	14 (36.8)	0.401	1.45 (0.61-3.46)	0.19	----
Genotype	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
CC	9 (37.5)	10 (52.6)	0.474	0.64 (0.19-2.16)	----	0.29
CT	6 (25.0)	4 (21.1)	1.000	1.25 (0.30-5.27)	0.12	----
TT	9 (37.5)	5 (26.3)	0.437	1.68 (0.45-6.25)	0.26	----
Allele	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	24 (50.0)	24 (63.2)	0.222	0.58 (0.24-1.39)	----	0.26
T	24 (50.0)	14 (36.8)	0.222	1.71 (0.72-4.09)	0.26	----

between MI and control groups (P=0.05), and EF (0.55). Thus, genotype CT was more frequently seen in risk group than in control group with OR (3.75).

In addition to that, allele C was seen in 26 (54.2%), and 24 (63.2%) of MI, and control groups, respectively. Allele T was seen in 22 (45.8%), and 14 (36.8%) of MI, and control groups, respectively. Neither allele C nor allele T showed significant difference in their frequency distribution among MI, and control groups (P>0.05), as shown in table (4-7).

Concerning Risk and control group, Genotype CC was seen in 9 (37.5%), and 10 (52.6%) of risk, and control groups respectively. Genotype CT was seen in 6 (25.0%), and 4 (21.1%) of risk, and control groups respectively. Genotype TT was seen in 9 (37.5%), and 5 (26.3%) of risk, and control groups respectively. There were no significant difference was seen in frequency distribution of **CC, CT, and TT** genotypes between risk and control groups (P=0.474, p= 1.00. p= 0.437) respectively, and EF (0.55). Thus, genotype CT was more frequently seen in risk group than in control group with OR(3.75).as shown in table (4-7).

In addition to that, allele C was seen in 24 (50.0%), and 24 (63.2%) of risk, and control groups, respectively. Allele T was seen in 24 (50.0%), and 14 (36.8%) of risk, and control groups, respectively. Neither allele C nor allele T showed significant difference in their frequency distribution among risk, and control groups (P>0.05), as shown in table (4-7).

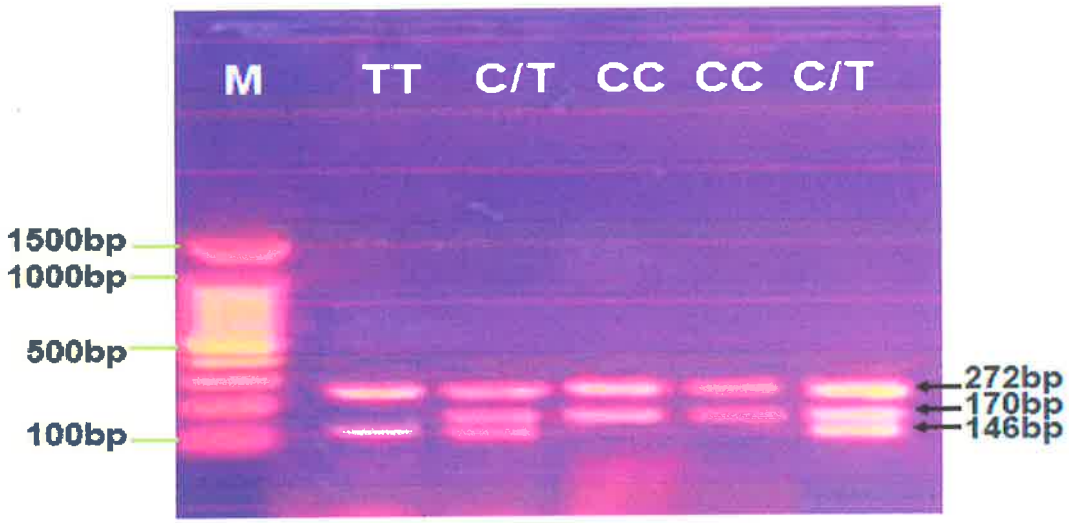


Figure (4-5): Agarose gel electrophoresis image that show the ARMS-PCR product analysis of SIRT3 gene (rs12293349) blood patients and healthy control samples. ARMS-PCR product was analysis by 1% agarose gel. Where M: marker (150-100bp), lane (CC) wild type homozygote was show at two bands (272bp internal control band and 170bp C allele bands), lane (TT) mutant type homozygote was show at two bands (272bp internal control band and 146bp T allele bands), and lane (C/T) heterozygote was show at three band digested at (272bp internal control band, 170bp C allele and 146bp T allele bands).

4.8 The frequency distribution of genotype and allele belonging to rs56312618.

The frequency distribution of genotype and allele belonging to **rs56312618** figure (4-6); according to groups is shown in table (4-8). Genotype GG was seen in 4 (16.7%), and 8 (42.1%) of MI, and control groups respectively. Genotype GA was seen in 7 (29.2%), and 7 (36.8%) of MI, and control groups respectively. Genotype AA was seen in 13 (54.2%), and 4 (21.1%) of MI, and control groups respectively.

There was significant difference in frequency distribution of AA, genotypes between MI and control groups (P=0.027), with an EF (0.59), and OR (4.43). Accordingly, genotype AA was more frequently seen in MI group than in control group.

Table 4-8: The frequency distribution of genotype and allele belonging to rs56312618 between MI, Risk, and control group.

Genotype	MI	Control	P	OR (95% CI)	EF	PF
GG	4 (16.7)	8 (42.1)	0.065	0.28 (0.067-1.12)	----	0.47
GA	7 (29.2)	7 (36.8)	0.594	0.71 (0.20-2.54)	----	0.17
AA	13 (54.2)	4 (21.1)	**0.027**	4.43 (1.13-17.43)	**0.59**	----
Allele	**MI**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
G	15 (31.3)	23 (60.5)	**0.007**	0.32 (0.13-0.77)	----	**0.46**
A	31 (64.7)	15 (60.5)	**0.007**	3.17 (1.29-7.76)	**0.46**	----
Genotype	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
GG	9 (37.5)	8 (42.1)	0.759	0.83 (0.24-2.82)	----	0.10
GA	5 (20.8)	7 (36.8)	0.245	0.45 (0.12-1.75)	----	0.34
AA	10 (41.7)	4 (21.1)	0.152	2.68 (0.68-10.53)	0.45	----
Allele	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
G	23 (47.9)	23 (60.5)	0.244	0.6 (0.25-1.42)	----	0.25
A	25 (52.1)	15 (39.5)	0.244	1.67 (0.7-3.95)	0.25	----

On the other hand, allele G was seen in 15 (31.3%), and 23 (60.5%) of MI, and control groups, respectively. Allele A was seen in 31 (64.7%), and 15 (60.5%) of MI, and control groups, respectively. Thus, Allele A was more frequently seen in patients with MI than in control group (P = 0.007), with an EF=0.46. While allele G was less frequently seen in patients with MI than in control group (p=0.007), with PF=0.46. Thus,

allele A was more frequently seen in MI group than in control group.as shown in table (4-8).

While, The frequency distribution of genotype and allele belonging to **rs56312618** belong to risk and control is shown in table (4-8). Genotype GG was seen in 9 (37.5%), and 8 (42.1%) of Risk, and control groups respectively. Genotype GA was seen in 5 (20.8%), and 7 (36.8%) of Risk, and control groups respectively. Genotype AA was seen in 10 (41.7%), and 4 (21.1%) of Risk, and control groups respectively. According to data of present study, There were no significant difference in frequency distribution of GG, GA, and AA, genotypes between Risk, and control groups (P> 0.05).Concerning allelic distribution, allele G was seen in 23 (47.9%), and 24 (63.2%) of risk, and control groups, respectively. Allele A was seen in 25 (52.1%), and 15 (39.5%) of risk, and control groups, respectively. Neither allele G nor allele A showed significant difference in their frequency distribution among risk, and control groups (P>0.05), as shown in table (4-8).

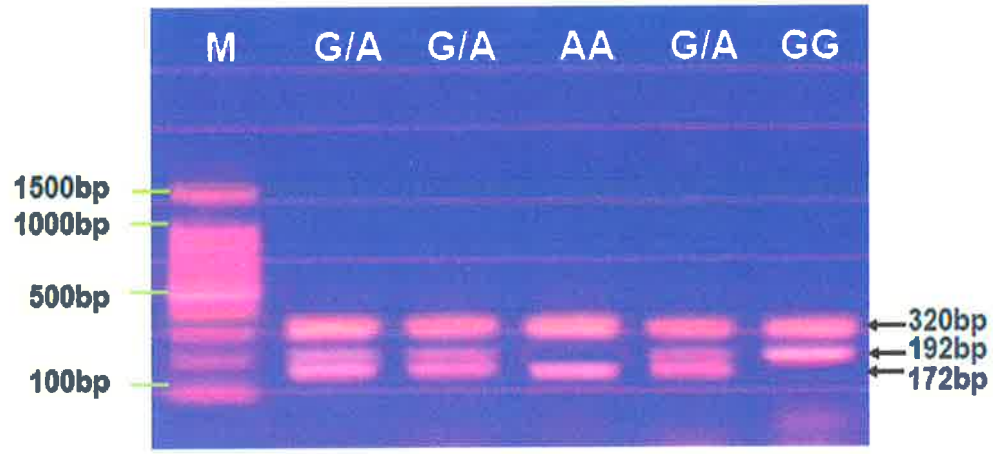


Figure (4-6): Agarose gel electrophoresis image that show the ARMS-PCR product analysis of SIRT3 gene (rs56312618) blood patients and healthy control samples. ARMS-PCR product was analysis by 1% agarose gel. Where M: marker (150-100bp), lane (GG) wild type homozygote was show at two bands (320bp internal control band and 192bp G allele bands), lane (AA) mutant type homozygote was show at two bands (320bp internal control band and 172bp A allele bands), and lane (G/A) heterozygote was show at three band digested at (320bp internal control band, 192bp G allele and 172bp A allele bands).

To date, few studies have linked the SIRT3 gene polymorphsim with coronary artery disease. Keeping in mind this is the first locally conducted study concerning such subject.

According to data of the above studded genotype , the only significant correlation concerning **rs11246029** genotype was seen in **CC** variant between MI and control group with an EF= 0.56. While in case of **rs12293349** genotype the significant difference was seen in **CT** variant,

between MI and control (p=0.05) group with an EF= **0.55** as mentioned previously.

In addition to that the **rs56312618** genotype show significant difference in **AA** variant, between MI and control group(p=0.027), also with EF=0.59. according to such data the **CC,CT,** and **AA** genotype were more frequently seen in MI group than in control group, thus people who have such genotype would be at risk for developing myocardial infarction.

Accordingly, Three SNPs were found in MI patients with significantly higher frequencies compared to controls SNPs, might be significantly decrease the transcriptional activities of the SIRT3 gene promoter in cells. Therefore, these SIRT3 gene promoter SNP may reduce SIRT3 levels, contributing to the MI development as risk factors. The human SIRT3 gene is localized to the chromosome 11p15.5, and encodes an NAD dependent mitochondrial deacetylase of 399-amino acids containing an N-terminal mitochondrial targeting signal and a central catalytic domain(Frye 1999, Schwer, North et al. 2002). SIRT3 gene is expressed in a variety of tissues with higher expression in adipose tissue, brain and heart in embryos and adults indicating the tissue-specific

regulation of the SIRT3 gene expression. The human SIRT3 gene promoter contains high GC contents and lacks the TATA box sequence and there are binding sites for activator protein 1 (AP1), GATA-binding factor,) and transcription factor ZF5, as well as multiple specificity protein 1 (SP1) binding sites(Bellizzi, Dato et al. 2007). Nuclear respiratory factor 2, a transcription factor that regulates mitochondrial genes, binds to the promoter of SIRT3 gene and induces its expression(Satterstrom, Swindell et al. 2015). In this study, the SNP

reduced the SIRT3 promoter transcriptional activity cells to different extents. Therefore, expression of the human SIRT3 gene may be manipulated for therapeutic purposes.

A series of downstream substrates of SIRT3, as well as histone, have been identified. SIRT3 deacetylates and activates several enzymes that are critical in maintaining cellular ROS levels and promote resistance to oxidative stress, including superoxide dismutase 2 (SOD2) and) (Chen, Zhang et al. 2011, Merksamer, Liu et al. 2013) (Tao, Coleman et al. 2010). Increased ROS levels and oxidative stress have been demonstrated to contribute to the atherogenesis(Li, Horke et al. 2014, V Goncharov, V Avdonin et al. 2015).

SIRT3 regulates fatty acid oxidation through heat shock protein 10(Lu, Chen et al. 2015). In human diploid fibroblasts, SIRT3 overexpression antagonizes high glucose-induced cellular senescence via the SIRT3- FOXO1 signaling pathway(Zhang, Cui et al. 2013). A recent study has shown that SIRT3 targets human verylong-chain acyl-CoA dehydrogenase, a key fatty acid oxidation enzyme(Verdin, Zhang et al. 2015). Therefore, decreased SIRT3 levels may contribute to MI development by: 1) affecting lipid metabolism, inflammation and other pathways, initiating the atherosclerosis; and 2) interfering with fatty acid oxidation, ROS generation and mitochondrial functions, leading to death of cardiomyocytes. Exact molecular mechanisms need further investigated and elucidated.

A previously conducted study found that, A SNP rs11555236 (C>A) in intron 5 of the SIRT3 gene, which increases expression of SIRT3 gene, has been associated with extended lifespan of humans(Albani, Ateri et al. 2014).

Like other candidate gene studies, there are several limitations in the present study. As the power to detect disease susceptibility genes is influenced by the number of patient's samples, the size of sample in the present study seemed to be relatively small. Thus the lack of significant differences between risk and control group related to all genotype under study (**rs11246029, rs12293349 and, rs56312618)** might be related to such factors mentioned above.

4.9 Median and inter-quartile range of mi-R 1 and 145 fold change values in control and study groups

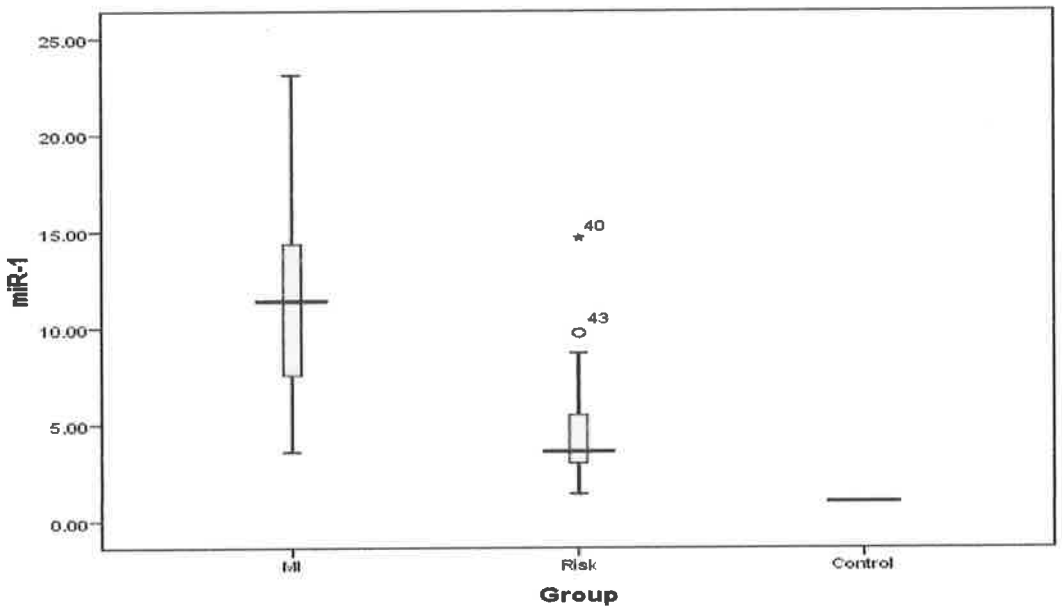


Figure (4-7): Median and inter-quartile values of miRNA- 1 in MI, risk and control groups

Since 2010, more than 30 studies have assessed the use of miRNAs as biomarkers for acute MI diagnosis, and their predictive values for post-MI outcomes, Protein biomarkers, based on cellular decay, such as TnI/T and creatine kinase MB (CK-MB), have been very well established in

clinical use, so the evaluation versus this current standard is of utmost importance.

The median fold change and inter-quartile range of miRNA-1 in patients groups was 11.4 (6.95), and 3.6 (2.7) of risk group respectively. and the value of fold change of control group was fixed at 1. Thus, the level of miRNA-1 fold change was significantly highest in the MI group followed by risk group and then by control group ($P<0.05$), as shown in figure (4-7).While, the median fold change and inter-quartile range of miRNA-145 in patients groups was 0.055 (0.12), 0.375 (0.78) of risk group respectively. and the value of fold change of control group was fixed at 1. The level of miRNA-145 fold change was significantly lowest in the MI group followed by risk group and then by control group ($P<0.05$), as shown in figure (4-8).

Circulating levels of miRNA-1 was significantly increased in patients with AMI, this result came in agreement with Pan Z, Sun X et al. (2012) since they mention that miRNA-1 positively correlates with serum CK-MB level in AMI patient.

miRNA-1, level was increased with risk group because the most of this group are hypertensive patients as a result of Atherosclerosis which consider as an inflammatory process of endothelial blood vessels wall that stimulating apoptosis, present study shows an increasing level of miRNA-1 in this group when compared with control group, but without increasing in level of ck-MB or others cardiac biomarker. (Creemers, Tijsen et al. 2012).

In the context of the cardiovascular system, the most discussed

are miRNA-1 and miRNA-133(Townley-Tilson, Callis et al. 2010). Moreover, A study done by Pavkova *et al.*, 2018 (Boldrini, Fulmore et al. 2018)found that, The expression of miRNA-1 in cardiogenic shock patients compared to controls was significantly higher. Also, in addition to that, results are in consensus with published studies describing increased circulating levels of miRNA-1 and miRNA-133 in patients with MI (Ai, Zhang et al. 2010, Cheng, Wang et al. 2014). The source of elevated levels of miRNA-1 and miRNA-133 is likely in the damaged cardiac tissue (Ai, Zhang et al. 2010, Kuwabara, Ono et al. 2011). The injury leads to deregulation of miRNA-1 and release from the damaged cardiomyocyte(Boštjančič, Zidar et al. 2010). In the model of chronic failing hearts, Kumarswamy et al have shown negative regulation of cardiomyocyte miRNA-1 expression and decreased miRNA-1 expression in vivo(Zhang, Li et al. 2012). Zhang et al showed the effect of miRNA-1 on cell cycle progression(Zhang, Li et al. 2012). Furthermore,miRNA-1 affects the apoptotic process, vasoactive mediators, and could be one of the mediators of cardiac hypertrophy(Fichtlscherer, Zeiher et al. 2011).

Most studies were focused on the group miRNA-1, -133a/b, -208a, -499. These so-called *myomirs* are heart specific, due to their regulatory interaction with the transcripts of different cardiac muscle myosin chains, similar to CK-MB(van Rooij, Quiat et al. 2009). Changes in the circulatory levels of these miRNAs specifically represent heart tissue. Release kinetics have been closely studied by Liebetrau *et al.* in an experimental setting, revealing significant elevation after 15 minutes and a peak at 85 minutes for miRNA-1 and -133, in close correlation with TnI/T(Fichtlscherer, De Rosa et al. 2010).

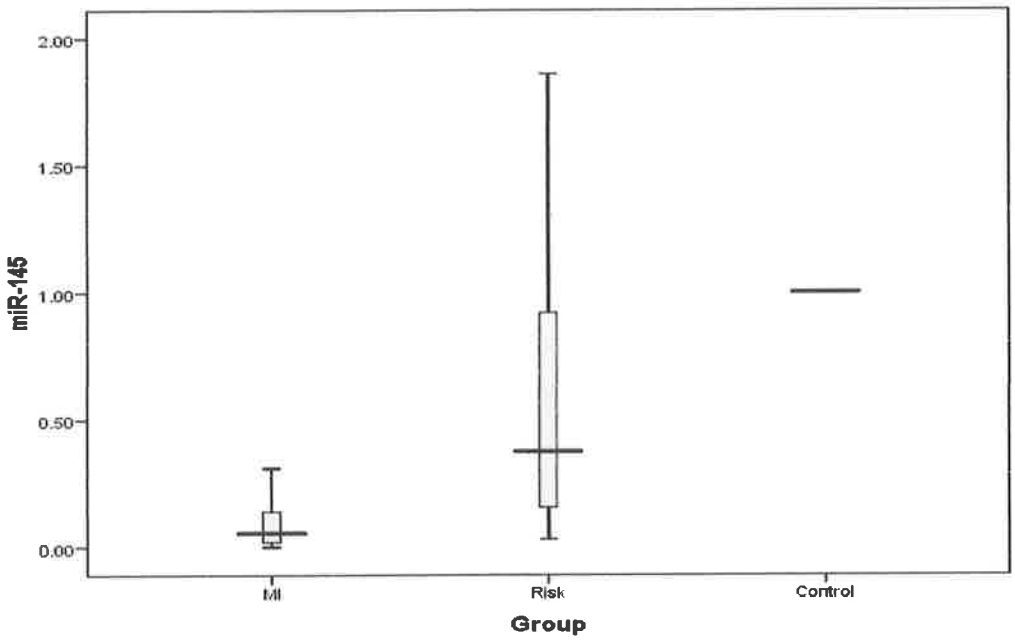


Figure (4-8): Median and inter-quartile values of miRNA-1 in MI, risk and control groups

The present study mention that the level of miRNA-145 fold change was significantly lowest in the MI group, which came in agreement with previous study that demonstrated a significantly lower miRNA-145 levels in coronary artery diseases patients(Fichtlscherer, De Rosa et al. 2010). Moreover, miRNA-145 fold change was significantly lower in the MI risk factor group (Hypertensive ,hyperlipidemia , DM and others risk factor) compared with control which confirm previously conducted study done by Cordes and colleagues since, they demonstrated that miRNA-145 promotes differentiation and represses proliferation of SMCs and the levels of this miRNA subtype were down regulated to nearly undetectable levels in atherosclerotic lesions containing neointimal hyperplasia(Cordes, Sheehy et al. 2009).

Moreover, These results reiterate findings from previous study that demonstrated significantly lower miRNA-145 levels in CAD patients(Fichtlscherer, De Rosa et al. 2010).

Circulating miRNA-145 has been shown to be regulated during coronary atherosclerosis (Cordes, Sheehy et al. 2009, Zhang 2009). Cheng Y et al (Cheng, Liu et al. 2009)reported that miRNA-145 is the most abundant miRNA subtype in the normal vascular walls and that it is selectively expressed in the Vascular smooth muscle cells VSMCs of vessel wall, and can regulate the phenotype of VSMC. Experimental animal studies have reported that carotid artery ligation was associated with markedly reduced miRNA-145 levels, as measured by quantitative PCR and subsequent carotid stenosis(Cordes, Sheehy et al. 2009). Mechanisms by which miRNA-145 regulates VSMC phenotype include KLF4, calmodulin kinase IIδ, myocardin, actin polymerization and angiotensin-converting enzyme(Albinsson and Sessa 2010). Restoration of miRNA-145 expression limits neointima formation in response to vascular injury by promoting KLF4 down regulation and VSMC contractile protein expression(Cheng, Liu et al. 2009, Albinsson and Sessa 2010).

Most studies failed to show discriminatory power for either a single or a subset of circulating miRNAs. However, lower levels of miRNA-145 and -155 showed an inverse correlation with CAD severity scores (Gensini and SYNTAX), Proliferative effects of miRNA-21, -199a, and antiproliferative effects of miRNA-145, add to the possible concerted hyperplastic ability of these candidates(Bhattachariya, Dahan et al. 2015).

4.10 Cutoff values of miRNA(1,145) that predict MI diagnosis.

In order to find a cutoff value of miRNA-1, and miRNA-145 that can predict a diagnosis of MI, More precisely, on receiver operating characteristic curves (ROC) curried out. The area under the curve (AUC), the usual endpoint measure in clinics to test the accuracy of a test in separating groups, were 0.94, and 0.92 for miRNA-1, and

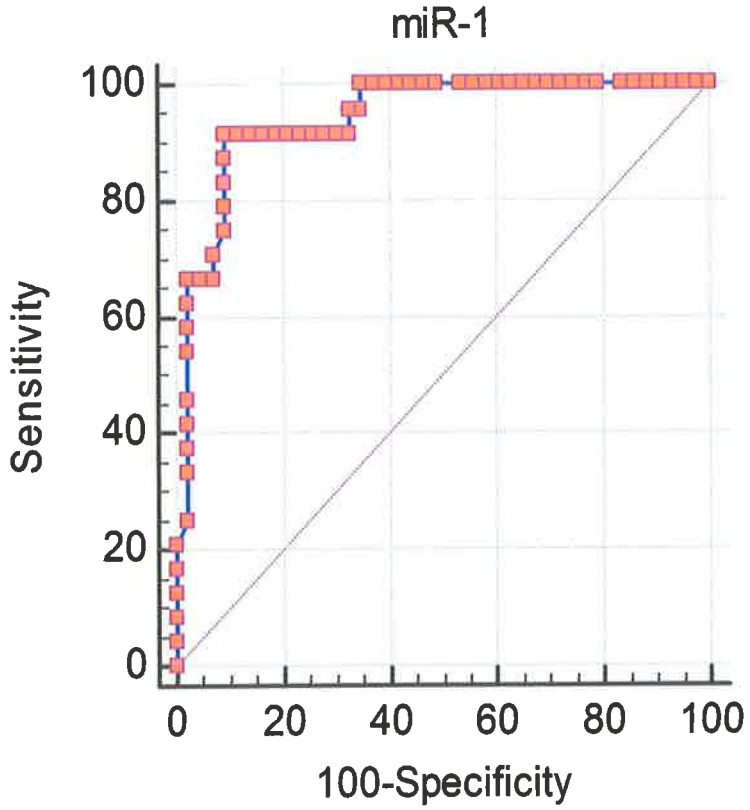


Figure (4-9): ROC curve to find miRNA-1 cutoff value that predict diagnosis of MI

Table (4-9): Characteristics of ROC curve shown in figure (4-9)

Cutoff value	>5.28 fold change
AUC (accuracy)	0.940 (94%)
P	<0.001
Sensitivity	91.67
Specificity	90.70

miRNA-145 respectively as shown in figure (4-9) and figure (4-10), for the separation between non-MI and AMI patients An area of 1 would represent a perfect test contrariwise to an area of 0.5 that would consist in a worthless test.

As shown in figure (4-9). The cut off value was identified at miRNA-1 level of >5.28 fold change with a sensitivity of 91.67 % and a specificity of 90.7%, as shown in table 4-8. Similarly, the cut off value was identified for miRNA-145 level of ≤0.7 fold change with a sensitivity of 95.83 % and a specificity of 89.47%, as shown in table (4-10). Furthermore, the combination of the two miRNAs managed to deliver an increased diagnostic power.

These results came in agreement with Guo-Kun 2010 his results from clinical samples in patients demonstrated that the levels of miRNA-1, miRNA-133a, miRNA-499, and miRNA-208a in blood from patients with AMI are elevated compared with those from individual without AMI. Receiver operating characteristic analysis further indicated that these four miRNAs might be good biomarkers for AMI diagnosis. These results are partially supported by others report that miRNA-1 level was significantly higher in plasma from AMI patients compared with non-AMI subjects(Ai, Zhang et al. 2010, Wang, Zhu et al. 2010).

Therefore these miRNAs could be used as AMI biomarker according to above result and others miRNA characterizes, which are miRNAs remain stable in serum and other body fluids(Reid, Kirschner et al. 2011). Circulating miRNAs remain stable even after exposure to severe conditions, such as high temperatures, extreme pH, and prolonged storage(Chen, Ba et al. 2008). Circulating miRNAs are protected

themselves from degradation by several mechanisms, including packing in membrane vesicles (such as microvesicles(Hunter, Ismail et al. 2010),

Exosomes, and apoptotic bodies(Zernecke, Bidzhekov et al. 2009), bounding to transporter proteins, and inclusion in macromolecular complexes (such as high density lipoproteins) (Vickers, Palmisano et al. 2011) so can using miRNA-1, miRNA-145 as AMI biomarkers.

Previous studied mention that, the plasma level of miRNA-1 was reported to markedly increased in in AMI patients, which was only mildly increased in patients with non-AMI coronary heart disease or patients with other cardiovascular diseases(Wang, Zhu et al. 2010). In a rat model of AMI induced by coronary ligation, serum miRNA-1 level was rapidly increased around 200-fold after AMI with a peak at 6 h, and the miRNA-1 level returned to basal levels at 3 days after AMI, which displayed a strong positive correlation with myocardial infarct size. In a clinical study, the level of circulating miRNA-1 was increased nearly 100-fold in AMI patients compared with healthy subjects and showed a positive correlation with serum creatine kinase-MB (CK-MB) level. Interestingly, the increased plasma miRNA-1 was not associated with age, gender, blood pressure, diabetes mellitus, or other established biomarkers for AMI including cTnI and CK-MB. Collectively, these results strongly support that circulating miRNA-1 might be a novel sensitive biomarker for AMI diagnosis(Cheng, Tan et al. 2010).

Current study confirm previously conducted study done by Long *et al.*, since, they demonstrated that the ability of the miRNA-1-score to differentiate the AMI group from the control group according to ROC curve with an AUC of 0.92, 0.90, 0.94, 0.92, 0.96 and 0.90. they achieved a sensitivity of 93%, 93%, 94%, 93%, 93% and 90% and a specificity of

90%, 90%, 93%, 90%, 90% and 90%, respectively, measured at six time interval for the identification of AMI patients(Long, Wang et al. 2012).

Several studies have investigated the circulating miRNA-145 levels in patients with coronary artery disease (Fichtlscherer, De Rosa et al. 2010, D'Alessandra, Carena et al. 2013). Fichtlscherer et al. reported that circulating miRNA-145 was downregulated in patients with stable coronary artery disease compared with healthy controls(Fichtlscherer, De Rosa et al. 2010). However,

The miRNA-145 level in total peripheral blood has been found to be elevated in patients with acute myocardial infarction and correlate with the infarction size estimated by troponin-T release(Meder, Keller et al. 2011). Since miRNA-145 is enriched in VSMCs, elevated miRNA-145 levels in AMI may reflect the vessel injury that occurs during atherosclerotic plague rupture. Consistent with this idea, upregulation ofmiRNA-145 expression is found in atherosclerotic plaques in hypertensive patients(Santovito, Mandolini et al. 2013). Although miRNA-145 levels correlated with infarction size, Meder et al. did not identify miRNA-145 as a good predictor for AMI using receiver operator characteristic curves(Meder, Keller et al. 2011).

Thus, present study demonstrate a unique signature of circulating miRNA for sensitive and specific diagnosis of MI that could be translated into non invasive blood-based biomarker panels for patients. Considering that miRNA are active molecules, our study also suggests that miRNA-145, miRNA-1 may be instrumental in MI pathology and could represent new targets for therapeutic interventions.

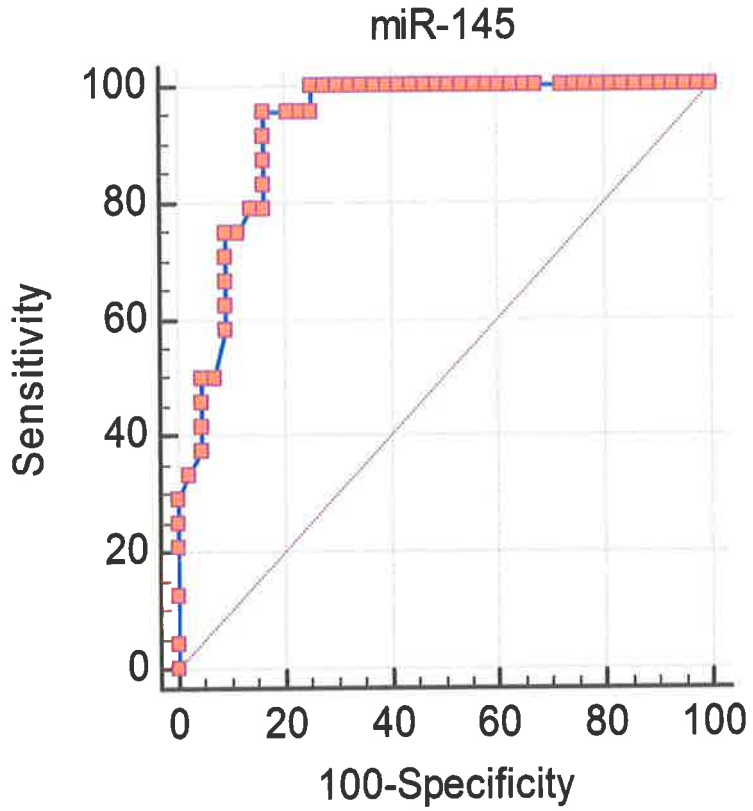


Figure 4-10: ROC curve to find miRNA-145 cutoff value that predict diagnosis of MI

Table (4-10): Characteristics of ROC curve shown in figure (4-10)

Cutoff value	≤0.7
AUC (accuracy)	0.927 (92.7%)
P	<0.001
Sensitivity	95.83
Specificity	83.72

Cardiac markers was using in AMI diagnosis as cTNT and cTNI can be released into the serum during necrosis occurs in the process of AMI However, the release of miRNA can be affected by any form of cellular stress, such as anoxia, lactic acidosis, or cellular edema. In AMI, miRNAs occur earlier than necrosis. Although several methods of RNA detection have been developed, both simple and complex methods tend to be expensive and time-consuming (Cheng, Wang et al. 2014). There are some promising methods, but they require improvement before they can be used in clinical settings or hospitals.

The characteristic of (miRNA-1,miR145) and theirs high sensitivity and Specificity in this study which was bushed to using them as alone biomarker or supported for Other biomarker in AMI diagnosis .

4.11 Assassment AMI prognosis depending on MiRNA-1, MiRNA-145

In order to find a cutoff value of miRNA-1 that can predict a recurrence of MI, a receiver operator characteristic (ROC) curve was carried out, The area under the curve (AUC), were 0.56, and 0.55 for miRNA-1, and miRNA-145 respectively as shown in figure (4-11) and figure (4-12). the miRNA-1 have predictive value of recurrence of MI with sensitivity and specificity (40% , 89.47 %) respectively as shown in table (4-11). Also, a receiver operator characteristic (ROC) curve was carried out for mi-145.

It was shown that miRNA-145 have a predictor of recurrence of MI with sensitivity and specificity (40% , 94.74 %) respectively, as shown in table (4-12).

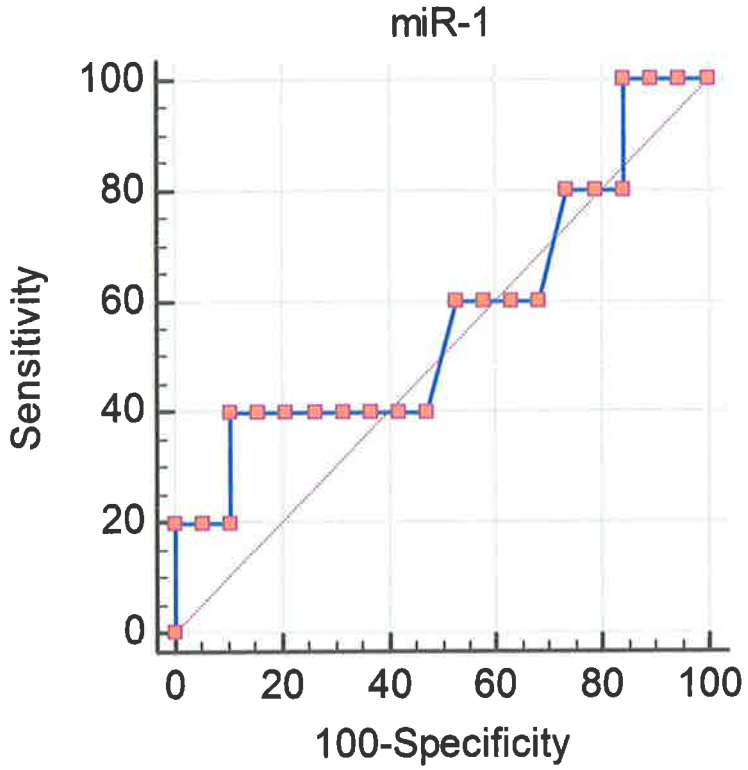


Figure (4-11): ROC curve to find miRNA-1 cutoff value that predict recurrence of MI

Table (4-11): Characteristics of ROC curve shown in figure 4-8

Cutoff value	≤6.06
AUC (accuracy)	0.568 (56.8%)
P	0.696
Sensitivity	40.00
Specificity	89.47

A study done by Long *et al.*, demonstrated that the ability of the miRNA-1-score to differentiate the AMI group from the control group according to ROC curve a sensitivity of 93%, 93%, 94%, 93%, 93% and

90% and a specificity of 90%, 90%, 93%, 90%, 90% and 90%, respectively, measured at six time interval for the identification of AMI patients. And they concluded that, miRNA-1 might be an especially rich source of diagnostic, prognostic and predictive information as biomarkers in AMI patients keeping in mind there was very little studies reporting the role or miRNA-1 in MI(Long, Wang et al. 2012).

In a study done by Zhang *et al.*, demonstrated that the levels of miRNA-145 was correlated with infarct size and peak levels of miRNA-145 was correlated with peak levels of troponin T. Collectively, their findings demonstrate a relationship between miRNA-145 levels and the degree of cardiac damage. And they concluded that Levels of miRNA-145 may be used as markers of acute cardiac myocyte death and in turn be used to estimate infarct size and residual cardiac function(Zhang, Cheng et al. 2017).

Recent studies by Li and Zhang and others have also shown that circulating miRNAs can act as novel biomarkers for the diagnosis and prognosis of many diseases(Li and Zhang 2015, Hyun, Kim et al. 2016). The low sensitivity of miRNA-1 and miRNA-145 as a prognostic marker might be explained according to several limitations present in current study. Firstly, it was a Limited in short time of study which involved a small sample size, and the distribution of patients in AMI and healthy groups was uneven. Therefore, the results of this study might be interpreted with caution, and another study the large based on large sample size would be required to further illustrate miRNA-1 ,145 as a prognosis marker in AMI.

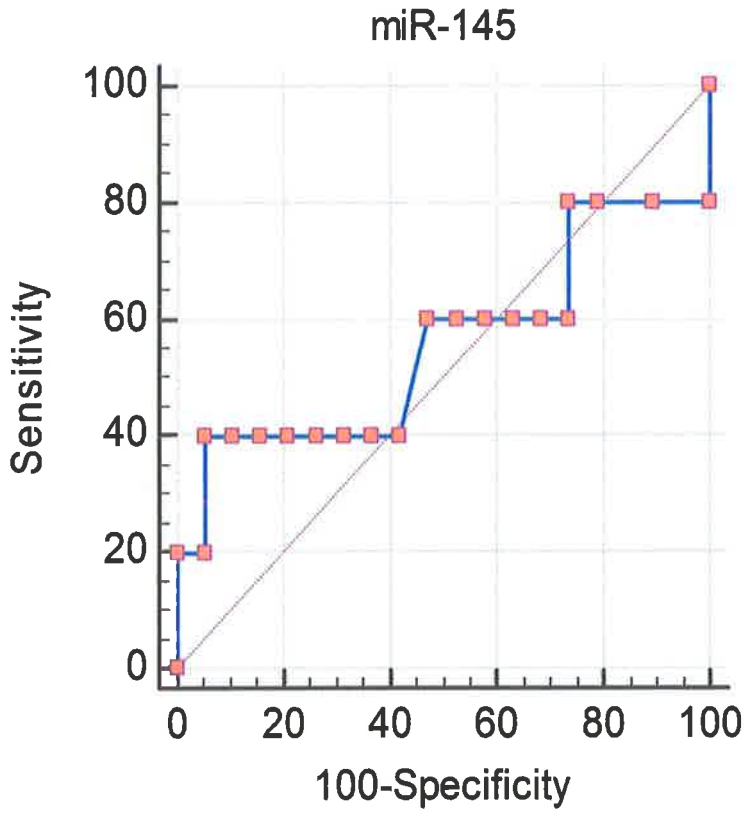


Figure (4-12): ROC curve to find miRNA-145 cutoff value that predict recurrence of MI

Table (4-12): Characteristics of ROC curve shown in figure (4-12)

Cutoff value	>0.54
AUC (accuracy)	0.553 (55.3%)
P	0.789
Sensitivity	40.00
Specificity	94.74

4.12 Association between SIRT3 promoter genotype frequency distribution and mi-R 1 or 145 level

The association between genotype frequency distribution and miRNA- 1 and 145 level is shown in table (4-13). There were no significant association between mi-RNA-1 and all three genotype

frequency rs11246029, rs12293349, rs56312618 in (P=0.182), (P=0.462), (P=0.074) respectively.

Table (4-12): Association between genotype frequency distribution and mi-R 1 and 145 level

MiRNA	rs11246029	Median (IQR)	P*	rs12293349	Median (IQR)	P*	rs56312618	Median (IQR)	P*
miRNA-1	CC	5.52 (7.29)	0.182	CC	3.75 (5.11)	0.462	GG	2.68 (4.14)	0.074
	CT	2.95 (5.80)		CT	5.18 (7.46)		GA	3.41 (8.73)	
	TT	3.33 (6.61)		TT	3.84 (5.45)		AA	6.19 (6.90)	
miRNA-145	CC	0.63 (2.22)	0.373	CC	1.37 (2.37)	0.273	GG	1.42 (2.72)	0.192
	CT	1.49 (3.28)		CT	0.31 (2.27)		GA	0.76 (3.09)	
	TT	1.14 (2.20)		TT	0.76 (1.99)		AA	0.54 (1.29)	

IQR: inter-quartile range;*: Kruskal Wallis test

More over there were no significant association between miRNA-145 and all three genotype frequency rs11246029, rs12293349, rs56312618 in (P=0.373) (P=0.273) (P=0.192) respectively

The result mean the fold change level of miRNA-1or miR145 not association with different SIRT3 promoter SNPs(rs12293349, rs56312618, rs11246029) ,or each one of polymorphism have same effect on the fold change level of miRNA-1, miR145. SIRT3 inhibits myocardial reperfusion injury and reduces oxidative stress-induced apoptosis (Chen, Fu et al. 2013, Bochaton, Crola-Da-Silva et al. 2015).Other studies reports increase an oxidative stress (H_2O_2) of cardiomyocytes with ischemia lead to reduce expression of miRNA-145 then over expression Bnip3 (miR145 target) . Bnip3is an initiation factor of mitochondrial apoptosis pathway and its over expression results in excessive ROS production ,that means reduce miR145,consequently leads to Bnip3 pathway induce cell death in those cell in pathway called

the intrinsic pathway of apoptosis (mitochondrial apoptosis pathway)and abnormal mitochondrial fission (Xu, Cao et al. 2017).

the harmful effects of miRNA-1 on the heart, include the myocardiocytes ischemia leads to increase oxidative stress which cause miRNA-1 overexpression leads to stimulates Bax and BCl2 which are involves in alter permeability of the mitochondrial outer membranes to cytochrome c out the mitochondria ,then its activation of caspase and stimulate PARP gene and apoptosis occurance (Xu, Gao et al. 2015). The miRNA-1 targets are Protein kinase C epsilon type (PKCe) and Heat shock protein 60 (HSP60) expression which are known to play a protective role against cardiac injury(Pan, Sun et al. 2012).

Unfortunately there is no previous study focus on the association between such miRNA and SIRT3 gene polymorphism ,however this result may be explained because small size of statistical sample which was chosen in this study and miRNA fold change deepened on severity of each disease stage which is not dependent in this study .

4.13 The correlation between miRNA-1, miRNA-145

In present study There was significant negative correlation between miRNA-1 and miRNA-145 ($r = -0.501$, $P<0.001$); however this correlation was able to explain only 15.6% of cases since R squared value was 0.156, as shown in figure (4-13).

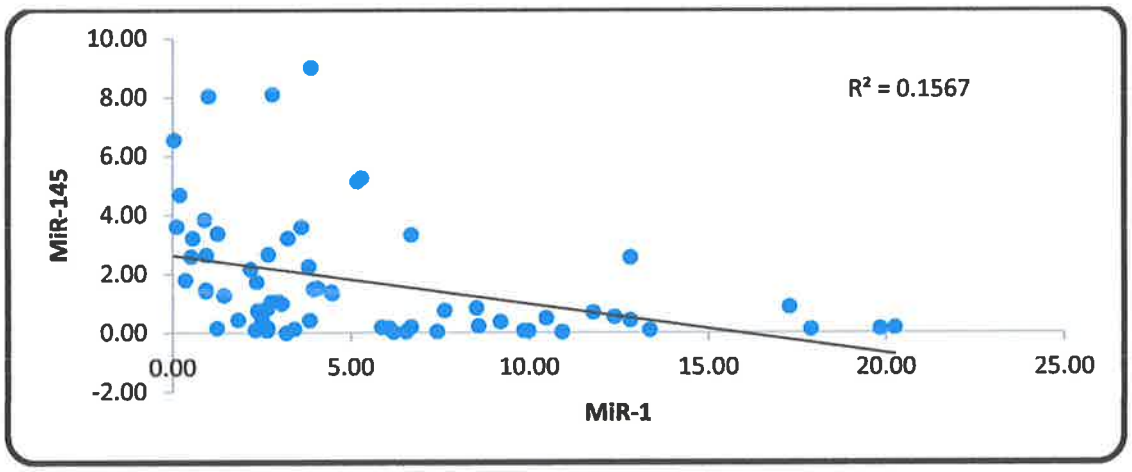


Figure (4-13): Correlation between mi-R 1 and 145

a weak correlation correlation between miRNA-1 and miRNA-145 level may be byond time of blood samples collection of MI patients not all patients with same stage of disease. There are other studies reported , that the level miRNA-1 associated with infract size of patients (Papageorgiou, Tousoulis et al. 2012) .other cause which is make these miRNA were not highly correlated is miRNAs secretion in two stage which are initial stage called active secretion product from apoptosis then miRNA-1 and miRNA-145 secretion in necrosis of myocardocytes occur called passive secretion these stages effect on association between miRNA-1 and miRNA145 correlation degree between them (Fichtlscherer, De Rosa et al. 2010) .

Conclusions and Recommendations

Conclusion

1. Myocardial infarction seem to be more common in age more than 53 years and the males more susceptible than females.
2. The individuals with SIRT3 gene promoter; **rs56312618** genotype(AAvariant); **rs12293349** genotype (CT variant);**rs11246029** (CC variant) were more frequently seem in MI patient than in control and risk group ,thus it has a role in disease susceptibility .
3. miRNA-1 fold change is significantly higher in MI patients followed by risk group and then by control group. while miRNA-145 fold change significantly lower in MI patients followed a risk group then control group.
4. ROC analysis of miRNA-1 diagnostic show a sensitivity of 91.67 and a specificity of 90.7 while ROC miRNA-145 show a sensitivity of 95.83 % and a specificity of 89.47%. , The characteristic of (miR-1,miR145) and theirs high sensitivity and Specificity in this study which was bushed to using them as alone biomarker or supported for Other biomarker in AMI diagnosis .
5. According to ROC analysis miRNA-1 show a predictive value of recurrence for MI with sensitivity(40%) and specificity(89.47 %) while miRNA-145 have a predictor of recurrence of MI with sensitivity(40%) and specificity (94.74 %).thus miRNA-1 , miRNA-145 can be using in MI prognosis interpretation but with caution.
6. There was significant negative correlation between mi-R1 and mi-R145 ($r = -0.501$, $P<0.001$).

Recommendation

1- Another study based on much reasonable sample number to get large data for determining role of SIRT3gene in a disease susceptible
2- Rt-PCR could be applied to detect the fold change difference in miRNA-1, miRNA-145 in MI patient.
3- Another study is recommend to find out another prognostic miRNAs for MI patient.
4- Large scale multicentric study required to further elucidate associated between (miRNA-1or miRNA-145) fold change and SIRT3 promoter gene polymorphism.

References

- Ai, J., R. Zhang, X. Gao, H.-F. Niu, N. Wang, Y. Xu, Y. Li, N. Ma, L.-H. Sun and Z.-W. Pan (2012). "Overexpression of microRNA-1 impairs cardiac contractile function by damaging sarcomere assembly." Cardiovascular research **95**(3): 385-393.
- Ai, J., R. Zhang, Y. Li, J. Pu, Y. Lu, J. Jiao, K. Li, B. Yu, Z. Li and R. Wang (2010). "Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction." Biochemical and biophysical research communications **391**(1): 73-77.
- Al-Asadi, J. and F. Kadhim (2014). "Day of admission and risk of myocardial infarction mortality in a cardiac care unit in Basrah, Iraq." Nigerian journal of clinical practice **17**(5): 579-584.
- Al-Gazally, M. E. and A. H. Al-Saadi (2016). "Effect of Uric Acid and Lipid Profile on Myocardial Infarction in Iraqi Population."
- AL-Oqaily, Q. A. (2014). "Assessment of Complete Blood Count in Patients with Coronary Artery Disease." Iraqi Journal of Medical Sciences **12**(1).
- Albani, D., E. Ateri, S. Mazzuco, A. Ghilardi, S. Rodilossi, G. Biella, F. Ongaro, P. Antuono, P. Boldrini, E. Di Giorgi, A. Frigato, E. Durante, L. Caberlotto, A. Zanardo, M. Siculi, M. Gallucci and G. Forloni (2014). "Modulation of human longevity by SIRT3 single nucleotide polymorphisms in the prospective study "Treviso Longeva (TRELONG)"." AGE **36**(1): 469-478.
- Albinsson, S. and W. C. Sessa (2010). "Can microRNAs control vascular smooth muscle phenotypic modulation and the response to injury?" Physiological genomics **43**(10): 529-533.
- Alderman, E. L., S. D. Corley, L. D. Fisher, B. R. Chaitman, D. P. Faxon, E. D. Foster, T. Killip, J. A. Sosa and M. G. Bourassa

(1993). "Five-year angiographic follow-up of factors associated with progression of coronary artery disease in the Coronary Artery Surgery Study (CASS)." Journal of the American College of Cardiology **22**(4): 1141-1154.

- Alhazzazi, T. Y., P. Kamarajan, E. Verdin and Y. L. Kapila (2011). "SIRT3 and cancer: tumor promoter or suppressor?" Biochimica et Biophysica Acta (BBA)-Reviews on Cancer **1816**(1): 80-88.
- Alrob, O. A., S. Sankaralingam, C. Ma, C. S. Wagg, N. Fillmore, J. S. Jaswal, M. N. Sack, R. Lehner, M. P. Gupta and E. D. Michelakis (2014). "Obesity-induced lysine acetylation increases cardiac fatty acid oxidation and impairs insulin signalling." Cardiovascular research **103**(4): 485-497.
- Apple, F. S. (1999). "Tissue specificity of cardiac troponin I, cardiac troponin T and creatine kinase-MB." Clinica chimica acta **284**(2): 151-159.
- Arroyo, J. D., J. R. Chevillet, E. M. Kroh, I. K. Ruf, C. C. Pritchard, D. F. Gibson, P. S. Mitchell, C. F. Bennett, E. L. Pogosova-Agadjanyan and D. L. Stirewalt (2011). "Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma." Proceedings of the National Academy of Sciences **108**(12): 5003-5008.
- Barger, J. L., R. M. Anderson, M. A. Newton, C. da Silva, J. A. Vann, T. D. Pugh, S. Someya, T. A. Prolla and R. Weindruch (2015). "A conserved transcriptional signature of delayed aging and reduced disease vulnerability is partially mediated by SIRT3." PloS one **10**(4): e0120738.
- Bayes-Genis, A., C. A. Conover, M. T. Overgaard, K. R. Bailey, M. Christiansen, D. R. Holmes Jr, R. Virmani, C. Oxvig and R. S.

Schwartz (2001). "Pregnancy-associated plasma protein A as a marker of acute coronary syndromes." New England Journal of Medicine **345**(14): 1022-1029.

- Bellenguez, C., S. Bevan, A. Gschwendtner, C. C. Spencer, A. I. Burgess, M. Pirinen, C. A. Jackson, M. Traylor, A. Strange and Z. Su (2012). "Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke." Nature genetics **44**(3): 328.
- Bellizzi, D., S. Dato, P. Cavalcante, G. Covello, F. Di Cianni, G. Passarino, G. Rose and G. De Benedictis (2007). "Characterization of a bidirectional promoter shared between two human genes related to aging: SIRT3 and PSMD13." Genomics **89**(1): 143-150.
- Bellizzi, D., G. Rose, P. Cavalcante, G. Covello, S. Dato, F. De Rango, V. Greco, M. Maggiolini, E. Feraco and V. Mari (2005). "A novel VNTR enhancer within the SIRT3 gene, a human homologue of SIR2, is associated with survival at oldest ages." Genomics **85**(2): 258-263.
- Bentwich, I., A. Avniel, Y. Karov, R. Aharonov, S. Gilad, O. Barad, A. Barzilai, P. Einat, U. Einav and E. Meiri (2005). "Identification of hundreds of conserved and nonconserved human microRNAs." Nature genetics **37**(7): 766-770.
- Bhattachariya, A., D. Dahan, M. Ekman, T. Boettger, T. Braun, K. Swärd, P. Hellstrand and S. Albinsson (2015). "Spontaneous activity and stretch-induced contractile differentiation are reduced in vascular smooth muscle of miR-143/145 knockout mice." Acta Physiologica **215**(3): 133-143.
- Blomberg, D. J., W. D. Kimber and M. D. Burke (1975). "Creatine kinase isoenzymes: Predictive value in the early diagnosis of acute

myocardial infarction." The American journal of medicine **59**(4): 464-469.

- Bochaton, T., C. Crola-Da-Silva, B. Pillot, C. Villedieu, L. Ferreras, M. Alam, H. Thibault, M. Strina, A. Gharib and M. Ovize (2015). "Inhibition of myocardial reperfusion injury by ischemic postconditioning requires sirtuin 3-mediated deacetylation of cyclophilin D." Journal of molecular and cellular cardiology **84**: 61-69.
- Boettger, T., N. Beetz, S. Kostin, J. Schneider, M. Krüger, L. Hein and T. Braun (2009). "Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster." The Journal of clinical investigation **119**(9): 2634.
- Boldrini, M., C. A. Fulmore, A. N. Tartt, L. R. Simeon, I. Pavlova, V. Poposka, G. B. Rosoklija, A. Stankov, V. Arango and A. J. Dwork (2018). "Human hippocampal neurogenesis persists throughout aging." Cell Stem Cell **22**(4): 589-599. e585.
- Bostjancic, E., N. Zidar, D. Stajer and D. Glavac (2010). "MicroRNA miR-1 is up-regulated in remote myocardium in patients with myocardial infarction." Folia biologica **56**(1): 27.
- Boštjančič, E., N. Zidar, D. Štajer and D. Glavač (2010). "MicroRNA miR-1 is up-regulated in remote myocardium in patients with myocardial infarction." Folia Biologica (Praha) **56**: 27-31.
- Bronze-da-Rocha, E. (2014). "MicroRNAs expression profiles in cardiovascular diseases." BioMed research international **2014**.
- Brown, K., S. Xie, X. Qiu, M. Mohrin, J. Shin, Y. Liu, D. Zhang, D. T. Scadden and D. Chen (2013). "SIRT3 reverses aging-associated degeneration." Cell reports **3**(2): 319-327.

- Bryant, R., T. Pawlowski, J. Catto, G. Marsden, R. Vessella, B. Rhees, C. Kuslich, T. Visakorpi and F. Hamdy (2012). "Changes in circulating microRNA levels associated with prostate cancer." British journal of cancer **106**(4): 768.
- Bueno, H., A. L. Caforio and F. Crea (2017). "2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation."
- Burgess, D. C., D. Hunt, L. Li, D. Zannino, E. Williamson, T. M. Davis, M. Laakso, Y. A. Kesäniemi, J. Zhang and R. W. Sy (2009). "Incidence and predictors of silent myocardial infarction in type 2 diabetes and the effect of fenofibrate: an analysis from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study." European heart journal **31**(1): 92-99.
- Cannon, C. P. (1998). Management of acute coronary syndromes, Springer Science & Business Media.
- Cardiology, T. F. o. t. M. o. S.-S. E. A. M. I. o. t. E. S. o. (2012). "Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation." Eur Heart J **33**(20): 2569-2619.
- Cavusoglu, E., C. Ruwende, C. Eng, V. Chopra, S. Yanamadala, L. T. Clark, D. J. Pinsky and J. D. Marmur (2007). "Usefulness of baseline plasma myeloperoxidase levels as an independent predictor of myocardial infarction at two years in patients presenting with acute coronary syndrome." The American journal of cardiology **99**(10): 1364-1368.
- Chan, D. and L. L. Ng (2010). "Biomarkers in acute myocardial infarction." BMC medicine **8**(1): 34.

- Chen, C.-J., Y.-C. Fu, W. Yu and W. Wang (2013). "SIRT3 protects cardiomyocytes from oxidative stress-mediated cell death by activating NF-κB." Biochemical and biophysical research communications **430**(2): 798-803.
- Chen, K. and N. Rajewsky (2007). "The evolution of gene regulation by transcription factors and microRNAs." Nature Reviews Genetics **8**(2): 93-103.
- Chen, X., Y. Ba, L. Ma, X. Cai, Y. Yin, K. Wang, J. Guo, Y. Zhang, J. Chen and X. Guo (2008). "Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases." Cell research **18**(10): 997.
- Chen, Y., J. Zhang, Y. Lin, Q. Lei, K. L. Guan, S. Zhao and Y. Xiong (2011). "Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS." EMBO reports **12**(6): 534-541.
- Cheng, A., Y. Yang, Y. Zhou, C. Maharana, D. Lu, W. Peng, Y. Liu, R. Wan, K. Marosi and M. Misiak (2016). "Mitochondrial SIRT3 mediates adaptive responses of neurons to exercise and metabolic and excitatory challenges." Cell metabolism **23**(1): 128-142.
- Cheng, C., Q. Wang, W. You, M. Chen and J. Xia (2014). "MiRNAs as biomarkers of myocardial infarction: a meta-analysis." PloS one **9**(2): e88566.
- Cheng, Y., X. Liu, J. Yang, Y. Lin, D.-Z. Xu, Q. Lu, E. A. Deitch, Y. Huo, E. S. Delphin and C. Zhang (2009). "MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation." Circulation research **105**(2): 158-166.

- Cheng, Y., N. Tan, J. Yang, X. Liu, X. Cao, P. He, X. Dong, S. Qin and C. Zhang (2010). "A translational study of circulating cell-free microRNA-1 in acute myocardial infarction." Clinical science **119**(2): 87-95.
- Chowdhury, R., S. Warnakula, S. Kunutsor, F. Crowe, H. A. Ward, L. Johnson, O. H. Franco, A. S. Butterworth, N. G. Forouhi and S. G. Thompson (2014). "Association of dietary, circulating, and supplement fatty acids with coronary riska systematic review and meta-analysis." Annals of internal medicine **160**(6): 398-406.
- Chua, J. H., A. Armugam and K. Jeyaseelan (2009). "MicroRNAs: biogenesis, function and applications." Current opinion in molecular therapeutics **11**(2): 189-199.
- Colburn, W., V. G. DeGruttola, D. L. DeMets, G. J. Downing, D. F. Hoth, J. A. Oates, C. C. Peck, R. T. Schooley, B. A. Spilker and J. Woodcock (2001). "Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Biomarkers Definitions Working Group." Clinical Pharmacol & Therapeutics **69**: 89-95.
- Colombo, M., G. Raposo and C. Théry (2014). "Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles." Annual review of cell and developmental biology **30**: 255-289.
- Cool, P. (2007). Medical Ststistics, Institute of Orthopaedics.
- Cordes, K. R., N. T. Sheehy, M. P. White, E. C. Berry, S. U. Morton, A. N. Muth, T.-H. Lee, J. M. Miano, K. N. Ivey and D. Srivastava (2009). "miR-145 and miR-143 regulate smooth muscle cell fate and plasticity." Nature **460**(7256): 705.

- Cortes, M., J. C. García-Cañaveras, E. Pareja and A. Lahoz (2017). "Liver Transplantation Biomarkers in the Metabolomics Era." Biomarkers in Liver Disease: 99-128.
- Cortez, M. A. and G. A. Calin (2009). "MicroRNA identification in plasma and serum: a new tool to diagnose and monitor diseases." Expert opinion on biological therapy **9**(6): 703-711.
- Creemers, E. E., A. J. Tijsen and Y. M. Pinto (2012). "Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease?" Circulation research **110**(3): 483-495.
- Cutlip, D. E., S. Windecker, R. Mehran, A. Boam, D. J. Cohen, G.-A. van Es, P. G. Steg, M.-a. Morel, L. Mauri and P. Vranckx (2007). "Clinical end points in coronary stent trials: a case for standardized definitions." Circulation **115**(17): 2344-2351.
- D'Alessandra, Y., M. C. Carena, L. Spazzafumo, F. Martinelli, B. Bassetti, P. Devanna, M. Rubino, G. Marenzi, G. I. Colombo and F. Achilli (2013). "Diagnostic potential of plasmatic MicroRNA signatures in stable and unstable angina." PloS one **8**(11): e80345.
- Davies, M. J. (2000). "The pathophysiology of acute coronary syndromes." Heart **83**(3): 361-366.
- Deacon, D. C., K. R. Nevis, T. J. Cashman, Y. Zhou, L. Zhao, D. Washko, B. Guner-Ataman, C. G. Burns and C. E. Burns (2010). "The miR-143-adducin3 pathway is essential for cardiac chamber morphogenesis." Development **137**(11): 1887-1896.
- Duffy, J. R. and M. Salerno (2004). "New Blood Test to Measure Heart Attack Risk: C-Reactive Protein." Journal of Cardiovascular Nursing **19**(6): 425-429.
- Elia, L., R. Contu, M. Quintavalle, F. Varrone, C. Chimenti, M. A. Russo, V. Cimino, L. De Marinis, A. Frustaci and D. Catalucci

(2009). "Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions." Circulation **120**(23): 2377-2385.

- Erbel, R. and G. Heusch (2000). Coronary microembolization, Journal of the American College of Cardiology.
- Falk, E., P. K. Shah and V. Fuster (1995). "Coronary plaque disruption." Circulation **92**(3): 657-671.
- Fang, Y.-C. and C.-H. Yeh (2015). "Role of microRNAs in vascular remodeling." Current molecular medicine **15**(8): 684-696.
- Fichtlscherer, S., S. De Rosa, H. Fox, T. Schwietz, A. Fischer, C. Liebetrau, M. Weber, C. W. Hamm, T. Röxe and M. Müller-Ardogan (2010). "Circulating microRNAs in patients with coronary artery diseasenovelty and significance." Circulation research **107**(5): 677-684.
- Fichtlscherer, S., A. M. Zeiher and S. Dimmeler (2011). "Circulating microRNAs: biomarkers or mediators of cardiovascular diseases?" Arteriosclerosis, Thrombosis, and Vascular Biology **31**(11): 2383-2390.
- Frye, R. A. (1999). "Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity." Biochemical and biophysical research communications **260**(1): 273-279.
- Galaup, A. and S. Germain (2012). "Protecting vascular integrity in acute myocardial ischemia-reperfusion." Medecine sciences: M/S **28**(2): 133.

- Gerhardt, W., H. Katus, J. Ravkilde, C. Hamm, P. Jørgensen, E. Peheim, L. Ljungdahl and P. Löfdahl (1991). "S-troponin T in suspected ischemic myocardial injury compared with mass and catalytic concentrations of S-creatine kinase isoenzyme MB." Clinical Chemistry **37**(8): 1405-1411.
- Gibbings, D. J., C. Ciaudo, M. Erhardt and O. Voinnet (2009). "Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity." Nature cell biology **11**(9): 1143-1149.
- Gibler, W. B., C. D. Gibler, E. Weinshenker, C. Abbottsmith, J. R. Hedges, W. G. Barsan, M. Sperling, I.-W. Chen, S. Embry and D. Kereiakes (1987). "Myoglobin as an early indicator of acute myocardial infarction." Annals of emergency medicine **16**(8): 851-856.
- Giralt, A. and F. Villarroya (2012). "SIRT3, a pivotal actor in mitochondrial functions: metabolism, cell death and aging." Biochemical Journal **444**(1): 1-10.
- Goretti, E. and Y. Devaux (2016). "Which future for circulating microRNAs as biomarkers of acute myocardial infarction?" Annals of translational medicine **4**(21).
- Goretti, E., D. R. Wagner and Y. Devaux (2014). "miRNAs as biomarkers of myocardial infarction: a step forward towards personalized medicine?" Trends in molecular medicine **20**(12): 716-725.
- Hafner, A. V., J. Dai, A. P. Gomes, C.-Y. Xiao, C. M. Palmeira, A. Rosenzweig and D. A. Sinclair (2010). "Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166

suppresses age-related cardiac hypertrophy." Aging (Albany NY) **2**(12): 914.

- Haltern, G., S. Peiniger, A. Bufe, G. Reiss, H. Gülker and T. Scheffold (2010). "Comparison of usefulness of heart-type fatty acid binding protein versus cardiac troponin T for diagnosis of acute myocardial infarction." The American journal of cardiology **105**(1): 1-9.
- Hergenreider, E., S. Heydt, K. Tréguer, T. Boettger, A. J. Horrevoets, A. M. Zeiher, M. P. Scheffer, A. S. Frangakis, X. Yin and M. Mayr (2012). "Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs." Nature cell biology **14**(3): 249-256.
- Higgins, J. P. and J. A. Higgins (2003). "Elevation of cardiac troponin I indicates more than myocardial ischemia." Clinical and investigative medicine **26**(3): 133.
- Hjortshøj, S., C. Dethlefsen, S. R. Kristensen and J. Ravkilde (2009). "Kinetics of ischaemia modified albumin during ongoing severe myocardial ischaemia." Clinica Chimica Acta **403**(1): 114-120.
- Hoffman, M., A. Blum, R. Baruch, E. Kaplan and M. Benjamin (2004). "Leukocytes and coronary heart disease." Atherosclerosis **172**(1): 1-6.
- Holmes, M. V., I. Y. Millwood, C. Kartsonaki, M. R. Hill, D. A. Bennett, R. Boxall, Y. Guo, X. Xu, Z. Bian and R. Hu (2018). "Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke." Journal of the American College of Cardiology **71**(6): 620-632.

- Hunter, M. P., N. Ismail, X. Zhang, B. D. Aguda, E. J. Lee, L. Yu, T. Xiao, J. Schafer, M.-L. T. Lee and T. D. Schmittgen (2008). "Detection of microRNA expression in human peripheral blood microvesicles." PloS one **3**(11): e3694.
- Hunter, M. P., N. Ismail, X. Zhang, B. D. Aguda, E. J. Lee, L. Yu, T. Xiao, J. Schafer, M.-L. T. Lee and T. D. Schmittgen (2010). "Correction: Detection of microRNA Expression in Human Peripheral Blood Microvesicles." PloS one **5**(3).
- Hyun, K.-A., J. Kim, H. Gwak and H.-I. Jung (2016). "Isolation and enrichment of circulating biomarkers for cancer screening, detection, and diagnostics." Analyst **141**(2): 382-392.
- Ikeda, S., A. He, S. W. Kong, J. Lu, R. Bejar, N. Bodyak, K.-H. Lee, Q. Ma, P. M. Kang and T. R. Golub (2009). "MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes." Molecular and cellular biology **29**(8): 2193-2204.
- Inoue, K., A. Sugiyama, P. C. Reid, Y. Ito, K. Miyauchi, S. Mukai, M. Sagara, K. Miyamoto, H. Satoh and I. Kohno (2007). "Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris." Arteriosclerosis, thrombosis, and vascular biology **27**(1): 161-167.
- Jaffe, A. S. and F. S. Apple (2000). It's time for a change to a troponin standard, Am Heart Assoc.
- Jennings, R. B. (2013). "Historical perspective on the pathology of myocardial ischemia/reperfusion injury." Circulation research **113**(4): 428-438.
- Joshi, K. (2009). "Early Myocardial Infarction Detection." San Jose State University.

- Karpinski, L., R. Plaksej, R. Derzhko, A. Orda and M. Witkowska (2009). "Serum levels of interleukin-6, interleukin-10 and C-reactive protein in patients with myocardial infarction treated with primary angioplasty during a 6-month follow-up." Pol Arch Med Wewn **119**(3): 115-121.
- Katus, H. A., A. Remppis, T. Scheffold, K. W. Diederich and W. Kuebler (1991). "Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction." The American journal of cardiology **67**(16): 1360-1367.
- Kempf, T., E. Björklund, S. Olofsson, B. Lindahl, T. Allhoff, T. Peter, J. Tongers, K. C. Wollert and L. Wallentin (2007). "Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction." European heart journal **28**(23): 2858-2865.
- Kempf, T., J.-M. Sinning, A. Quint, C. Bickel, C. Sinning, P. S. Wild, R. Schnabel, E. Lubos, H. J. Rupprecht and T. Münzel (2009). "Growth-differentiation factor-15 for risk stratification in patients with stable and unstable coronary heart disease." Circulation: Cardiovascular Genetics **2**(3): 286-292.
- Khalil, A., A. Kamar and G. Nemer (2017). "MicroRNAs and Myocardial Infarct: Investigating the Controversial Role of Second Generation Biomarkers." Journal of Cholesterol and Heart Disease **1**(1).
- Khan, S. Q., D. Kelly, P. Quinn, J. E. Davies and L. L. Ng (2007). "Myeloperoxidase aids prognostication together with N-terminal pro-B-type natriuretic peptide in high-risk patients with acute ST elevation myocardial infarction." Heart **93**(7): 826-831.

- Khandekar, M., A. Khurana, S. Deshmukh, A. Kakrani, A. Katdare and A. Inamdar (2006). "Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: an Indian scenario." Journal of clinical pathology **59**(2): 146-149.
- Kim, Y.-K. (2015). "Extracellular microRNAs as biomarkers in human disease." Chonnam medical journal **51**(2): 51-57.
- Kivimäki, M., S. T. Nyberg, G. D. Batty, E. I. Fransson, K. Heikkilä, L. Alfredsson, J. B. Bjorner, M. Borritz, H. Burr and A. Casini (2012). "Job strain as a risk factor for coronary heart disease: a collaborative meta-analysis of individual participant data." The Lancet **380**(9852): 1491-1497.
- Kong, X., R. Wang, Y. Xue, X. Liu, H. Zhang, Y. Chen, F. Fang and Y. Chang (2010). "Sirtuin 3, a new target of PGC-1α, plays an important role in the suppression of ROS and mitochondrial biogenesis." PloS one **5**(7): e11707.
- Kong, Y. and J.-H. Han (2005). "MicroRNA: biological and computational perspective." Genomics, proteomics & bioinformatics **3**(2): 62-72.
- Kosaka, N., H. Iguchi, K. Hagiwara, Y. Yoshioka, F. Takeshita and T. Ochiya (2013). "Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis." Journal of Biological Chemistry **288**(15): 10849-10859.
- Kosaka, N., H. Iguchi, Y. Yoshioka, F. Takeshita, Y. Matsuki and T. Ochiya (2010). "Secretory mechanisms and intercellular transfer of microRNAs in living cells." Journal of Biological Chemistry **285**(23): 17442-17452.

- Kumari, S., S. N. Chaurasia, M. K. Nayak, R. L. Mallick and D. Dash (2015). "Sirtuin inhibition induces apoptosis-like changes in platelets and thrombocytopenia." Journal of Biological Chemistry **290**(19): 12290-12299.
- Kuwabara, Y., K. Ono, T. Horie, H. Nishi, K. Nagao, M. Kinoshita, S. Watanabe, O. Baba, Y. Kojima and S. Shizuta (2011). "Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate the existence of myocardial damage." Circulation: Genomic and Precision Medicine: CIRCGENETICS. 110.958975.
- Lanas, F., A. Avezum, L. E. Bautista, R. Diaz, M. Luna, S. Islam and S. Yusuf (2007). "Risk factors for acute myocardial infarction in Latin America: the INTERHEART Latin American study." Circulation **115**(9): 1067-1074.
- Lanktree, M. B., M. Dichgans and R. A. Hegele (2010). "Advances in genomic analysis of stroke: what have we learned and where are we headed?" Stroke **41**(4): 825-832.
- Lee, C. D., A. R. Folsom, F. J. Nieto, L. E. Chambless, E. Shahar and D. A. Wolfe (2001). "White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study." American journal of epidemiology **154**(8): 758-764.
- LeLeiko, R. M., C. S. Vaccari, S. Sola, N. Merchant, S. H. Nagamia, M. Thoenes and B. V. Khan (2009). "Usefulness of elevations in serum choline and free F 2-isoprostane to predict 30-day cardiovascular outcomes in patients with acute coronary syndrome." The American journal of cardiology **104**(5): 638-643.

- Levine, B., J. Kalman, L. Mayer, H. M. Fillit and M. Packer (1990). "Elevated circulating levels of tumor necrosis factor in severe chronic heart failure." New England Journal of Medicine **323**(4): 236-241.
- Lewis, B. P., C. B. Burge and D. P. Bartel (2005). "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets." cell **120**(1): 15-20.
- Li, H., S. Horke and U. Förstermann (2014). "Vascular oxidative stress, nitric oxide and atherosclerosis." Atherosclerosis **237**(1): 208-219.
- Li, M. and J. Zhang (2015). "Circulating microRNAs: potential and emerging biomarkers for diagnosis of cardiovascular and cerebrovascular diseases." BioMed research international **2015**.
- Li, Q., X.-W. Song, J. Zou, G.-K. Wang, E. Kremneva, X.-Q. Li, N. Zhu, T. Sun, P. Lappalainen and W.-J. Yuan (2010). "Attenuation of microRNA-1 derepresses the cytoskeleton regulatory protein twinfilin-1 to provoke cardiac hypertrophy." J Cell Sci **123**(14): 2444-2452.
- Liang, Q., G. A. Benavides, A. Vassilopoulos, D. Gius, V. Darley-Usmar and J. Zhang (2013). "Bioenergetic and autophagic control by Sirt3 in response to nutrient deprivation in mouse embryonic fibroblasts." Biochemical Journal **454**(2): 249-257.
- Liebetrau, C., H. Möllmann, O. Dörr, S. Szardien, C. Troidl, M. Willmer, S. Voss, L. Gaede, J. Rixe and A. Rolf (2013). "Release kinetics of circulating muscle-enriched microRNAs in patients undergoing transcoronary ablation of septal hypertrophy." Journal of the American College of Cardiology **62**(11): 992-998.

- Liu, G., M. Cao, Y. Xu and Y. Li (2015). "SIRT3 protects endothelial cells from high glucose-induced cytotoxicity." International journal of clinical and experimental pathology **8**(1): 353.
- Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method." methods **25**(4): 402-408.
- Long, G., F. Wang, Q. Duan, F. Chen, S. Yang, W. Gong, Y. Wang, C. Chen and D. W. Wang (2012). "Human circulating microRNA-1 and microRNA-126 as potential novel indicators for acute myocardial infarction." International journal of biological sciences **8**(6): 811.
- Lu, Z., Y. Chen, A. M. Aponte, V. Battaglia, M. Gucek and M. N. Sack (2015). "Prolonged fasting identifies heat shock protein 10 as a Sirtuin 3 substrate elucidating a new mechanism linking mitochondrial protein acetylation to fatty acid oxidation enzyme folding and function." Journal of Biological Chemistry **290**(4): 2466-2476.
- Lund, J., Q. P. Qin, T. Ilva, K. Nikus, M. Eskola, P. Porela, S. Kokkala, K. Pulkki, K. Pettersson and L. M. Voipio-Pulkki (2006). "Pregnancy-associated plasma protein A: A biomarker in acute ST-elevation myocardial infarction (STEMI)." Annals of medicine **38**(3): 221-228.
- Mad, P., H. Domanovits, C. Fazelnia, K. Stiassny, G. Russmüller, A. Cseh, G. Sodeck, T. Binder, G. Christ and T. Szekeres (2007). "Human heart-type fatty-acid-binding protein as a point-of-care test in the early diagnosis of acute myocardial infarction." Journal of the Association of Physicians **100**(4): 203-210.

- Madjid, M., I. Awan, J. T. Willerson and S. W. Casscells (2004). "Leukocyte count and coronary heart disease: implications for risk assessment." Journal of the American College of Cardiology **44**(10): 1945-1956.
- Mair, J., D. Morandell, N. Genser, P. Lechleitner, F. Dienstl and B. Puschendorf (1995). "Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction." Clinical chemistry **41**(9): 1266-1272.
- Malovini, A., R. Bellazzi, C. Napolitano and G. Guffanti (2016). "Multivariate methods for genetic variants selection and risk prediction in cardiovascular diseases." Frontiers in cardiovascular medicine **3**: 17.
- Mastella, A. K., R. N. Moresco, D. B. da Silva, A. M. Becker, M. M. Duarte, L. L. Giovelli, S. H. da Silva, L. Rossato, M. B. Moretto and J. E. P. da Silva (2009). "Evaluation of ischemia-modified albumin in myocardial infarction and prostatic diseases." Biomedicine & Pharmacotherapy **63**(10): 762-766.
- Matsushima, S. and J. Sadoshima (2015). "The role of sirtuins in cardiac disease." American Journal of Physiology-Heart and Circulatory Physiology **309**(9): H1375-H1389.
- Meder, B., A. Keller, B. Vogel, J. Haas, F. Sedaghat-Hamedani, E. Kayvanpour, S. Just, A. Borries, J. Rudloff and P. Leidinger (2011). "MicroRNA signatures in total peripheral blood as novel biomarkers for acute myocardial infarction." Basic research in cardiology **106**(1): 13-23.

- Medrano, R. F. V. and C. A. de Oliveira (2014). "Guidelines for the tetra-primer ARMS–PCR technique development." Molecular biotechnology **56**(7): 599-608.
- Mei, Y., M. D. Thompson, R. A. Cohen and X. Tong (2015). "Autophagy and oxidative stress in cardiovascular diseases." Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease **1852**(2): 243-251.
- Members, A. T. F., P. G. Steg, S. K. James, D. Atar, L. P. Badano, C. B. Lundqvist, M. A. Borger, C. Di Mario, K. Dickstein and G. Ducrocq (2012). "ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC)." European heart journal **33**(20): 2569-2619.
- Mendis, S., K. Thygesen, K. Kuulasmaa, S. Giampaoli, M. Mähönen, K. Ngu Blackett, L. Lisheng and W. g. o. b. o. t. p. e. o. t. W. c. f. r. o. W. d. o. m. infarction (2010). "World Health Organization definition of myocardial infarction: 2008–09 revision." International journal of epidemiology **40**(1): 139-146.
- Merksamer, P. I., Y. Liu, W. He, M. D. Hirschey, D. Chen and E. Verdin (2013). "The sirtuins, oxidative stress and aging: an emerging link." Aging (Albany NY) **5**(3): 144.
- Mestl, H. E. and R. Edwards (2011). "Global burden of disease as a result of indoor air pollution in Shaanxi, Hubei and Zhejiang, China." Science of the total environment **409**(8): 1391-1398.
- Mitchell, P. S., R. K. Parkin, E. M. Kroh, B. R. Fritz, S. K. Wyman, E. L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K. C. O'Briant and A. Allen (2008). "Circulating microRNAs as

stable blood-based markers for cancer detection." Proceedings of the National Academy of Sciences **105**(30): 10513-10518.

- Morrow, D. A., J. A. de Lemos, M. S. Sabatine, S. A. Murphy, L. A. Demopoulos, P. M. DiBattiste, C. H. McCabe, C. M. Gibson, C. P. Cannon and E. Braunwald (2003). "Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non–ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18." Journal of the American College of Cardiology **41**(8): 1264-1272.
- Mythili, S. and N. Malathi (2015). "Diagnostic markers of acute myocardial infarction." Biomedical reports **3**(6): 743-748.
- Nymark, C. (2015). Afflicted by an acute myocardial infarction: patients' thoughts, feelings and actions prior to care-seeking, Inst för kliniska vetenskaper, Danderyds sjukhus/Dept of Clinical Sciences, Danderyd Hospital.
- Organization, W. H. (2016). Noncommunicable diseases (NCD) country profiles 2014: Indonesia. 2014.
- Ozaki, K. and T. Tanaka (2016). "Molecular genetics of coronary artery disease." Journal of human genetics **61**(1): 71.
- Pan, Z., X. Sun, J. Ren, X. Li, X. Gao, C. Lu, Y. Zhang, H. Sun, Y. Wang and H. Wang (2012). "miR-1 exacerbates cardiac ischemia-reperfusion injury in mouse models." PloS one **7**(11): e50515.
- Papageorgiou, N., D. Tousoulis, E. Androulakis, G. Siasos, A. Briasoulis, G. Vogiatzi, A.-M. Kampoli, E. Tsiamis, C. Tentolouris and C. Stefanadis (2012). "The role of microRNAs in cardiovascular disease." Current medicinal chemistry **19**(16): 2605-2610.

- Perridon, B. W., H. G. Leuvenink, J.-L. Hillebrands, H. van Goor and E. M. Bos (2016). "The role of hydrogen sulfide in aging and age-related pathologies." Aging (Albany NY) **8**(10): 2264.
- Pillai, V. B., N. R. Sundaresan, G. Kim, M. Gupta, S. B. Rajamohan, J. B. Pillai, S. Samant, P. Ravindra, A. Isbatan and M. P. Gupta (2010). "Exogenous NAD blocks cardiac hypertrophic response via activation of the SIRT3-LKB1-AMP-activated kinase pathway." Journal of Biological Chemistry **285**(5): 3133-3144.
- Pleister, A., H. Selemon, S. M. Elton and T. S. Elton (2013). "Circulating miRNAs: novel biomarkers of acute coronary syndrome?" Biomarkers **7**(2): 287-305.
- Rangrez, A. Y., Z. A. Massy, V. Metzinger-Le Meuth and L. Metzinger (2011). "MiR-143 and MiR-145." Circulation: Cardiovascular Genetics **4**(2): 197-205.
- Rawal, S., P. Manning and R. Katare (2014). "Cardiovascular microRNAs: as modulators and diagnostic biomarkers of diabetic heart disease." Cardiovascular diabetology **13**(1): 44.
- Reichlin, T., W. Hochholzer, C. Stelzig, K. Laule, H. Freidank, N. G. Morgenthaler, A. Bergmann, M. Potocki, M. Noveanu and T. Breidthardt (2009). "Incremental value of copeptin for rapid rule out of acute myocardial infarction." Journal of the American College of Cardiology **54**(1): 60-68.
- Reid, G., M. B. Kirschner and N. van Zandwijk (2011). "Circulating microRNAs: Association with disease and potential use as biomarkers." Critical reviews in oncology/hematology **80**(2): 193-208.
- Ridker, P. M., N. Rifai, M. Pfeffer, F. Sacks, S. Lepage and E. Braunwald (2000). "Elevation of tumor necrosis factor-α and

increased risk of recurrent coronary events after myocardial infarction." Circulation **101**(18): 2149-2153.

- Roberts, R. (2014). "Genetics of coronary artery disease." Circulation research **114**(12): 1890-1903.
- Roger, V. L. (2015). "Cardiovascular diseases in populations: secular trends and contemporary challenges—Geoffrey Rose lecture, European Society of Cardiology meeting 2014." European heart journal **36**(32): 2142-2146.
- Rosengren, A., S. Hawken, S. Ôunpuu, K. Sliwa, M. Zubaid, W. A. Almahmeed, K. N. Blackett, C. Sitthi-amorn, H. Sato and S. Yusuf (2004). "Association of psychosocial risk factors with risk of acute myocardial infarction in 11 119 cases and 13 648 controls from 52 countries (the INTERHEART study): case-control study." The Lancet **364**(9438): 953-962.
- Ruseva, A. (2005). "MYOCARDIAL INFARCTION." Trakia journal of sciences **3**(1): 8-14.
- Russo, F., S. Di Bella, V. Bonnici, A. Laganà, G. Rainaldi, M. Pellegrini, A. Pulvirenti, R. Giugno and A. Ferro (2014). "A knowledge base for the discovery of function, diagnostic potential and drug effects on cellular and extracellular miRNAs." BMC genomics **15**(3): S4.
- Russo, F., S. Di Bella, G. Nigita, V. Macca, A. Lagana, R. Giugno, A. Pulvirenti and A. Ferro (2012). "miRandola: extracellular circulating microRNAs database." PloS one **7**(10): e47786.
- Ruzgar, O., A. K. Bilge, Z. Bugra, S. Umman, E. Yilmaz, B. Ozben, B. Umman and M. Meric (2006). "The use of human heart-type fatty acid-binding protein as an early diagnostic biochemical marker of myocardial necrosis in patients with acute coronary

syndrome, and its comparison with troponin-T and creatine kinase–myocardial band." Heart and vessels **21**(5): 309-314.

- Samant, S. A., H. J. Zhang, Z. Hong, V. B. Pillai, N. R. Sundaresan, D. Wolfgeher, S. L. Archer, D. C. Chan and M. P. Gupta (2014). "SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress." Molecular and cellular biology **34**(5): 807-819.
- Samples, P. (2012). "A Role for miR-145 in Pulmonary Arterial Hypertension."
- Sanchis-Gomar, F., C. Perez-Quilis, R. Leischik and A. Lucia (2016). "Epidemiology of coronary heart disease and acute coronary syndrome." Annals of translational medicine **4**(13).
- Santovito, D., C. Mandolini, P. Marcantonio, V. De Nardis, M. Bucci, C. Paganelli, F. Magnacca, S. Ucchino, D. Mastroiacovo and G. Desideri (2013). "Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients." Expert opinion on therapeutic targets **17**(3): 217-223.
- Satterstrom, F. K., W. R. Swindell, G. Laurent, S. Vyas, M. L. Bulyk and M. C. Haigis (2015). "Nuclear respiratory factor 2 induces SIRT3 expression." Aging cell **14**(5): 818-825.
- Satterstrom, F. K., W. R. Swindell, G. Laurent, S. Vyas, M. L. Bulyk and M. C. Haigis (2015). "Nuclear respiratory factor 2 induces SIRT 3 expression." Aging Cell **14**(5): 818-825.
- Sayed, D., C. Hong, I.-Y. Chen, J. Lypowy and M. Abdellatif (2007). "MicroRNAs play an essential role in the development of cardiac hypertrophy." Circulation research **100**(3): 416-424.
- Schömig, A., J. Mehilli, D. Antoniucci, G. Ndrepepa, C. Markwardt, F. Di Pede, S. G. Nekolla, K. Schlotterbeck, H.

Schühlen and J. Pache (2005). "Mechanical reperfusion in patients with acute myocardial infarction presenting more than 12 hours from symptom onset: a randomized controlled trial." Jama **293**(23): 2865-2872.

- Schwarzenbach, H., N. Nishida, G. A. Calin and K. Pantel (2014). "Clinical relevance of circulating cell-free microRNAs in cancer." Nature reviews Clinical oncology **11**(3): 145-156.
- Schwer, B., B. J. North, R. A. Frye, M. Ott and E. Verdin (2002). "The human silent information regulator (Sir) 2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide–dependent deacetylase." J Cell Biol **158**(4): 647-657.
- Shi, T., F. Wang, E. Stieren and Q. Tong (2005). "SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes." Journal of Biological Chemistry **280**(14): 13560-13567.
- Shingu, M., Y. Nagai, T. Isayama, T. Naono and M. Nobunaga (1993). "The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase production by human chondrocytes and TIMP production by synovial cells and endothelial cells." Clinical & Experimental Immunology **94**(1): 145-149.
- Signorile, A., A. Santeramo, G. Tamma, T. Pellegrino, S. D'Oria, P. Lattanzio and D. De Rasmo (2017). "Mitochondrial cAMP prevents apoptosis modulating Sirt3 protein level and OPA1 processing in cardiac myoblast cells." Biochimica et Biophysica Acta (BBA)-Molecular Cell Research **1864**(2): 355-366.
- Skyschally, A., R. Schulz and G. Heusch (2008). "Pathophysiology of myocardial infarction." Herz Kardiovaskuläre Erkrankungen **33**(2): 88-100.

- Smith, M., R. Saunders, L. Stuckhardt and J. M. McGinnis (2013). "Imperative: Managing Rapidly Increasing Complexity."
- Smith, S. C., J. Allen, S. N. Blair, R. O. Bonow, L. M. Brass, G. C. Fonarow, S. M. Grundy, L. Hiratzka, D. Jones and H. M. Krumholz (2006). "AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update." Circulation **113**(19): 2363-2372.
- Srivastava, R. K., S. Tiwari, P. Singh, A. Puri, G. Chaudhary, W. Ali and A. Verma (2014). "Gender Risk Profile In Acute Myocardial Infarction-A Prospective Study in Indian Population." Int J Sci Res Publ **4**: 1-3.
- Stanley, W. C., F. A. Recchia and G. D. Lopaschuk (2005). "Myocardial substrate metabolism in the normal and failing heart." Physiological reviews **85**(3): 1093-1129.
- Stefano De Servi, M., M. Mariani, G. Mariani and A. Mazzone (2005). "C-reactive protein increase in unstable coronary disease." Journal of the American College of Cardiology **46**(8).
- Steptoe, A. and M. Kivimäki (2012). "Stress and cardiovascular disease." Nature Reviews Cardiology **9**(6): 360-370.
- Strimbu, K. and J. A. Tavel (2010). "What are biomarkers?" Current Opinion in HIV and AIDS **5**(6): 463.
- Sun, W., C. Liu, Q. Chen, N. Liu, Y. Yan and B. Liu (2018). "SIRT3: A New Regulator of Cardiovascular Diseases." Oxidative medicine and cellular longevity **2018**.
- Sundaresan, N. R., M. Gupta, G. Kim, S. B. Rajamohan, A. Isbatan and M. P. Gupta (2009). "Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense

mechanisms in mice." The Journal of clinical investigation **119**(9): 2758-2771.

- Sundaresan, N. R., S. A. Samant, V. B. Pillai, S. B. Rajamohan and M. P. Gupta (2008). "SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70." Molecular and cellular biology **28**(20): 6384-6401.
- Suzuki, S., Y. Takeishi, T. Niizeki, Y. Koyama, T. Kitahara, T. Sasaki, M. Sagara and I. Kubota (2008). "Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure." American heart journal **155**(1): 75-81.
- Tao, R., M. C. Coleman, J. D. Pennington, O. Ozden, S.-H. Park, H. Jiang, H.-S. Kim, C. R. Flynn, S. Hill and W. H. McDonald (2010). "Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress." Molecular cell **40**(6): 893-904.
- Taylor, D. D. and C. Gercel-Taylor (2008). "MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer." Gynecologic oncology **110**(1): 13-21.
- Thygesen, K., J. S. Alpert, A. S. Jaffe, M. L. Simoons, B. R. Chaitman and H. D. White (2013). "Expert Consensus Document. Third Universal Definition of Myocardial Infarction." Revista Española de Cardiología **66**(02): 132-132.
- Thygesen, K., J. S. Alpert, A. S. Jaffe, M. L. Simoons, B. R. Chaitman, H. D. White, W. G. o. b. o. t. J. E. A. A. W. T. F. f. t. U. D. o. M. Infarction, A. T. F. M. Chairpersons, K. Thygesen and J.

S. Alpert (2012). "Third universal definition of myocardial infarction." European heart journal **33**(20): 2551-2567.

- Tijsen, A. J., Y. M. Pinto and E. E. Creemers (2012). "Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases." American journal of physiology-heart and circulatory physiology **303**(9): H1085-H1095.
- Toma, M., Y. Fu, J. A. Ezekowitz, F. A. McAlister, C. M. Westerhout, C. B. Granger and P. W. Armstrong (2010). "Does silent myocardial infarction add prognostic value in ST-elevation myocardial infarction patients without a history of prior myocardial infarction? Insights from the Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) Trial." American heart journal **160**(4): 671-677.
- Topol, E. J. and J. S. Yadav (2000). "Recognition of the importance of embolization in atherosclerotic vascular disease." Circulation **101**(5): 570-580.
- Townley-Tilson, W. D., T. E. Callis and D. Wang (2010). "MicroRNAs 1, 133, and 206: critical factors of skeletal and cardiac muscle development, function, and disease." The international journal of biochemistry & cell biology **42**(8): 1252-1255.
- Tseng, A. H.-H., L.-H. Wu, S.-S. Shieh and D. L. Wang (2014). "SIRT3 interactions with FOXO3 acetylation, phosphorylation and ubiquitinylation mediate endothelial cell responses to hypoxia." Biochemical Journal **464**(1): 157-168.
- Tucker, J. F., R. A. Collins, A. J. Anderson, J. Hauser, J. Kalas and F. S. Apple (1997). "Early diagnostic efficiency of cardiac troponin

I and troponin T for acute myocardial infarction." Academic Emergency Medicine **4**(1): 13-21.

- Turchinovich, A., L. Weiz, A. Langheinz and B. Burwinkel (2011). "Characterization of extracellular circulating microRNA." Nucleic acids research **39**(16): 7223-7233.
- V Goncharov, N., P. V Avdonin, A. D Nadeev, I. L Zharkikh and R. O Jenkins (2015). "Reactive oxygen species in pathogenesis of atherosclerosis." Current pharmaceutical design **21**(9): 1134-1146.
- Vaidya, H. (1994). "Myoglobin: an early biochemical marker for the diagnosis of acute myocardial infarction." J. Clin. Immunossay **17**: 35-39.
- Valadi, H., K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee and J. O. Lötvall (2007). "Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells." Nature cell biology **9**(6): 654-659.
- Van de Werf, F., D. Ardissino, A. Betriu, D. V. Cokkinos, E. Falk, K. A. Fox, D. Julian, M. Lengyel, F.-J. Neumann and W. Ruzyllo (2003). "Management of acute myocardial infarction in patients presenting with ST-segment elevation." European heart journal **24**(1): 28-66.
- van Rooij, E., D. Quiat, B. A. Johnson, L. B. Sutherland, X. Qi, J. A. Richardson, R. J. Kelm and E. N. Olson (2009). "A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance." Developmental cell **17**(5): 662-673.
- Varkonyi-Gasic, E. and R. P. Hellens (2011). Quantitative stem-loop RT-PCR for detection of microRNAs. RNAi and Plant Gene Function Analysis, Springer: 145-157.

- Vella, K., R. Vella and C. Mifsud (2015). Sensitivity and specificity of Troponin I in the detection of acute myocardial infarction at the Emergency Department, Mater Dei Hospital.
- Verdin, E., Y. Zhang, S. Bharathi, M. Rardin, R. Uppala, B. Gibson and E. Goetzman (2015). "SIRT3 and SIRT5 regulate the enzyme activity and cardiolipin binding of very long-chain acyl-CoA dehydrogenase."
- Vickers, K. C., B. T. Palmisano, B. M. Shoucri, R. D. Shamburek and A. T. Remaley (2011). "MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins." Nature cell biology **13**(4): 423.
- Villarroya-Beltri, C., C. Gutiérrez-Vázquez, F. Sánchez-Cabo, D. Pérez-Hernández, J. Vázquez, N. Martin-Cofreces, D. J. Martinez-Herrera, A. Pascual-Montano, M. Mittelbrunn and F. Sánchez-Madrid (2013). "Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs." Nature communications **4**: 2980.
- Voors, A. A., S. von Haehling, S. D. Anker, H. L. Hillege, J. Struck, O. Hartmann, A. Bergmann, I. Squire, D. J. van Veldhuisen and K. Dickstein (2009). "C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study." European heart journal **30**(10): 1187-1194.
- Wang, G.-K., J.-Q. Zhu, J.-T. Zhang, Q. Li, Y. Li, J. He, Y.-W. Qin and Q. Jing (2010). "Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans." European heart journal **31**(6): 659-666.

- Wang, J., S. Zhang, Y. Jin, G. Qin, L. Yu and J. Zhang (2007). "Elevated levels of platelet–monocyte aggregates and related circulating biomarkers in patients with acute coronary syndrome." International journal of cardiology **115**(3): 361-365.
- Wang, K., S. Zhang, J. Weber, D. Baxter and D. J. Galas (2010). "Export of microRNAs and microRNA-protective protein by mammalian cells." Nucleic acids research **38**(20): 7248-7259.
- Wang, T., H. Lee, J. Hwang, J. Oh, J. Lim, H. Kang, J. Joo and K. Lee (2013). "Myoglobin: A promising exogenous reference marker using in proteomics analysis." Food Science and Biotechnology **22**(2): 393-398.
- Weber, C., A. Schober and A. Zernecke (2010). "MicroRNAs in arterial remodelling, inflammation and atherosclerosis." Current drug targets **11**(8): 950-956.
- Weber, J. A., D. H. Baxter, S. Zhang, D. Y. Huang, K. H. Huang, M. J. Lee, D. J. Galas and K. Wang (2010). "The microRNA spectrum in 12 body fluids." Clinical chemistry **56**(11): 1733-1741.
- Weber, M., M. B. Baker, R. S. Patel, A. A. Quyyumi, G. Bao and C. D. Searles (2011). "MicroRNA expression profile in CAD patients and the impact of ACEI/ARB." Cardiology research and practice **2011**.
- Wei, Y., M. Nazari-Jahantigh, P. Neth, C. Weber and A. Schober (2013). "MicroRNA-126,-145, and-155." Arteriosclerosis, thrombosis, and vascular biology **33**(3): 449-454.
- Xu, C., Y. Lu, Z. Pan, W. Chu, X. Luo, H. Lin, J. Xiao, H. Shan, Z. Wang and B. Yang (2007). "The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting

HSP60, HSP70 and caspase-9 in cardiomyocytes." Journal of cell science **120**(17): 3045-3052.

- Xu, H., H. Cao, G. Zhu, S. Liu and H. Li (2017). "Overexpression of microRNA-145 protects against rat myocardial infarction through targeting PDCD4." American Journal of Translational Research **9**(11): 5003.
- Xu, J., T. R. Kimball, J. N. Lorenz, D. A. Brown, A. R. Bauskin, R. Klevitsky, T. E. Hewett, S. N. Breit and J. D. Molkentin (2006). "GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation." Circulation research **98**(3): 342-350.
- Xu, J., J. Zhao, G. Evan, C. Xiao, Y. Cheng and J. Xiao (2012). "Circulating microRNAs: novel biomarkers for cardiovascular diseases." Journal of molecular medicine **90**(8): 865-875.
- Xu, M.-C., X.-F. Gao, C. Ruan, Z.-R. Ge, J.-D. Lu, J.-J. Zhang, Y. Zhang, L. Wang and H.-M. Shi (2015). "miR-103 regulates oxidative stress by targeting the BCL2/adenovirus E1B 19 kDa interacting protein 3 in HUVECs." Oxidative medicine and cellular longevity **2015**.
- Yang, B., H. Lin, J. Xiao, Y. Lu, X. Luo, B. Li, Y. Zhang, C. Xu, Y. Bai and H. Wang (2007). "The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2." Nature medicine **13**(4): 486.
- Yin, X., S. Pang, J. Huang, Y. Cui and B. Yan (2016). "Genetic and functional sequence variants of the SIRT3 gene promoter in myocardial infarction." PloS one **11**(4): e0153815.
- Yusuf, S., S. Hawken, S. Ôunpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen, A. Budaj, P. Pais and J. Varigos (2004). "Effect of

potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study." The lancet **364**(9438): 937-952.

- Zernecke, A., K. Bidzhekov, H. Noels, E. Shagdarsuren, L. Gan, B. Denecke, M. Hristov, T. Köppel, M. N. Jahantigh and E. Lutgens (2009). "Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection." Sci. Signal. **2**(100): ra81-ra81.
- Zhang, B., S. Cui, X. Bai, L. Zhuo, X. Sun, Q. Hong, B. Fu, J. Wang, X. Chen and G. Cai (2013). "SIRT3 overexpression antagonizes high glucose accelerated cellular senescence in human diploid fibroblasts via the SIRT3–FOXO1 signaling pathway." Age **35**(6): 2237-2253.
- Zhang, C. (2008). "MicroRNAs: role in cardiovascular biology and disease." Clinical Science **114**(12): 699-706.
- Zhang, C. (2008). "MicroRNomics: a newly emerging approach for disease biology." Physiological genomics **33**(2): 139-147.
- Zhang, C. (2009). "MicroRNA-145 in vascular smooth muscle cell biology: a new therapeutic target for vascular disease." Cell Cycle **8**(21): 3469-3473.
- Zhang, D., X. Li, C. Chen, Y. Li, L. Zhao, Y. Jing, W. Liu, X. Wang, Y. Zhang and H. Xia (2012). "Attenuation of p38-mediated miR-1/133 expression facilitates myoblast proliferation during the early stage of muscle regeneration." PloS one **7**(7): e41478.
- Zhang, M., Y.-J. Cheng, J. D. Sara, L.-J. Liu, L.-P. Liu, X. Zhao and H. Gao (2017). "Circulating MicroRNA-145 is associated with acute myocardial infarction and heart failure." Chinese medical journal **130**(1): 51.

- Zhao, Y., E. Samal and D. Srivastava (2005). "Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis." Nature **436**(7048): 214.

Appendix

Questionnaire

Name:

Serum Symbol : plasma Symbol

Living : rural city

Age:

Weight:

History diseases :

Clinical feature:

Hematology features :

Lipid profile:

MI frequency:

Troponine test :

Myoglobin test:

CK test :

ECG result:

Final diagnosis and Not:

الخلاصة

احتشاء العضلة القلبية هو مرض متعدد العوامل ، تدريجي الحدوث ، من الإمراض المعقد ، ناتج عن فقدان مفاجئ لإمدادات الدم لجزء من عضلة القلب ويتبع بنخر العضلة مما ينتج عنة ظهور بروتين التروبونين القلبي في الدم. وهذا ناتج عن تجلط الدم في الشرايين التاجية و عادتا ما يكون الانسداد التدفق الأولي بسبب تراكم الصفائح الدموية ، ولكن الفيبرين مهم لتحقيق الاستقرار اللاحق لخثرة الصفائح الدموية المبكرة والهشة. ولذلك ، فإن كل من الصفائح الدموية والفيبرين تشارك في تطور الجلطة التاجية الدائمة.هناك العديد من العوامل الوراثية التي تجعل الفرد أكثر عرضة لحدوث احتشاء العضلة القلبية كتباين النمطي لبادئ جين.*SIRT3*

وقد تضمنت هذه الدراسة على دراسة تباين النمطي لبادئ جين *SIRT3* لأن له وظيفة مهمة في احتشاء العضلة القلبية ، وينظم SIRT3 النشاط الأنزيمي للأنزيمات الرئيسية للفسفرة التأكسدية عبر عملية إزالة الاستيل ، وكذلك من خلال تنظيم الايض الطاقة في الميتوكوندريا. التدابير العلاج لهذا المرض يجب تتم بسريعة وأن وقت التشخيص يكون ضروري للعلاج. هناك العديد من المعلمات الحيوية المستخدمة في تشخيص احتشاء العضلة القلبية ولكنها تكون محدودة الفائدة كما في التروبونين وهو مؤشر حيوي جيد ولكن يفرز بعد حدوث تلف في عضلة القلب . الدراسات الحديثة تقترح استخدام (miRNAs)كدلائل لتشخيص احتشاء العضلة القلبية .إن (MiRNAs)عبارة عن RNAs غير مشفرة ، أحادية الشريط ، تتكون من تقريبا من اثنان وعشرون من النيوكليوتيدات وتشكل فئة جديدة من منظمات الجينات.

الهدف من الدراسة الحالية هو التنبؤ بخطر احتشاء عضلة القلب من خلال دراسة تعدد الأشكال SIRT3promoter وإيجاد معلمات غير جراحية مثل (miRNA-1، miRNA-145). لتحقيق هذا الهدف ، تم جمع عينة دم من ثلاثة مجموعات. كانت المجموعة الأولى ٢٤ مريضًا مصابًا بمرض عضلة القلب والتي تشمل (١٦ رجل و ٨ نساء) ، والذين كانوا تحت المشاهدة في وحدة الرعاية المركزية بمستشفى الديوانية التعليمي ، وكانت المجموعة الثانية تضم ٢٤ مريضًا لديهم عامل خطورة لاحتشاء العضلة القلبية (فرط ضغط الدم وفرط الدهون بالدم والسكري). كانت المجموعة الثالثة تضم ١٩ متطوعاً أصحاء (من أمراض الشرايين التاجية). تم جمع عينة الدم عن طريق بزل الوريد من هذه المجموعات (خمسة مليمترات من الدم الوريدي). تم جمع عينة دم لكل فرد من هذة المجموعات ثم قسمت إلى ٢ مل من الدم تم جمعها مباشرة في أنبوب معقم يحتوي على EDTA لاستخراج الحمض النووي منقوص الأوكسجين ، ثم تطبيق طريقة

ARMS(نظـام طفـرة التضـخيم الحـراري) لدراسـة تعـدد الأنمـاط لبـادى الجينـيSIRT3 (rs56312618) ؛ (rs12293349)؛ (rs11246029). ثلاثـة مليلتـر كانـت تـم جمعهـا فـي أنبوب لا تحتوي على مانع التخثر EDTA ثم تم فصل مصل الدم في أنبوب Eppendrof ثم استخدامها للقياس الكمي miRNA-1 و miRNA-145 بجهاز RT-PCR

فيمـا يتعلـق بتوزيـع الجـنس (٦٦,٧٪) مـن مرضـى احتشـاء العضـلة القلبيـة كـانوا ذكـورًا ، و (٣٣,٣٪) مـن المرضـى كـانوا مـن الإنـاث ، بينمـا تضـمنت المجموعـة المعرضـة للمـرض (٤١,٧٪) ذكورًا و (٥٨,٣٪) من الإناث ومجموعة السيطرة (٦٨,٤٪). الذكور و (٣١,٦ ٪) من النساء. لم يكن هناك فرق معنوي في متوسط العمر بين المجموعات الثلاث فيما يتعلق بتوزيع المرضى حسب النوع (P = 0.121) ، والذي يضمن تطابق الجنس الذي يعد إلزاميا لمثل هذه الدراسة وفقا لنتيجة العمل الحالي.

كان هناك ارتباط كبير فيما يتعلق بنمط وراثي rs11246029 في متغير CC بين مجموعة المرض ومجموعة السيطرة EF = 0.56. بينما في حالة rs12293349 كما تم ملاحظة الفرق الكبير في المتغير CT ، بين مجموعة المرض والسيطرة (p = 0.05) EF = 0.55. يظهر النمط الوراثي rs56312618 اختلاف كبير في متغير AA ، بين مجموعة المرض ومجموعة السيطرة (p = 0.027) ، أيضا مع EF = 0.59. وكان مستوى miRNA-1 أعلى بشكل ملحوظ في مجموعة المرض تليها مجموعة الخطورة (المعرضين للمرض) ثم من تليها مجموعة السيطرة (P <0.05). و كان التغير في مستوى miRNA-1 أعلى بشكل ملحوظ في مجموعة المرض متبوعًا بالمجموعة الخطورة ومن ثم مجموعة السيطرة (P <0.05) بينما كان مستوى miRNA-145 أدنى بشكل ملحوظ في مجموعة المرض متبوعًا بمجموعة الخطورة ومن ثم يتبعة مجموعة السيطرة (P <0.05).

تحليل (ROC) تم الاستعانة به لغرض أيجاد فحص الحساسية والخصوصية لـmiRNA-1 ؛ miRNA-145 .تـم تحديـد القيمـة عنـد miRNA-1 مـن> ٥,٢٨ مـع حساسـية ٩١,٦٧٪ وخصوصية ٩٠,٧٪ ، بينما خفضت قيمة لـ miRNA-145 ≥٠,٧ مع حساسية ٩٥,٨٣٪ وخصوصية قدرها ٨٩,٤٧ ٪. miNAR-1 لديها القيمة التنبؤية لتكرار المرض مع حساسية وخصوصية (٤٠ ٪ ، ٨٩,٤٧ ٪) على التوالي. في حين أن miRNA-145 لها مؤشر مع تكرار حدوث المرض ب حساسية وخصوصية (٤٠٪ ، ٩٤,٧٤٪) على التوالي.

الاستنتاج: الأفراد الذين لديهم نوع الجين SIRT3 rs56312618 . النمط الوراثي (AA البديل) ؛ rs12293349 التركيب الوراثي (CT النوع) ؛ كان(CC) rs11246029 أكثر عرضة للإصابة باحتشاء عضلة القلب.

خصائص (miRNA-1، miRNA-145) حساسيتهما ونوعيتها العالية في هذه الدراسة تدفعنا لاستخدامها كمؤشر حيوي وحيد أو مدعم بمعلمات حيوية أخرى في تشخيص احتساء العضلة القلبية الحاد.

يمكن استخدام (miRNA-1، miRNA-145) كمؤشرات حيوية في متابعة تطور المرض لكن بحذر. علما إن الدراسة الحالية لم تستطع تحديد ارتباط كبير بين (miRNA-1 أو miRNA-145) والتغير النمطي في بادئ جين SIRT3.