T. C.

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DEPARTMENT OF MOLECULAR MEDICINE

INVESTIGATION OF THE EFFECT OF HUMAN INFLAMMATORY CYTOKINES (IL-1β AND IL-6) IN CARDIOVASCULER DISEASE

MASTER THESIS

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I declare this written dissertation which is about " Investigation of the Effect of Human Inflammatory Cytokines (IL-1β and IL-6) in Cardiovascular Disease" is a result of almost my own work and research that represent with my own words, ideas, data, and measurements except for the cited parts which are referenced to their original provenance.

I confess that this thesis has not been submitted or used by any study before nor accepted for any other degree studies before.

25 / 05 / 2021

Dilveen Dlawer Omer OMER

DEDICATION

I dedicate my humble effort and work to the reason behind my success and who I have become today, the ones who spent all their lives to afford me an honest and great life so I could achieve all my goals, the ones who made all my dreams come true, the ones who were always with me even through their unstoppable prayers day and night for my accommodation and prosperity. To my guardian angles;

Father and Mother

These perfect, gentle, and pure souls made me believe that everything in life is achievable if I believed in myself. Those amazing two souls whose care, love, affection, encouragement, and endless support made me able to get to who I am today with such honor and success. They taught me that success comes to those who work hard no matter how small they started.

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TABLE OF CONTENTS

THESIS APPROVAL FORM.....ii

DECLARATION.....iii

DEDICATION.....iv

ACKNOWLEDGEMENTS.....v

TABLE OF CONTENTS.....vi

LIST OF TABLES.....x

LIST OF FIGURES.....xi

LIST OF SYMBOLS AND ABBREVIATIONS.....xii

ABSTRACT.....xvi

ABSTRACT (Turkish).....xvii

1. INTRODUCTION AND PURPOS.....1

2. LITERATURE REVIEW.....4

2.1. Overview of Cardiovascular diseases.....4

2.2. Topography of Disease.....4

2.2.1. Coronary Heart Disease 4

2.2.2. Heart Attack.....6

2.2.3. Stroke.....6

2.2.4. Heart Failure.....8

2.2.5. Arrhythmia....8

2.2.6. Heart Valve Problems....10

2.2.7. Cardiomyopathy....10

2.2.8. Congenital Heart Defects....11

2.2.9. Peripheral Artery Disease....12

2.2.10. Other Cardiovascular Diseases....12

2.3. The Oxidative Stress Role and the Cardiovascular Disease....12

2.4. Pathophysiology of Cardiovascular Diseases....16

2.4.1. Atherosclerotic Pathophysiology....20

2.4.2. Cell Death Programs: Apoptosis and Necrosis....23

2.5. Role of Antioxidants....26

2.5.1. Ascorbic Acid (Vitamin C)....28

2.5.2. Glutathione....28

2.5.3. Vitamin E (Tochopherols)....29

2.5.4. Coenzyme Q 10 (CoQ10)....29

2.5.5. Carotenoids....29

2.5.6. Polyphenols....30

2.6. Role of Interleukin-6 in Cardiovascular Diseases....30

2.7. Role of Interleukin- 1 beta (IL-1β) in Cardiovascular Diseases....32

3. MATERIALS AND METHODS.....39

3.1. Study Aim.....39

3.2. Study Design and Patients.....39

3.3. Specimen Collection.....40

3.4. Material and Devices.....40

3.5. Assay Protocol and Laboratory Indices.....40

3.5.1. Samples Preparation.....40

3.5.2. Reagent Preparation.....41

3.5.2.1. Preparation of Mix Diluent 41

3.5.2.2. Preparation of Standard Solution.....41

3.5.2.3. Preparation of the Wash buffer.....42

3.5.2.4. Biotinylated Human Interleukin-1 beta Antibody Preparation.....42

3.5.2.5. Streptavidin-Peroxidase Conjugate Preparation.....42

3.5.3. Assay Procedure.....43

3.5.3.1. Phase 1.....43

3.5.3.2. Phase 2.....44

3.5.3.2. Phase 3.....45

3.5.3.2. Phase 4.....45

3.5.3.2. Phase 5.....45

3.5.4. Absorbance Reading.....46

3.5.5. Statistical Analysis.....46

4. RESULTS.....47

4.1. The Demographic Data of the Working Groups.....47

4.2. Smoking and Diabetes History Linked to Interleukon-1 Beta.....48

4.3. The Study Group Risk Factors Related to Interleukon-1 Beta.....50

4.4. The Cholesterol Effect on The Gender and Other Parameters.....51

5. DISCUSSION AND CONCLUSION.....52

6. REFERENCES.....59

7. APPENDICES.....89

7.1. ETHICAL APPROVAL.....89

7.2. FORMS.....90

7.3. RAW DATA.....98

LIST OF TABLES

Table 2.2.3. Definition and Types of Stroke.....7

Table 2.2.6. Heart Valves Classification.....10

Table 2.7. Interleukin-1 Family Members.....34

Table 3.5.2.2. Standard Solution Tubes Preparation.....42

Table 3.5.3.1. Assay Templet.....43

Table 4.1. Demographic Results of The Study Population.....47

Table 4.2-1. Smoking and Diabetes History Linked to the IL-1β.....48

Table 4.2-2. Smoking and Gender Linked to the IL-1β.....48

Table 4.2-3. Diabetes History Linked to IL-1β of the working group.....49

Table 4.2-4. Smoking, Gender, Diabetes History of the Working Group Linked to IL-1β.....49

Table 4.3. Total Plasma Cholesterol, Triglycerides, LDL, HDL, Linked to the I IL-1β Results in CVD Patients and Healthy Control Groups.....50

Table 4.4. Cholesterol Linked to Smoking, Gender, Diabetes History.....51

LIST OF FIGURES

Figure 2.2.1. The difference between a narrowing artery and normal one.....5

Figure 2.2.3. Stroke Categories.....7

Figure 2.2.5. Cardiac Circulatory System.....9

Figure 2.3. Heart Failure and Cardiovascular Disease linked to Oxidative stress.....14

Figure 2.4-1. Different Risk Factors of ROS.....16

Figure 2.4-2. Representative Events Following MI Schematic Depiction.....17

Figure 2.4-3. Mitochondrial Electron Transfer Chain Schematic Illustration.....19

Figure 2.4-4. Formation Of Atherosclerotic Plaque.....21

Figure 2.4.2. Mitochondria Role in Cell Death Pathways.....23

Figure 2.5-1. Antioxidants Major Classes Implicated in The CVD Management.....27

Figure 2.5-2. Antioxidants Mechanisms in CVD.....28

Figure 2.6. The Inhibitory and Trans-signaling Mechanisms of IL-6R Complex.....31

Figure 2.7-1. Inflammasome Activates IL-1β and Views Danger Signals by the Proteolytic Action on the Mature Form Via Caspase-1.....35

Figure 2.7-2. IL-1β Actions Correlated to Atherosclerosis.....36

Figure 2.7-3. Inflammasome, IL-1, and HF after Acute Myocardial Infarction (AMI).....37

Figure 3.5.4. Standard Curve of Human IL-1β.....46

LIST OF SYMBOLS AND ABBREVIATIONS

$^{\bullet}O_2^-$: Superoxide Anion

$^{\bullet}OH$: Hydroxyl Radical

AF: Atrial Fibrillation

AMI: Acute Myocardial Infarction

Ang II: Angiotensin-II

ARVC/D: Arrhythmogenic Right Ventricular Cardiomyopathy/dysplasia

AT1R: : Angiotensin-1 Receptor

ATP: Adenosine Triphosphate

AV: Atrioventricular Node

AVSDs: Atrioventricular Septal Defects

bpm: Beats Per Minute

CAD: Coronary Artery Disease

CASP1: Caspase 1

CAT: Catalase

CHD: Coronary Heart Disease

CHDs: Congenital Heart Defects

CHF: Cardiac Heart Failure

CNS: Central Nervous System

CRP: C-Reactive Protein

CVD: Cardiovascular Disease

DAMP: Danger-Associated Molecular Pattern

DCM: Dilated Cardiomyopathy

ECC: Excitation-Contraction Coupling

ECT: Electron Transport Chain

gp130: IL-1β-Receptor Glycoprotein 130

GPx: Glutathione Peroxidase

$H2O2^{-}$: Hydrogen Peroxide

HCM: Hypertrophic Cardiomyopathy

HDL: High-Density Lipoproteins

HF: Heart Failure

HMGB: High Motility Group Box

HSP: Heat Shock Proteins

ICH: Intracerebral Hemorrhage

IL-1ra: IL-1 receptor antagonist

IL-1β: Interleukin-1beta (IL-1β)

IL-6: Interleukin 6

IL-6R: Interlukin-6 Receptor

ISFC: International Society and Federation of Cardiology

LDL: Low-Density Lipoproteins

MCP-1: Monocyte Chemoattractant Protein

MI: Myocardial Infraction

MMPs: Matrix Metalloproteinases

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

NOX: NADH Oxidase

NOXs: NADPH Oxidases

OH^-: Hydroxyl Anions

OMM: Outer Mitochondrial Membrane

$ONOO^-$: Peroxynitrite

oxLDL: Oxidized Low-Density Lipoprotien

PAD: Peripheral Artery Disease

PKC: Protein Kinase C

proIL-1β: Precursor Interleukin -1 beta

RCM: Restrictive Cardiomyopathy

ROS: Reactive Oxygen Species

SA: Sinoatrial Node

SD: Standard Deviation

sIL-6R: Soluble Form of the IL-6R

SMCs: Smooth Muscle Cells

SNP: Single Nucleotide Polymorphism

SOD: Superoxide Dismutase

TIA: Transient Ischemic Attack

TSI: Turkey Statistics Institution

TLR: Toll-Like Receptor

TNF: Tumor Necrosis Factor

VLDL: Very-Low Density Lipoprotein

WHO: World Health Organization

ABSTRACT

Omer, D. D. Investigation Of The Effect Of Human Inflammatory Cytokines (IL-1β And IL-6) In Cardiovascular Disease. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. M.Sc. Thesis, Istanbul, 2021.

Although deaths due to cardiovascular diseases have decreased in the last 30 years worldwide, they are still among the most common causes of death in North America and Europe. The Turkish Society of Cardiology (TEKHARF) pioneered since 1990, has conducted according to a study data that, two million cases of coronary heart disease were recorded and about 160,000 people are dying from coronary heart disease every year in Turkey. Cytokines are biological mediators that regulate immunological, local, or systemic inflammatory and restorative host responses and act as signals between cells. IL-1β is a type of cytokine that can have multiple effects, such as growth and differentiation, on many cell types simultaneously. In our study, we evaluated a comparison between a patient group (n=39) with cardiovascular diseases and a healthy, who were not previously diagnosed with this disease a control group (n=30) about several risk factors including diabetes, obesity, smoking, and hyperlipidemia and their correlation between Interleukin-1 beta (IL-1β), and cardiovascular diseases. A statistically significant relationship was found between triglycerides and the expression of IL-1β in the study groups (p=0.012). However, the complete statistical analysis of the absence of clear significant differences between the pathological and non-pathological states in most of the studied traits except for triglycerides, and the variables used such as gender, age, weight, BMI have no significant effects, except in very few replications. The relationship of cytokines, which are biological response modifying molecules that can show activity even at very low concentrations, with cardiovascular events is still being investigated.

Key words: Cardiovascular Disease, Atherosclerosis, Interleukin-1 beta (IL-1β).

ÖZET

Omer, D. D. İnsan İnflamator Sıtokınleri (IL-1β Ve IL-6) Kardıovasküler Hastalıklara Etkisinin Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp Bölüm. Yüksek Lisans Tezi. İstanbul, 2021.

Kardiyovasküler hastalıklara bağlı ölümler dünya genelinde son 30 yılda azalmış olsa da, hala Kuzey Amerika ve Avrupa'da en yaygın ölüm nedenleri arasında yer almaktadır. 1990 yılından bu yana öncülük eden Türk Kardiyoloji Derneği (TEKHARF), Türkiye'de her yıl 2 milyon koroner kalp hastalığı vakasının kaydedildiği ve yaklaşık 160.000 kişinin koroner kalp hastalığından hayatını kaybettiği çalışma verilerine göre gerçekleştirdi. Sitokinler, immünolojik, lokal veya sistemik enflamatuar ve restoratif konakçı tepkilerini düzenleyen ve hücreler arasında sinyal görevi gören biyolojik aracılardır. IL-1β, aynı anda birçok hücre türü üzerinde büyüme ve farklılaşma gibi birden çok etkiye sahip olabilen bir sitokin türüdür. Çalışmamızda, kardiyovasküler hastalığı olan bir hasta grubu (n = 39) ile daha önce bu hastalık tanısı almamış sağlıklı bir kontrol grubu (n = 30) arasında diyabet, obezite, sigara gibi çeşitli risk faktörleri hakkında bir karşılaştırmayı değerlendirdik. ve hiperlipidemi ve bunların Interleukin-1 beta (IL-1β) ve kardiyovasküler hastalıklar arasındaki korelasyonu. Çalışma gruplarında trigliseridler ile IL-1β ekspresyonu arasında istatistiksel olarak anlamlı bir ilişki bulundu (p = 0,012). Bununla birlikte, trigliseridler haricinde incelenen özelliklerin çoğunda patolojik ve patolojik olmayan durumlar arasında açık anlamlı farklılıkların yokluğunun tam istatistiksel analizi ve cinsiyet, yaş, kilo, VKİ gibi kullanılan değişkenler dışında anlamlı bir etki yoktur. çok az kopyada. Çok düşük konsantrasyonlarda bile aktivite gösterebilen biyolojik tepkiyi modifiye eden moleküller olan sitokinlerin kardiyovasküler olaylarla ilişkisi halen araştırılmaktadır.halen araştırılmaktadır.

Anahtar Kelimeler: Kardiyovasküler Hastalık, Ateroskleroz, İnterlökin-1 beta (IL-1β).

1. INTRODUCTION AND PURPOSE

The cardiovascular disease “CVD” is a vital foundation of death in the industrialized world. Modern approaches to managing the disease epidemic, are grounded on advanced medical techniques and powerful engineering models. Innovative researchers have identified several CVD risk factors and hypertension. However, our ability to manage the epidemic our understanding of these disorders remains limited (1). CVD consequences are the most crucial health issue that accounts for major third of deaths in the United States. The medical and burden costs of cardiovascular disease will notably increase worldwide in the coming decades (2). Diabetes, hyperlipidemia, smoking, high blood pressure, obesity, and high cholesterol are risk factors that could produce a stroke and heart failure (HF). Million Hearts plan is meant to prevent 1 million strokes and heart attacks over the next 5 years (3,4). The disease interrupts the blood flow around the body, posing serious cardiovascular complications (5). The annual mortality of CVD is expected to extent 23.6 million by 2030 (6).

Coronary artery disease (CAD) is a common reason for deaths among myocardial infarction (MI) and CVD patients, MI is influenced via obstruction of the coronary arteries which results in nutrients and oxygen insufficiency at the infarction spot and following cardiomyocytes death often leads to heart failure (HF) development. The danger-associated molecular patterns (DAMPs) exposure from passed away heart muscle cells, prompts the relocation of monocytes, lymphocytes, dendritic cells, besides other cells with following launch of inflammation, tissue rejuvenation, oxidative stress, and cardiac restoration. Yet, the process overdue these outcomes remains not understood (7-10).

Expression of cytokines is a result of a response to various kinds of cardiac stress, submitting an associated response to heart damage. They are increased in diverse circumstances such as cardiac ischemic states, allograft rejection, inflammatory myocarditis, reperfusion injury, and cardiac heart failure (CHF). However, elevated levels of their pattern of expression parallels are noticeable previous to activation of neurohormonal signal in initial HF making them possibly more irritable elements of heart function (11).

Proinflammatory cytokines were understood to be concealed by classical immune cells only. It is now recognized that nearly all single nucleated cardiac muscle cell varieties are capable to secrete proinflammatory cytokines as a result of different stress forms. The viscus expression of cytokines can arise in an absolute lack of systemic immune stimulation. At this point, we underline the character of the proinflammatory cytokines Interleukin-1beta (IL-1β) and interleukin-6 (IL-6) as these are the foremost well-studied and represented within the HF context (12). Raised basal c-reactive protein has been linked with an elevated risk of CVD. Moreover, a cascade of inflammation comprising tumor necrosis factor (TNF), IL-1β, and IL-6 have a crucial role in the pathogenesis of mutual postoperative problems related to cardiac surgery including a cardiopulmonary bypass (13). IL-1β is a pro-inflammatory cytokine and it is expressed by a variety of cells involving neutrophils, macrophages, and monocytes. IL-1β belongs to the IL-1 group mass. The caspase 1 (CASP1) gene proteolytically stimulates IL-1β. This gene is engaged in proliferation, differentiation, and cell death. IL-1β has also been associated with wound healing and septic shock (14).

IL-1β, the chief circulating form of IL-1, it is primarily produced as a precursor-IL-1β (pro-1β) that is initiated by CASP1 fissure in a macromolecular structure identified as inflammasome (15,16). CASP1 also involve in the excretion of active IL-1β that can later attach to the IL-1 receptor on the membrane of the neighboring cells (paracrine), same cell (autocrine), or go into the circulation aiming a far cells (endocrine). The practical expression of pro-1β is extremely cell-type specific (15,17). After tissue injury, the initiation of the inflammasome generates a local flow of IL-1β that radically strengthens the inflammatory process, employing extra inflammatory cells, inducing metalloproteinase actions, and eventually stimulating inflammatory cell death (pyroptosis) within resident cells, and leukocyte (18-20).

Turkish population has specifically decreased levels of total cholesterol and the good cholesterol high-density lipoprotein (HDL), correlated thru elevated levels of fasting triglycerides. Also, about 60% of males practicing smoking, physical quietness is mutual in both genders; and obesity is usual among Turkish females indicating an increased occurrence of diabetes and hypertension (21).

These influences possibly become a reason for the unsuspected truth that Turks consume morbidity reasons design alike developed population, while the industrialization procedure still ongoing, its population structure quite fresh, and total cholesterol levels are relatively low (21). Furthermore, gene polymorphisms are linked through the early inception of acute myocardial infarction and atherosclerosis (22).

The leading independent predictors of death and coronary consequences are linked to the metabolic syndrome, measured to overcome about 4% of adults over the age of thirty years and one-eight of cases of CVD. Since various elements display an elevated tendency, prevention of CVD should undertake extra higher importance in abundant problems in Turkey (23).

The goal of this research, was to discover and study the action of human inflammatory cytokine IL-1β levels on cardiovascular diseases by using serum samples of CVD diagnosed patients and free-CVD individuals among the Turkish population, and also to provide a relatively comprehensive association with various inflammatory parameters related to CVD such as diabetes, smoking, cholesterol, triglycerides, LDL, and HDL, as well as aging and gender.

2. LITERATURE REVIEW

2.1. Overview of Cardiovascular Diseases

CVD is the principal morbidity reason worldwide and in the United States. Lots of patients submit to the concerns of arrhythmias, HF (impaired contractility/relaxation), and myocardial ischemia (triggered primarily by acute coronary syndromes) (24). CVD mortality, over 17 million deaths in 2008 in the world, which is valued to elevate by 2030 to around 23 million and more to keep on the leading source of mortality (25,26). CVD is not a single disease, nevertheless a cluster of injuries and diseases that affect the cardiovascular system (27,28).

2.2. Topography of the Disease

CVD is actually a group of diseases disturbing the heart and blood vessels. However, they include: coronary heart disease, stroke, congenital and rheumatic heart disease, angina, peripheral arterial disease, deep vein thrombosis, and other less common cardiovascular diseases (28).

2.2.1. Coronary Heart Disease

Coronary heart disease (CHD) is identified as Ischemic heart disease, CAD, obstructive coronary artery disease as well. However, it is a term used for a wide range of heart conditions that alters its function and structure. CHD is a form of a disease that influence the heart and progresses once the cardiac arteries are unable to provide enough oxygen-rich blood to the cardiac muscle. It is the major trigger of mortality in the U.S. CHD, usually begins by plaque buildup (atherosclerosis) within the inside layer of the large coronary arteries. This plaque is able to somewhat block the blood flow completely or moderately in the heart's bulky arteries. Some kinds of this condition may be due to an injury/damage or a disease influencing the arteries function in the heart (29).

As the artery thickens, the oxygen-rich blood circulation becomes compromised, leading to CVDs such as stroke and heart attacks. When atherosclerotic plaque takes place in the coronary arteries there is a blocking of the arterial lumen results in a reduction in

the myocardial perfusion which ends up as myocardial ischemia. This condition is identified as coronary artery disease (Figure 2.2.1) (30).

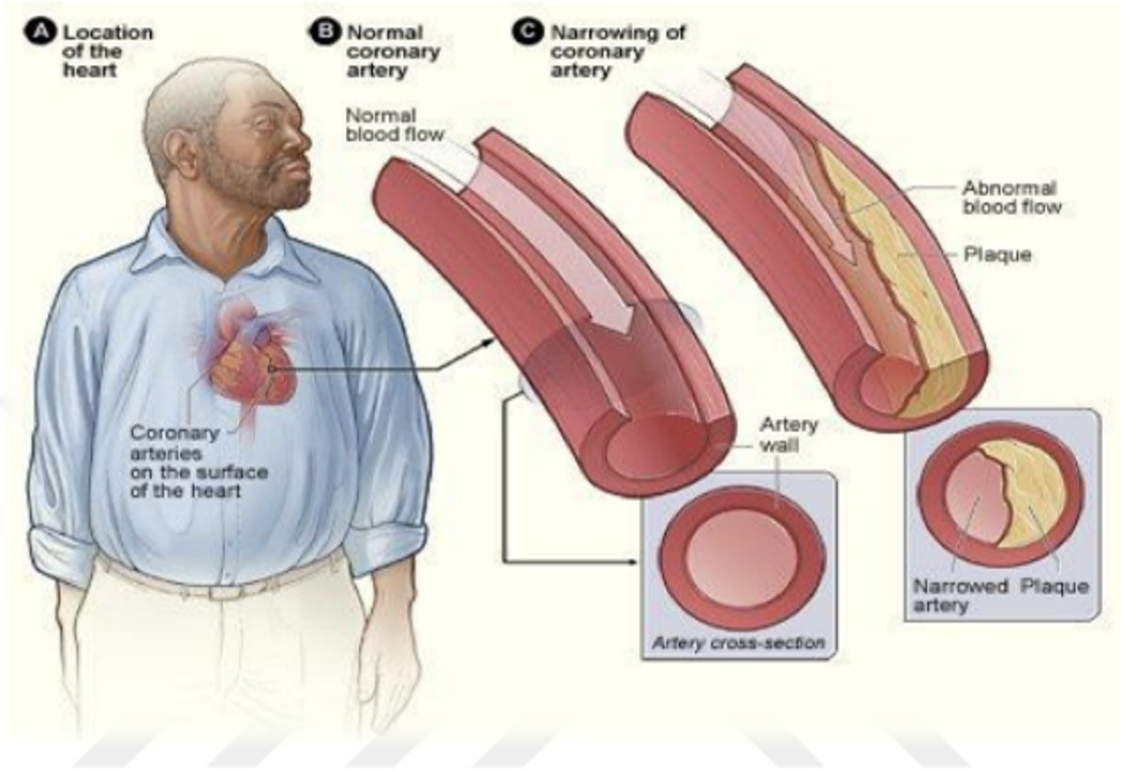

Figure 2.2.1. The Difference Between a Narrowing Artery and Normal One

CHD remains the most common pathology with which cardiac surgeons and cardiologists are facing. It is the foremost reason of death among the Turkish population in 2013 (31). The Turkish Heart Study was accepted out to describe the lipoprotein and lipid changes in Turkey. Turkish population has an elevated incidence of CHD approximated at 37% of deaths according to the Turkish Ministry of Health (32). The typical signs and symptoms of CHD include (33):

- Angina (chest pain) – This pain may develop or move to the jaw, neck, back or arm and other types of discomfort.
- Breath shortness
- Nausea
- Sweating
- Irregular heartbeat

2.2.2. Heart Attack

A heart attack is usually owing to an disturbance of the blood supply to the heart. Without this supply, the heart muscle may be damaged and begin to die, if a large part of the heart is damaged in this way, cardiac arrest occurs once the heart stops beating, resulting in heart death (33). The main reason for heart attacks is atherosclerosis. Before a heart attack, a plaque bursts (ruptures), causing a blood clot to form at the rupture site. The clot may block the blood supply to the heart, resulting in a heart attack (33). Heart attacks also known as MI, and are chief contributors to heart disease, with an predictable 750,000 taking place once a year (34). The symptoms can vary in their duration and order, they may come and go suddenly or last several days, the symptoms include (35):

- Tightness, pain, pressure, sore in the chest
- pain spreads to the jaw, arms, neck other parts
- heartburn or indigestion similar feelings
- feeling dizzy or lightheaded, sometimes vomiting and nausea
- feeling clammy and sweaty
- breath shortness
- wheezing or coughing, if fluid accumulates in the lungs
- Hypoxemia: involves low oxygen levels.
- Pulmonary edema: Occurs when fluid builds up around and inside the lungs.
- Cardiogenic shock: The blood pressure falling swiftly due to the heart does not source enough blood to the the body to perform properly.

2.2.3. Stroke

A stroke is an acute or moderate neurological injury due to blood interruption supply to a part of the brain, either by rupture (hemorrhage) or arterial blockage. Strokes might be organized into two major categories: hemorrhagic and ischemic (Figure 2.2.3) (36-38). Stroke is traditionally characterized as a neurological failure attributed by a vascular origin into moderate pivotal damage of the central nervous system (CNS), involving intracerebral hemorrhage (ICH), cerebral infarction, and subarachnoid hemorrhage, and is the chief reason for death globally (Table 2.2.3) (39,40).

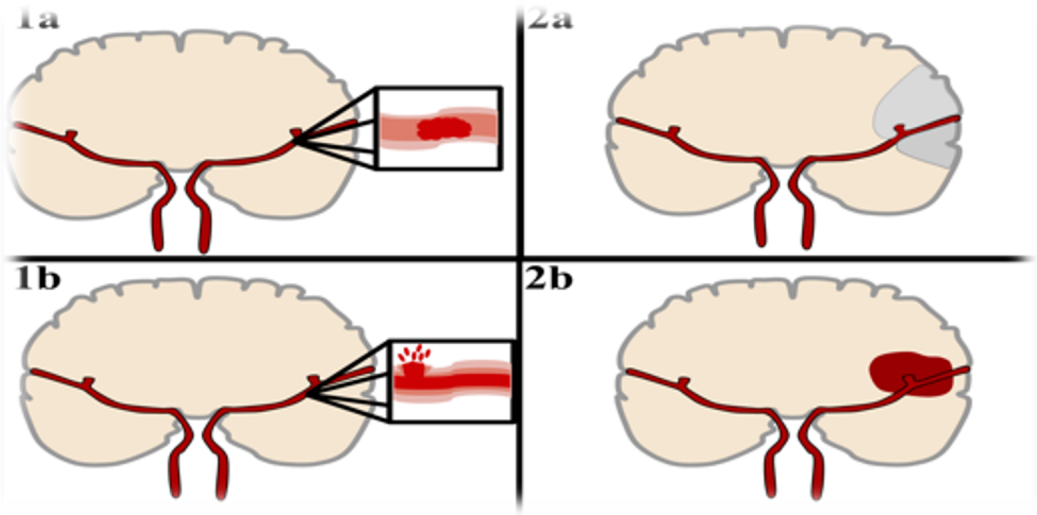

Figure 2.2.3. Stroke Categories

There are two stroke types. Ischemic, due to an artery clotting (1a) rising in brain death of the injured area (2a). Hemorrhagic, due to blood seeping into the brain caused by blood vessel rupture (1b) permitting blood to flow into the injured area (2b) therefore raising the pressure in the brain.

Table 2.2.3. Definition and Types of Stroke

Stroke is a series of acute and moderate neurological defects believed to be caused by hemorrhage or ischemia, lasting ≥24 hours, classified as one of the following	
Transient Ischemic Attack (TIA)	A series of focal and temporary dysfunction of vascular root, that are different in period, commonly persisting from 3-15 minutes, yet sometimes, lasting for 24 hours. No persistent neurological deficits are left
Hemorrhagic Stroke (CNS Infraction)	A spinal cord, retinal cell, or brain passing attributable to ischemia. It includes type I and II hemorrhagic infarctions 1. Intracerebral Hemorrhage: A main collection of blood within the ventricular system or brain parenchyma which is not related to trauma. 2. Subarachnoid hemorrhage: Bleeding within the subarachnoid space.
Ischemic stroke	A series of neurological dysfunction due to retinal infarction, spinal or focal cerebral. It can be subclassified into 4 types: 1. Systemic hypoperfusion. 2. Thrombosis 3. Embolism. 4. Cerebral venous sinus thrombosis.

2.2.4. Heart Failure

Inflammation is a crucial manner in the pathophysiology of heart failure (HF). Elevated levels of pro-inflammatory cytokines are correlated with poorer outcomes and unfavorable heart remodeling in HF patients (41). It is a serious medical condition which results from any mechanical or functional disturb of the heart, wounding ventricular ejection and the blood circulation of general needs. HF can be a result due to diseases of the myocardium, endocardium, pericardium, metabolic disorders, heart valves, or vessels (42,43). Symptoms due to a lessening in heart muscle output include (weakness and fatigue) that is most marked with physical exertion and symptoms due to accumulation of excess fluid include (orthopnea, edema, dyspnea, abdominal distention, and hepatic congestion pain) (43).

Furthermore, significant associations between HF-associated mortality and IL-6 have been discussed in minor HF cohorts (42,43). For example, IL-6 is of special attention in HF as pharmacological agents affecting IL-6 signaling at present have successfully been used and developed in a variety of autoimmune diseases (44-48). IL-6 levels are known to increase with aging and IL-6 signaling has been involved in the pathophysiology of HF co-morbidities involving anemia, renal disease, atrial fibrillation (AF), and frailty (45-47).

2.2.5. Arrhythmia

The heartbeat offers the mechanical force for the pumping of deoxygenated blood away from and oxygenated blood to the peripheral tissues (Figure 2.2.5) (49,50). The heart action comes from the successive closing and opening of ion channel proteins which cross the individual myocytes plasma membrane. Its transference within the heart hangs on the electrical connection within these cells, which is facilitated by gap junctions (51). Differences in the properties and expression of ion channels ensue heterogeneities in potential waveforms action in different heart cell types and regions, and in the expand direction through the heart (52).

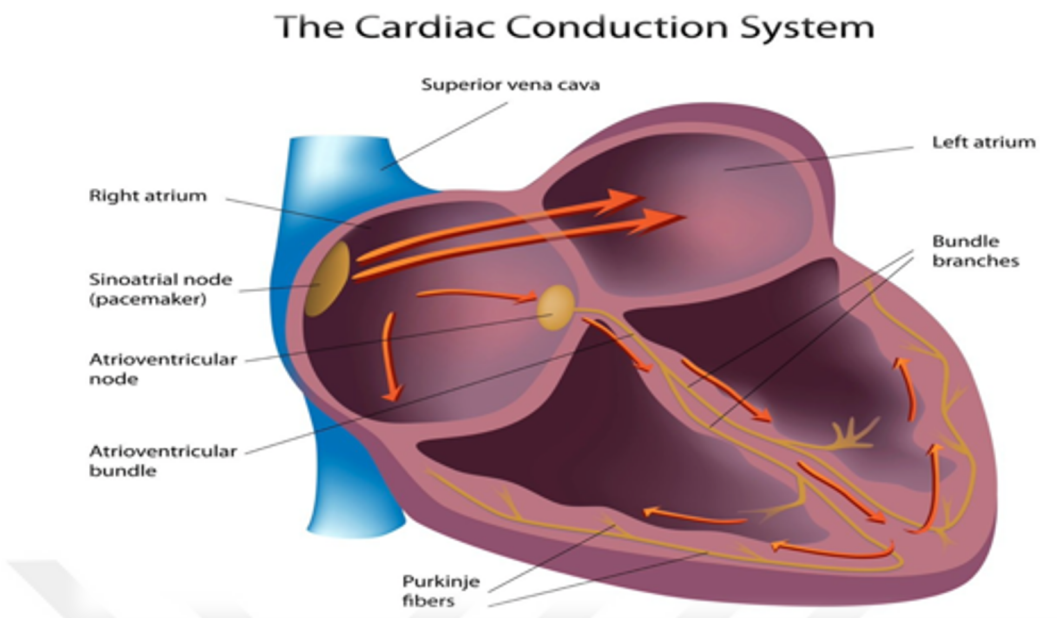

Figure 2.2.5. Cardiac Circulatory System

Arrhythmia is an abnormal heartbeat arrangement. The only natural heart pace is a normal sinus rhythm. In this rhythm, an impulse is created in the sinoatrial (SA) node, which is shows and slows down whereas passing through the atrioventricular node (AV) (53). Any abnormality from this managed route results in Arrhythmia. That can be classified according to numerous conditions. The best way to categorize them is according to the rate of conveyance as a tachyarrhythmia with a beating rate higher than 100 beats per minute (bpm) and bradyarrhythmia with a rate fewer than 60 bpm (53). The occurrence of this problem is supposed to be 1.5-5% in the general population, with AF presence most common (54).

AF may occur without evident organic heart disease and in these cases may lead to heart failure if the ventricular rate is very fast for a very long time (55). Any irregular state affecting any process interfering with the normal work of the heart valves and the myocardium function may be responsible for Arrhythmia. The general risk factors for AF are high blood pressure, age, diabetes, heart valve abnormalities, and heart failure (56).

Cardiac arrhythmias sometimes are classified according to their source as ventricular arrhythmias (originating in ventricles) or supraventricular arrhythmias (originating from above the ventricles, typically the atria) (56).

2.2.6. Heart Valves Problems

It is any cardiovascular disease including one or might be more of the four heart valves. This disorder occur frequently as an outcome of aging (57) yet might also be as a result of a specific disease or congenital abnormalities or physiologic processes involving pregnancy and rheumatic heart disease (58). Insufficiency/regurgitation and stenosis represent the dominant anatomic and functional consequences associated with valve heart disease Stenosis is characterized by a valvular orifice narrowing that prevent adequate blood outflow (Table 2.2.6) (59).

Table 2.2.6. Heart Valves Classification

Valve	Stenotic disease	Insufficiency
Pulmonary	Pulmonary valve narrowing	Pulmonary insufficiency
Tricuspid	Tricuspid valve narrowing	Tricuspid insufficiency
Aortic	Aortic valve narrowing	Aortic insufficiency
Mitral	Mitral valve narrowing	Mitral insufficiency

2.2.7 Cardiomyopathy

Cardiomyopathy is a diverse cluster of myocardium disorders related to electrical and mechanical malfunction that often show improper dilatation or chamber hypertrophy due to several reasons that recurrently are inherited. Cardiomyopathies are both a part of simplified systemic disorders or are limited to the heart, regularly leading to CVD or gradual cardiac failure-linked disability (60). In 1980, the WHO described Cardiomyopathy as "a cardiac muscle disorder of unidentified cause," to differentiate cardiac dysfunction from Cardiomyopathy due to known cardiovascular units for example hypertension, or valvular disease (58,61).

Consequently, the 1995 WHO/International Society and Federation of Cardiology (ISFC) Task Force on the classification and definition of the Cardiomyopathy types extended to involve all heart muscle disorders and to catch etiology into consideration, besides the leading pathophysiology (62). In 1995 classification, the disease was defined as "A myocardium disease linked with heart malfunction". They are categorized based on the physiology and anatomy into the following forms, every one of them has few distinct causes (62):

- Restrictive cardiomyopathy (RCM)
- Dilated cardiomyopathy (DCM)
- Hypertrophic cardiomyopathy (HCM)
- Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC)
- Unclassified cardiomyopathies

Most reasons of HF generating from DCM about 70% happens from pump failure, whereas sudden or unexpected heart muscle death from Arrhythmias measures for 30% (63-68).

2.2.8. Congenital Heart Defects

Congenital heart defects (CHDs) are the primary cause of neonatal mortality and the most common birth disorder worldwide. The principal subtype of CHDs is the nonsyndromic atrioventricular septal defects (AVSDs) (69). less than 20% of CHD can be ascribed to huge structural chromosomal alternates or single-gene alterations initiating monogenic syndromes (70). The majority of CHDs are nonsyndromic and are of unidentified etiology (71).

Studies have demonstrated a connection between birth defects and air pollution. Among these defects CHDs, which might be related to revelation particularly during serious embryological progress periods such as weeks 2-8 when the heart organ is formed (72-74).

Frequently called the infant's "serious exposure window", revelation to toxins including air pollutants during this phase may affect growth and result in a congenital defect (75,76). Yet, environmental risk factors linked to specific pollutants and air pollution correlated with a CHD remain unclear (77).

2.2.9. Peripheral Artery Disease

Peripheral artery disease (PAD) is a chronic health condition, it is described as a lower extremities atherosclerotic vascular disease. PAD is the most undertreated, and underdiagnosed of vascular diseases even with its pitiable prediction. In 2010, it is measured that 70% of the two hundred million people in low to middle-income countries are living with PAD (78).

The presentation of symptoms of PAD varies from asymptomatic, intermittent claudication, atypical leg symptoms, ischemic rest pain, to tissue loss (79). Unrelated to the presentation, on individual testing, PAD patients have intensified prevalence of foremost cardiovascular series (e.g. stroke, MI, or cardiovascular death) and besides walking impairment likened to age-matched members population (80,81).

2.2.10. Other Cardiovascular Diseases

These could include brain vascular tumors, disorders of the heart muscle, tumors of the heart, heart valve disorders, and diseases of the heart wall (82).

2.3. The Oxidative Stress Role and the Cardiovascular Disease

It is identified as an inequity among the detoxification and creation of reactive oxygen species (ROS) (82-84). ROS at biological levels is valued to operate as molecules of signaling to provide the maintenance of cardiovascular homeostasis and to normalize a broad array of activities in the cardiovascular system (85). In comparison, the sustained and excessive elevation of ROS has a crucial part in the development and initiation of clinical series of CVD (86-88). These mechanical signals trigger signaling paths that result in endothelium coating dysfunction which is a pro-inflammatory, cooperated, and thrombotic barrier (86-89).

Oxidative stress in vascular and cardiac muscle cells labels the cells' damage rising from the low antioxidant reserve or elevated generation of ROS. The increase in the ROS formation seems to be due to elevated generation of ROS by WBC, endothelial dysfunction, impaired or diminished mitochondrial reduction of molecular oxygen, besides radiation and pollution exposure (90).

In addition to mechanical stimuli, activation of endothelial cells is elevated by different molecular stimuli, including cytokines, oxidized low-density lipoprotein (LDL), pathogen-associated molecules, and advanced glycosylation outcome (89-92). Nevertheless, an atheroprotection purpose of high-density lipoproteins (HDL) is to enhance nitric oxidase (NO) production and prevent endothelial activation to maintain barrier integrity. Enzymatic sources for ROS, E.g. nitric oxide synthase (NOS), NADPH oxidases (NOXs), nicotinamide adenine dinucleotide phosphate (NADPH), and mitochondria, all these are considered important roots of ROS causing heart failure, myocardial and vascular dysfunction (82).

Oxidative stress stress is a outcome of the inability of the antioxidant system to neutralize the extreme production of ROS, which have a critical part in remodeling and damaging heart tissues. ROS are oxygen-involving molecules, for example, hydrogen peroxide ($H_2O_2^-$), superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$ and largely formed by neutrophils in the heart, endothelial cells, and cardiac muscle cells (93).

Oxidative stress causes up-regulation of adhesion molecules, endothelial cell dysfunction, increasing endothelial cell permeability, the formation of pro-inflammatory cytokines, and neutrophils migration (94-97). Many kinds of research and reports have founded the prognostic strength of the levels of cholesterol as the main factor for developing CAD in different populations. In addition, elevated levels of triglyceride (TG) have been linked with high CAD incidence (96-99).

Obesity is nowadays a universal rise in adults and children and it is related to plenty of CVD episodes such as stroke, hypertension, lipid faults, and atherosclerosis, heart disease, and diabetes mellitus type 2. An analysis by González-Muniesa et al. confirmed the hereditary contextual approving the chronic disorder of the metabolic homeostasis which scores in regulation of oxidative stress-related and pro-inflammatory

proteins, which could underline the progression of overweight-linked disorders (100). It is been counseled that the inflammatory condition associated with obesity appears to be mostly elicited by extreme nutrient ingestion (101) or an unhealthy diet (102).

Nutrient supplementation and dietary strategies are used for administration and prevention of obesity and obesity-linked disorders. De la Iglesia et al. explored the value of a healthy eating approach (energy limit, high antioxidant capacity, and specific macronutrient distribution) in obese individuals. The researchers revealed that this new eating approach weakened oxidative stress biomarkers points, low blood pressure in patients with obesity, and reduced android fat mass (103).

When the production of ROS offsets the antioxidant capacity, then endothelial dysfunction and cell damage levels, which lead to atherosclerosis development. Furthermore, HF rises by different mechanisms, involving obesity, diabetes, aging, hyperglycemia, and hypertension, impose extra oxidative stress on the cardiac muscle (Figure 2.3) (104).

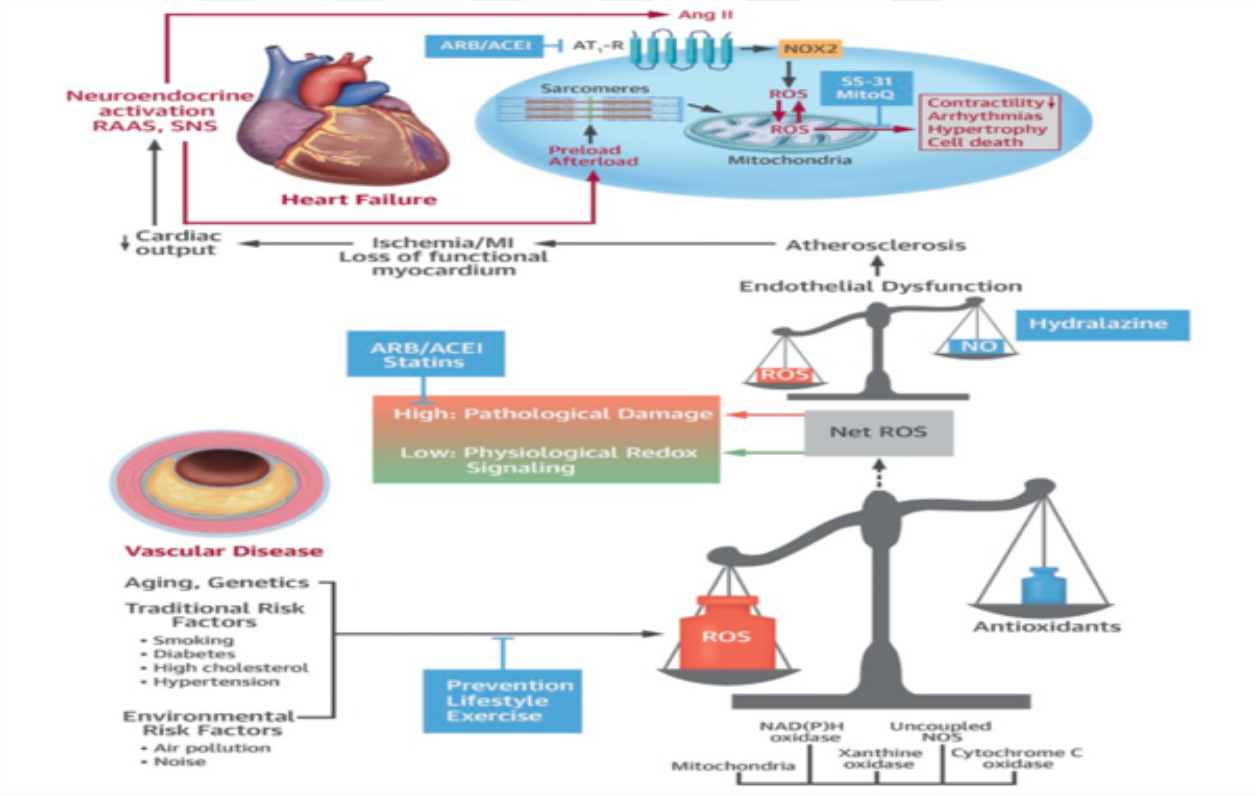

Figure 2.3. Heart Failure and Cardiovascular Disease Linked to Oxidative Stress

Vascular disease or dysfunction indicates to diverse pathological conditions affecting the arterial and/or venous system that might yield arise to unfavorable vascular and cardiac events. Among those pathological conditions are arterial modification, atherosclerosis, restenosis, and thrombosis. Various risk factors influencing the CVD, such as aging, diabetes, hypercholesterolemia, and obesity, lead to vascular disease and dysfunction, somewhat through oxidative stress, which is a common risk factor of cardiac disease, as well as an appealing preventive and therapeutic aim (105,106).

Oxidative stress is an important constitute of hypertension too, where NADPH oxidase is the main origin of ROS. Automatically, Ang II, through angiotensin-1 receptor (AT1R) mediates, indicating the NADPH oxidase activation and vascular up-regulation (107,108**)** promoted by phosphorylation and membrane translocation or via a quick GTPase translocation to the cellular membrane (109,110).

Hyperglycemia controls cellular oxidative stress with mitochondrial respiratory chain as $O_2^{\bullet -}$ (111). evolved glycation final yields, in line, activate NADPH oxidase and through post-translational remodeling, inhibit eNOS (112), whereas NADPH oxidase-linked uncoupling eNOS upholds oxidative stress in advance (113).

Smoking is also identified to affect endothelial NADPH oxidase (114) and reduce mitochondrial performing, therefore, prompting mitochondrial oxidative stress (115). high $O_2^{\bullet -}$ and peroxy nitrite ($ONOO^-$) formation assess inflammation and damage of DNA, thus, rushing cardiovascular aging. Through oxidation of LDL, fag smoke improves its pro-oxidative capacity (116). In vascular aging, two ROS-related characteristics are described, central arterial stiffness and endothelial dysfunction (117). Endothelial dysfunction is mediated either through increased degradation or decreased synthesis of NO bioavailability. furthermore, latest data have been suggesting that an age-dependent eNOS uncoupling (118), increasing oxidative stress via increased $O_2^{\bullet -}$ and $ONOO^-$ and decreased availability of NO formation. The disturbed redox balance controls inflammatory process, that rises high O_2^- production and NADPH oxidase expression via mediators (119).

2.4. Pathophysiology of Cardiovascular Diseases

ROS also regulates many vascular cell purposes, e.g. smooth and endothelial myocyte proliferation, development, migration; apoptosis,, host defens, and genetic firmness. Nevertheless, elevated levels of ROS progress vascular disorder via disruption of redox signaling processes of the vascular wall, also, direct and permanent oxidative effect on macromolecules (104). So, as consequences, the oxidation of the mitochondrial NADPH hands rise to $H_2O_2^-$, which has a fundamental effect in arrhythmia, contract dysfunction, and eventually cardiac makeover via cell death and hypertrophy. Potential marks of interference are by the means of exercise, medication, and lifestyle revolution to decrease the environmental stressors and risk factors (Figure 2.3) (104).

Most importantly, is to notice that risk factors of CVD all related specifically to endothelial dysfunction and increased oxidative stress, making it an appealing therapeutic target (Figure 2.4-1) (104).

Figure 2.4-1. Different Risk Factors of ROS

MI is occurred by obstruction of a heart artery, that leads to nutrients and oxygen shortage at the infarction position and following death of heart muscle cells. Inflammatory MI phenotype macrophages show increased oxidative stress, enhanced chemokine/cytokine secretion, impaired cholesterol efflux, and leading to more endothelial cell activation, remnant/LDL oxidation, foam cell formation, and monocyte recruitment. The revealing of danger-associated molecular patterns (DAMPs) from finished cardiomyocytes, causes the traveling of lymphocytes, monocytes, in addition to other cells with tracking inflammation, cardiac remodeling, and oxidative stress; nevertheless, the procedures behind those routes are not fully agreed yet (9,10,120,121). The DAMPs that are included in MI are generally high motility group box 1 (HMGB1) (Figure 2.4-2) (121).

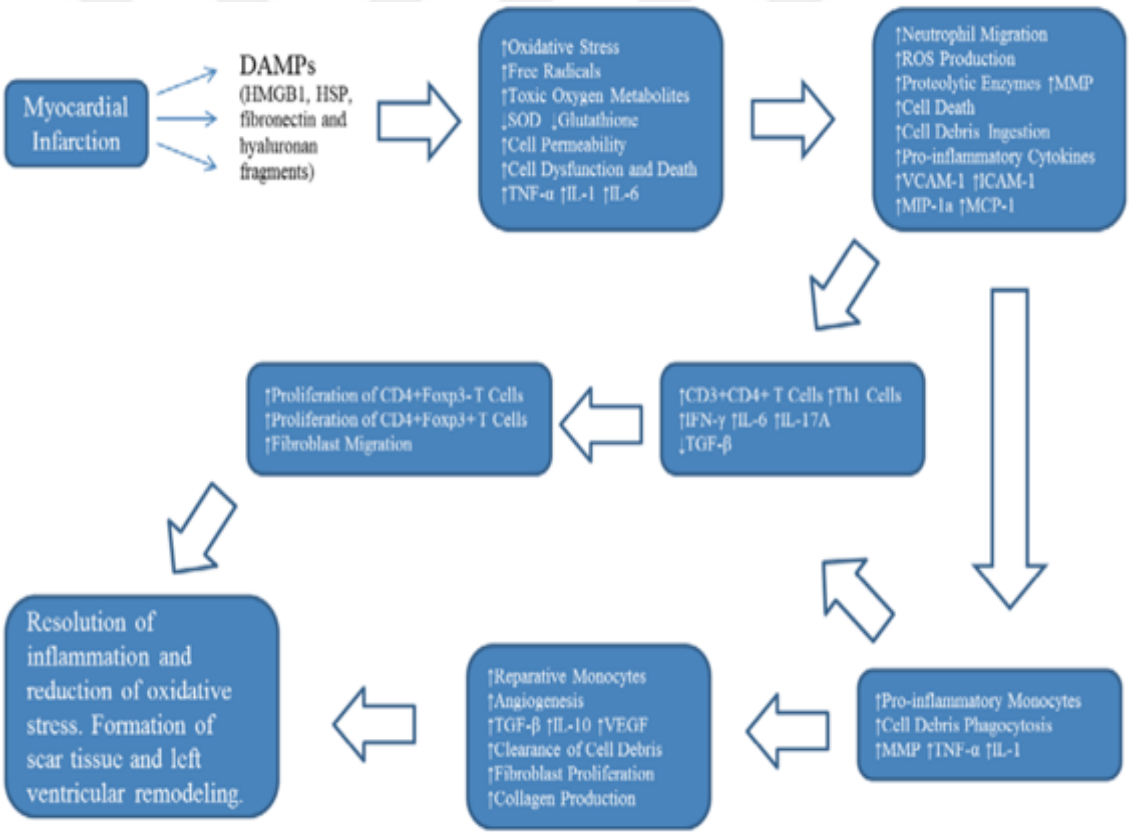

Figure 2.4-2. Representative Events Following MI Schematic Depiction

MI results in the, inflammation and initiation of oxidative stress, exposure of DAMPs and can lead to the scar tissue development, resolution of inflammation, left ventricular remodeling, and reduction of oxidative stress. Yet, the pathological procedure after MI is too complex

HMGB1 is chromatin linked protein found in the nucleus, and it is secreted from altered cells and form heterodimers with IL-1β (122), activates the macrophages to generate pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α after its binding to toll-like receptor4 (TLR4) (123,124). In addition, HMGB1 forming a heterocomplex with chemokine to enhances cell migration (125). After the hypoxic shock, the stress protein HSP60, is released from cardiac myocytes, can activate caspase 3 and 8, signals the formation of pro-inflammatory cytokines and cause DNA fragmentation and binding to TLR4 triggers apoptosis of cardiac myocytes (126,127). DAMPs are identified by TLR on the external part of endothelial cells, heart muscle cells, and immune system cells. TLR in humans sharing the innate immune system, and pattern recognition receptors (128). Moreover, components of DAMPs and the apoptotic heart muscle cells can trigger the complement system which has a vital part in the MI pathogenesis (129,130) .

The heart ought to cause a great quantity of high-energy adenosine triphosphate (ATP) on permeant grounds (131). Notably, the heart functions utilizes all presented fuel elements. Mitochondrial fatty acid oxidation is the major predominant and efficient energy generation factor in the typical cardiac muscle, even though glycolysis, lactate, glucose, ketone, and amino acid oxidation supply to overall myocardial ATP production (132). Maladaptative or adaptative metabolic variants in the cardiovascular system are proposed to influence to various disorders states, e.g. cardiac hypertrophy, heart failure, cardiomyopathy-linked diabetes, , and MI (133,134,135)

Mitochondria generate energy, in ATP form, via a multitude process, which is presented via electron travel through the electron transport chain (ETC) and oxidative phosphorylation, consisting five protein assembly (complexes I-IV and the ATP-synthase complex) and two shuttles (cytochrome C and coenzyme Q) (131). ROS origination in mitochondria is correlated to the restricted reduction of O_2 to O_2^- by I and III complexes of the ETC (131). The last common pathway of ATP generation for mitochondrial oxidative metabolism via the chronological route of substrate oxidation decreasing equivalents (FAD or NAD) which later, offer electrons downward the ETC among the parallel operation of oxygen to produce ATP (136,137).

The full means inside the mitochondria named as oxidative phosphorylation, and the schematic design of the biochemical responses which create ATP in the ETC is revealed in (Figure 2.4-3) (138). The genomic disturbance of the oxidative phosphorylation route of the enzymes been linked to heart disturbance and diseases that usually manifest with cardiomyopathic characteristics (138). The electrons transfer within the ETC is frequently incomplete coupled with ATP production and oxygen depletion. Therefore, a tiny amount (<0.1%) of electrons is leaking from the ETC and results in the limited decrease of O_2 into O_2^- and ROS intermediates production for instance $H_2O_2^-$and hydroxyl radicals. As well as, more enzymes within mitochondria have the ability to produce H_2O_2 as direct and required products of the catalytic process (139). The NADH oxidase (NOX) is a group of 7 coating-bound enzymes and characterize the main origin of ROS in the cardiovascular system (134,140,141).

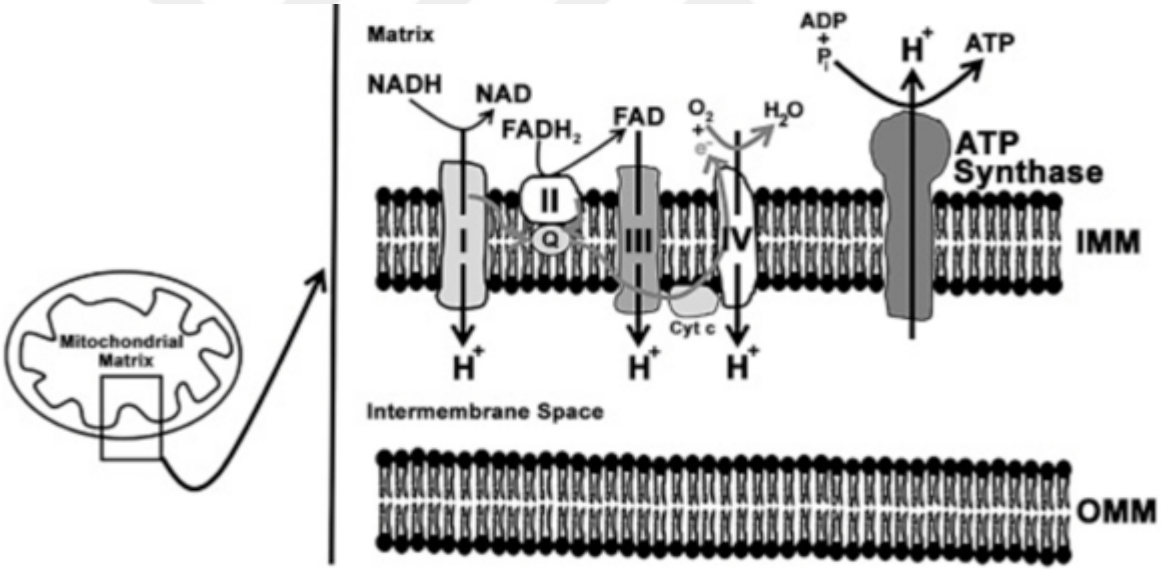

Figure 2.4-3. Mitochondrial Electron Transfer Chain Schematic Illustration

The left panel rectangular box shows the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) structure, while the right panel displays the protein complexes (I through IV) arrangements that setup the ETC. The last common way for oxidative metabolism, is the sequential route of electrons to low (molecular oxygen) redox from high ($FADH_2$ or NADH) potentials down complexes I through IV of the ETC which produces the bulk of ATP. This stepping electron transfer leads active pumping of hydrogen ions to the intermembrane space. The electrons move down the complexes illustrated as the gray arrow and finally react with oxygen to produce water at complex IV. The "escape" of electrons along the ETC gives substrate for ROS generation. The consequent electrochemical gradient produced across in the IMM eases the translocation of protons from the intermembrane space through the ATP-synthase complex again into the mitochondrial matrix. The proton translocation is attached to the phosphorylation of ADP to generate ATP.

The bulk of laboratory researches linked to disruption of ROS breakdown or excess mitochondrial ROS establishment provide their effect in worsening HF or ischemia, and diabetic cardiomyopathy (135,142,143). Notably, mitochondria increase ROS generated from NOXs and perhaps thereby works as “redox hubs” in the heart physiology of diseases (144,145).

Also, Also, ROS plays an important part in the inflammation process of atherosclerosis, where they assist inflammasome development, which activates Caspase-1 and supports secreting of the proinflammatory cytokines IL-1β and IL-8. The proinflammatory enzyme caspase-1, also, named interleukin altering enzyme, is the main manager of converting inactive pro-IL-1β to the active IL-1β. A theory had suggested the inflammasome is a vital content by which physical and psychological mediators can control depression progression. Therefore, suggested inflammasome, and caspase-1 might be a good aim for new depression therapies (146). Modified lipoproteins, oxidative stress, and other lesion factors are able of initiating the inflammation process by receptor recognition (120,147). Besides, the plasma membrane cholesterol in macrophage foamcytes develops indicating by inflammatory receptors (148,149). Lately, activation of the inflammasome and IL-1β and IL-18 has been implicated in atherogenesis (150,151).

2.4.1. Atherosclerotic Pathophysiology

Atherosclerosis is a chronic vascular inflammation which related to oxidative stress (152). Also, it is the principal reason of strokes, peripheral vascular disease, and heart attack. Nevertheless, It is an important reason of morbidity and death in the developed countries and the U.S. (153).

Changing in apoB lipoproteins via glycation and oxidation develops their realization through some receptors which are not regulated by plasma cholesterol (154,155). Throughout the initial phase of atherosclerosis fatty streak (Figure 2.4-4), the monocyte-generated macrophages affect the engaged apoB-consisting lipoproteins, that are destroyed in lysosomes, and the ending results of cholesteryl ester (CE) is wrapped up within cytoplasmic fat droplets, that are foam cells features (155-157).

Cholesterol dependent inflammatory receptor signaling and formation of macrophage foam cell is lessened by the clearing the cholesterol thru apoA-I and atheroprotective HDL by means of a several mechanisms (156-159). Moreover, macrophages are the major insightful cells, different cells are also control the progression of injury involving dendritic cells (160,161), B, mast, and T cells (Figure 2.4-4) (162). Dendritic cells secrete cytokines and controls the training reactive T cell clones, working in a large pro-inflammatory volume (163). Moreover, they response to lipids, which leads to elevated pro-inflammatory cytokines creation and inflammasome activation (164).

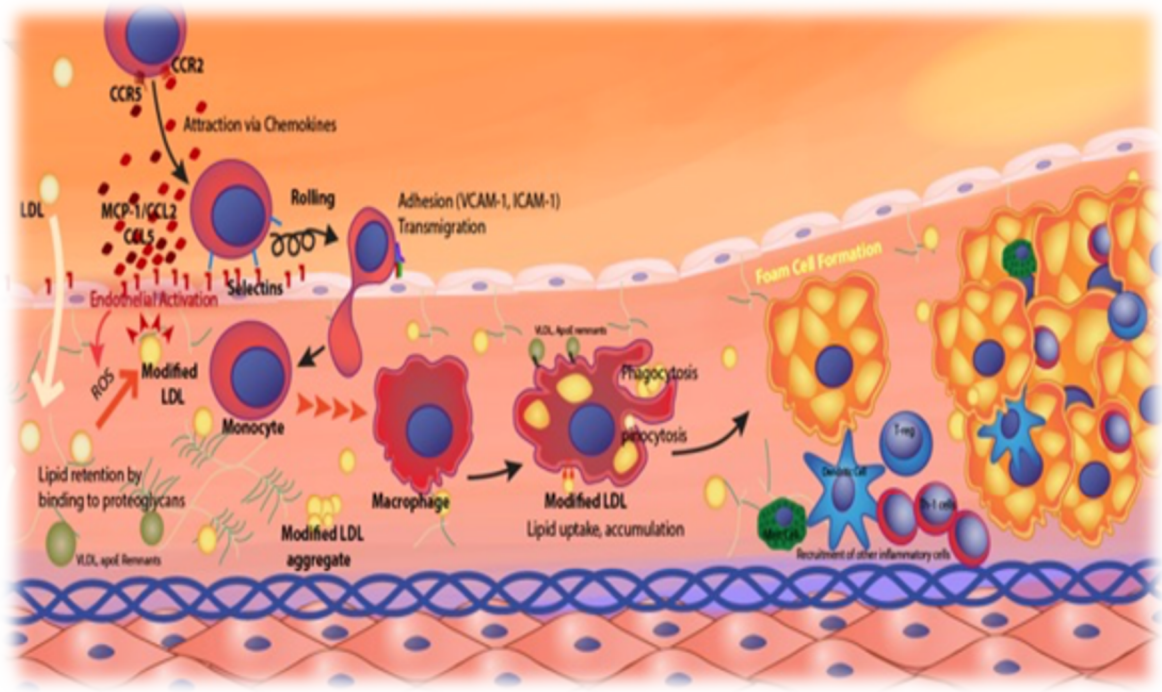

Figure 2.4-4. Formation of Atherosclerotic Plaque.

Atherosclerotic lesion initiation. The fatty aspect stage of atherosclerosis starts with the retaining of apoB-contains lipoproteins (LDL, VLDL, and apoE remnants) and endothelial cells dysfunctional in the subendothelial space. Retained lipoproteins are modified (enzymatic, glycation, oxidation), which, along with other atherogenic factors, controls endothelial cell activation. Endothelial cell activation promotes the enrollment of other immune cells as well involving regulatory T cells (T-reg), dendritic cells, T helper 1 (Th-1) cells, and mast cells. The monocytes segregate into macrophages and show receptors that referee the internalization of modified LDL apoE remnants, and VLDL to turn into foam cells. Further, inflammatory waving processes are turned on in macrophage foam cells guiding to additional cell enrollment and LDL modification.

Also, small non-coding RNAs have been discovered to affect atherosclerosis events by cholesterol homeostasis or adjusting inflammation in various cell types in injuries (165,166)

Furthermore, approval of native LDL via pinocytosis perhaps impact to foam cell creation (167-169). Oxidation of polyunsaturated fatty acids (PUFA) phospholipids existing in plasma lipoproteins gives the arise of various of reactive oxidized phospholipids which turn these lipoproteins into harmful elements (170). Significant relationship is seen between oxLDL and the scope of atherosclerosis in human patients (171). oxLDL range forecast the progression and presence of symptomatic atherosclerosis (172). Thus, the circulating LDL in the plasma is sheltered from oxidation, through dietary antioxidants such as vitamins e.g. E and C (173), protective enzymes such as peroxiredoxins, glutathione peroxidases (174-177). LDL Penetration inside the arteries wall happens at separate spots into the aorta and other. Holding of LDL in the intima, as a result of the relation with the extracellular environment. A wide range of peroxidases and oxidases forms powerful oxidants which readily oxidize LDL. These include NOXs, inducible xanthine oxidase (XO), and nitric oxide synthase (iNOS),. Oxygenase is also been realized to oxidize LDL in vitro (178-183).

In atherosclerosis receptive areas, diminishes expression of endothelial nitric oxide synthase (eNOS) and SOD heads to negotiated endothelial barrier integrity (Figure 2.4-4) guiding to elevated gathering of subendothelial atherogenic apoB-containing lipoproteins LDL and chylomicrons and leftovers of very low-density lipoproteins (VLDL) (183,184).

2.4.2. Cell Death Programs: Apoptosis and Necrosis

ROS can lead to functional alterations in nucleus, proteins, and lipids, and the buildup of these molecules is considered to be one cause that contributes the neurodegenerative changes (185,186). ROS physiological levels performing as waving particles, to adjust lipids or proteins parts within the mitochondria, and transform cardiomyocyte and mitochondrial activity (187). This action of ROS generated from the mitochondria leads alteration of transcripting elements that normalize targeted gene transcription (188).

The main types of cell fatality in the cardiac muscle split into two particularly regulated molecular routes known as necrosis and apoptosis (Figure 2.4.2). In spite of their variations, both are activated through inducing cell "death" receptors on the surfaces, or via calcium overload and ROS affects mitochondria (189-191). The programmed cell death is initiated by various stressors in the cardiac muscle cells involving oxidative stress, DNA damage, and cytokines. A lot of studies have verified that blocking apoptosis is a cardioprotective and can also inhibit HF progression (190).

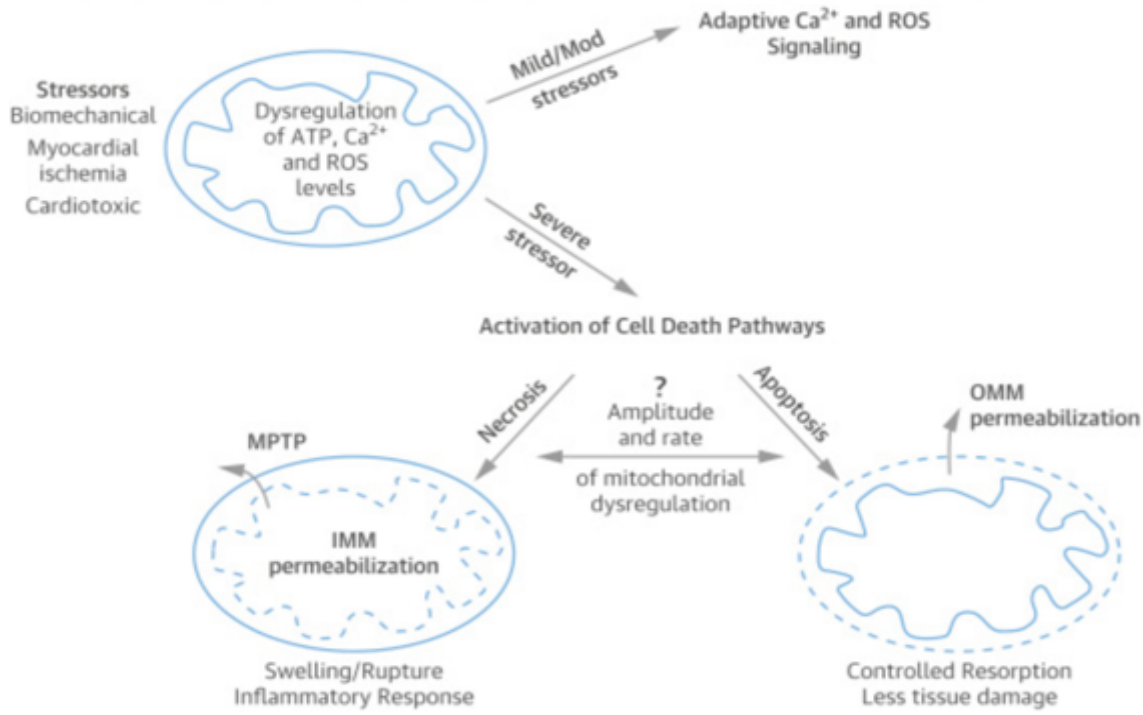

Figure 2.4.2. Mitochondria Role in Cell Death Pathways

Apoptosis is an extremely regulated programmed cell death and is initiated by death receptors of the plasma membrane, also in the endoplasmic reticulum and mitochondria. Plasma membrane receptor–starting apoptosis via stimulating cytokine receptors. The generating episode in mitochondrial-mediated apoptosis ends by the permeability of the outer mitochondrial membrane (OMM), letting the discharge of apoptosis molecules named *apoptogens* (191).

However, once they are released, they are toxic in the cytosolic section. A traditional example of apoptogens is the cytochrome c, that is usually joins as a part of the mitochondrial ETC; though, once freed into the cytosol it activates apoptosis. Whatever of inducting route, apoptosis stimulates the caspases. Caspase-linked proteolysis of several cellular molecules later it reaches about the finishing of the cell. (190-193).

In HF, apoptosis and although a proof of necrosis is also been submitted (189,194). In comparison to regulated cellular resorption in apoptosis case, organelle and cellular tissue breakdown is an important characteristic of necrosis, causing extrusion of inflammatory molecules that results in leukocyte recruitment and damaging tissue. Beginning of necrosis is through cellular damage that educes constant permeabilization of the inner mitochondrial membrane (IMM) or due to receptors of death pathway (necroptosis) which triggers receptor-cooperating protein homologous threonine kinases or serine (195,196).

It must be noticed in which the mitochondrial principles in the cellular death pathway are not totally different in apoptosis and necrosis (190,197). This pathophysiological overlay among necrosis and apoptosis is a clue as well when evaluating histological episodes of heart diseases (198,199). Additionally, regardless to the signals of death receptors, mitochondria is usually a critical sire of the clarification loop arranging these cellular death series (200).

The presence of TG lipolysis in atherosclerotic damages is able to increase toxic free fatty acid rates in the vascular walls, which could lead to cell death and ends with inflammation processes. Both elevated inflammatory signaling and elevataed cell death are key controllers that attributes of atherogenesis (201).

As mitochondria has a vital part in these episodes, more researchs into perception for the quality control of those routes that endure mitochondrial truth, involving mitochondrial mitophagy, mitochondrial dynamics, and the mitochondrial proteins unfolding and banding reactions, will become of more importance to expose more goals to diminish or eliminate cell death from going further (202,203).

2.5. Role of Antioxidants

At reduced levels of ROS, they are included in physiological procedures, involving cell multiplying and differentiation, and excitation-contraction coupling (ECC). Equally, when these levels become higher and elevated, they are able to alter the molecular structure and activity of intracellular substrates. For instance, ROS have the power to modify the genomic of DNA integrity by stimulating mutations, to affect the structural parts of the proteins via enzymatic inactivation or alterations, and modify intracellular lipids via lipid peroxidation as well (204). Mitochondria also include antioxidant mechanisms to remove ROS (205). The physiological scheme of the humans has appropriate proficiency for battling versus "disturbed redox homeostasis". Additionally, antioxidant therapy had shown to have beneficial effects in atherosclerosis congestive HF, hypertension, cardiomyopathies, and ischemic heart disease (167,206).

In the oxidative stress state, the body's "antioxidant defense" system typically led to the creation of the natural antioxidant (207). These mainly involve various enzymatic antioxidants; glutathione peroxidase, (GPx),), catalase (CAT), superoxide dismutase (SOD and non-enzymatic antioxidants (glutathione, Vitamin E, and C) (208). , Under normal physiological circumstances, the "antioxidant defense" is usually responsible for the exclusion of toxic oxygen species. However, in the case of a debilitated or diseased state of the antioxidant defense system, an exterior supply of antioxidants is crucial to reduce the oxidative stress and CVD subsequent initiation (209). An inclusive range of dietary antioxidants such as polyphenols, ubiquinone, ascorbic acid, carotenoids, vitamin E, and their combination has been investigated for the therapy purposes and prevention of CVDs. In the prior years, several experimental studies have been conceded to prove the therapeutic effect of antioxidant enhancements and enzymes/vitamins for managing CVDs (210).

Moreover, the reaction of a certain antioxidant with one type of ROS might potentially create extra ROS, which the earliest antioxidant cannot find. As a result, to the character of ROS as signaling molecules, serious exposure of antioxidants brings about redeeming upregulation of ROS production (211).

About considering the usage of dose, there might been discrepancies among various revisions, as no info is made presented about vascular antioxidant efficacy or concentrations. Such info is precarious, allowing the extreme quantities of the retorts among endogenous substrates such as ROS and NO; certainly, for an antioxidant making its function properly, its outcomes should out-compete that of the ROS through its aimed molecule (212). Various of antioxidants from lots of chemical or biological roots, are existing for therapeutic control purposes (Figure 2.5-1) provides a wide grouping of antioxidants mostly involved in CVD treatment (212).

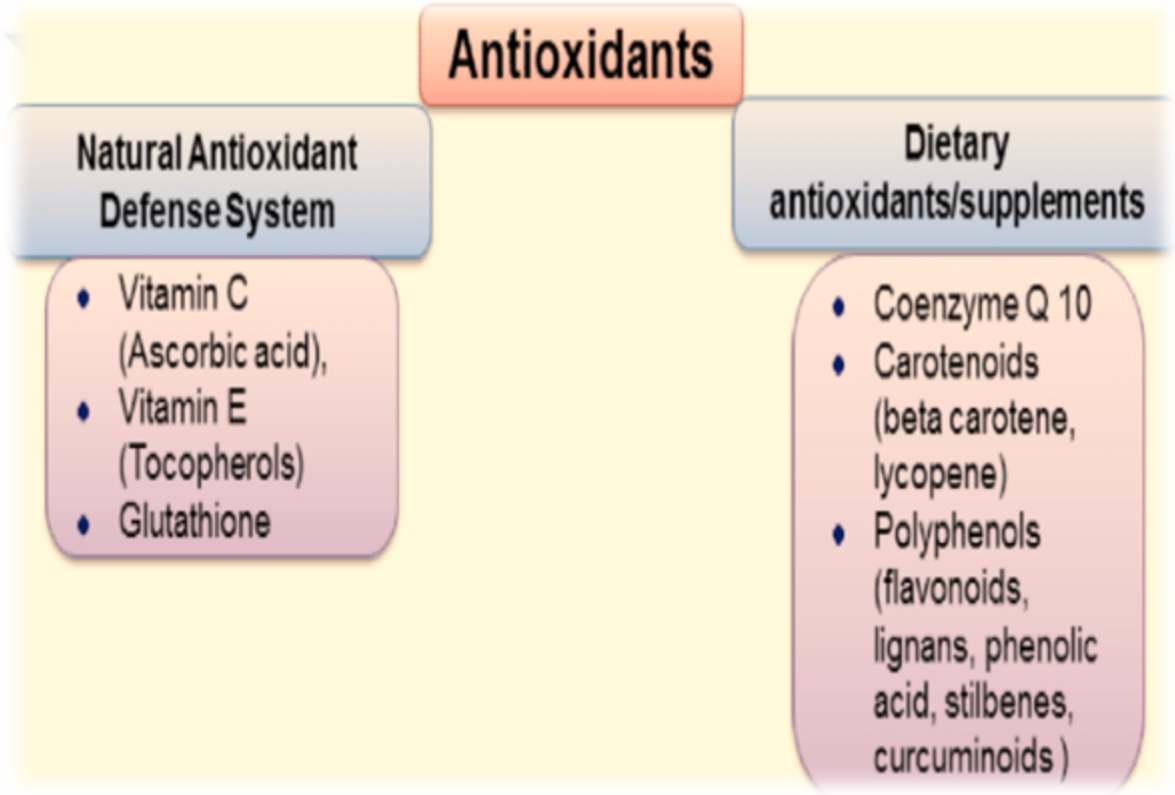

Figure 2.5-1. Antioxidants Major Classes implicated in the CVD Management

2.5.1. Ascorbic Acid (Vitamin C)

Many nutritional antioxidants consisting of antioxidant enzymes and vitamins work by exclusive mechanisms to manage CVD (Figure 2.5-2) describes the role of antioxidants actions in managing CVD (213). Vitamin C, is the main constitute of antioxidant system, which is found in a its lowered status by bonding with glutaredoxin and glutathione (214). It is soluble in water, monosaccharide antioxidant. Its plasma values depend only on nutritious intake. Vitamin C is reliable for prevention and neutralization of ROS e.g. $H_2O_2^-$. Chronic vitamin C deficiency may lead to increased atherogenesis and mortality (215). Vitamin C also delay the adherence of monocyte with the endothelium, and with that it reduces apoptosis and control the vasodilation of vascular SMCs (216).

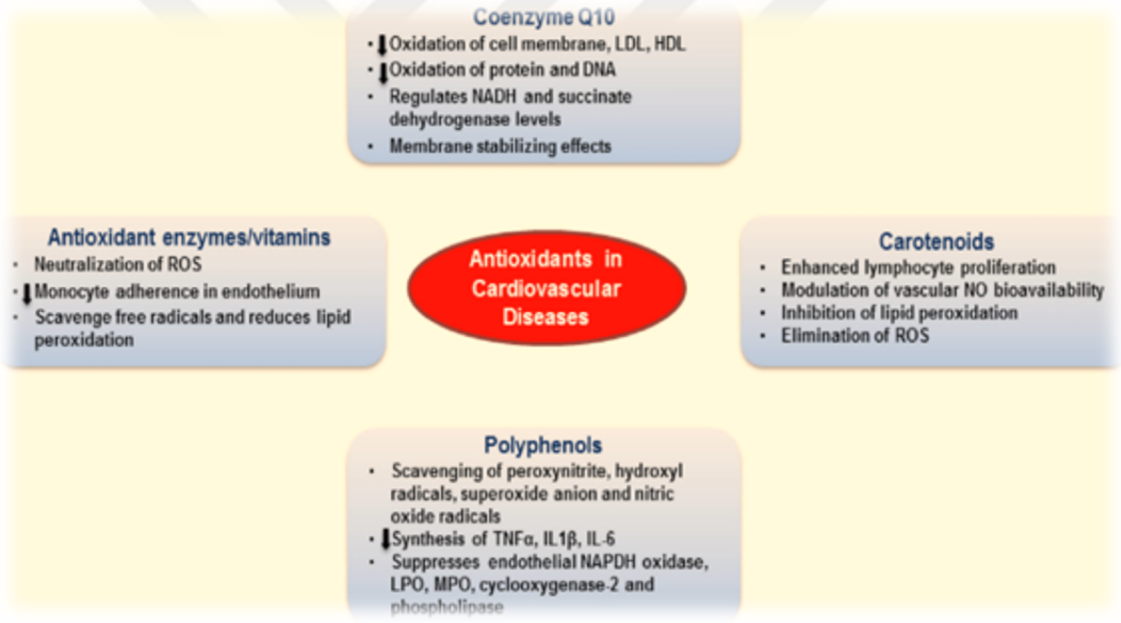

Figure 2.5-2. Antioxidants Mechanisms in CVD

2.5.2.Glutathione

It is a natural antioxidant, synthesized from the essential amino acids in the cells. It is a peptide molecule which has antioxidant features due to the existence of thiol group, glutathione is considered among the vigorous antioxidants in managing redox homeostasis for preventing CVD (217).

2.5.3. Vitamin E (Tochopherols)

In nutrition, the largest mode of vitamin E is γ-tocopherol, while in plasma it is mostly discovered as α-tocopherol (218). The vitamin E antioxidant property is comprising the ability of ultimately terminating free radical chain reactions and hunting the free radicals created from lipids biomolecule (219). After its reaction with lipid peroxyl radicals, it is replaced to its previous intuitive form through many other antioxidants. However, the action of of vitamin C with tocopheroxyl radical leads to α-tocopherol regeneration. This become a clue for the presence of an “antioxidants network” within the cells for best protection versus different ROS and antioxidant radicals itself (220,221).

2.5.4. Coenzyme Q 10 (CoQ10)

Named “ubiquinone” as well. CoQ10 mostly involve as an essential co-factor in mitochondrial ETC reaction. Besides, it is a vital substrate for ATP production and cellular respiration (222). As an antioxidant, CoQ10 has a vital role in prevention of the LDL, HDL, cell membrane, lipids, proteins, DNA and various blood components oxidation (223). CoQ10 deficiency starts a variety of CVD symptoms such as heart failure, endothelial function disorder, ischemic heart disease, and hypertension (224-227). The diminished form of CoQ10 inhibits lipids, membrane proteins, circulatory lipoproteins peroxidation, decreases the inflammatory pointers (IL-6), and increases conflict against LDL oxidation (228-230).

2.5.5. Carotenoids

Carotenoids are chemical mixtures that are mainly recognized in microorganism and plants. The human’s biological system cannot create them, thus obtained from other supplements and diet (231). The key mechanisms involved in this antioxidant are; better lymphocyte spread, elimination of ROS, lipid peroxidation elimination, and making the immune system stronger (232-236).

2.5.6. Polyphenols

Polyphenols are chemical compounds containing multiple phenol units. It is mostly found in tea, coffee, cocoa, and olive oil as their dietary sources (237). They act as the inhibitor of reactive nitrogen species and ROS by direct scavenging of $^{\bullet}OH$, $^{\bullet}O2^{-}$, NO, and peroxynitrite (238).

2.6. Role of Interleukin-6 in Cardiovascular Diseases

IL-6 has a multi purposes that are better-defined with anti- and pro-inflammatory characteristics(239). IL-6 initiates intracellular signaling pathway after it is bind to its specific membrane-bound receptor (IL-6R). Transduction of the signals is facilitated by the β-receptor glycoprotein 130 (gp130) (239). On the marked cell, binding of IL-6 to IL-6R. this complex is associated with another protein, directly initiates and dimerizes intracellular stimulating (240). IL-6 contains regulation functions of the of the nervous and immune systems. Moreover, IL-6 is included in the metabolic control of the body and liver regeneration (240). The implication of IL-6 in CVD has only recently been fully recognized. A large meta-analysis investigated the IL-6 receptor single nucleotide polymorphism (SNP) (241). gp130 is extracted by almost every cell in the body, while IL-6R is mostly originated by neutrophils hepatocytes, some lymphocy, monocytes and macrophages. A naturally happening, soluble form of the IL-6R (sIL-6R), which is obtained from different fluids in the body, for instance blood and urine (242), and is originated through two liberated process: an alternatively spliced mRNA translation and limited proteolysis of the membrane protein (243,244). It is interesting that IL-6 both with soluble IL-6R stimulate cells, which just express gp130 (245,246)

Gp130 expressing cells able to be stirred by the means of IL-6 and sIL-6R even when the IL-6R is absent (246) in mechanism known as trans-signaling (Figure 2.6A) (245-247). It was published that peeling of the IL-6R from neutrophils, and inflammatory processes, are leading to the endothelial cell stimulation. Later, it stimulates the mononuclear cell drawing cytokine MCP-1 secretion. Hence, the starting point of damage is reflected through number of attracted neutrophils (248).

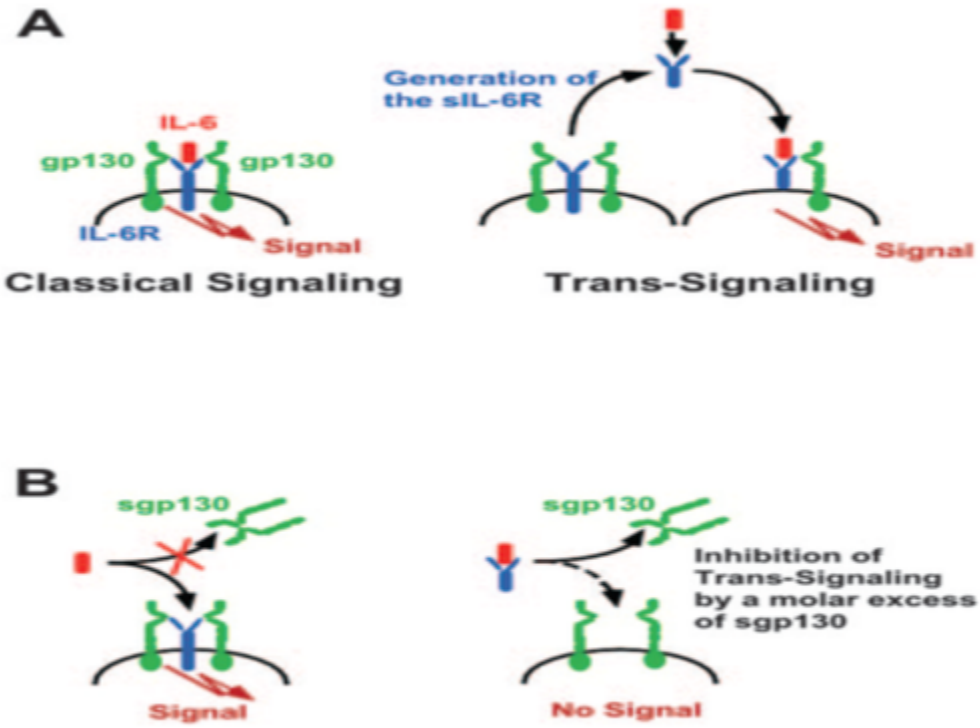

Figure 2.6. The Inhibitory and Trans-signaling Mechanisms of IL-6R Complex.

(A) both styles of activation of IL-6 are shown as traditional activation of IL-6 through the membrane-attached IL-6R and sIL-6R-mediated cell signaling (IL-6 trans-signaling). In both situations, responses are stimulated via positioning with membrane-bound gp130. (B) traditional IL-6 signaling does not affected by sgp130 yet favorably binds the sIL-6R/IL-6 complex to irritate IL-6 trans-signaling.

A lot of cells, involving hematopoietic progenitor cell, endothelial cell, SMCs, embryonic stem cells, and neuronal cells that are not responding just to the IL-6, but have noticeable reply to IL-6/sIL-6R (247,249-251). The molecular mechanism thru which sgp130 employs inhibition selectively to the IL-6/sIL-6R complex is shown in (Figure 2.6B) (247,252).

Further down physiological circumstances, IL-6 activity is assumed to be a paracrine way (253), yet in recent reports, IL-1 and IL-6, are major participants downstream of the vascular inflammatory surge (254,255). Cardiovascular results are more common in patients with high circulating points of different inflammatory markers, and treatment based on inflammatory parameters (256).

Modern genetic polymorphism reports state that IL-6R signaling appears to consume noticeable outcome on CAD (257). The IL-6 cytokine is originated from macrophages, T lymphocytes, and adipocytes, and show its activity through its soluble receptor or membrane-bound inducing fibrinogen hepatic creation, accelerating atherosclerosis, CRP, and joint inflammation (258). Raised levels of IL-6 are found to be connected with a high danger of MI in healthy men (259). Furthermore, IL-6 and the receptor levels of IL-6 contain an early peek at the critical phase of MI, similarly linked to instability of the plaque (260,261). Similar to fibrinogen, and CRP and whose creation is stimulated by IL-6R signalling, excessive flowing IL-6 levels might be linked to high CHD risk events in future experimental trials (259,262,263). IL-6R signaling might be an important therapeutic target for CHD prevention (264).

Atherosclerosis is a serious inflammation-related disease, through IL-6 as an important participant of the inflammation cascade. IL-6 blockade might lower CVD hazards, yet current therapeutic agents to inhibit IL-6 perhaps activates dyslipidemia, a discovery with an unsure prediction (265).

2.7. Role of Interleukin- 1β in Cardiovascular Diseases

Inflammation has a vital part in the cardiovascular events development, most notably congestive heart failure and coronary atherosclerosis (266,267). Some inflammatory particles are occupied in those procedures, involving interleukin-1(268). IL-I is a multipotent mediator in inflammation processes that might have an important effect on cardiovascular pathophysiology. E.g. IL-1 endorses all leukocytes types adhesion to cultivated endothelial cells (269-271) and enhances their procoagulant activity formation (272), and plasminogen activator inhibitor (273,274). IL-1 stimulates the expression and synthesis of a lot of hundreds of derived inflammatory mediators and also stimulates its processing and production, and this action is chief in the pathogenesis of many autoinflammatory problems (275).

The gene of the IL-1 family involves 3 proteins, IL-1 receptor antagonist (IL-1ra), IL-1α, and IL-1β, IL-1α and IL-1β produce their actions by engaging to the IL-1 type I receptor. The IL-1 type II receptor also binds IL-1β and IL-1α but is not included in signal transduction, thus, balancing the inflammatory response of IL-1α and IL-1β. IL-1ra is an endogenous blocker of IL-1β, which combative binds to the IL-1 type I receptor deprived of stimulating it (276). Both IL-1 and IL-1ra are created by macrophages, SMCs, and endothelial cells. IL-1 production is generated via certain endogenous elements, such as microbial products and uric acid generated throughout cell death. Both kinds of agonists induce a cytosolic assembly named the inflammasome, which stimulates caspase-1 to activate IL-1β emission. Polymorphisms in the IL-1 gene cluster that result in the larger or decreased expression of the agonists and antagonist respectively been related to increased severity, premature onset, or worsen consequences in patients with several inflammatory cases, varying from Alzheimer disease, periodontitis, rheumatoid arthritis, bowel inflammation and diseases (277,278).

Atherothrombosis and atherosclerosis are the highlighted mechanisms of the vast majority of the frequent coronary episodes. The level of which plaques are created, rupture, and progress is determined, however in a small part, on the degree of inflammation. In atherosclerotic plaques, the IL-1β expression is linked to the plaques progression, with little expression in healthy coronary arteries (279,280). As it is frequently, several points of regulation ruling the activity of IL-1 (281).

The balance between endogenous inhibitor IL-1ra and IL-1 isoforms (1β, 1α) introduce one main level of control. The IL-1 isoforms contain different functional outlines. IL-1α mainly exist on the surface of the cells and induces signals by direct contact at short spaces. While, IL- 1β able to induce action at a distance, IL-1R1 transduce IL-1β signaling (Table 2.7) (282.383).

Table 2.7. Interleukin-1 Family Members

Cytokines	Comments
IL-1β	IL-1β acts primarily extracellularly as a soluble mediator
IL-1α	typically exist on the cell surface and signals by direct contact at short distances by juxtacrine or paracrine signaling
IL-ra	IL-1ra blocks the signaling receptor that binds either IL-1 β or α
Receptor	
Interleukin-1 receptor 1 (IL-r1)	IL-1 receptor 1 binds to both isoforms
Interleukin-1 receptor 2 (IL-r2)	IL-1 receptor 2 acts as an inhibitory decoy and does not signal

Several elements of control set the IL-1β activation. Pro-IL-1β experiences proteolytic cleavage to create mature IL-1β which owns biological activity (Figure 2.7-1) (283,284). The proteolytic enzyme liable for the secretion and production of IL-1β, before named IL- 1β converting enzyme, and nowadays carries the term Caspase-1. Human atherosclerotic plaque macrophages contain caspase-1 (284).

Caspase-1 on its turn is activated through a structure assembly identified as inflammasome (Figure 2.7-1) (285,286). The inflammasome converts inflammatory signals of procaspase-1 to their vigorous mode, which consecutively stimulates IL-1β and activates it. Several rare inherited diseases reveal the value of the inflammasome which controls the pro-inflammatory potential of the active cytokine. Cryopyrin-associated periodic syndrome and Muckle-Wells syndrome occur from mutations of gain-of-function within the inflammasome (287). Those alterations significantly make devastating inflammatory diseases among children and augment IL- 1β maturation, unquestionably due to over-production of energetic IL-1β. The inflammasomes commonly require 2 signals to act and assemble (285).

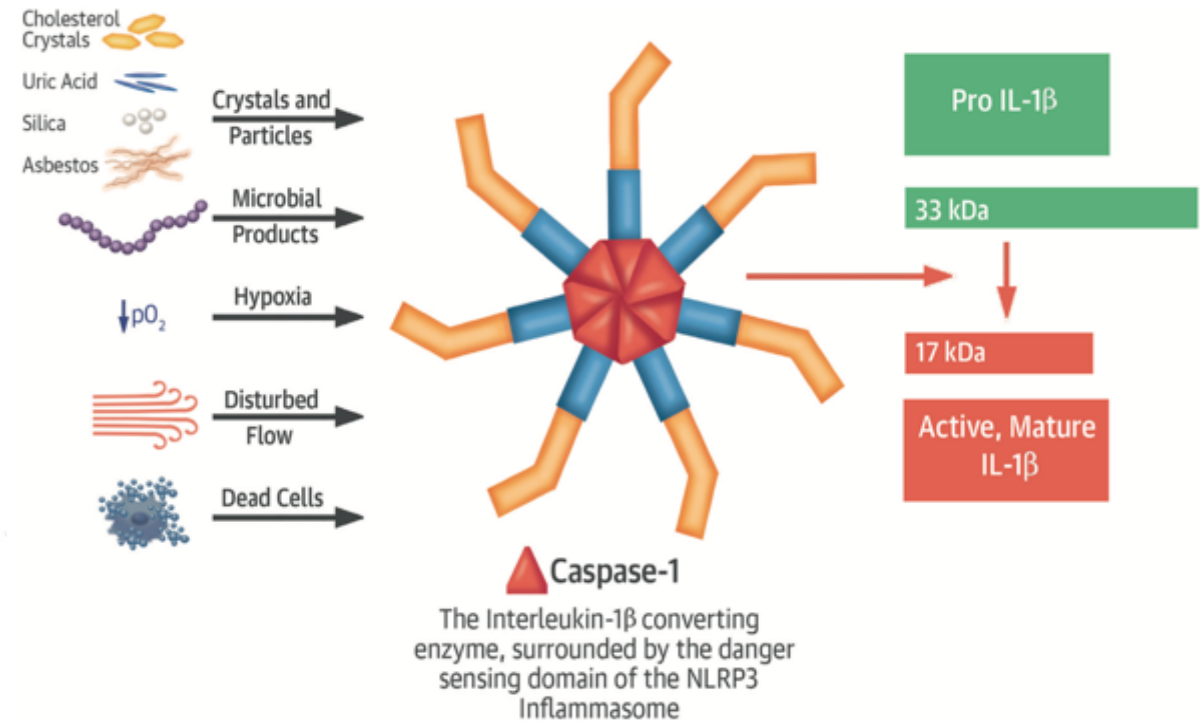

Figure 2.7-1. Inflammasome Activates IL-1β and Views Danger Signals by the Proteolytic action on the Mature Form Via Caspase-1

The triggers or co-activators of the inflammasome involove constituents of infectious mediators. Crystals is also can co-activate the inflammasome. Such as, urate crystals that are caught up in the gout pathogenesis also work within the inflammasome to boost mature IL-1β creation (288). Cholesterol crystals as well, can yield a sign to the inflammasome (289,290). Human atheromata protect stimulated inflammasomes (291). Additional atherosclerosis-related stimuli that able to expand inflammasome activity involve distressed flow and chronic acidosis and hypoxia (mostly in the plaques core) (Figure 2.7-1) (292,293). IL-1 develops multiple actions on vascular SMCs (Figure 2.7-2). IL-1 can stimulates autocrine assmembly of platelet-derived growth factors that can activates SMC propagation (294). IL-1β madiates endothelial damage, contributing to the proliferative tunica response (280,295). Therefore, the drifts of IL-1β might be different on the vascular district basis and might control both outward vessel remodeling and plaque burden (296).

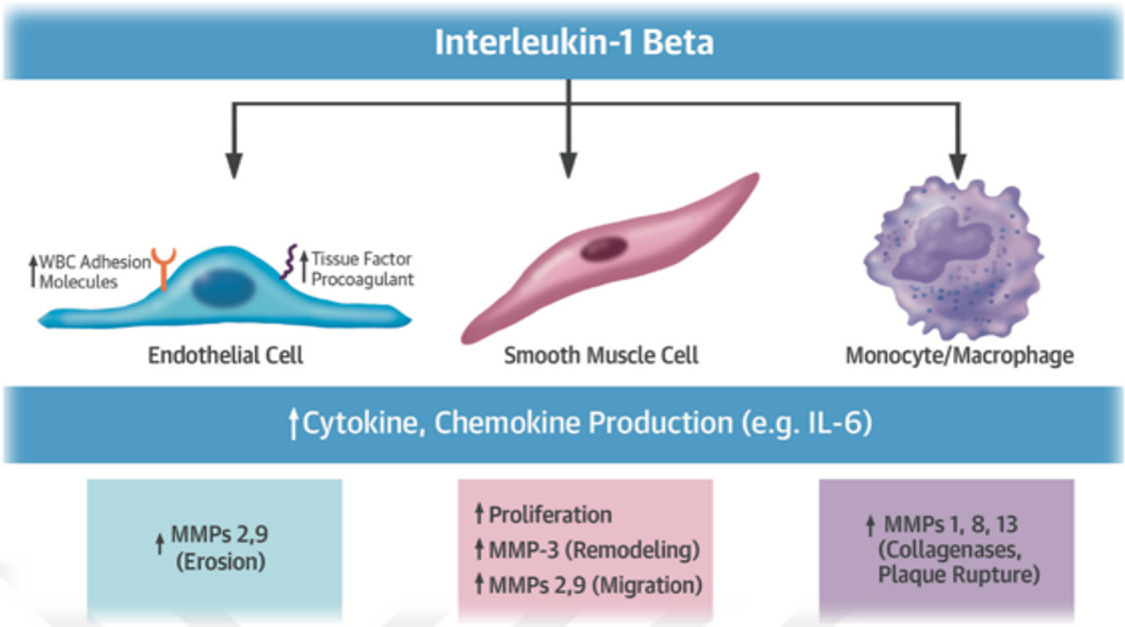

Figure 2.7-2. IL-1β Actions Correlated to Atherosclerosis

IL-1β acts on different organs and cell kinds, including vascular endothelial cells, macrophages, and SMCs. IL-1β provokes the functions shown, among many organs. Obstructing IL-1-induced MMP-2 (type IV collagenase) could control the mortality and noticeable decline in incidence of cancer by delaying tumor invasion by the collagen type IV of the basement membrane. WBC: white blood cell, MMP: matrix metalloproteinase.

IL-1β also applies direct effects on insulin resistance, thrombosis, and obesity-related metabolic derangements (297-299). In the conclusion of the trials of direct detection of plasma IL-1β values (frequently unnoticeable even in cases with clear proof of elevated IL-1β action), very little reports have detected IL-1β levels and showed they are intensified in individuals with a higher atherosclerotic load (300-302).

Sustainable wounded local cells produce active IL-1β too following inflammasome activation. Once WBC has been enrolled to the damage, the activated WBC is the main origin of IL-1β. The creation of an active inflammasome in the cardiac regulates and hunts the therapeutic procedures. In preclinical reports, genomic removal of the framework constitutes of the inflammasome (a speck-like, apoptosis-associated protein comprising a caspase staffing area (ASC)) heads to a noticeable progression in heart curing after reperfusion/ischemia (303).

Another study confirmed that the cardiac muscle cells extracted all the inflammasome elements, but obtained no proof of full-grown IL-1β in cardiomyocytes, suggesting that the manners of caspase-1 activation, inflammasome creation, and IL-1β growth might be disconnected and differences among various cell forms (304). Furthermore, the triggering of caspase-1 in the cardiomyocytes, possibly in other cells too, might result in IL-1β–autonomous properties such as pyroptosis which causes inflammatory cell death (304-306). The findings that patients with Cryopyrin-related periodic diseases are greatly receptive to IL-1β barriers has guided to the hypothesis that effects are intermediated by elevated IL-1β action (Figure 2.7-3) (307,308).

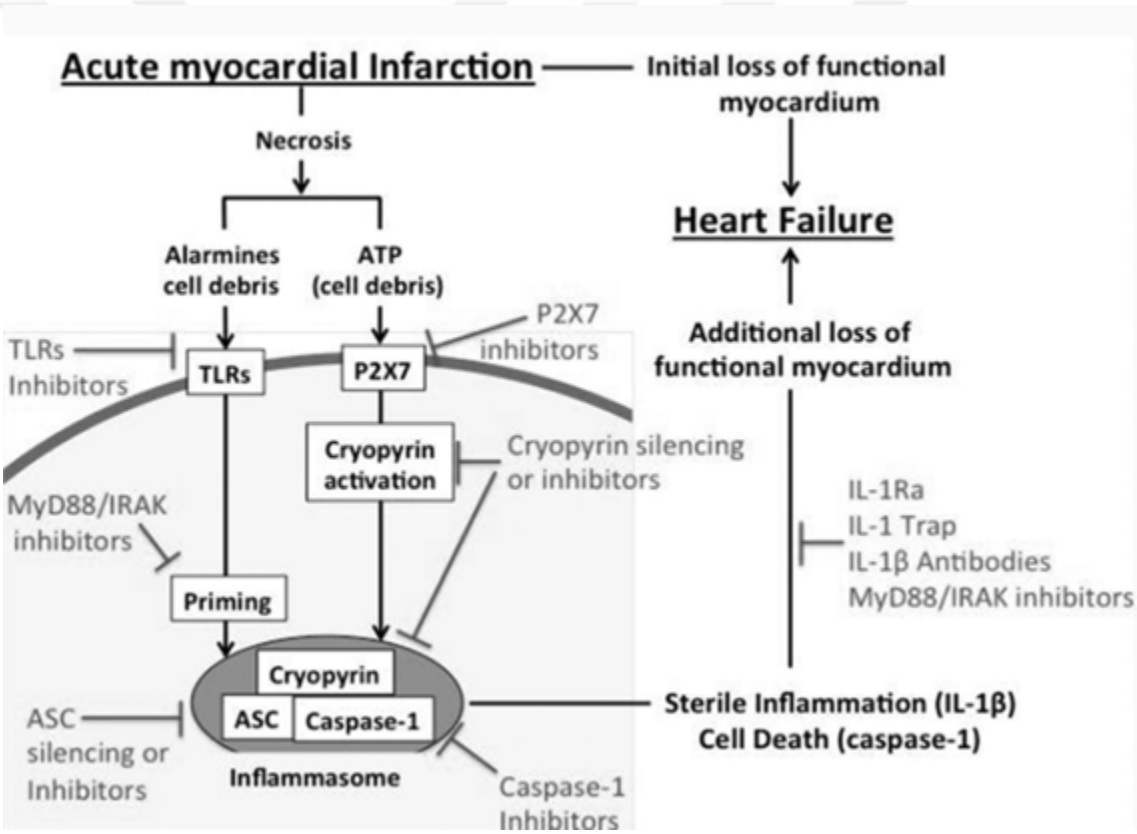

Figure 2.7-3. Inflammasome, IL-1, and HF after Acute Myocardial Infarction (AMI).

The receptors which control inflammasome TLR activation, purinergic receptor 2X7 [P2X7], cryopyrin), the components of the inflammasome are (caspase-1, cryopyrin, dot-like, an apoptosis-associated protein containing a caspase domain [ASC]), and the product of inflammasome IL-1β also manage to the extension of the inflammatory events and apoptosis that are expressed as a potential targets for the AMI treatment and the prevention of HF. The subsequent activation of caspase-1 and cryopyrin inflammasome formation endorse HF after AMI. , IRAK: interleukin-1 receptor–associated kinase, MyD88: myeloid differentiation factor-88 IL-1Ra indicates interleukin-1 receptor antagonist.

In auto-inflammatory disorders, IL-1β provokes the processing, creation, and publishing of more IL-1β (282). When tried in the MI model, IL-1β obstruction with an IL-1β Ab improved the heart muscle altering process yet did not disturb the power of the caspase-1 activity in the tissue or the inflammatory response (309) additional activates (such as ATP, cell debris) should then be more or equally necessary for the determination of the sterile inflammatory reaction intensity (Figure 2.7-3) (307) is a graphic illustration of the intermediaries included in the sterile inflammation path following AMI, the inflammasome formation, and the later discharge of interleukin1, also shows usage that is demonstrated to dull the inflammatory response and also to improve the modifying in pre-clinical sorts of AMI (307).

Therapy with IL-1 powerfully prompts SMCs and further cells to improve other cytokines (Figure 2.7-4) (310). IL-6 causes the mild phase response: liver cells aroused by IL-6 enhance their production of mild phase reactants, as well as plasminogen and fibrinogen inhibitor activator, molecules quietly elaborate in limiting fibrinolysis or promoting occlusion, likewise that of c-reactive protein, an indicator of inflammatory standing (311,312).

IL-1, therefore, initiates an elaboration circle whereby a particular IL-1 can imitate several particles of IL-6, which consecutively stimulates atherothrombosis mediators over-production, for example, the revealed patterns previously. Human genetic studies encourage IL-6 destiny in CHD. Therefore, IL-1 exist upstream in the path which makes IL-6, a fundamental molecule in atherothrombosis (313,314).

Several mechanisms are able to conflict with IL-1 activity. Management of the trap receptor has not experienced practical medical progress. Canakinumab, a human monoclonal antibody, carefully deactivates IL-1β only, yet IL-1ra blocks IL-1β and IL-1α. An anti-IL-1α antibody is similarly placed in clinical estimation (315). Canakinumab aids inflammation interacted by IL-1β, involving scarcer forms of immature inflammatory arthritis and Muckle-Wells syndrome (316,317) and acute gouty arthritis as well (318).

3. MATERIALS AND METHODS

3.1. The Study Aim

The current research is to investigate and explore the effect of human inflammatory cytokine IL-1β and the utmost focus of IL-1β effect on cardiovascular disease among the Turkish population.

3.2. Study Design and Patients

We have conducted a prospective study and research with patients who have cardiovascular disease equated with the control group (healthy people who don't experience any disease or relevant cardiovascular disease). All the patients and control volunteers who fulfilled inclusion and exclusion criteria of the research and accepted to participate were included in this study. All volunteers and participants were in agreement to have their data published. The study practice is revised and permitted via Turkish ethics committee.

The work in this research is conducted on human serum samples. The analysis was performed on 39 serum samples of patients who diagnosed with atherosclerosis, All patient’s age range were between 40-65 years, with a cardiovascular-relevant disease without having any other disease rather than mentioned one. For the control group, 30 volunteers were enrolled and were in the same age range as the patient's group, but healthy participants who do not have any disease including cardiovascular diseases, both groups are fulfilled the TEKHARF (Heart Disease and Risk Factors in Turkish Adults) criteria for diagnosis and classification of CVD.

The blood collection in this study was gathered from patients who had gone to cardiologist polyclinic, who were diagnosed with atherosclerosis at Yeditepe University Hospital, Department of Cardiology. The blood samples after collection that were taken from the patients, were centrifuged to obtain the serum samples, frozen at -80°C after centrifugation and saved till the testing time. Blood specimens of the control group were managed similarly and applying equal methods (procedures). The specimens are all categorized through patient's names and registration numbers to ensure identification and

to avoid any confusion. The research was accepted by the Yeditepe University Hospital's ethics committee.

3.3. Specimen Collection

Visiting the mentioned hospital above, and a detailed explanation of the study was provided to the patients. A face-to-face survey then blood collection was performed. The samples were collected into a serum separator tube and centrifuged for 10 minutes at 3000 rpm and saved at -80°C till the period of testing.

3.4. Material and Devices

Human IL-1β ELISA kit (AssayPro LLC) which contains the following: Mix Diluent, Wash buffer, Human Interleukin- 1 beta Standard (IL-1β Standard), Biotinylated Human Interleukin- 1 beta Antibody (Biotin-IL-1β Ab), Streptavidin-Peroxidase Conjugate (SP Conjugate), Chromogen Substrate, Stop Solution, Precoated 96 well ELISA Human IL-1β Microplate (AssayPro), Adhesive Plate Covers or Sealing tapes (AssayPro), Centrifuge robot (Microfuge 16), Vortex (CS Cleaver), Automatic Pipette set, Disposable Pipette tips, Distilled or Deionized water, Absorbent paper, Vials Holder, ELISA Microplate Reader able in measuring absorbance at 450nm (WHYM201), Automated Plate washer (Paramedical PKL (PPC), 240 Automatic ELISA Washer), Incubator, Plate Shaker device (KJ-201B Microplate Shaker).

3.5. Assay Protocol and Laboratory Indices

All the reagents were brought into the laboratory before using to reach normal temperature of the working area and then diluted with sterile water. Exception for the Biotinylated Human Interleukin- 1 beta Antibody (Biotin-IL-1β Ab) and Streptavidin-Peroxidase Conjugate (SP Conjugate), which will be mentioned later below.

3.5.1. Samples Preparation

The work was performed in the medical laboratory of the Molecular Medicine department at Yeditepe University. We brought the patient's samples that were frozen in the refrigerator at -80°C and centrifuged them into (Microfuge 16 Centrifuge) at 6380

rpm for 5 minutes to obtain the pure serum. After finishing the centrifugation process of the patient's samples, the control group then is processed similarly.

3.5.2. Reagent Preparation

3.5.2.1. Preparation of Mix Diluent

2 tubes were prepared of Mix Diluent Solution. The Mix Diluent Concentrate first left to reach the room temperature and mixed it gently to dissolve any participated salts (crystals). In the first tube, 3 ml (3000µl) of Mix Diluent Concentrate (The Mix Diluent Concentrate tube contains 30ml) diluted with 27 ml distilled water. In the second tube 1.5ml. of Mix Diluent Concentrate was diluted with 13.5ml of distilled water. So in both tubes, the ratio was 1:10 as it should be prepared in the protocol and by mastering this ratio, the Mix Diluent tubes were ready to use.

3.5.2.2. Preparation of Standard Solution

We brought 8 Microtube and labeled them as (ST1, ST2, ST3,... etc). We reconstituted 2 ml (2000µl) of the Mix diluent with 500pg of Standard concentrate , which is (IL-1β Standard), to generate a standard solution of 250pg. However, in this step we handled the Standard vial carefully and without pipetting up and down when first diluted, after we finished adding the Mix diluent, we swirled and inverted it several times and let the Standard solution Microtube set for 10 minutes to ensure complete reconstitution . By this step we generated the standard solution. Then, performed a one to two 1:2 serial dilution of the standard solution by adding 50µl of Mix Diluent to the each labeled microtube, then added 250pg/ml of the reconstituted standard solution into the first microtube, mixed it throughly by pipetting up and down and changed the pipette tips between the tubes. With this step we produced several standard points (250 ,125, 62.5, 31.25, 15.63, 7.81, 3.91, 0 pg/ml solutions. The 0 zero standard served as a Background (Mix Diluent) as shown in (Table 3.5.2.2).

We worked with 2 standard groups so we avoid mistakes in reading later and to obtain accurate readings and results.

Table 3.5.2.2. Standard Solution Tubes Preparation

STANDARD TUBE	DILUTION	IL-1B (PG/ML)
ST1	Standard 250pg/ml	250
ST2	ST1 + Mix Diluent	125
ST3	ST2 + Mix Diluent	62.5
ST4	ST3 + Mix Diluent	31.25
ST5	ST4 + Mix Diluent	15.63
ST6	ST5 + Mix Diluent	7.81
ST7	ST6 + Mix Diluent	3.91
ST8	Mix Diluent	0

3.5.2.3. Preparation of the Wash buffer

The Wash buffer solution is needed for washing purposes during each step of the ELISA assay, by placing it within the ELISA automated washer bottles. Mixed the Wash Buffer gently to dissolve any participated salts (crystals) before using. mixing the Wash Buffer Concentrate with 500ml of distilled water and connected the bottles of the Wash Buffer solution to the Automatic ELISA washer (Paramedical PKL (PPC) Automatic ELISA Washer). Accordingly, the Wash buffer is generated.

3.5.2.4. Biotinylated Human Interleukin- 1 beta Antibody Preparation

As we should perform 1:100 as the desired amount of the antibody and Mix Diluent of the protocol. For that, diluted 50µl of Biotinylated Human Interleukin- 1 beta Antibody (Biotin-IL-1β Ab) with 4950µl of Mix Diluent, and the tube of the Ab solution was generated and swirled many times and let it set in room temperature to ensure complete constitute.

3.5.2.5. Streptavidin-Peroxidase Conjugate Preparation

Mixed 50µl of Streptavidin-Peroxidase Conjugate (SP-Conjugate) with 4950µl of Mix diluent, again because the chosen quantity of the conjugate with Mix Diluent was

1:100 of the protocol. The tube of the Conjugate solution was generated and swirled many times and let it set at room temperature to ensure complete constitute.

3.5.3. Assay Procedure

3.5.3.1. Phase 1

First of all, After diluting the Mix Diluent Concentrate, Standard Concentrate, and Wash Buffer Concentrate. As we have 39 patient samples and 30 control samples, and we prepare 16 standard solutions (because as mentioned above we will work on 2 rows of standards) this will give us a total of 96-wells will be work on, which covered the whole wells of the Microplate. Hence, we brought the Precoated 96 well ELISA Human IL-1β Microplate (AssayPro), put into the first two rows of the ELISA Microplate the prepared solution of the 16 standard tubes as shown in the highlighted area in (Table 3.5.3).

Table 3.5.3.1. Assay Templet

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Then started to add 50µl of the patient's serum per well starting from 3A, changing the pipette tips between each sample and kept adding accordingly 3B, 3C, 3D until the whole patient's samples are done at 7G, and we added in 7H Mix Diluent so it will take the place of a patients serum, so we can start with a new column of the control serums to avoid confusion in reading the results later. Likewise, changing the pipette tip started with the control samples at 8A.

After finishing the phase of loading the standards, patients and control samples covered the pre-coated ELISA Microplate wells with adhesive plate covers (adhesive sealing tape) and placed the plate on a shaker device (KJ-201B Microplate Shaker) for 2 minutes at 500 rpm speed to ensure thorough mixing of the well.

Least but not last, after the wells have been mixed well, The Microplate was hatched at 37ºC for 2 hours then moved to be washed using an Automated ELISA Plate washer (Paramedical PKL (PPC), 240 Automatic ELISA Washer). We checked the Wash bottle in which we added the wash buffer into it, checked the Waste Bottle in which it should be empty, and after turning the ELISA washer On, we set up the program of the ELISA Washer as the following:

1. We Chose (Plate wash) as the washing method of the device.
2. Setting the number of washing cycles by selecting 4 cycles.
3. Wash volume was selected on (200y/n).
4. On the Plate option we entered Incubated Well then selected (Flat).
5. Cross wash was selected Y/N,
6. Entered Strip Position (POZ1).
7. Selected the number of the well strips (12).
8. After entering the well in the correct position the process was about to start after selecting Done and then Start. The process of programmable Dispensing/Aspirating starts. The washing and incubation process of the ELISA Washer took 10 minutes.

In this phase, the reaction occurred between the immobilized (capture) target antibody that has been pre-coated in the Microplate wells will bind to the Standards, Patients, Controls.

3.5.3.2. Phase 2

While the plate was incubating and before the washing process started we started to prepare the diluted Biotin-IL-1β Ab, and SP-Conjugate tubes (which are mentioned in the Reagent Preparation part) and we put them aside at room temperature to ensure complete constitute.

After the 10 minutes of washing process were done, we placed the Microplate on the working bench and started to add 50µl of the Biotin-IL-1β Ab per well and covered it with an adhesive plate cover and hatched at 37ºC for 2 hours. Then we washed it again by using the Automatic ELISA Washer. In this phase, the addition of this second antibody (Biotin-IL-1β Ab) which was a detector antibody, formed the principle of a Sandwich reaction in which it bound to the antigen-antibody complex.

3.5.3.3. Phase 3

After 10 minutes of Dispensing and Aspirating process we took the plate out and started to add 50µl of the diluted SP-Conjugate per well, sealed it with an adhesive plate cover, and incubate it at the room temperature for 30 minutes. Furthermore, washed the microplate for the last time. In this phase, SP-Conjugates are utilized for the detection of bound Biotinylated antibodies on the samples.

3.5.3.4. Phase 4

In this phase, after the last washing method, we added 50µl of the substrate of Chromogen per well then left it to incubate 10 minutes till the optimum density of the blue color develops, and then tapped the microplate slightly to guarantee complete blending and break any bubble into the wells via the tips of the pipette. While the plate is incubated we set up the microplate reader program in advance.

In this phase, the addition of the Chromogen substrate solution worked with the enzyme-antibody-target complex to create a noticeable sign which was the light blue color development.

3.5.3.5. Phase 5

The step before the reading procedure was the addition of 50µl of the Stop Reagent per well. We saw the blue color changed into yellow within minutes and then, the reading process begun immediately after the addition of this solution.

In this phase, the addition of stop solution as the name refers to, so stops further reactions.

3.5.4. Absorbance Reading

The two-layer sandwich ELISA method is used to measure inflammatory cytokine levels. Their level is determined by reading the color changes by the ELISA Microplate Reader, capable of measuring absorbance at 450nm (WHYM201) and compared to the standard curve in the photometer. We used 2 readings of absorbance. The first reading used 450nm as a primary wavelength and the second reading used 492nm as the reference wavelength. The standard curve of human IL-1β was generated as it described below in (Figure 3.5.4-1) as the results entered into an excel sheet first then pasted them using the ELISA analysis website to calculate my results.

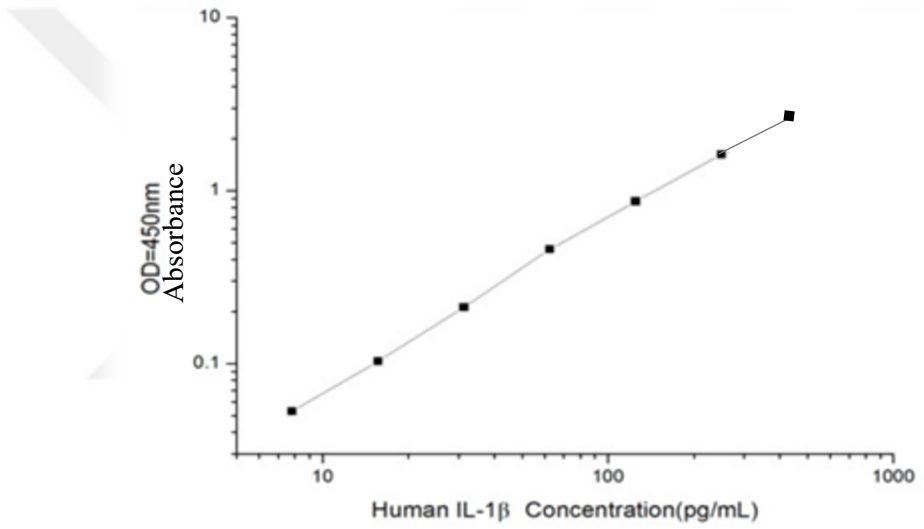

Figure 3.5.4. Standard Curve of Human IL-1β

3.5.5. Statistical Analysis

Performing the statistical analysis using the IBM SPSS 22.0 software (SPSS, Inc, Chicago, IL, USA) and the values were stated as (X±SD): mean of value ± Standard deviation. Student T-test, Chi-square tests, and Fisher's Tests have been used to compare the demographic records. The values significance level of $p < 0.05$ were considered to reveal statistical significance. The variables with parametric data analysis summarized as numbers, percentages, and ROC analysis with 95% confidence interval (Cl). Correlations were determined by using the Pearson Correlation test. The student T-test was used to examine the significance differences.

4. RESULTS

4.1. The Demographic Data of the Working Groups

The study was accomplished by using cardiovascular disease biochemical parameters, and demographics. 69 individuals were included in this research sorted into two groups, patients and control groups; the patient group consisted of 39 individuals (8 females and 31 male), and the control group consisted of 30 individuals (4 females and 26 males). The distribution of patient's risk factors and control group and identifying information are all delivered in Table 4.1.

Table 4.1. Demographic Results of The Study Population

	Patient (n=39)		Control (n=30)	*P* Values
Age X±SD	62.95±10.968		59.96±9.361	0.143 (NS)
Gender % (n)	Male %	79.0% (31)	87.0% (26)	0.533 (NS)
	Female%	21.0% (8)	13.0% (4)	
History of Diabetes % (n)	Yes %	33.0% (13)	17.0% (5)	0.698 (NS)
	No %	67.0% (26)	83.0% (25)	
Smoking % (n)	Yes %	38.0% (15)	53.0% (16)	0.470 (NS)
	No %	62.0% (24)	47.0% (14)	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases, (NS): non-significant (p>0.05), student t-test used for comparison, chi square and other demographic characteristics.

In the analysis mentioned above, we can notice they are not showing any statistical significance between both groups. As it has seen the age (p=0.143), gender (p=0.533), diabetes (p=0.698), and smoking (p=0.470). CVDs were observed to be extra common in men (79.0%) than in women (21.0%).

4.2. Smoking and Diabetes History Linked to Interleukin-1 Beta

In another statistical analysis, we calculated the relationship between the smoking and history of diabetes in both patients and the healthy control group linked to the IL-1β as it is shown in Table 4.2-1.

Table 4.2-1. Smoking and Diabetes History Linked to the IL-1β.

IL-1β						
Case		Patients X±SD (n=39)		Controls X±SD (n=39)		*P* Value
Diabetes		Yes	No	Yes	No	
Smoking	Yes	12.12±1.45	18.40±151	15.10±1.32	14.03±139	0.143 (NS)
	No	14.70±1.55	7.65±1.48	16.26±1.40	14.75±147	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases, (NS): non-significant (p>0.05), student t-test used for comparison of manage values and chi square and other demographic characteristics.

According to this analysis of the case, diabetes history, smoking of the mentioned table above (Table 4.2-1) we can notice that there is no statistical significance in this replication between both groups. The p-value of this replication of the case, diabetes history, smoking (p=0.143) was observed, and not show any significance.

Also, we studied the relationship between gender and smoking and it was calculated as it is shown in Table 4.2-2. We can notify from this table that there is no significant relationship between smoking and gender (p=0.541).

Table 4.2-2. Smoking and Gender Linked to the IL-1β.

IL-1β					
Gender	Female X±SD		Male X±SD		*P* Value
Smoking	Yes	No	Yes	No	0.541 (NS)
	14.90±3.01	12.00±2.14	14.92±3.05	14.68±1.51	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases, (NS): non-significant (p>0.05), student t-test used for comparison of manage values and chi square and other demographic characteristics.

Moreover, another extra data was analyzed between the study group cases and diabetes history which involved 2 replications and they were the case and diabetes history, as it is shown in the Table 4.2-3.

Table 4.2-3. Diabetes History Linked to IL-1β of the working group

IL-1β				
		Patient : X±SD (n=39)	Control: X±SD (n=30)	*P* Value
History of Diabetes	Yes	13.41±1.51	15.68±1.50	0.833 (NS)
	No	13.03±1.40	14.39±143	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases. NS: non-significant (p>0.05), advanced chi square tests and double independent sample student t-test was used.

As it can be noticed in the terms of there was also no statistical significance between the groups as the p-value (p=0.833) was observed.

Furthermore, another replication was analyzed to obtain a statistical analysis with a significant difference, and this time we selected another replication which was gender, diabetes, smoking, as it is shown in Table 4.2-4.

Table 4.2-4. Smoking, Gender, Diabetes History of the Working Group Linked to IL-1β

IL-1β						
Gender		Female X±SD		Male X±SD		*P* Value
Smoking		Yes	No	Yes	No	0.050*
Diabetes History	Yes	12.27±3.03	17.18±3.01	14.95±3.11	13.78±3.05	
	No	17.53±3.22	6.82±3.20	14.90±3.01	15.58±2.98	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases. *significant difference (p≤0.05), advanced chi square tests and double independent sample student t-test was used.

The significant difference (p=0.050) of the replication of gender, diabetes, smoking criteria of both groups have increased risk to CVD as was mentioned in Table 4.2-4.

4.3. The Study Group Risk Factors Related to Interleukin-1 Beta

The study was accomplished with the statistical investigation of the interleukin concentration, total plasma cholesterol, triglycerides, LDL, and HDL parameters in CVD related to IL-1 Beta genotype and evocative information of the patient's group who are diagnosed with CVD and a control group who are not diagnosed with any type of this disease. These groups both are used for investigating and understanding the expression levels of IL-1β on human serum samples. The results are expressed as it is shown in Table 4.3.

Table 4.3. Total Plasma Cholesterol, Triglycerides, LDL, HDL, Linked to the I IL-1β Results in CVD Patients and Healthy Control Groups

IL-1β			
	Patient : X±SD (n=39)	Control: X±SD (n=30)	*P* Values
Cholesterol (mg/dl)	185.15±7.977	174.40±6.252	0.34 (NS)
Triglycerides (mg/dl)	132.31±6.681	142.33±14.63	0.012*
LDL (mg/dl)	120.61±6.721	105.23±6.191	0.329 (NS)
HDL (mg/dl)	36.74±1.142	40.23±1.555	0.685 (NS)

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases, *significant difference ($p<0.05$), (NS): non-significant ($p>0.05$), one-way ANOVA test was used.

As we can notice from the table above (Table 4.3) we can understand that there is no significant difference in the study groups, in which Cholesterol ($p=0.34$), LDL ($p=0.329$), HDL ($p=0.685$). Nevertheless, we obtained a clear sign of the triglycerides ($p=0.012$) of our study groups.

4.4. The Cholesterol Effect On The Gender and Other Parameters

As we performed the statistical analysis of the different variants, we noticed that there was a correlation between the cholesterol and the replication of gender, diabetes, smoking of the study group's samples with a significant degree of (p=0.015). As it is shown in Table 4.3-1.

Table 4.4. Cholesterol linked to Smoking, Gender, Diabetes history

Cholesterol						
Gender		Female X±SD		Male X±SD		*P* Value
Smoking		Yes	No	Yes	No	
Diabetes History	Yes	166.5±12.15	194.8±12.11	201.4±12.13	159.3±12.16	0.015*
	No	214.8±12.11	203.2±12.14	165.2±12.09	172.3±12.12	

The results are expressed as: X±SD: mean of value ± Standard deviation, n: number of cases. *significant difference ($p<0.05$), advanced chi square tests and double independent sample student t-test was used.

5. DISCUSSION AND CONCLUSION

Our study has gone through several limitations. We couldn't study the effect of IL-6 due to the COVID-19 pandemic prevalence we couldn't bring the kits from abroad. Although most standard features of cardiovascular risk were alike between the two study groups. However, it should be recognized that our study was restricted to few cases only; due to the pandemic period, and it was hard to guess whether the anti-inflammatory effect would remain to control the proatherogenic effect over the long term.

Epidemiological studies have proved the role of risk factors for example hypertension, triglycerides, smoking, cholesterol, and diabetes mellitus in the development of CVDs. Experimental researches have shown that these risk factors play an extensive body reaction by triggering a general inflammatory response. Both systemic and acute phase reactors become active due to the response to the risk factors, and an endothelium signal transduction begins (319).

Oxidative stress is promoted by stimulation of various cardiovascular risk factors and pro-oxidant enzyme systems, knocking the redox balance in oxidation favor, with $O2^{-}$ as the key mediator. Hyperglycemia links primarily with ROS production of the mitochondrial and as seen in diabetes will secondarily trigger NADPH oxidase activation. The cigarette smoke components activate NOX which intercedes mitochondrial dysfunction and therefore ROS creation. Epidemiological studies proved that smoking, cholesterol, diabetes, and triglycerides are risk factors for the atherosclerosis development (104,320).

NCEP published in 2001 III. Factors for example age, smoking, gender, hyperlipidemia, and family genetic history related to CVD (321). For adults over 40 years, the incidence of atherosclerosis and CAD is increasing in parallel with the aging process. The most common age of CVD is 50-60 for males and 60-70 for females. Advanced atherosclerotic lesions occur in males about 20 years earlier than females, According to the Framingham Heart Study results, the incidence of CAD in men was 2 times higher than in women (322). In a study of 14786 people aged 25-64 years, the incidence of CAD in men was three times higher and the mortality was five times higher in women (323).

In our study, these risk factors were obtained by a questionnaire done with the patient and control groups. According to the results we found, the age of the patient and control group related to CVD is (p=0.143) and not significant.

Smoking is the foremost risk factor in the progression of CVD that showed in large numbers of studies. Other studies compared the non-smokers with smokers that smoke 20 cigarettes and/or more a day and the CAD prevalence was 2-3 times more in smokers (324). However, according to our observation, no significant difference was observed between smokers and non-smokers between the patients and control groups, and according to our results (p=0.470) of the smoking risk factor. Inhaling low levels of CO gas has the potential to supply adverse health effects, particularly in folks with CAD (325). The binding of carbon monoxide gas to hemoprotein ends up in carboxyhemoglobin, that reduces the number of oxygens to the guts muscle and alternative tissues for adenosine triphosphate production (326,327).

According to data from the COHORT study, and (Heart Disease and Risk Factors in Turkish Adults) mortality of Turkish women from CAD due to smoking, is the highest among European countries (328). However, in our study, we noticed there was not any significance between smoking and gender-related to CVD among individuals that are included in our work, no significant differences between women and men (p=0.541).

CHD is the major cause of mortality and morbidity in Turkey, also United States and Europe. According to Turkey Statistics Institution (TSI), heart disease mortality data has gradually tended to rise. Heart disease rate in 1989 was 40%, in 1993 was 45% (169,329). The prevalence in 1990 reports compared to the results of 2009 and 2014 reports for individuals over the age of 50 has increased 80% (330).

Epidemiological reports have shown that cigarette increases the risk of atherosclerosis (331). The results of smoking are caused by nicotine which acts on the nervous system, causing the rising of the cardiovascular system impairment. This leads to blood vessel narrowing and causes hypertension in the heart (332).

Diabetes Mellitus is an autonomous risk factor. Risk increases twice in diabetic men and increases four times in diabetic women according to Framingham Heart Study. Patients with diabetes mellitus have two times increased risk of CVD (333). The direct effect of diabetes and hyperinsulinemia on endothelial cells can manage the emergence of atherosclerosis (334).

The prevalence of diabetes in developing world has been documented to rise rapidly, and the number of adults with diabetes in the world by 2025 will be 300 million in developing countries (335,336). In Turkey, especially with aging, people tend to have a more sedentary lifestyle, gaining more weight and changes of diet habits in a negative way, which can result in the disease (337).

In our study, the patients with diabetes were about 33% of CVD while 67% of the patients do not suffer from diabetes in terms of diabetes history (p=0.833) have revealed no significant difference between the control and CVD patients as shown in (Table 4.2-3).

Obesity, one of the adaptable risk factors, is known to be a complex multifactorial chronic disease and it is thought that it is developed due to social, behavioral, physiological, metabolic, cultural, and genetic factors as a temporary result. Obesity is considered a crucial risk factor for CAD (338,339).

In the heart, all those harmful processes could prompt heart muscle dysfunction and apoptosis that lead to impaired heart remodeling, contractile dysfunction, fibrosis, cardiac failure, and hypertrophy (340). The inflammatory signaling of macrophage foam cells and endothelial persist to endorse the enrollment of more immune cells and monocytes within the subendothelial gap. A subset of SMCs direct macrophage receptors and adopt lipoproteins to develop foam cells and to travel into the intima and proliferate. (341).

LDL is a major CVD risk factor that had established through genetic, epidemiological, and pharmacological studies (342). Measurement of oxLDL was high in patients with CAD, that increased levels of oxLDL indicated future heart episodes in patients suffering from diabetes with CAD (343). Elevation of blood levels of LDL, cholesterol, and TG are high-risk factors for rising CVD (344).

This study included Total cholesterol, Triglycerides, LDL and HDL development of atherosclerosis and CVD, total cholesterol (p=0.34), HDL levels (p=0.685), and LDL (p=0.329) were showing no significant variance between the control and patients with CVD related to IL-1β, but for the TG (p=0.012) it has shown a significant degree related CVD linked to IL-1β as it was explained previously in Table 4.3.

Kathiresan and his colleagues' (2013) research in the Center for Human Genetic Research (CHGR) found a pattern of association between CVD and triglycerides similar

to what they found in LDL: the effects of triglyceride levels were highly related with their effect on CAD risk. The data strongly submitted that triglycerides are a fundamental factor in CVD (345). The exact mechanism of triglycerides and how they contribute to CAD is still undetermined. LDL is known to cause CAD by causing a build-up of “plaque" along the artery walls, As these molecules are accumulated, they block the blood flow through the arteries, and can lead to an ischemic heart attack (346).

In the contrast, an atheroprotective procedure of the HDL is to inhibit endothelial stimulation and boost NO construction to provide barrier integrity (347). Nevertheless, the number of blood monocytes deriving from the bone marrow and spleen will increase in an exceeding response to hypercholesterolemia and coronary artery disease (348,349). Furthermore, it protects against atherosclerosis plaque by directly inhibiting monocyte migration into the subendothelial gap. In the endothelium, NO protects against inflammation and activation. In an exceedingly amount of prospective Cohort studies, HDL has been shown to has a negative association with the risk of cardiac diseases. For each increase of HDL by (1mg/dL), the total risk of CVD reduced by 2-3% (350)

Oxidative stress and inflammation play major functions in tissue injury, cardiac muscle fibrosis, the makeover of heart tissue following MI, and clearance of cell fragments; though the main points of the development, initiation, and management of those processes have not been absolutely taken. ROS causes apoptosis via destroying DNA and activating pro-apoptotic signal pathways. Besides, it stimulates the creation of MMPs and pro-inflammatory cytokines; therefore, irritates inflammation and pathological makeover (94,351).

Also, mitochondria are a part of regulating injury-initiated programmed death. Though we have a tendency to currently acknowledge that mitochondria are specialized for diverse functions in several cell sorts and organs (352).

Apoptosis is a genetically programmed death that is expedited by the activation of the proteolytic enzyme cascade and leads to the macromolecule substrate's cleavage and DNA breakup. Programmed cell death has become more and more recognized as a united mechanism of cell death throughout reperfusion/ischemia injury (353). IL-1β makes changes in cardiac muscle cell functions. Also, to the blood vessel wall cells. IL-

1 spoils contractile function and is able to worsen ischemia or reperfusion injury and critical cardiac remodeling subsequently experimental MI (354,355).

Treatment with IL-1 blockers prevents atherosclerosis progression. Most treatments of cancer entail cardiovascular risk (273,355).

This study certainly contained some limitations as we mentioned earlier that should be discussed, this study has relatively short-term outcomes and the number of CVD patients is insufficient. These factors, together with differences in sample characteristics and study designs, could elucidate much of the discrepancy among the study (356). The identification of different alleles might allow better identification of new stroke mechanisms that may be a target of new therapeutic approaches (357).

Conclusion and Recommendation

- In our study we have investigated cardiovascular diseases in patients related to diabetes, smoking, gender, LDL, HDL, cholesterol, and triglycerides compared to the healthy free CVD control group; had shown no significance due to the very slight difference which was almost notable of IL-1β concentration between both groups.
- With our investigation of the IL-1β effect, we have found that the smoking and history of diabetes have significant difference among the study groups in with (p=0.050) as it was mentioned in Table 4.2-4.
- However, our statistical analysis has shown a significant relationship between the IL-1β expression and the triglycerides concentration as (p=0.012) of the study group.
- In the earlier discussion afford some examples of the evolution of molecular mechanisms of cardiovascular diseases have improved our standing of this episode of diseases at many levels, and several major common topics arise from this research work.
- We have realized how the stability and balance among the positive and negative regulatory factors can seriously affect and induce all cardiovascular disease events.
- Although, inflammation is the main process in the cause of pathophysiology of CVD episodes involving atherosclerosis, heart failure, and myocardial ischemia.
- CVDs are the chief reason of morbidity around the world. It is a serious health problem with a very high-cost treatment, yet, primary and secondary prevention attempts can be achieved.

- Inflammatory proteins, cells, and responses of the vascular cells have essential parts in formation, progression, and rupture of plaques.
- Cytokines are biological mediators and modifying molecules that are able to show activity even at very low concentrations to regulate immunological, local, or systemic inflammatory and restorative host responses and act as signals between cells. One type of cytokine can have multiple effects, such as growth and differentiation, on many cell types simultaneously.
- Elevated degrees of pro-inflammatory cytokines mainly (IL-1β) are aimed with aggravate consequences and opposing cardiac remodeling in individuals with CVD.

- Atherosclerosis, which is among the diseases that threaten human life with the most consequences and complications it creates, which in later stages, it can lead to critical complications for instance; MI, abdominal aortic aneurysms, and stroke by causing thrombi with the rupture of atherosclerotic plaque.
- IL-1 is the ideal proinflammatory intermediary in chronic and acute inflammation and a vigorous part in induction innate immune reaction.
- The activity and production of IL-1β, are sharply normalized at various levels, and a very actual minor concentrations can induce shock and syndromes such as sepsis-like syndrome.
- The relationship of cytokines, which are biological response modifying molecules that can show activity even at very low concentrations, with cardiovascular events is still being investigated.
- Though the risk factors leading to the disease have been recognized with broad epidemiological reports, yet these factors alone are not enough sufficient to clarify the prevalence of CVD, thus scholars need to explore more about the classical risk factors that were included in this study to take important steps in early detection of cardiovascular disease.
- Authenticating the awareness of individuals to know their own risk factors has vital importance in terms of preventive medicine. In this respect, every new knowledge and genetic study will contribute to the creation of new approaches in pharmacogenetics protocols.

- Controlling obesity is among the basic principles of protection against CVD. It will be possible to prevent a significant part of the deaths due to eliminating diabetes progression through weight gain from a young age, increasing physical activity, and raising awareness of eating habits.
- New markers to be found with CVD may be used as risk factors that can help to detect the disease more accurately.
- New genetic studies predisposition by identifying markers will enable early detection of cardiovascular disease which will facilitate an early diagnosis and detection, and prevent the progression of CVD.
- Inflammation intervention at a molecular level by determining the genetic profile expose a critical approach in the pharmaceutical pathway for treating various types of cardiovascular diseases
- Currently, it has been documented that additional reduction of LDL has been related to further CVD risk, and it's proposing a strategy of "the lower, the better" for CVD treatment and prevention.
- New evidence from genetic studies on this matter is added to the literature every day, therefore able to reach new therapeutic approaches and modified treatment protocols in the early stages of the patient life.
- Lifestyle changes and pharmacogenetics will contribute to the creation of customized therapy.
- Nowadays, nearly 50 cytokines related to inflammation have been identified and most of them are detected in human atherosclerotic plaque.
- The issues discussed in our work also explain how the usage of molecular and cellular approaches has raised a richer understanding of the pathogenesis of CVD. This amplified knowledge of the essential mechanisms helps us to sympathize with how current therapies for CVD may act.
- Further studies of cytokine gene expression will be a proof to best identification of the disease and will support the detection of individuals who’s at risk.

6. REFERENCES

1. Landsbergis P, Baker D, Schnall P. Why the workplace and cardiovascular disease. *Pub Med.gov.* 2000; 15(1): 1-6.
2. Lloyd-Jones D, Adams R, Carnethon M, de Simone G, et al. Heart disease and stroke statistics—2009 update: A report from the american heart association statistics committee and stroke statistics subcommittee. *Circulation*. 2009; 119: 480–486. (3)
3. CDC. Million Hearts: Strategies to reduce the prevalence of leading cardiovascular disease risk factors—United States. MMWR. 2011; 60(36): 1248–51. 2011
4. Frieden TR, Berwick DM. The "Million Hearts" Initiative—Preventing heart attacks and strokes. *N Engl J Med*. 2011; 365(13): e27.
5. Saçlı H, Kara I, Kırali MK. Atherosclerosis - Yesterday, Today and Tomorrow. Focus on Coronary Atherosclerosis. Luigi Gianturco; 2018.
6. Alissa EM, Ferns GA. Heavy metal poisoning and cardiovascular disease. *J Toxicol*, 2011; 2011: 870125.
7. Arslan, F, de Kleijn, D.P, Pasterkamp G. Innate immune signaling in cardiac ischemia. *Nat. Rev. Cardiol.* 2011; 8: 292–300.
8. Coggins M, Rosenzweig A. The fire within: Cardiac inflammatory signaling in health and disease. *Circ Res.* 2012; 110: 116–125.
9. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. *Circ Res.* 2012; 110: 159–173. (11)
10. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science*. 2013; 339: 161–166. (12)
11. Mann, D. L. Mechanisms and models in heart failure: a combinatorial approach. *Circulation* 1999; 100: 999 –1008.
12. Kapadia S, Lee J, Torre-Amione G, et al. Tumor necrosis factor-α gene and protein expression in adult feline myocardium after endotoxin administration. *J. Clin. Invest*. 1995; 96: 1042–1052.
13. Hage FG, Szalai AJ. The role of C-reactive protein polymorphisms in inflammation and cardiovascular risk. *Curr Atheroscler Rep*. 2009; 11(2): 124-30.

14. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics.* 1994; 19(2): 382-4.
15. Dinarello CA., Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011; 117: 3720–373.
16. Stutz A., Golenbock DT., Latz E. et al. Inflammasomes: too big to miss. *J Clin Invest.* 2009; 119:3502–3511.
17. Dinarello CA, Simon A, Vander-Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov.* 2012; 11: 633–652.
18. Terkeltaub R, Sundy JS, Schumacher HR, et al. The interleukin 1 inhibitor rilonacept in treatment of chronic gouty arthritis: results of a placebo-controlled, monosequence crossover, non-randomised, single-blind pilot study. *Ann Rheum Dis.* 2009; 68: 1613–1617
19. Lamkanfi M, Kanneganti TD. Nlrp3: an immune sensor of cellular stress and infection. *Int J Biochem Cell Biol.* 2010; 42: 792–795.
20. Hurme M, Lahdenpohja N, Santtila S. Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. *Ann Med.* 1998; 30: 469–473.
21. Onat, A. Risk factors and cardiovascular disease in Turkey. *Atherosclerosis.* 2001; 156(1): 1-10.
22. Andreotti F, Porto I, Crea F, et al. Inflammatory gene polymorphisms and ischemic heart disease: review of population association studies. *Heart.* 2002; 87: 107–112.
23. Razum O. Is mortality from ischaemic heart disease in Turkey among the highest in Europe? Atherosclerosis. 2001; 158(2): 499-500.
24. Mozaffarian D, Lsabeth LD, Mussolino ME, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. *Circulation.* 2013; 27: e6–e245.
25. Alwan, A. Global Status Report on Noncommunicable Diseases 2010. World Health Organization: Geneva, Switzerland, 2011; 9–11.

26. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006; 3: e442.
27. From interview with a BHF professor of cardiovascular sciences, on 15th May 2006
28. Nason E. An overview of cardiovascular disease and research. *RAND Europ.* 2007; WR-467-RS
29. NHLBI. Coronary Heart Disease.NIH 26.8.2019, https://www.nhlbi.nih.gov/health-topics/coronary-heart-disease.
30. Go AS, Mozaffarian D, Roger VL, et al. Executive summary: Heart disease and stroke statistics—2013 update: A report from the American Heart Association. *Circulation.* 2013; 127(1): 143-152.
31. Mahley RW, E. E. Palaoglu KE, Atak Z, et al. Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. *J Lipid Res.* 1995; 36: 839-859.
32. Onat A, G. Surdum-Avci M, Senocak E, et al. Plasma lipids and their interrelationship in Turkish adults . *J . Epidemiol. Community Health* 1992; 46: 470-476.
33. Wedro B, Heart Disease (Cardiovascular Disease, CVD). *MedicineNet* 2020.
34. Benjamin EJ, Blaha MJ, Chiuve SE, et al. ; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. *Circulation.* 2017; 135: e146–603.
35. Newman T, How to spot and treat a heart attack. Medical News Today 29.9.2020, https://www.medicalnewstoday.com/articles/151444#Causes
36. "Brain Basics: Preventing Stroke". National Institute of Neurological Disorders and Stroke. 8.10.2009. https://www.ninds.nih.gov/disorders/all-disorders/stroke-information-page
37. "What Is a Stroke?". www.nhlbi.nih.gov/. March 26, 2014.
38. "What Are the Signs and Symptoms of a Stroke?". www.nhlbi.nih.gov. March 26, 2014.
39. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. *The Lancet.* 2008; 371(9624): 1612–23.
40. A Classification and outline of cerebrovascular diseases. *Stroke* 1975; 6:564–616.

41. Shirazi LF, Bissett J, Romeo F, Mehta JL Role of inflammation in heart failure. *Curr Atheroscler Rep.* 2017; 19: 27.
42. Maeda K, Tsutamoto T, Wada A, Mabuchi N, et al. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll Cardiol* 2000; 36: 1587–1593.
43. Hamzic-Mehmedbasic A. Inflammatory cytokines as risk factors for mortality after acute cardiac events. *Med Arch.* 2016; 70: 252–255.
44. Garbers C, Heink S, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nat Rev Drug Discov.* 2018; 17:395–412.
45. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: a magnificent pathway. *J Gerontol A Biol Sci Med Sci.* 2006; 61: 575–584.
46. Jackson K, Hanberg J, Mahoney D, et al. Inflammation and cardio-renal interactions in heart failure: a potential role for interleukin-6. *Eur J Heart Fail.* 2018; 20: 933–934.
47. Marcus GM, Whooley MA, Glidden DV. Interleukin-6 and atrial fibrillation in patients with coronary artery disease: data from the Heart and Soul Study. *Am Heart J.* 2008; 155: 303–309.
48. Whitteridge G. The anatomical lectures of William Harvey. Edinburgh and London, UK: E. & S.Livingstone; 1964.
49. Kanno S, Saffitz JE. The role of myocardial gap junctions in electrical conduction and arrhythmogenesis. *Cardiovasc Pathol*. 2001; 10: 169–77.
50. Nerbonne JM, Guo W. Heterogeneous expression of voltage-gated potassium channels in the heart: roles in normal excitation and arrhythmias. *J Cardiovasc Electrophysiol.* 2002; 13: 406–9.
51. Desai D. Arrhythmias. Arrythemias *CME*. 2020.
52. Lakshminarayan K, Anderson DC, Herzog CA,Qureshi AI, Clinical epidemiology of atrial fibrillation and related cerebrovascular events in the United States. *The neurologist.* 2008.
53. Resnekov L. Cardiac Arrythmias. *Postgrad. Med. J.* 1964; 40, 381

54. Harvard Medical School. Cardiac Arrhythmias. Harvard Health Publishing 2019.

55. Nkomo VT, Gardin JM, Skelton TN, et al. Burden of valvular heart diseases: a population-based study 2006; 368(9540): 1005-11.

56. Kovacs AH, Harrison JL, Colman JM, et al. Pregnancy and contraception in congenital heart disease: what women are not told. *J Am Coll Cardiol.* 2008; 52(7): 577.

57. Ragavendra R. Baliga, Kim A. et al. Practical Cardiology, Lippincott Williams & Wilkins, 2008; pp.452.

58. Abelmann, WH. Classification and natural history of primary myocardial disease. *Prog Cardiovasc Dis.* 1984; 27: 73.

59. Report of the WHO/ISFC task force on the definition and classification of cardiomyopathies. *Br Heart J.* 1980; 44: 672.

60. Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation.* 1996; 93: 841.

61. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J.* 2008; 29: 270.

62. McKenna WJ, Maron BJ, Thiene G. Classification, epidemiology, and global burden of cardiomyopathies. *Circ. Res.* 2017; 121: 722–730.

63. Saxon LA, De Marco T. Arrhythmias associated with dilated cardiomyopathy. *Card. Electrophysiol. Rev*. 2002; 6: 18–25.

64. Fairweather D, Cooper LT, Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy.*Curr. Probl. Cardiol.* 2013;38:7–46.

65. McGoon MD, Miller DP. REVEAL: a contemporary US pulmonary arterial hypertension registry. *Eur. Respir. Rev.* 2012; 21: 8–18.

66. Halliday BP, et al. Sex- and age-based differences in the natural history and outcome of dilated cardiomyopathy. *Eur. J. Heart Fail.* 2018; 20: 1392–1400.

67. Al Turki S, Manickaraj AK, Mercer CL. Et al. Rare Variants in NR2F2 Cause Congenital Heart Defects in Humans. *Am J Hum Genet.* 2014; 94(4).

68. Blue G.M., Kirk E.P., Sholler G.F., Harvey R.P., Winlaw D.S. Congenital heart disease: current knowledge about causes and inheritance. *Med. J. Aust*. 2012; 197: 155–159.
69. Pierpont ME, Basson CT, Benson DW, et al. American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation* 2007; 115: 3015–3038.
70. Ritz B, Yu F, Fruin S, et al. Ambient air pollution and risk of birth defects in Southern California. *Am J Epidemiol.* 2002; 155(1): 17-25.
71. Dolk H, Armstrong B, Lachowycz K, et al. Ambient air pollution and risk of congenital anomalies in England, 1991-1999. *Occup Environ Med.* 2010; 67(4): 223-7.
72. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. Larsen's human embryology. Philadelphia 2015. https://www.clinicalkey.com.ezproxy2.umc.edu/#!/content/book/3-s2.0 B9781455706846000126.
73. Dadvand P, Rankin J, Rushton S, Ambient air pollution and congenital heart disease: a register-based study. *Environ Res.* 2011; 111(3): 435-41.
74. Patel SS, Burns TL. Nongenetic risk factors and congenital heart defects. Pediatr Cardiol. 2013; 34(7): 1535-55.
75. Reller MD, Strickland MJ, Riehle-Colarusso T, et al. Prevalence of congenital heart defects in metropolitan Atlanta,1998-2005. *J Pediatr.* 2008; 153(6): 807-13.
76. Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015; 386(9995): 743-800.
77. McDermott MM. Lower extremity manifestations of peripheral artery disease: the pathophysiologic and functional implications of leg ischemia. *Circ Res.* 2015; 116(9): 1540-50.

78. Schiano V, Brevetti G, Sirico G. Et al. Functional status measured by walking impairment questionnaire and cardiovascular risk prediction in peripheral arterial disease: results of the Peripheral Arteriopathy and Cardiovascular Events (PACE) study. *Vasc Med.* 2006 Nov; 11(3):147-54.

79. Morris DR, Rodriguez AJ, Golledge J.Association of Lower Extremity Performance with Cardiovascular and All-Cause Mortality in Patients with Peirpheral Artery Disease: A Systematic Review and Meta-analysis. *J Am Heart Assoc*, 2014; 3: e001-105

80. Thom et al. "Heart Disease and Stroke Statistics - 2006 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee", *Circulation* 2006; 113: e85-e151

81. Gori T, Münzel T. Oxidative stress and endothelial dysfunction: therapeutic implications. Ann Med. 2011; 43: 259-272

82. Gori T, Münzel T, Taddei S. Et al. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J.* 2010; 31: 2741-2748.

83. Droge W. Free radicals in the physiological control of cell function. Physiol. Rev 2002; 82: 47–95.

84. Csanyi G, Yao M, Rodriguez AI. Thrombospondin-1 regulates blood flow via CD47 receptor-mediated activation of NADPH oxidase 1. *Arterioscler. Thromb. Vasc. Biol.* 2012; 32: 2966–2973.

85. Sugiyama S, Kugiyama K, Aikawa M, et al. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: Involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. *Arterioscler. Thromb. Vasc. Biol.* 2004; 24: 1309–1314.

86. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: Implications in human hypertension. *Hypertens. Res.* 2011; 34: 5–14.

87. Gimbrone MA, Garcia-Cardena G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. Cardiovascular pathology : *the official journal of the Society for Cardiovascular Pathology.* 2013; 22: 9-15.

88. Netticadan, Thomas, Naranjan S. et al. Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension.* 2000; 18(6): 655-673

89. Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. *The Journal of biological chemistry.* 2000; 275:12633–12638.
90. Collins T, Read MA, Neish AS, et al. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers: official publication of the Federation of American Societies for Experimental Biology. *FASEB journal.* 1995; 9: 899–909.
91. Park S, Yoon SJ, Tae HJ, et al. RAGE and cardiovascular disease. *Frontiers in bioscience*. 2011;16: 486–497.
92. Dauphinee SM, Karsan A. Lipopolysaccharide signaling in endothelial cells. Laboratory investigation; *J. of technical methods and pathol.* 2006; 86: 9–22.
93. Ferrari R, Guardigli G, Mele D, et al. Oxidative stress during myocardial ischaemia and heart failure. Curr. Pharm. Des 2004; 10: 1699–1711.
94. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. *Oxid. Med. Cell. Longevity* 2009; 2: 259–269.
95. Tsutsui, H.; Kinugawa, S.; Matsushima, S. Oxidative stress and heart failure. *Am. J. Physiol.* 2011; 301: H2181–H2190.
96. National Cholesterol Education Program. Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel 11). *Circulation*. 1994; 89: 1329-1445.
97. Gonzalez-Muniesa, P, Marrades MP, Martinez JA, Moreno-Aliaga MJ. Differential proinflammatory and oxidative stress response and vulnerability to metabolic syndrome in habitual high-fat young male consumers putatively predisposed by their genetic background. *Int. J. Mol. Sci.* 2013; 14: 17238–17255.
98. Hotamisligil, GS. Inflammation and metabolic disorders. *Nature.* 2006; 444: 860–867.
99. Robertson LT, Mitchell JR. Benefits of short-term dietary restriction in mammals. E*xp. Gerontol* 2013.

100. De la Iglesia R, Lopez-Legarrea P, Celada P, et al. Beneficial effects of the resmena dietary pattern on oxidative stress in patients suffering from metabolic syndrome with hyperglycemia are associated to dietary tac and fruit consumption. *Int. J. Mol. Sci.* 2013; 14: 6903–6919.
101. Mittal M, Siddiqui MR, Tran K. Et al. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal*. 2014; 20(7): 1126-67.
102. Thomas SR, Witting PK, Drummond GR Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. *Antioxid Redox Signal.* 2008 ; 10(10): 1713-65.
103. Saparov A, Wen Chen C, Wang Y. Et al. The Role of Antioxidation and Immunomodulation in Postnatal Multipotent Stem Cell-Mediated Cardiac Repair. *Int. J. Mol. Sci.* 2013; 14(8): 6258-16279.
104. Nickenig G, Harrison DG. The AT1-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. *Circulation*. 2002; 105: 393-396
105. Zalba G, San José G, Moreno MU, et al. Oxidative stress in arterial hypertension: role of NADPH oxidase. *Hypertension*. 2001; 38: 1395-1399.
106. Laufs U, Kilter H, Konkol C, Wassmann S, et al. Impact of HMG CoA reductase inhibition on small GTPases in the heart. *Cardiovasc Res*. 2002; 53: 911-920.
107. Touyz RM, Yao G, Schiffrinc-Src EL. induces phosphorylation and translocation of p47phox: role in superoxide generation by angiotensin II in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2003; 23: 981-987.
108. Camici GG, Schiavoni M, Francia P, et al. Genetic deletion of p66Shc adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. Proc Natl Acad Sci U S A. 2007; 104: 5217-5222.
109. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* 107; (2010): 1058-1070.
110. Funk A, Yurdagul Jr, Orr AW. Hyperglycemia and endothelial dysfunction in atherosclerosis: lessons from type 1 diabetes. *Int J Vasc Med.* 2012; 2012: 569654
111. Jaimes EA, DeMaster EG, Tian RX, Raij L. Stable compounds of cigarette smoke induce endothelial superoxide anion production via NADPH oxidase activation. *Arterioscler Thromb Vasc Biol*. 2004; 24: 1031-1036.

112. Csiszar A, Podlutsky A, Wolin MS, et al. Oxidative stress and accelerated vascular aging: implications for cigarette smoking Front Biosci (Landmark Ed). 2009; 14: 3128-3144
113. Steffen Y, Vuillaume G, Stolle K, et al. Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase Nitric Oxide. 2012: 27; 176-184.
114. Heitzer T, Brockhoff C, Mayer B, et al. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. *Circ Res.* 2000; 86: E36-E41.
115. Camici GG, Savarese G, Akhmedov A, Lüscher TF, Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease. *Eur Heart J.* 2015; 36: 3392-3403.
116. Donato AJ, Gano LB, Eskurza I, et al. Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *Am J Physiol Heart Circ Physiol.* 2009; 297: H425-H432.
117. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. *Physiol Rev*. 2004; 84: 1381-1478
118. Arslan F, deKleijn DP, Pasterkamp G. Innate immune signaling in cardiac ischemia. *Nat. Rev. Cardiol.* 2011; 8; 292–300.
119. Colin S, Chinetti-Gbaguidi G, Staels B. Macrophage phenotypes in atherosclerosis. *Immunological reviews.* 2014; 262: 153–166.
120. Adamson S, Leitinger N. Phenotypic modulation of macrophages in response to plaque lipids. *Current opinion in lipidology.* 2011; 22: 335–342.
121. Bolick DT, Skaflen MD, Johnson LE, et al. G2A deficiency in mice promotes macrophage activation and atherosclerosis. *Circulation research.* 2009;104:318–327.
122. Li Y, Si R, Feng Y, Chen HH, et al. Myocardial ischemia activates an injurious innate immune signaling via cardiac heat shock protein 60 and Toll-like receptor 4. *J. Biol. Chem.* 2011; 286: 31308–31319.
123. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the drosophila Toll protein signals activation of adaptive immunity. *Nature.* 1997; 388: 394–397.

124. Diepenhorst GM, Van-Gulik, TM, Hack CE. Complement-mediated ischemia-reperfusion injury: Lessons learned from animal and clinical studies. *Annu. Surg.* 2009; 249: 889–899.
125. Banz Y, Rieben R. Role of complement and perspectives for intervention in ischemia-reperfusion damage. *Annu. Med.* 2012; 44: 205–217
126. Leuschner F, Rauch PJ, Ueno T, Gorbatov R, et al. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. *J. Exp. Med.* 2012; 209: 123–137.
127. Murphy E, Ardehali H, Balaban RS, et al. American Heart Association Council on Basic Cardiovascular Sciences, Council on Clinical Cardiology, and Council on Functional Genomics and Translational BiologyMitochondrial function, biology, and role in disease: a scientific statement from the American Heart Association. *Circ Res.* 2016; 118: 1960-1991.
128. Schaper J, Meiser E, Stammler G. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice, and from human hearts. *Circ Res.* 1985; 56: 377-391.
129. Taegtmeyer, H. Energy metabolism of the heart: from basic concepts to clinical applications. *Curr Probl Cardiol.* 1994; 19: 59-113
130. Lopaschuk GD, Ussher JR, Folmes CD, et al. Myocardial fatty acid metabolism in health and disease. *Physiol Rev*. 2010; 90: 207-258.
131. Orsini F, Migliaccio E, Moroni M, et al. The life span determinant p66shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. *The Journal of Biological Chemistry.* 2004; 279(24): 25689–25695.
132. Wallace, DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet. 2005; 39: 359-407
133. Chandel, NS. Evolution of mitochondria as signaling organelles. *Cell Metab.* 2015; 22: 204-206

134. Li J. M., Shah A. M. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2004; 287(5): R1014–R1030.

135. Sack, MN. Type 2 diabetes, mitochondrial biology and the heart. *J Mol Cell Cardiol.* 2009; 46: 842-849.

136. Sack, MN. Mitochondrial depolarization and the role of uncoupling proteins in ischemia tolerance. Cardiovasc Res. 2006; 72: 210-219.

137. Kröller-Schön S, Steven S, Kossmann S. et al. Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models. *Antioxid Redox Signal* 2014; 20: 47-266

138. Maack C, Böhm M. Targeting mitochondrial oxidative stress in heart failure throttling the after burner. *J. Am Coll Cardiol* 2011; 58: 83-86.

139. Allen T, Hopper KA, Jones BM, Campbell GL. Neuroinflammation in Mood Disorders: Mechanisms and Drug Targets. *Annual Reports in Medicinal Chemistry* 2013; 48: 317-331

140. Ye D, Lammers B, Zhao Y, Meurs I, et al. ATP-binding cassette transporters A1 and G1, HDL metabolism, cholesterol efflux, and inflammation: important targets for the treatment of atherosclerosis. Current drug targets. 2011; 12: 647–660.

141. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nature reviews Immunology. 2015; 15: 104–116.

142. Evrard SM, Lecce L, Michelis KC, et al. Endothelial to mesenchymal transition is common in atherosclerotic lesions and is associated with plaque instability. *Nat Commun.* 2016; 7: 11853.

143. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 2015; 131: e29–322.

144. Rogacev KS, Cremers B, Zawada AM, Seiler S, et al. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. *Journal of the American College of Cardiology.* 2012; 60: 1512–1520.
145. Brown MS, Goldstein JL, Krieger M, et al. Reversible accumulation of cholesteryl esters in macrophages incubated with acetylated lipoproteins. *J Cell Biol.* 1979; 82: 597–613.
146. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem.* 1983; 52: 223–261.
147. Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arterioscler thromb vasc biol.* 2006; 26: 1702–1711.
148. Younis N, Sharma R, Soran H, et al. Glycation as an atherogenic modification of LDL. *Current opinion in lipidology.* 2008; 19: 378–384.
149. Torzewski M, Lackner KJ. Initiation and progression of atherosclerosis--enzymatic or oxidative modification of low-density lipoprotein? *Clinical chemistry and laboratory medicine : CCLM / FESCC* 2006; 44: 1389-1394.
150. Yancey PG, Jerome WG, Yu H, Griffin EE, et al. Severely altered cholesterol homeostasis in macrophages lacking apoE and SR-BI. *J Lipid Res*. 2007; 48: 1140–1149.
151. Fioravanti J, Medina-Echeverz J, Berraondo P. Scavenger receptor class B, type I: a promising immunotherapy target. *Immunotherapy*. 2011; 3: 395–406
152. Kellner-Weibel G, de la Llera-Moya M. Update on HDL receptors and cellular cholesterol transport. *Current atherosclerosis reports*. 2011; 13: 233–241
153. Choi JH, Do Y, Cheong C, Koh H, Boscardin SB, et al. Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves. *The Journal of experimental medicine*. 2009; 206: 497–505.
154. Paulson KE, Zhu SN, Chen M, Nurmohamed S, et al. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. *Circulation research.* 2010; 106: 383–390.

155. Veillard NR, Kwak B, Pelli G, Mulhaupt F, et al. Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. *Circulation research.* 2004; 94: 253–261

156. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. *Nature reviews Cardiology*. 2009; 6: 399–409

157. Gil-Pulido J, Zernecke A. Antigen-presenting dendritic cells in atherosclerosis. *Eur J Pharmacol.* 2017; 816: 25–31.

158. Pirillo A, Bonacina F, Norata GD, Catapano AL. The Interplay of Lipids, Lipoproteins, and Immunity in Atherosclerosis. *Curr Atheroscler Rep*. 2018; 20: 12.

159. Abdolmaleki F, Gheibi Hayat SM, Bianconi V, Johnston TP, Sahebkar A. Atherosclerosis and immunity: A perspective. *Trends Cardiovasc Med.* 2019; 29(6): 363-371

160. Donaldson CJ, Lao KH, Zeng L. The salient role of microRNAs in atherogenesis. *Journal of molecular and cellular cardiology*. 2018; 122: 98–113

161. Michell DL, Vickers KC. Lipoprotein carriers of microRNAs. *Biochimica et biophysica acta.* 2016; 1861: 2069–2074.

162. Ridker PM, MacFadyen JG, Everett BM, Libby P, et al. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet*. 2018; 391: 319–328.

163. Schwartz EA, Reaven PD. Lipolysis of triglyceride-rich lipoproteins, vascular inflammation, and atherosclerosis. *Biochimica et biophysica acta.* 2012; 1821: 858–866.

164. Fujioka Y, Cooper A, Fong L. Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. *J Lipid Res.* 1998; 39: 2339–2349.

165. Anzinger JJ, Chang J, Xu Q, Barthwal MK, et al. Murine bone marrow-derived macrophages differentiated with GM-CSF become foam cells by PI3Kgamma-dependent fluid-phase pinocytosis of native LDL. *J Lipid Res*. 2012; 53: 34–42.

166. Brame CJ, Salomon RG, Morrow JD, Roberts LJ 2nd. Identification of extremely reactive gamma-ketoaldehydes (isolevuglandins) as products of the isoprostane pathway and characterization of their lysyl protein adducts. *J Biol Chem.* 1999; 274(19): 13139-46.

167. Tsimikas S, Bergmark C, Beyer RW, et al. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol*. 2003; 41: 360–370.

168. Tanaga K, Bujo H, Inoue M, Mikami K, Kotani K, Takahashi K, Kanno T, Saito Y. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arteriosclerosis, thrombosis, and vascular biology.* 2002; 22: 662–666.

169. Wang J, Hu B, Meng Y, et al. The level of malondialdehyde-modified LDL and LDL immune complexes in patients with rheumatoid arthritis. *Clin Biochem.* 2009; 42: 1352–1357.

170. Esterbauer H, Rotheneder M, Striegel G, et al. Vitamin E and other lipophilic antioxidants protect LDL against oxidation. *Fat Sci Technol.* 1989; 8: 316–324.

171. Hussein O, Rosenblat M, Refael G, Aviram M. Dietary selenium increases cellular glutathione peroxidase activity and reduces the enhanced susceptibility to lipid peroxidation of plasma and low-density lipoprotein in kidney transplant recipients. *Transplantation*. 1997; 63: 679–685.

172. Guo Z, Van Remmen H, Yang H, et al. Richardson A. Changes in expression of antioxidant enzymes affect cell-mediated LDL oxidation and oxidized LDL-induced apoptosis in mouse aortic cells. *Arteriosclerosis, thrombosis, and vascular biology.* 2001; 21: 1131–1138.

173. Steinbrecher UP, Pritchard PH. Hydrolysis of phosphatidylcholine during LDL oxidation is mediated by platelet-activating factor acetylhydrolase. *J Lipid Res*. 1989; 30: 305–315.

174. George J, Struthers AD. Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress.*Vascular health and risk management.* 2009; 5: 265–272.

175. Aviram M, Rosenblat M, Etzioni A, Levy R. Activation of NADPH oxidase required for macrophage-mediated oxidation of low-density lipoprotein. *Metabolism*. 1996; 45(9): 1069–1079.
176. Miyoshi T, Li Y, Shih DM, Wang X, et al. Deficiency of inducible NO synthase reduces advanced but not early atherosclerosis in apolipoprotein E-deficient mice. *Life sciences.* 2006; 79: 525–531.
177. Upston JM, Neuzil J, Stocker R. Oxidation of LDL by recombinant human 15-lipoxygenase: evidence for alpha-tocopherol-dependent oxidation of esterified core and surface lipids. *J Lipid Res.* 1996; 37: 2650–2661.
178. Ezaki M, Witztum JL, Steinberg D. Lipoperoxides in LDL incubated with fibroblasts that overexpress 15-lipoxygenase. *J Lipid Res*. 1995; 36: 1996–2004.
179. Lei J, Vodovotz Y, Tzeng E, Billiar TR. Nitric oxide, a protective molecule in the cardiovascular system. *Nitric oxide* : *biology and chemistry / official journal of the Nitric Oxide Society* 2013; 35:175-185.
180. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology.* 2007; 27: 2292–2301.
181. Topper JN, Cai J, Falb D, Gimbrone Jr MA. Identification of vascular endothelial genomes variation responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci USA.* 1996; 93: 10417–10422.
182. Gerrity RG, Naito HK, Richardson M, Schwartz CJ. Dietary induced atherogenesis in swine. Morphology of the intima in prelesion stages. *The American journal of pathology.* 1979; 95: 775–792.
183. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis* 1989; 9: 908–918.
184. Ungvari Z, Wolin MS, Csiszar A. Mechanosensitive production of reactive oxygen species in endothelial and smooth muscle cells: role in microvascular remodeling? *Antioxidants & redox signaling* 2006; 8: 1121-1129.
185. Steinbrecher, UP. Role of superoxide in endothelial-cell modification of low-density lipoproteins. *Biochim Biophys Acta*. 1988; 959: 20–30.

186. Tonks, NK. Redox redux: revisiting PTPs and the control of cell signaling Cell. 2005; 121: 667-670.
187. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species *Mol Cell.* 2012; 48: 158-167.
188. McLeod CJ, Jeyabalan, Minners JO, Clevenger R, et al. Delayed ischemic preconditioning stimulates nuclear-encoded ETC genome expression in parallel with enhanced postanoxic mitochondrial respiratory recovery. *Circulation*. 2004; 110: 534-539.
189. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell*. 2004; 116: 205-219
190. Kung G, Konstantinidis K, Kitsis RN. Programmed necrosis, not apoptosis, in the heart *Circ Res*. 2011; 108: 1017-1036. (272)
191. Whelan RS, Kaplinskiy V, Kitsis RN. Cell death in the pathogenesis of heart disease: mechanisms and significance. *Annu Rev Physiol.* 2010; 72: 19-44.
192. Lee Y, Gustafsson AB. Role of apoptosis in cardiovascular disease. *Apoptosis*. 2009; 14: 536–548.
193. Saelens X, Festjens N, Parthoens E, et al. Protein synthesis persists during necrotic cell death. *J Cell Biol*. 2005; 168: 545-551.
194. Soldani C, Scovassi AI. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. *Apoptosis*. 2002; 7: 321-328.
195. Williams, RS. Apoptosis and heart failure. N Engl J Med. 1999; 341: 759-760.
196. Nakayama H, Chen X, Baines CP, et al. Ca2+- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. *J Clin Invest.* 2007; 117: 2431-2444.
197. Leist M, Single B, Castoldi AF, Kühnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med.* 1997; 185: 1481-1486.
198. Karch J., Kwong J.Q., Burrm A.R. et al.Bax and Bak function as the outer membrane component of the mitochondrial permeability pore in regulating necrotic cell death in mice. *Elife*. 2013; 2: e00772.
199. Gottlieb R.A, Burleson K.O, Kloner R.A. Et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J. Clin Invest.* 1994; 94: 1621-1628.

200. Jennings RB, Sommers HM, Smyth GA. et al. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch Pathol.* 1960; 70: 68-78.

201. Lieberthal W, Menza SA, Levine JS. Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. *Am J Physiol.* 1998; 274: F315-F327.

202. Chang W, Lin J, Dong J. Et al. Pyroptosis: an inflammatory cell death implicates in atherosclerosis. *Medical hypotheses*. 2013;81:484–486.

203. Dai DF, Hsieh EJ, Liu Y, et al. Mitochondrial proteome remodelling in pressure overload-induced heart failure: the role of mitochondrial oxidative stress. *Cardiovasc Res.* 2012; 93: 79-88

204. Ikeda Y, Shirakabe A, Brady C. Et al. Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system *J. Mol Cell Cardiol*. 2015;78:116-122

205. Moris D, Spartalis M, Tzatzaki E, et al. The role of reactive oxygen species in myocardial redox signaling and regulation. *Annals of Translational Medicine*.2017;5(16): 324

206. Dai DF, Santana LF, Vermulst M, et al. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation*. 2009; 119(21): 2789-97

207. Nuttall SL, Kendall MJ, Martin U. Antioxidant therapy for the prevention of cardiovascular disease. QJM 1999; 92: 239-44. (298)

208. Cadenas, E. Sies, H. The lag phase. *Free Radic Res* 1998; 28: 601-9.

209. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007; 39: 44-84.

210. Ratnam D.V, Ankola D.D, Bhardwaj V, et al. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *J Control Release* 2006; 113: 189-207.

211. Kris-Etherton PM, Lichtenstein AH, Howard BV, et al. Antioxidant vitamin supplements and cardiovascular disease. *Circulation* 2004; 110: 637-41.

212. Witting P.K, Willhite C.A, Davies M.J, et al. Lipid oxidation in human low-density lipoprotein induced by metmyoglobin/H2O2: involvement of α-tocopheroxyl and phosphatidylcholine alkoxyl radicals *Chem Res Toxicol.* 1999;12:1173-1181.

213. Drummond G.R, Selemidis S, Griendling K.K, et al.Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov.* 2011; 10: 453-471.

214. Jain A.K, Mehra N. K, and Swarnakar N. K. Role of Antioxidants for the Treatment of Cardiovascular Diseases: Challenges and Opportunities. *Current Pharmaceutical Design* 2015; 21(30).

215. Bliznakov EG. Cardiovascular diseases, oxidative stress and anti-oxidants: the decisive role of coenzyme Q10. *Cardiovasc Res.* 1999; 43: 248-9.

216. Ginter E, Simko V, Panakova V. Antioxidants in health and disease. *Bratisl Lek Listy*. 2014; 115: 603-6.

217. Honarbakhsh S, Schachter M. Vitamins and cardiovascular disease. *Br J Nutr* 2009; 101: 1113-31.

218. Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983; 52: 711-60.

219. Vardi M, Levy N. S, Levy A. P. Vitamin E in the prevention of cardiovascular disease: the importance of proper patient selection. *J Lipid Res* 2013; 54: 2307-14.

220. Wang X, Quinn P. J. Vitamin E and its function in membranes. *Prog Lipid Res.* 1999; 38: 309-36.

221. Oguntibeju O, Esterhuyse A, Truter E. Cardiovascular disease and the potential protective role of antioxidants. *African J Biotechnol*. 2009; 8: 3107-17.

222. Packer L, Weber SU, Rimbach G. Molecular aspects of α-tocopherol-tocotrienol antioxidant action and cell signalling. *J. Nutr*. 2001; 131: 369S-73S.

223. Swarnakar N. K, Jain A. K, Singh RP, et al. Oral bioavailability, therapeutic efficacy and reactive oxygen species scavenging properties of coenzyme Q10-loaded polymeric nanoparticles. *Biomaterials*. 2011; 32: 6860-74.

224. Crane FL. Biochemical functions of coenzyme Q10. *J Am Coll Nutr.* 2001; 20: 591-8.

225. Adarsh K, Kaur H, Mohan V. Coenzyme Q10 (CoQ10) in isolated diastolic heart failure in hypertrophic cardiomyopathy (HCM). *Bio-factors* 2008; 32: 145-9.
226. Celik T, Iyisoy A. Coenzyme Q10 and coronary artery bypass surgery: what we have learned from clinical trials. *J Cardiothorac Vasc Anesth*. 2009; 23: 935-6.
227. Rosenfeldt FL, Haas SJ, Krum H, et al. Coenzyme Q10 in the treatment of hypertension: a meta-analysis of the clinical trials. *J Hum Hypertens.* 2007; 21: 297-306.
228. Belardinelli R, Mucaj A, Lacalaprice F, et al. Coenzyme Q10 and exercise training in chronic heart failure. *Eur Heart J.* 2006; 27: 2675-81.
229. Mohr D, Bowry VW, Stocker R. Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within flowing lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation. *Biochim Biophys Acta.* 1992; 1126: 247-54.
230. Witting PK, Pettersson K, Letters J, er al. Anti-atherogenic effect of coenzyme Q10 in apolipoprotein E gene knockout mice. *Free Radic Biol Med.* 2000; 29: 295-305.
231. Lee BJ, Huang YC, Chen SJ, et al. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with coronary artery disease. *Nutrition*. 2012; 28: 250-5.
232. McNulty H, Jacob RF, Mason RP. Biologic activity of carotenoids related to distinct membrane physicochemical interactions. *Am J Cardiol.* 2008; 101: 20D-29D.
233. Jialal I, Norkus EP, Cristol L, Grundy SM. beta-Carotene inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta.* 1991; 1086: 134-8.
234. Robbins M, Topol EJ. Inflammation in acute coronary syndromes. *Cleve Clin J Med*. 2002; 69(2): SII130-42.
235. Schwartz SJ, Hadley CW, Clinton SK. The depletion of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. *J Nutr.* 2003; 133: 727-32.

236. Rissanen TH, Voutilainen S, Nyyssonen K, et al. Serum lycopene concentrations and carotid atherosclerosis: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr*. 2003; 77: 133-8.
237. Rissanen TH, Voutilainen S, Nyyssönen K, et al. Low plasma lycopene concentration is associated with increased in-tima-media thickness of the carotid artery wall. *Arterioscler Thromb Vasc Biol*. 2000; 20: 2677-2681.
238. Arranz S, Chiva-Blanch G, Valderas-Martinez P, et al. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. *Nutrients*. 2012; 4: 759-81.
239. Tangney CC, Rasmussen HE, Polyphenols, inflammation, and cardiovascular disease. *Curr Atheroscler Rep*. 2013; 15: 324-34.
240. Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: a highly regulated and dynamic system. *Cytokine*. 2014; 70(1): 11-20.
241. Rose-John, S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci.* 2012;8(9):1237-47.
242. IL6R Genetics Consortium Emerging Risk Factors Collaboration. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*. 2012; 379: 1205–1213.
243. Novick D, Engelmann H, Wallach D, Rubinstein M. Soluble cytokine receptors are present in normal human urine. *J Exp Med.* 1989 ; 170(4): 1409-14.
244. Rose-John S, Scheller J., Elson G., Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *JLB* 2006. https://doi.org/10.1189/jlb.1105674
245. Lust, JA, Donovan KA, Kline MP, et al. Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. *Cytokine* 1992; 4: 96–100.
246. Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S. Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. *J. Immunol.* 1992; 149: 2021–2027.
247. Taga T, Hibi M, Hirata Y, et al. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell*. 1989; 58: 573–581.
248. Jones SA, Richards PJ, Scheller J, Rose-John S. IL-6 transsignaling: the in vivo consequences. *J. Interferon Cytokine Res*. 2005;25:241–253.
249. DeLeo FR. Attractive shedding. *Blood*. 2007; 110(6): 1711-2.

250. Wang XP, Schunck M, Kallen KJ, The interleukin-6 cytokine system regulates epidermal permeability barrier repair in wild-type and IL-6-deficient mice. *J. Invest. Dermatol*. 2004; 123: 124–131.

251. Rose-John, S. Designer cytokines for human haematopoietic progenitor cell expansion: impact for tissue regeneration. *Handb. Exp. Pharmacol.* 2006; 174: 229–247.

252. Hacker C, Kirsch RD, Ju XS, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat. Immunol.* 2003; 4: 380–386.

253. Rose-John S, Neurath M. IL-6 trans-signaling: the heat is on. *Immunity.* 2004; 20: 2–4.

254. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta*. 2011; 1813: 878–88

255. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation*. 2003; 108: 2957–2963.

256. McInnes IB, Thompson L, Giles JT, et al. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. *Ann Rheum Dis*. 2013; 1–9.

257. Ridker PM, Danielson E, Fonseca FAH, et al. JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.

258. The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. *Lancet*. 2012; 379: 1214–1224.

259. Davies R, Choy E. Clinical experience of IL-6 blockade in rheumatic diseases—implications on IL-6 biology and disease pathogenesis. *Semin Immunol.* 2014; 26: 97–104.

260. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101:1767–1772.

261. Anderson DR, Poterucha JT, Mikuls TR, et al. IL-6 and its receptors in coronary artery disease and acute myocardial infarction. *Cytokine*. 2013; 62: 395–400.

262. Gabriel AS, Martinsson A, Wretlind B, Ahnve S. IL-6 levels in acute and post myocardial infarction: their relation to CRP levels, infarction size, left ventricular systolic function, and heart failure. *Eur J Intern Med*. 2004; 15: 523–528.

263. Danesh J, Kaptoge S, Mann AG. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med.* 2008; 5: e78.

264. Sattar N, Murray HM, Welsh P. Are markers of inflammation more strongly associated with risk for fatal than for nonfatal vascular events? *PLoS Med.* 2009; 6: e1000099.

265. Keavney B, Danesh J, Parish S. Fibrinogen and coronary heart disease: test of causality by "Mendelian randomization" Int J Epidemiol. 2006; 35: 935–943.

266. Bacchiega BC, Bacchiega AB, Usnayo MJG, Interleukin 6 Inhibition and Coronary Artery Disease in a High-Risk Population: A Prospective Community-Based Clinical Study. *J Am Heart Asso*c. 2017; 6(3): e005038.

267. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004; 109(suppl II): II-2–II-10.

268. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res*. 2002; 91: 988–998.

269. Fearon WF, Fearon DT. Inflammation and Cardiovascular Disease; Role of the Interleukin-1 Receptor Antagonist. *Circulation* 2008; 117: 2577–2579.

270. Pober JS, Bevilacqua MP, Majeau GR, et al. Interleukin-1 acts on cultured human vascular endothelium to elevates the adhesion of polymorphonuclear monocytes, leukocytes, and related WBC cell lines. *J .Clin.Invest.* 1985; 76: 2003.

271. Cavender DE, Haskard DO, Joseph B, and Ziff M. Interleukin 1 increases the binding of human B and T lymphocytes to endothelial cell monolayers. *J.Immunol.* 1986; 136: 203.

272. Pohlman TH, Stanness PG, Beatty HD, et al . A n endothelial cell surface factor(s) prompted in vitro by lipopolysaccharide, IL-1, TNF-alpha elevates neutrophil adherence by a CDw 18- dependent mechanism. *J.Immunol*. 1986; 136: 4548.

273. Bevilacqua MP, Pober JS, et al. Interleukin-I (IL-1) induces biosynthesis and cell surface expression of proco- agulant activity in human vascular endothelial cells. *J.Exp.Med.* 1984; 160: 618-623.

274. Nachman RL, Hajjar KA, R .L .et al. Interleukin 1induces endothelial cell synthesis of plasminogen activator inhibitor. *J.Exp.Med*. 1986 ;163: 1595.

275. Kooistra J, and Emeis JJ. Interleukin 1 and lipopolysaccharide induce an inhibitor of tissue-type plasminogen activator in vivo and in cultured endothelial cells. *J.Exp.Med.* 1986; 163: 1260.

276. Doria A, Zen M, Bettio S, et al. Autoinflammation and autoimmunity: bridging the divide. *Autoimmun Rev*. 2012; 12: 22–30.

277. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood*.1996; 87: 2095–2147.

278. Tabrizi AR, Zehnbauer BA, Freeman BD, et al. Genetic markers in sepsis. *J Am Coll Surg*. 2001; 192: 106–17.

279. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. *Clin Infect Dis.* 2002; 34: 204–209.

280. Dewberry R, Holden H, Crossman D, and Francis S. Interleukin-1 receptor antagonist expression in human endothelial cells and atherosclerosis. *Arterioscler Thromb Vasc Bio.* 2000; 20: 2394–2400.

281. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood*. 2011; 117: 3720–32.

282. Dinarello CA, Ikejima T, Warner SJC, et al. Interleukin-1 induces interleukin-1. I. Induction of circulating interleukin-1 in rabbits in vivo and in human mononuclear cells in vitro. *J Immunol.* 1987; 139: 1902–1910.

283. Warner SJC, Auger KR, Libby P. Interleukin-1 induces interleukin-1. II. Recombinant human interleukin-1 induces interleukin-1 production by adult human vascular endothelial cells. *J Immunol.* 1987; 139: 1911–7.

284. Geng Y-J, Libby P. Evidence for apoptosis in advanced human atheroma. Co-localization with interleukin-1 b-converting enzyme. *Am J Pathol* 1995; 147: 251–66.

285. Latz E, Xiao TS, Stutz A. Activation and regu- lation of the inflammasomes. *Nat Rev Immunol.* 2013; 13: 397–411.

286. Patel MN, Carroll RG, Galvan-Pena S, et al. Inflammasome priming in sterile inflammatory disease. *Trends Mol Med.* 2017; 23: 165–80.

287. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol.* 2007; 7: 31–40.

288. Martinon F, Petrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature.* 2006; 440: 237–41.

289. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature.* 2010; 464: 1357–61.

290. Rajamaki K, Lappalainen J, Oorni K, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link be- tween cholesterol metabolism and inflammation. *PLoS One* 2010;5:e11765.

291. Paramel Varghese G, Folkersen L, Strawbridge RJ, et al. NLRP3 inflammasome expression and activation in human atherosclerosis. *J Am Heart Assoc* 2016;5.

292. Folco EJ, Sukhova GK, Quillard T, Libby P. Moderate hypoxia potentiates interleukin-1beta production in activated human macrophages. *Circ Res* 2014;115:875–83.

293. Rajamaki K, Nordstrom T, Nurmi K, et al. Extracellular acidosis is a novel danger signal alerting innate immunity via the NLRP3 inflammasome. *J Biol Chem* 2013; 288: 13410–9.

294. Chamberlain J, Evans D, King A, et al. Interleukin-1beta and signaling of interleukin-1 in vascular wall and circulating cells modulates the extent of neointima formation in mice. *Am J Pathol.* 2006; 168: 1396–1403.

295. Alexander MR, Moehle CW, Johnson JL, et al. Genetic inactivation of IL-1 signaling enhances atherosclerotic plaque instability and reduces outward vessel remodeling in advanced atherosclerosis in mice. *J Clin Invest*. 2012; 122:70–79.

296. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol.* 2011; 11: 98–107.

297. Chamberlain J, Francis S, Brookes Z, et al. Interleukin-1 regulates multiple atherogenic mechanisms in response to fat feeding. *PLoS One.* 2009; 4: e5073.

298. Ikonomidis I, Andreotti F, Economou E, et al. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation*. 1999; 100: 793–798.

299. Ikonomidis I, Lekakis J, Revela I, et al. Increased circulating C-reactive protein and macrophage-colony stimulating factors are complementary prophets of long-standing consequence in patients with chronic coronary artery disease. *Eur Heart J.* 2005; 26: 1618–1624.

300. Saitoh T, Kishida H, Tsukada Y, et al. Clinical significance of increased plasma concentration of macrophage colony-stimulating factor in patients with angina pectoris. *J Am Coll Cardiol*. 2000; 35: 655–665.

301. Fantuzzi G, Liuzzo G, Biasucci LM, et al. High levels of interleukin (IL)-1Ra and IL-6 among the first 2 days of hospitalization in unbalanced angina are associated with increased risk of in-hospital coronary events.*Circulation*. 1999; 99:2079–2084.

302. Biasucci LM, Vitelli A, Liuzzo G, et al. Elevated levels of interleukin-6 in unstable angina. *Circulation*. 1996; 94: 874–877.

303. Mezzaroma E, Toldo S, Farkas D, et al. The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. *Proc Natl Acad Sci U S A*. 2011; 108: 19725–19730.

304. Mezzaroma E, Toldo S, Abbate A. Role of NLRP3 (cryopyrin) in acute myocardial infarction. *Cardiovasc Res.* 2013; 99: 225–226.

305. Benjamin W, Van T, Toldo S, et al. Targeting Interleukin-1 in Heart Disease. *Circulation.* 2013; 128: 1910–1923

306. Brydges SD, Mueller JL, McGeough MD, et al. Inflammasome-mediated disease animal models reveal roles for innate but not adaptive immunity. *Immunity*. 2009; 30: 875–887.

307. Toldo S, Mezzaroma E, Van Tassell BW, et al. Interleukin-1ß blockade improves cardiac remodelling after myocardial infarction without interrupting the inflammasome in the mouse. *Exp Physiol*. 2013; 98: 734–745.

308. Loppnow H, Libby P. Proliferating or inter- leukin 1-activated human vascular smooth muscle cells secrete copious interleukin-6. *J Clin Invest* 1990;85:731–8.

309. Le JM, Vilcek J. Interleukin 6: a multifunc- tional cytokine regulating immune reactions and the acute phase protein response. *Lab Invest*. 1989; 61: 588–602.

310. Castell JV, Gomez-Lechon MJ, David M, et al. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. Hepatology. 1990; 12: 1179–86.

311. Sarwar N, Butterworth AS, Freitag DF, et al., for the IL6R Genetics Consortium Emerging Risk Factors Collaboration. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*. 2012; 379: 1205–13.

312. Hingorani AD, Casas JP, for the Interleukin-6 receptor mendelian randomisation analysis (IL6R MR) consortium. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* 2012;379:1214–24.

313. Suzuki K, Murtuza B, Smolenski RT, et al. Overexpression of interleukin-1 receptor antago- nist provides cardioprotection against ischemia- reperfusion injury associated with reduction in apoptosis. *Circulation*. 2001; 104: I308–13.

314. Abbate A, Salloum FN, Van Tassell BW, et al. Alterations in the interleukin-1/interleukin-1 receptor antagonist balance modulate cardiac remodeling following myocardial infarction in the mouse. *PLoS One* 2011; 6: e27923.

315. Hong DS, Janku F, Naing A, et al. Xilonix, a novel true human antibody targeting the inflammatory cytokine interleukin-1 alpha, in non-small cell lung cancer. *Invest New Drugs.* 2015; 33: 621–31.

316. Lachmann HJ, Kone-Paut I, Kuemmerle- Deschner JB, et al. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med.* 2009; 360: 2416–25.

317. Ruperto N, Brunner HI, Quartier P, et al. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. *N Engl J Med.* 2012; 367: 2396–406.

318. Schlesinger N, Alten RE, Bardin T, et al. Can- akinumab for acute gouty arthritis in patients with limited treatment options: results from two randomised, multicentre, active-controlled, double-blind trials and their initial extensions. *Ann Rheum Dis*. 2012; 71: 1839–48.

319. Turhan S, Erol Ç. Kardiyovasküler hastalıklarda epidemiyoloji. In: Gliçin G, Biberoğlu K, Süleymanlar G, Ünal S. Eds. Iç hastalikları, 2. Baskı, Ankara: Güneş Kitabevi, 2005; 241-243.

320. Cantin B, Gagnon F, Moorjani S, Despres JP, et al. Is lipoprotein an independent risk factor for ischemic heart disease in men The Quebec Cardiovascular Study. *JACC.* 1999; 31: 519-525.

321. Morris JA and Gardner MJ. Calculating confidence intervals for relative risks and standardised ratios and rates. *Br. Med. J*.1988; 296: 1313-1456.

322. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, et al. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the European side . *BMJ*, 1996; 313: 84-90.

323. Hacettepe University Institute of Population Studies. Turkey Demographic and Health Survey, 2013, Hacettepe University Institute of Population Studies. Ankara, Turkey; T.R. Ministry of Development and TÜBITAK; 2014

324. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. *Ann Intern Med*. 2003; 138: 891-897.

325. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol*. 2002; 89: 1117-1119.

326. Price JF, Mowbray PI, Lee AJ, et al. Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial discase and coronary artery disease: Edinburgh. Artery study. *Eur Heart J.* 1999; 20: 344-353.

327. Powell JT. Smoking Epidemiology of Peripheral Vascular Disease. Berlin: Springer-Verlag. 1991; 141-53.

328. Mark DE, Marcus SC. Reactive Oxygen Species And Death: Oxidative DNA Damage In Atherosclerosis. *Circ Res.* 1995; 41:139-146. 24.

329. Ezzati M, Henley J, Thun MJ, Lopez AD. Role of smoking in global and regional cardiovascular mortality. *Circulation.* 2005; 112: 489-497.

330. World Health Organization. World Health Report 1998, Translation edited by: Metin B, Akın A, Güngör İ. Turkish Ministry of Health, Department of Foreign Relations, Ankara, 1998;53.

331. Barua RS, Ambrose JA, Srivastava S. Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an in vitro demonstration in human coronary artery endothelial cells. *Circulation*. 2003; 107(18): 2342-2347.

332. Smith FB, Lowe GDO, Fowkes FGR. Smoking haemostatic factors and lipid peroxides in a population case control study of peripheral arterial diseases. *Atherosclerosis*.1993; 102: 155-162.

333. Simon C, Earl SF, Janet BC , Julia C. Subsequently developed and refined a diabetic and CHD mortality model. *JAMA.* 2010; 88(2): 120-130.

334. Muller DC, Elahi D, Tobin JD, Andres R. The effect of age on insulin resistance and secretion: a review. *Semin Nephrol*. 1996; 16: 289-298.

335. Kleemann R, Zadelaar S, Kooistra T. Cytokines and atherosclerosis: a comprehensive review of studies in mice. *Cardiovascular Research*. 2008; 79: 360

336. Stern M. Diabetes And Cardiovascular Disease. The "Common Soil" Hypothesis. *Diabetes.* 1995; 44(4): 369-74.

337. Barber C, Aragaki A, Rivera-Chavez F, Purdue J, Hunt W. TLR4 and TNF-alpha polymorphisms are associated with an increased risk for severe sepsis following burn injury. *J. Med. Genet*. 2004; 808-813.

338. Nathan DM, Cleary PA, Backlund JY, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med.* 2005; 353(25): 2643-2653.

339. Gerick JE, John E. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev*. 1998; 19: 491-503.

340. Libby, P. Changing concepts of atherogenesis. *JIM*. 2000; 247: 349-358.

341. Robbins CS, Hilgendorf I, Weber GF, Theurl I, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nature medicine*. 2013; 19: 1166–1172.

342. Spriggs S, Deutsch DW. Genomic structure, induction, and production of TNF-alpha. *Immunol. Ser*.1992; 334

343. Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest.* 1997; 99: 2075–2081.

344. Karabina SA, Liapikos TA, Grekas G, Goudevenos J, Tselepis AD. Distribution of PAF-acetylhydrolase activity in human plasma low-density lipoprotein subfractions. Biochim Biophys Acta, 1994; 1213: 34-38.

345. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ. Schaefer EJ, Wilson PWF, D'Agostino RB, Vasan RS, Robins SJ. Increased small low-density lipoprotein particle number: A prominent feature of the metabolic sydrome in the Framingham Heart Study. *Circulation.* 2006; 113: 20-29.

346. Gonzales TK, Vargas-Barrón J, Vallejo M Myocardial infarction in the Wisconsin Longitudinal Study: the interaction among environmental, hcalth, social. behavioural and genetic factors. *BMJ.* 2013; 7: 1152.

347. Christensen JJ, Osnes LT, Halvorsen B, Retterstol K, et al. Altered leukocyte distribution under hypercholesterolemia: A cross-sectional study in children with familial hypercholesterolemia. *Atherosclerosis.* 2017; 256: 67–74.

348. Van der Valk FM, Kuijk C, Verweij SL, Stiekema LCA, et al. Increased haematopoietic activity in patients with atherosclerosis. *European heart journal.* 2017; 38: 425–432.

349. Kruth, HS. Fluid-phase pinocytosis of LDL by macrophages: a novel target to reduce macrophage cholesterol accumulation in atherosclerotic lesions. *Current pharmaceutical design.* 2013; 19: 5865–5872.

350. Peters DL, Barber RC, Flood EM. Methodologic quality and genotyping reproducibility in studies of tumor necrosis factor -308 G-A single nucleotide polymorphism and bacterial sepsis: implications for studies of complex traits. *Crit Care Med.* 2003: 31(6): 1691-6.

351. Madamanchi NR, Hakim ZS, Runge MS.Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. *J. Thromb Haemost.* 2005; 3: 254-267.

352. Münzel T, Gori T, Keaney JF, et al. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. *Eur Heart J.* 2015; 36: 2555-2564.

353. Yaoita H, Ogawa K, Maehara K, et al. Apoptosis in relevant clinical situations: contribution of apoptosis in myocardial infarction. *Cardiovasc Res.* 2000; 45: 630–641.

354. 414 Abbate A, Van Tassell BW, Biondi-Zoccai G, et al. Effects of interleukin-1 blockade with ana- kinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) (VCU-ART2) pilot study]. *Am J Cardiol.* 2013; 111: 1394–400.

355. 415 Abbate A, Dinarello CA. Anti-inflammatory therapies in acute coronary syndromes: is IL-1 blockade a solution? *Eur Heart J*. 2015; 36: 337–9.

356. Bemic S, Ann C, Nancy S, Amy R, Xiao-Y. BiEing and Functional Comparisons of Two Types of Tunor Necerosis Factor Autaigonists Jourmail of Pharmacolegy and Experimen Therapentics 2002; 301(2): 4IS-426.

357. Beutler B, Krochin N. Milsark IW. Lncdike C. Cenam A Control of cachectin (tumor necrosis factor) synthesis: mech of cndotoxin nesistance. *Seience*. 2006; 3(3):

7. APPENDICES

7.1. ETHICAL APPROVAL

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1811 **30/01/2020**
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makama (Dilveen Dlawer Omer)

Yeditepe Üniversitesi Moleküler Tıp ABD Prof. Dr. Turgay İsbir'in Proje Koordinatörü olduğu Kardiyoloji ABD Dr. Öğr. Üyesi Ayça Türer Cabbar'ın sorumlu araştırmacı olduğu **"İnsan İnflamator Sitokinleri (IL – 1ß, IL-6) Kardiyovasküler Hastalıklara Etkisinin İncelenmesi Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1793)** kayıt Numaralı KAEK Başvuru Dosyası,Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **29.01.2020** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 1152**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
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7.2. FORMS

Biyolojik Materyal Transfer Formu 1
(Biological Material Transfer Form)
01.09.2015 Versiyon 1.0

KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER ANLAŞMASI

Araştırmanın Açık Adı: İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesi

Protokol Numarası :

Araştırmanın Özeti :

Kardiyovasküler hastalıklara bağlı ölümler dünya genelinde son 30 yılda azalmakla birlikte Kuzey Amerika ve Avrupa'da halen en sık ölüm nedenleri arasında yer almaktadır. Türk Kardiyoloji Derneği öncülüğünde 1990 yılından beri yürütülen TEKHARF (Türk EriÇkinlerinde Kalp Hastalığı ve Risk Faktörleri) çalıÇması verilerine göre, Türkiye'de iki milyon koroner kalp hastasının bulunduğu ve yılda 160.000 bireyin koroner kalp hastalığından öldüğü tahmin edilmektedir. TEKHARF izlem raporunda koroner arter hastalığı (KAH) sıklığı, erişkin nüfusta % 2.7, 45-74 yaş arasında ise %10.2 olarak bildirilmektedir.

Oluşturduğu komplikasyonlarla insan hayatını en fazla tehdit eden hastalıklar arasında yer alan ateroskleroz, başlangıçta sadece akım sınırlayıcı stenozlar ile karakterize iken, ilerleyen dönemlerde aterosklerotik plağın parçalanmasıyla trombüslere yol açarak miyokard infarktüsü, inme ve abdominal aort anevrizmaları gibi insan hayatını tehdit eden ciddi komplikasyonlara yol açabilmektedir. Plak oluşumunda, ilerlemesinde ve yırtılmasında, inflamatuar hücrelerin, inflamatuar proteinlerin ve damar hücrelerinin inflamatuar yanıtlarının önemli rolleri vardır. Sitokinler, immünolojik, lokal veya sistemik inflamatuar ve onarıcı konak cevabını düzenleyen ve hücreler arasında sinyal görevi gören biyolojik mediyatörlerdir. Tek bir çeşit sitokinin aynı anda pek çok hücre tipi üzerinde büyüme ve farklılaşma gibi multipl etkileri olabilir. Çok düşük konsantrasyonlarda bile etkinlik gösterebilen ve genel anlamda biyolojik yanıt değiştirici moleküller olan sitokinlerin kardiyovasküler olaylarla ilişkisi halen araştırılmaktadır. Günümüzde inflamasyonla ilişkili 50'ye yakın sitokin tanımlanmış ve bunların önemli bir kısmına insan aterosklerotik plaklarında saptanmıştır. Çalışmamızda, İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesini amaçladık.

İşbu anlaşma ile biyolojik materyali gönderen araştırmacı ve kurum "**İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesi** " isimli araştırmada kullanılmak üzere gönderilecek "**5ml ve araştırma**" amacı ile kullanılması üzere **26 Ağustos Yerleşkesi Yeditepe Üniversitesi Kayışdağı / İSTANBUL** adresindeki **Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı** ' ndaki merkezine göndermeden önce GONDERİCİ ve ALICI'dan aşağıdaki koşulları kabul etmesi istenmektedir;

1. Gönderilen biyolojik materyaller yalnızca yukarıda yazılı amaç için kullanılabilir. ALICI biyolojik materyallerin alınma amacından dışında ikincil amaç için kullanılma isteklerini GÖNDERİCİ'ye yazılı olarak bildirecektir.
2. Biyolojik materyaller ALICI'ya gönderilmeden önce biyolojik materyalin sağlandığı gönüllülere ait Türkiye İlaç ve Tıbbi Cihaz Kurumu ve Etik Kurul'un onayladığı tüm kullanım amaçlarına yönelik olarak düzenlenmiş bilgilendirilmiş gönüllü olur formunun her bir gönüllüden alınmış olması gerekmektedir.
3. ALICI biyolojik materyali GÖNDERİCİ'nin yazılı izni olmadan üçüncü kişi/kurumlara vermeyecektir.
4. Biyolojik materyaller GÖNDERİCİ tarafından bireyin kimlik ve tanımlayıcı bilgileri olmaksızın ALICI'ya gönderilecektir.
5. ALICI biyolojik materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel

AGREEMENT FOR TRANSFER OF BIOLOGICAL MATERIAL TO BE USED IN CLINICAL TRIALS

Full name of the clinical trial : Investigation of The Effect of Human Inflammatory Cytokines (IL-1β, IL-6) on cardiovascular diseases
Protocol code :

Summary of the clinical trial:

Although deaths related to cardiovascular diseases have decreased in the last 30 years worldwide, they are still among the most common causes of death in North America and Europe. Turkish Society of Cardiology pioneered conducted since 1990 TEKHARF (Heart Disease and Risk Factors in Turkish eriğkin) According to Study data, which two million coronary heart disease was found and 160,000 people in a year are estimated to have died from coronary heart disease in Turkey. The incidence of coronary artery disease (CAD) is reported to be 2.7% in the adult population and 10.2% in the 45-74 age groups.

Atherosclerosis, which is one of the most life-threatening diseases due to its complications, is initially characterized only by flow-limiting stenosis. The inflammatory responses of inflammatory cells, inflammatory proteins, and vascular cells have important roles in plaque formation, progression and tearing. Cytokines are biological mediators that regulate immunological, local or systemic inflammatory and restorative host response and serve as signals between cells. A single type of cytokine can have multiple effects such as growth and differentiation on many cell types at the same time. The association of cytokines, which are generally biologically responsive molecules, which can show efficacy even at very low concentrations, is still under investigation. Currently, about 50 cytokines associated with inflammation have been identified and a significant portion of them have been detected in human atherosclerotic plaques. In this study, we aimed to investigate the effect of human inflammatory cytokines (IL-1β, IL-6) on cardiovascular diseases.

By this agreement, the investigator and the institution who send the biological material requires the CONSIGNEE and CONSIGNOR to agree on the below terms before sending *(Specify biological material type and amount)* which shall be used for to be dispatched to the address of .

1. Delivered biological materials shall be used only for the above-mentioned purposes. CONSIGNEE shall use those materials only for secondary purposes, which are initially approved by the CONSIGNOR in written.
2. Prior to the dispatch of the biological materials to the CONSIGNEE, Turkish Medicines and Medical Devices Agency and Ethics Committee approved informed consent forms, which belong to the persons for whom the biological material is provided, should be obtained. This consent form should explain all the purposes of use of the biological samples.
3. CONSIGNEE cannot provide the biological material to the third parties without prior written approval of the CONSIGNOR.
4. Biological materials shall be dispatched by the CONSIGNOR to the CONSIGNEE without the identity or any descriptive information of the individuals.
5. CONSIGNEE shall use the biological materials in accordance with as the United Nations Human Genome and Universal Declaration of Human Rights.

5. ALICI biyolojik materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel Beyannamesine uygun olarak kullanacaktır.
6. Bu anlaşma ile gönderilecek biyolojik materyalin araştırma için kullanılacak olduğu ve biyolojik materyal kullanımına ait risklerin var olduğu ALICI tarafından kabul edilmektedir. Söz konusu risklere karşı uygun önlemlerin alınması gerekmektedir.
7. GÖNDERİCİ ve ALICI gönderilen biyolojik materyalin herhangi bir şekilde ticari kazanç kaynağı olarak kullanılamayacağı ancak elde edilebilecek fikri mülkiyet ve patent haklarının bu durumdan istisna olduğu kabul etmektedir. GÖNDERİCİ ve ALICI söz konusu haklarını araştırma başlangıcında karşılıklı olarak belirleyecektir.
8. ALICI bu anlaşmanın sonlanması veya biyolojik materyalin sağlandığı gönüllülere ait anlaşmanın 2. maddesinde belirtilen olurun geri çekilmesi halinde bütün materyalleri geri vermeyi veya ortadan kaldırmayı ve bunu belgelemeyi kabul eder.
9. Bu anlaşma, araştırmanın sonlanması, ilgili mevzuat hükümlerine uyulmaması veya ilgili tarafların anlaşma hükümlerine uymaması durumlarında son bulacaktır.
10. Bu anlaşmanın yürütülmesinde ALICI ve GÖNDERİCİ yetkilileri sorumludur. Anlaşmazlık halinde ihtilafın çözümü için her iki ülke mahkemeleri de yetkilidir.

5. CONSIGNEE shall use the biological materials in accordance with as the United Nations Human Genome and Universal Declaration of Human Rights.
6. CONSIGNEE acknowledges and agrees that the biological materials to be dispatched under this agreement shall be utilized for research purposes and have some risks associated with their usage. Appropriate preventive actions should be taken for those risks.
7. CONSIGNOR and CONSIGNEE shall mutually agree that the biological materials cannot be used as a source for any commercial profit and the rights relating to a joint publication or a patent right that may arise may be the only exception for that. CONSIGNOR and CONSIGNEE shall mutually agree on those rights prior to trial initiation.
8. CONSIGNEE agrees to return or dispose of all materials and to evidence such acts accordingly in the event of termination of the agreement or withdrawal of written consent of the volunteer referred in Item 2.
9. This agreement shall be terminated in the event of, termination of the trial, violation on the terms of related regulations or noncompliance with agreement clauses of either of the parties.
10. CONSIGNEE and CONSIGNOR shall be responsible from the execution of this Agreement and performances hereunder. In case of conflict, both countries of the parties' courts are authorized.

BİYOLOJİK MATERYALİ GÖNDEREN ARAŞTIRMACI BİLGİSİ

Adı Soyadı ve Unvanı	: Dr.Öğr.Üyesi Ayça Türer Cabbar
Uzmanlık Alanı	: Kardiyoloji
Kurumu	: Yeditepe Üniversitesi Hastanesi
Adresi	: İçerenköy Mahallesi Hastane Sokak No:4 D:4/1, 34854 İstanbul
Telefon	: 05333649282
Faks	:
E-posta	:

BİYOLOJİK MATERYALİ ALAN ALICI BİLGİSİ

Adı Soyadı ve Unvanı	: Prof.Dr.Turgay İsbir
Uzmanlık Alanı	: Biyokimya
Kurumu	: Yeditepe Üniversitesi
Adresi	: İnönü Mah. Kayışdağı Cad. Teditepe Üniversitesi 26 Ağustos Yerleşkesi Ataşehir
Telefon	: 05332823726
Faks	:
E-posta	: turgay.isbir@yeditepe.edu.tr

Bu anlaşmada belirtilen koşulları okudum ve anladım. Gönderilen materyalde bu anlaşmada belirtilen koşullara uyacağımı taahhüt ederim.

INFORMATION REGARDING THE INVESTIGATOR SENDING THE BIOLOGICAL MATERIAL

Name Surname and Title	:
Specialization	:
Institution	:
Address	:
Telephone	:
Fax	:
E-mail	:

INFORMATION REGARDING THE CONSIGNEE RECEIVING THE BIOLOGICAL MATERIAL

Name Surname and Title	:
Specialization	:
Institution	:
Address	:
Telephone	:
Fax	:
E-mail	:

I read and understood the terms under this agreement. I hereby agree and undertake that I will act in accordance with the terms of this agreement with respect to the dispatched materials.

	CONSIGNOR	CONSIGNEE

Note: Instead of the signature of the consignee representative, a signed "end use certificate" including clauses similar to this agreement's to be issued by the consignee institution may also be accepted.

	GÖNDERİCİ				ALICI
	Gönderen Araştırmacı	Gönderen Destekleyici Firma Yetkilisi veya Yasal Temsilcisi	Eğitim Görevlisi / Ana Bilim Dalı Başkanı	Kurum Amiri / Rektör veya Yetkilendirdiği Makam	Alıcı Kurum Yetkilisi
El Yazısı ile Adı Soyadı Unvanı					
Tarih					
İmza					

Not: Bu anlaşmada yer alan alıcı kurum yetkilisinin imzası yerine alıcı kurum tarafından verilecek olan ve içerik olarak bu anlaşmadaki hükümlere benzer hükümleri içeren imzalı "end use certificate" "son kullanım sertifikası" de kabul edilebilir.

	Consignor Investigator	Consignor Sponsor Company Official or Legal Representative	Chief / Head of the Department	Chief Officer of the Institution / Rector or Assigned Person	Consignee Institution Official
Name Surname and Title in Handwriting					
Date					
Signature					

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	TARİH: 02.01.2020 VERSİYON: 2

Araştırmanın Adı: İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesi

Araştırmaya Katılımcı Sayısı: 80

Bu araştırmanın Amacı: Kardiyovasküler hastalıklara bağlı ölümler dünya genelinde son 30 yılda azalmakla birlikte Kuzey Amerika ve Avrupa'da halen en sık ölüm nedenleri arasında yer almaktadır. Türk Kardiyoloji Derneği öncülüğünde 1990 yılından beri yürütülen TEKHARF (Türk EriGkinlerinde Kalp Hastalığı ve Risk Faktörleri) çalıGması verilerine göre, Türkiye'de iki milyon koroner kalp hastasının bulunduğu ve yılda 160.000 bireyin koroner kalp hastalığından öldüğü tahmin edilmektedir. TEKHARF izlem raporunda koroner arter hastalığı (KAH) sıklığı, erişkin nüfusta % 2.7, 45-74 yaş arasında ise %10.2 olarak bildirilmektedir.

Oluşturduğu komplikasyonlarla insan hayatını en fazla tehdit eden hastalıklar arasında yer alan ateroskleroz, başlangıçta sadece akım sınırlayıcı stenozlar ile karakterize iken, ilerleyen dönemlerde aterosklerotik plağın parçalanmasıyla trombüslere yol açarak miyokard infarktüsü, inme ve abdominal aort anevrizmaları gibi insan hayatını tehdit eden ciddi komplikasyonlara yol açabilmektedir. Plak oluşumunda, ilerlemesinde ve yırtılmasında, inflamatuar hücrelerin, inflamatuar proteinlerin ve damar hücrelerinin inflamatuar yanıtlarının önemli rolleri vardır. Sitokinler, immünolojik, lokal veya sistemik inflamatuar ve onarıcı konak cevabını düzenleyen ve hücreler arasında sinyal görevi gören biyolojik mediyatörlerdir. Tek bir çeşit sitokinin aynı anda pek çok hücre tipi üzerinde büyüme ve farklılaşma gibi multipl etkileri olabilir. Çok düşük konsantrasyonlarda bile etkinlik gösterebilen ve genel anlamda biyolojik yanıt değiştirici moleküller olan sitokinlerin kardiyovasküler olaylarla ilişkisi halen araştırılmaktadır. Günümüzde inflamasyonla ilişkili 50'ye yakın sitokin tanımlanmış ve bunların önemli bir kısmına insan aterosklerotik plaklarında saptanmıştır. Çalışmamızda, İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesini amaçladık

Süresi: ***12Ay***

İzlenecek Yöntem/Yöntemler:

Araştırmam insan serum örnekleri üzerinde gerçekleştirilecektir. Analiz, Ateroskleroz tanısı konulan hastalardan alınan 40 serum örneğinde yapılacaktır. Serum numuneleri santrifüj edildikten sonra -80°C'de dondurulacak ve test zamanına kadar depolanacaktır. Kontrol grubunun kan örnekleri aynı yöntem ve prosedürler kullanılarak benzer şekilde işlenecek. Numuneler, tanımlanmasını sağlamak ve herhangi bir karışıklığı önlemek için hastaların kayıt numaraları ile etiketlenecektir. İnflamatuar sitokin düzeylerinin ölçülmesinde iki katlı sandviç ELISA yöntemi kullanılacaktır. Seviyeleri, fotometrideki standart eğriye kıyasla renk değişimlerini okuyarak belirlenecektir.

İstatistiksel analizler için IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) paket programı kullanılacaktır. İstatistiksel anlamlılık $p<0.05$ olarak kabul edilir. Toplanan veriler sayılar, yüzdeler ve ortalama±standart sapma olarak özetlenereki Chi-Kare analizi verilerini değerlendirmede kullanılacaktır. Kardiyovasküler hastalığa sahip bireylerden ve kontrol gruplarından toplanan kantitatif parametreler student t-testi kullanılarak analiz edilecektir

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	TARİH: 02.01.2020 VERSİYON: 2

Araştırma Sonunda Beklenen Fayda
Çalışmamızda beklenen fayda; İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin belirlenmesi olacaktır

Bu Çalışmada Herhangi Bir Alternatif Tedavi yada Girişimde Bulunulmayacaktır.
Bu Araştırma Gönüllüler İçin Hiçbir Risk Teşkil Etmemekte ve Hiçbir Rahatsızlığa Sebep olmamaktadır.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim. Tarafımdan alınan kan ve doku örneklerinin daha sonra başka araştırma çalışmalarında kullanılmasında herhangi bir sakınca yoktur.

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün:

Adı Soyadı:

İmzası:

Tarih:

Açıklamaları Yapan Kişinin:

Adı Soyadı:

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	TARİH: 02.01.2020 VERSİYON: 2

İmzası:

Tarih:

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Yasal Temsilcinin :

Adı Soyadı:

İmzası:

Tarih:

Klinik Araştırma Proje Koordinatörü İletişim Bilgileri:

Adı Soyadı:
Uzmanlık alanı:
Kurumu:
E-posta adresi:
Telefon numarası:

Sorumlu Araştırmacı: Dr.Öğr.Üyesi Ayça Türer Cabbar

	YEDİTEPE ÜNİVERSİTESİ OLGU RAPOR FORMU

ÇALIŞMANI ADI: **İnsan inflamator sitokinleri (IL-1β, IL-6) kardiyovasküler hastalıklara etkisinin incelenmesi araştırılması**

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Grupları için;

- Gönüllü Olma
- Koroner Arter hastası olma
- 40 – 60 yaş aralığında olma

Yukarıda belirtilenler haricinde bir hastalığa sahip olmama

Kontrol Grupları için,

- Gönüllü olma
- Sağlıklı olma (yukarıda belirtilenlerde dahil olmak üzere hiçbir hastalığa sahip olmama)
- 40 – 60 yaş aralığında olma

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü olmama
- 40 – 60yaş aralığı dışında olma
- Yukarıda belirtilenler dışında bir hastalığa sahip olma

7.3. RAW DATA

TABLES= CASE BY (GENDER -DIABETES –SMOKING)

Case*Gender **Crosstab**

			Gender		
			Female	Male	Total
case	control	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
	patient	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
Total		count	24	24	48
		%within case	50.0%	50.0%	100.0%
		%within gender	100.0%	100.0%	100.0%
		%of total	50.0%	50.0%	100.0%

Chi-Square Tests

Value		df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.000	1	1.000		
Continuity Correction[b]	0.000	1	1.000		
Likelihood Ratio	0.000	1	1.000		
Fisher's Exact Test				1.000	0.613
Linear-by-Linear Association	0.000	1	1.000		
N of Valid Cases	48				

Risk Estimate

		95% Confidence Interval	
	value	Lower	Upper
Odds Ratio for case (control/patient)	1.000	0.323	3.101
For Cohort gender= Female	1.000	0.568	1.761
For cohort gender= Male	1.000	0.568	1.761
N of Valid Cases	48		

Case*Diabetes

Crosstab

			Diabetes		
			No	Yes	Total
case	control	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
	patient	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
Total		count	24	24	48
		%within case	50.0%	50.0%	100.0%
		%within gender	100.0%	100.0%	100.0%
		%of total	50.0%	50.0%	100.0%

Chi-Square Tests

Value		df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.000	1	1.000		
Continuity Correction[b]	0.000	1	1.000		
Likelihood Ratio	0.000	1	1.000		
Fisher's Exact Test				1.000	0.613
Linear-by-Linear Association	0.000	1	1.000		
N of Valid Cases	48				

Risk Estimate

		95% Confidence Interval	
	value	Lower	Upper
Odds Ratio for case (control/patient)	1.000	0.323	3.101
For Cohort diabetes= no	1.000	0.568	1.761
For cohort diabetes= yes	1.000	0.568	1.761
N of Valid Cases	48		

Case*Smoking

Crosstab

			Smoking		
			No	Yes	Total
case	control	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
	patient	count	12	12	24
		%within case	50.0%	50.0%	100.0%
		%within gender	50.0%	50.0%	100.0%
		%of total	25.0%	25.0%	50.0%
Total		count	24	24	48
		%within case	50.0%	50.0%	100.0%
		%within gender	100.0%	100.0%	100.0%
		%of total	50.0%	50.0%	100.0%

Chi-Square Tests

Value		df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.000	1	1.000		
Continuity Correction[b]	0.000	1	1.000		
Likelihood Ratio	0.000	1	1.000		
Fisher's Exact Test				1.000	0.613
Linear-by-Linear Association	0.000	1	1.000		
N of Valid Cases	48				

Risk Estimate

		95% Confidence Interval	
	value	Lower	Upper
Odds Ratio for case (control/patient)	1.000	0.323	3.101
For Cohort smoking= no	1.000	0.568	1.761
For cohort smoking= yes	1.000	0.568	1.761
N of Valid Cases	48		

T-TEST GROUPS

Group Statistics

	Case	N	Mean	Std. Deviation	St. Error Mean
Cholesterol	Control	24	1.903	48.738	9.948
	Patient	24	1.791	23.313	4.758
Triglycerides	Control	24	1.315	32.963	6.728
	Patient	24	1.349	49.329	10.069
LDL	Control	24	1.235	43.474	8.874
	Patient	24	1.130	22.799	4.653
HDL	Control	24	36.125	5.501	1.122
	Patient	24	38.833	5.968	1.218

Independent Sample Test

		Lavene's Test for Equality of Variances		T-test for Equality Means	
		F	Sig.	t	df
Cholesterol	Equal variances assumed	13.527**	0.001	1.018	46
	Equal variances not assumed			1.018	33.002
Triglycerides	Equal variances assumed	0.948	0.335	-0.286-	46
	Equal variances not assumed			-0.286-	40.126
LDL	Equal variances assumed	9.412**	0.004	1.051	46
	Equal variances not assumed			1.051	34.762
HDL	Equal variances assumed	0.033	0.856	-1.635-	46
	Equal variances not assumed			-1.635-	45.698

ONE WAY "CHOLESTROL"" TRIGLYCERIDES"" HD""L LDL "BY IL-1β

ANOVA

		Sum of Squuares	df	Mean Square	F	Sig.
Cholesterol	Between Groups	2210.549	2	11105.274	0.749	0.479
	Within Groups	66438.904	45	1476.420		
	Total	68649.453	47			
Triglycerides	Between Groups	1508.302	2	755.151	0.426	0.655
	Within Groups	79596.177	45	1768.804		
	Total	81104.479	47			
LDL	Between Groups	5180.973	2	2590.436	0.260	0.116
	Within Groups	51575.332	45	1146.118		
	Total	56756.205	47			
HDL	Between Groups	172.982	2	86.491	2.721	0.077
	Within Groups	1430.498	45	31.789		
	Total	1603.479	47			