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“Association of Tumor Necrosis Factor (TNF) Alpha and Beta polymorphism in Coronary Artery Disease”

DOCTOR OF PHILOSOPHY THESIS

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DECLARATION

I have gained all the information in this thesis in academic and ethical rules, I have sourced all the information and interpretations not obtained by this thesis, and I have taken these sources to the list of sources, again, I have learned that this thesis work is my own work, from the planning of the thesis until the writing, I declare that there is no violation of the patent and copyrights during the study and writing of this thesis.

M.D.Mohanad FATLAWI

DEDICATION

This Belong to the honour of Molecular Medicine in Turkey, My Precious Teacher Prof. Dr. Turgay Isbir.

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As my life is at every stage, I would like to thank my family my father Adnan Fatlawi and my mother who continuously support me spiritually during this study.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

DM : Diabetes Mellitus

HMG-CO : 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase

EDTA : Ethylene Diamine Tetraacetic Acid

HLA : Human Leukocyte Antigen

LTA : Lymphotoxin Alpha

SNP : Single Nucleotide Polymorphism

APO B : Apolipoprotein B

MmLDL : Minimal modified LDLIL Interleukin

OX LDL : Oxidized Low-Density Lipoprotein

PTK : Protien Tyrosine Kinase

TNFR1 : Tumor Necrosis Factor Receptor 1

TNFR2 : Tumor Necrosis Factor Receptor 2

SAPK : Stress-Activated Protein Kinases

RT PCR : Real-Time Polymerase Chain Reaction

JNK : c-Jun N-terminal Kinases

NO : Nitric Oxide

MHC II : Major Histocompatibility Complex

OLIG3 : Oligodendrocyte Transcription Factor 3

NCEP ATP III :National Cholesterol Education Program Adult Treatment Panel III

SPSS : Stastistical Package for Social Sciences

NCHS : National Center for Health Statistics

NHANES : National Health and Nutrition Examination Survey

HDL : High -Density Lipoprotein

LDL : Low-Density Lipoprotein

TG : Triglyceride

BMI : Body Mass Index

CRP : C-Reactive Protein

ATP : Adenosine Triphosphate

IFN : Interferon

GM CSF : Granulocyte-Macrophage Colony-Stimulating Factor

PGE2 : Prostaglandin E2

NF-κB : Nuclear Factor Kappa BSCRIP Stanford Coronary Risk Intervention Project

PROCAM : Prospective Cardiovascular Munster

MRFIT : Multiple Risk Factor Intervel Trial

CI : Confidence Interval

OR : Odd ratio

CAD : Coronary Artery Disease

TNF α : Tumor Necrosis Factor Alpha

TNF-β : Tumor Necrosis Factor Beta

TEKHARF : Heart Disease and Risk Factors in Turkish Adults

ITS : Institute of Turkish Studies

TNFAIP3 : TNF Alpha Induced Protein 3

PDG : Platelet-Derived Growth Factor

MCP-1 : Monocyte Chemotactic Protein 1

Mg : Microgram

MI : Myocardial Infarction

ML : Microliter

Fsh : Framingham Hart Study

NHLBI : National Heart Lung and Blood Institute

M : : Mutant

VLDL : Very Low Density Lipoprotein

VEGF : Vascular Endothelial Growth Factor

ÖZET

FATLAWI. M. A. Koroner arter hastalığı tümör nekroz faktörü (TNF) alfa ve beta polimorfizm birliği. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, moleküler tıp bölümü. Doktora tezi. İstanbul, 2017

Koroner Arter Hastalığı veya Ateroskleroz, dinamik ve ilerleyici bir inflamatuar patolojidir ve dünyadaki en sık görüen ölüm nedenidir. Bu çalışmada, Ateroskleroz gelişiminde, ilerlemesinde ve komplikasyonlarında anti-inflamatuar sitokinlerin rolüyle ilgili güncel bilgileri bir araya getirerek, Tümör Nekroz Faktörü (TNF) Alfa ve Beta polimorfizmlerinin KAH riski ile ilişkili olup olmadığı araştırdık. Genotiplendirme, Gerçek Zamanlı Polimeraz Zincir Reaksiyonu temelli teknik ile gerçekleştirilmiştir.

Çalışmamızda koroner arter hastalığı olan hasta grubu (n:50) ile kontrol grubu (n:50) arasında hiperlipidemi, sigara kullanımı, obezite ve diyabet gibi risk faktörlerinin ve Tümör Nekroz Faktörü (TNF) Alfa ve Beta Polimorfizmlerının karşılaştırılması amaçlandı.

Hasta ve kontrol gruplarının karşılaştırılmasında erkek cinsiyet (p=0.0860) ve sigara tüketimi (p=0.110) ve diyabet (p=0.026) risk faktörü olduğu gözlendi. Hasta grubunda total kolestrol (p=0.102) ve LDL (p=0.178) düzeyleri yüksek, HDL (p=0,040) düzeyleri ise daha düşük olarak saptanmıştır.

Hasta ve kontrol örneklerinin Tümör Nekroz Faktörü Alpha (TNFα) genotip dağılımları incelendiğinde gruplar arasında anlamlı olarak farklılık saptanmadı. Homozigot Mutant Genotipin değerlendirmesi hasta grubunda anlamlı seviyede fazla olduğu belirlenmesi (p=0.004). Bu genotipin kardioyovasküler riski 12.2-kat arttırdığı gözlendi (OR=12.250, 95% CI=1.504-99.798; p=0.004). Tümör Nekroz Faktörü (TNF) Beta polimorfizmleri ile KAH arasında bir ilişki saptanmamıştır.

Bireylerin genetik yatkınlığı erken yaşam evrelerinde yeni yaklaşımlarla belirlenebilir ve böylece koroner arter hastalığına karşı alınan yeni tedbirlerle hastalık oluşumunun önlenebileceği izlenimi edinilmiştir.

Anahtar Kelimeler: Ateroskleroz, Koronar Arter Hastalığı, TNF-α, TNF-β, Polimorfizm.

ABSTRACT

FATLAWI. M. A. Association of Tumor Necrosis Factor (TNF) Alpha and Beta polymorphism in Coronary Artery Disease. Yeditepe University Health Sciences Institute, Department of Molecular Medicine. Ph.D. Thesis. İstanbul, 2017.

Coronary Artery Disease or Atherosclerosis is a dynamic and progressive inflammatory pathology and is the major cause of death and disability worldwide. The purpose of this study is to bring together the current information concerning the role of pro- and anti-inflammatory cytokines in the development, progression, and complications of atherosclerosis. The aim of the study was to assess whether these Tumor Necrosis Factor (TNF) Alpha and Beta polymorphisms were related to the risk of CAD or not Genotyping was performed with techniques based on Real Time-Polymerase Chain Reaction.

In our study we compared patient group (n:50) which have coronary artery disease and control group (n:50) about risk factors such as hyperlipidemia, smoking, obesity and diabetes and their association between Tumor Necrosis Factor (TNF) Alpha and Beta polymorphisms and Coronary Artery Disease.

In the comparison of the patient and control groups, male gender (p=0.0860) and cigarette consumption (p=0.110) and diabetes (p=0.026) were assumed to be the risk factors . Total cholesterol (p=0.102) and LDL (p=0.178) levels were high, HDL (p=0,040) levels were found to be lower in patients group.

Tumor Necrosis Factor Alpha (TNFα) genotype was significantly higher in patients group compared to control group. The frequency of the Hemozygote Mutant Genotype was found to be significantly higher in patients compared to controls (p=0.004). The cardiovascular risk for Homozygote Mutant Genotype was 12.2-times increased in patients compared with the control group (OR=12.250, 95% CI=1.504-99.798; p=0.004). No association was found about those Tumor Necrosis Factor (TNF) Beta polymorphisms and CAD between the groups.

Genetic predisposition of individuals can be determined with new approaches in the early life stages and thus disease formation can be prevented with new measures taken against Coronary Artery Disease .

Key Words: Atherosclerosis, Coronary Artery Diseas , TNF-α, TNF-β, Polymorphism, RT-PCR.

1. INTRODUCTION and PURPOSE

Coronary artery disease (CAD) is the most common cause of morbidity and mortality around the world as well as in Turkey. Also, CAD is developed in the large area of atherosclerosis surface (1).

Atherosclerosis is a systemic inflammatory disease. The initial steps of atherogenesis involve cholesterol accumulation in the intima that is thought to mediate recruitment of inflammatory leucocytes, followed by development of a fibro-fatty plaque comprising a lipid core and macrophages that ultimately evolve into lipid-rich foam cells. Process of atherosclerosis takes several years to develop coronary artery disease, when the Coronary artery disease inducing agents are reduced or diagnosed at an early stage, the patient can still maintain a healthy life with primary treatment (2).

For this reason, researchers are working on the prevention of Coronary artery disease and also atherosclerosis and new early diagnosis markers. Early detection of silent atherosclerotic plaques and at-risk patients; Myocardial infarction and angiogenesis. If determined, serious CAD could be found detected before any symptoms or complaints . Traditional cardiovascular risk factors are not significant in the majority of patients who actually experience the first myocardial infarction. Therefore, there is a need for new cardiovascular risk markers, their early identification and risk classification (3).

All around the world in 2001, Coronary artery disease (CAD) was responsible for 54% of all cardiovascular deaths . CAD was found to be the most common cause leading to deaths in males and females (more than one in five deaths caused by CAD). In those who are older than 35 years, we are responsible for the incidence of CAD (4, 5). For females, the age-related risk profile was found to be even higher in non-angina outbreaks is because women who had Coronary artery disease often had more moderate risk factors (6).

In Turkey, coronary artery disease and related diseases are similar to other countries in terms of prevalence. In (COHORT) study; adult with CAD was found to be 3.8% and 14% of patients at aged 60-69 years who were diagnosed with the disease as clinically. Again, in relation to the subject, the Turkish Society of Cardiology (TSC) has investigated the atherosclerotic connective tumors (CAD and stroke) in our country, accounting for 43% of all cases (7).

Tumor necrosis factor-alpha (TNF α) plays a key role in pathophysiology of Coronary artery disease and genetic variants of TNF-a promoter region was reported to be increased the (TNF α) serum levels associated with coronary artery disease. These levels are correlated with the first-time CAD and also markers for recurrent coronary events after a previous Coronary Artery Disease (8, 9).

Tumor necrosis factor-alpha polymorphisms is most studied variants of TNF-α, because several studies have found that patients with coronary artery disease the A allele show high levels of (TNF α) - in serum. However, the association between coronary artery

disease and TNF-α genes polymorphisms must be validated using larger and independent sample populations, using case-case control studies, in order to better understand the incidence of cardiovascular events, a case-control study is contemplated (10).

Tumor necrosis factor-beta (TNF -β), one of the mediators of inflammation, may play an important role in the pathogenesis of coronary artery disease. And to study and investigate the association between common polymorphisms (G>A) of the (TNF-β) gene and susceptibility to coronary artery disease have produced contradictory and inconclusive results (11).

The aims of our study were: to be compare the role of TNF-α and TNF-β in coronary artery disease, and to identify the TNF-α receptor subtype responsible for coronary artery disease. And also to provide a relatively comprehensive account of the association of TNF-α and TNF-β polymorphisms with susceptibility to coronary artery disease (12).

Health Study and Health Professionals Follow-Up Study Molecular genetics data along with the intracellular signaling cascade mechanisms may have important clinical implications in the treatment of Coronary Artery Disease. and may lead to use Tumor Necrosis Factor as a Pharmacological Target to further treatment and to use of anti-TNF molecules in coronary artery disease (13).

Our aim was to investigate whether the common polymorphisms of the TNF-α and TNF-β which were associated with inflammatory parameters and cell adhesion molecules and whether these polymorphisms are related to coronary artery disease in turkey.

2. LITERATURE REVIEW

2.1. Structure, Histological and Physiological Properties of Coronary Arteries

Coronary Arteries are divided into two: elastic and muscular arteries. Elastic arteries are usually large arteries and the expandable capacities are high due to the elastic fibers in the constructions. The arteries that expand during systole provide the continuity of blood flow by restoring to their original state during diastole. Examples of elastic arteries include aorta, carotid, and iliac arteries (14). Muscle arteries are medium-large vessels, contain fewer smooth muscle cells, and have fewer expandable capacities. However, unlike elastic arteries, when the metabolic needs change, it can increase or decrease the vessel cap by autoregulation in response. Muscle arteries may be coronary, brachial, femoral, and radial arteries (15).

Coronary arteries have well-developed, three-layered structure. This foundation on the wall of the artery forms the layers of intima, media and adventitia. Intima; is endothermic in the icus and internal elastic lamina outside. Media; It is the layer between the internal elastic lamina and the external elastic lamina. Adventitia is the layer outside the external elastic laminate (16).

The endothelium is a single-stratified cell layer of extracellular matrix, which can be known as the largest paracrine organ of the body, it's exposed to trauma throughout life, in direct contact with blood components, which propagate on the inner surface of vascular structures. The inner surface of intima is intact and active metabolically (17).

The extracellular matrix in endothelial cells are consists of collagen type IV, fibronectin, laminin, and other extracellular matrix molecules. This matrix is produced by the vascular smooth muscles. Endothelial cells also have important implications in hemostatic mechanisms. Through the heparan sulfate in the surface, antithrombin III binds to the thrombin and makes it inactive. In addition, thrombomodulin, located on the endothelial cell surface, acts antithrombotic by activating proteins S and C, binding to thrombin (18).

When thrombus begins to build up, normal endothelial cells invite physiological fibrinolytic mechanisms. In this case, the tissue produces plasminogen activators (19). These enzymes catalyze the activation of plasminogen, which is a fibrinolytic enzyme, plasmin. Thanks to these defined mechanisms, the endothelium ensures the continuity of blood flow in the veins (19).

The Media layer: is a layer between internal and external elastic laminae formed almost entirely in the smooth muscle cells. The main function of this layer and smooth muscle cells is to adjust the arterial tone and regulate the gore blood flow for metabolic needs. Another function is to produce the above-mentioned extracellular matrix proteins. As the artery cap increases, the number of smooth muscle cells in the media also increases. There between each layer is also an elastic layer. The inner 1/3 area of the media layer is fed from the vessel lumen, and the outer 2/3 part is fed by the vasa vasorum; Loose connective tissue comes to the front and forms the outermost layer of the arteries. It includes fibroelastic tissue, vasa vasorum and nerve tissue (20).

2.2. Coronary Artery Disease

Coronary Artery Disease (CAD) is among the most important causes leading to morbidity and mortality in the whole world. Among causes of death in the population of the world, CAD is ranked first for those under 45 years old, and for the age of 45 years old. Each age is a significant morbidity and mortality factor and its prevalence is increasing each day. Occlusive CAD usually manifests itself as the narrowing of the epicardial coronary arteries to atheromatous plaques. Rarely, congenital anomalies, myocardial bridges, radiation and arthritis involving coronary arteries can be used to diagnose coronary artery disease other than atheromatous (21).

The main underlying cause of CAD is impaired coronary endothelial function leading to atherosclerosis. Endothelial dysfunction results from inflammation, accumulation of lipid, and also fibromuscular hyperplasia leading to coronary atherosclerotic plaque formation. This plaque is highly prone to tearing and subsequent clot formation (22).

Not all of patients have a single finding or symptoms, some patients may be asymptomatic. Coronary artery disease may be caused by socioeconomic condition, myocardial infarction, cardiac insufficiency, sudden cardiac death and similar health problems, which are common in the community (23).

2.2.1. Epidemiology

The global rise in Coronary Artery Disease (CAD) is the result of an unprecedented transformation in the causes of morbidity and mortality during the twenty-first century. known as the epidemiologic transition, this shift is driven by industrialization, urbanization, and associated lifestyle changes and is taking place in every part of the world among all races, ethnic groups, and cultures. The epidemiologic transition is divided into four stages: (i) pestilence and famine, (ii) receding pandemics, (iii) degenerative and human-made diseases, and (iv) delayed degenerative diseases. A fifth stage, characterized by an epidemic of inactivity and obesity, may be emerging in some countries (24).

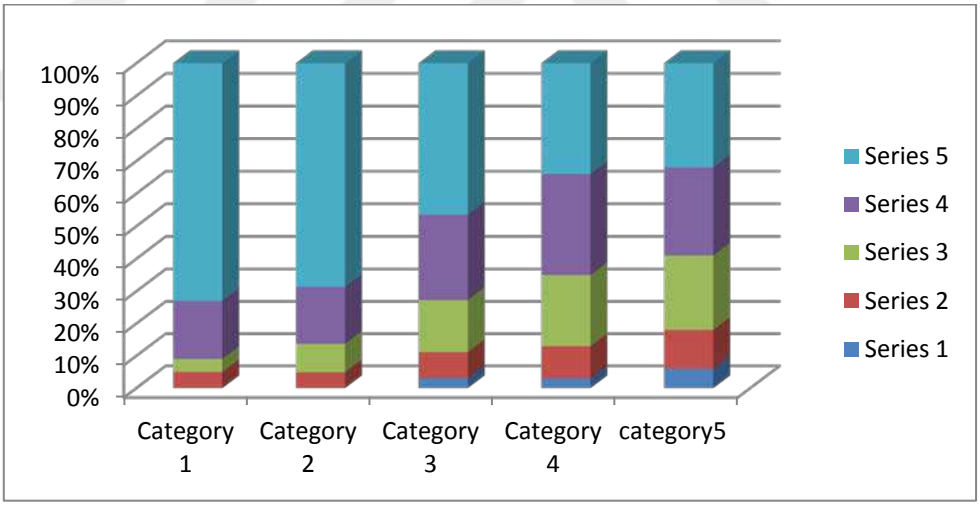
The Framingham study may be beneficial and also maybe in understanding the clinical activity and prognosis of CAD. The data collection was began in 1949, moreover, has continued in one regimen where the treatment options in the CAD are still not sufficient due to inadequate data and the treatment options are not enough. .The important part of the CAD may be unstable angina , its complications, myocardial infarction and even progression (25).

In society, the association of these temporal affinities with risky, changeable, risky risk factors is a deterrent. In the United States in 2001, CAD was responsible for 54% of all Coronary Artery Disease. CAD is the most common cause of deaths in males and

females (more than one in every five deaths). In those who are older than 35 years, it's responsible for the incidence of CAD. For females, the age-related risk profile was found to be even higher in non-angina outbreaks. The reason for this was that women who had myocardial infarction often had more moderate risk factors. Coronary Artery Disease is the most prevalent cause of death in the world, and in the next decade, the increasing incidence of cardiovascular disease will be increasing related to the growing societies, Diabetes Mellitus (DM) and obesity (26).

In Figure 2.1. has been showen: 445,687 deaths occurred due to Coronary Artery Disease (CAD) in 2015. It is estimated that 785,000 Americans will have a new coronary attack in 2009 and about 470,000 will have a recurrent attack of heart diseases . It is estimated that an additional 195,000 silent first MIs occur each year. About every 25 seconds an American will have a coronary event, and about every minute, someone will die from one. On the basis of the National Heart, Lung and Blood Institute (NHLBI)-sponsored Framingham Heart Study (FHS), CAD makes up more than half of all cardiovascular events in men and women older than <75 years of age (Figure 2.1) (27).

In the lifetime risk of developing CAD after 40 years of age is increased to 49to 49% for men and about 32% for women. The incidence of CAD in women behind men by 10 years for total CAD and by 20 years for more serious clinical events such as MI and sudden death (28).



Portugal	USA	Brazil	India	S. Africa
(10%)	(12%)	(28%)	(35%)	(41%)

Figure 2.1. Projected CAD mortality from 2000–2030 (29).

In Table 2.1 Overall death rate from Coronary Artery disease (CAD) (International Classification of Diseases) in 2015 per 100,000 was 278.9. The rates were 324.7 per 100,000 for white males, 438.4 for black males, 230.4 for white females, and 319.7 for black females. From 1995 to 2015 death rates from CAD declined 26.4%.

Mortality data for 2015 show that CAD accounted for 34.2% of all 2,425,900 deaths in 2015 (30).

Coronary Artery Disease CAD includes stable angina , unstable angina and acute MI , other acute ischemic (coronary) heart disease , , atherosclerotic and (CAD), and all other forms of (CAD). Indicated data is not clear or available. Sources: Prevalence: NHANES 2005-2006 (NCHS) and NHLBI. Total data are for Americans ≥ 20 years of age; percentages for racial/ethnic groups are age adjusted for ≥20 years of age. Data is based on self-reports. Evaluation from NHANES 2005-2006 (NCHS) was applied to 2006 population estimates (≥20 years of age). Incidence: ARIC (1987-2004), NHLBI. Mortality: NCHS (data represent underlying cause of death only). Hospital discharges: NHDS, NCHS (data include those inpatients discharged alive, dead, or status unknown). Cost: NHLBI; data include estimated direct and indirect costs for 2009. Mortality data are for whites and blacks, and Hispanics (31)

Table 2.1. Prevalence of Coronary Heart Disease (32).

Table -1 • Prevalence of Coronary Heart Disease

Population Group	Prevalence, CAD 2015 Age ≥ 20 yr	Prevalence, MI, 2015 Age ≥ 20 yr	New and Recurrent MI and Fatal CAD Age ≥ 35 yr	New and Recurrent MI 2015 Age ≥ 35 y	Mortality,* CAD 2014 All Ages	Mortality,* MI 2015 All Ages	Hospital Discharges, All Ages	Cost, CAD
Both sexes	16,800,000 (7.6%)	7,900,000 (3.6%)	1 255,000	935,000	445,687	151,004	1,760,000	$165.4 billion
Males	8,700,000 (8.6%)	4,700,000 (4.7%)	740,000	565,000	232,115 (52.1%)‡	80,079 (53.0%)‡	1 056,000	.....
Females	8,100,000 (6.8%)	3,200,000 (2.7%)	515,000	370,000	213,572 (47.9%)‡	70,925 (47.0%)‡	704,000	.....
NH white MALES	8.8%	4.9%	675,000§	...	203,924	70,791	.....	.....
NH white females	6.6%	3.0%	445,000§	...	186,497	61,573	.....	.....
NH black males	9.6%	5.1%	70,000§	...	22,933	7527	.....	.....
NH black females	9.0%	2.2%	65,000§	...	23,094	8009	...	...
Mexican males	5.4%	2.5%	...	...	...	...	...	...
Mexican females	6.3%	1.1%	...	...	... .	.. ..	. ...	.....
Hispanic or Latino,† age ≥18 yr	5.7%	...	...	...	...	...	...	...
Asian,† age ≥18 yr	4.3%	...	...	...	...	...	...	...

2.2.2. Atherosclerosis

Atherosclerosis is a disease that causes and leads to impaired flexibility and antithrombotic properties of the arterial wall, and due to progressive arterial stenosis and

occlusion due to intimal plaques involving lipids, fibroblasts, macrophages, smooth muscle cells and extracellular materials at various ratios. Atherosclerosis ,is a multifactorial and complex disease, which starts in fetal life, leading to impaired blood flow in various organs . In that case, atherosclerosis can be stopped or regressed if its causes can be detected and treated (33).

It slowly progressesd during childhood and adolescence life . In adult and alderly life, it leads to a faster progression, leading to higher morbidity and possible clinical conditions. Dyslipidemia is a primary risk factor for the development of atherosclerosis. However, the presence of an inflammatory response is associated with lipid accumulation in the atherosclerotic arterial wall (34).

2.2.3. Risk Factors of Coronary Artery Disease

As indicated earlier, the global variation in coronary artery disease rates is related to temporal and regional variations in known risk behaviors and factors. Ecological analyses of major CAD risk factors and mortality demonstrate high correlations between expected and observed mortality rates for the three main risk factors: smoking, serum cholesterol, and hypertension and suggest that many of the regional variations are based on differences in conventional risk factors (35).

The correct identification of the risk factors of the disease and their contribution to the formation of CAD will be beneficial to public health. Some of risk factors can be changed during life style such as (smoking, physical inactivity, obesity, etc.) or can maybe controlled by treatment and drug therapy (DM, hypertension, infection, etc.) and some can notcannot be controlled (family history, age, genetic and racial factors) (36).

Following the findings of suspected risk factors determined with epidemiological studies, we found that CAD is detected from signs and symptomes and also risk factors. When the patient is admitted to the hospital, the disease develops and contributes to its prognosis (37).

There are several studies on this subject such as; The Framingham Study , the Multiple Risk Factor Intervention Trial (MRFIT), the Prospective Cardiovascular Munster (PROCAM), "The Collaborative Trial on Multifactorial Prevention of Coronary Artery Disease " "The Oslo Diet-Heart Study" and "The Stanford Coronary Risk Intervention Project" (SCRIP) . However, the current studies on the management of risk factors are generally a one-factor researches (38).

2.3. Pathogenesis of Coronary Artery Disease:

Pathology of Coronary Artery Disease is characterized by the formation of intrinsic fat fibrous lesions leading to narrowing of vessel lumen. It it’s called

atherosclerotic plaques, and plaques are associated with degenerative changes in the media and adventitia layers . Some of the plaques are largely fibrous, and others maybe soft, oily, and small which leads to secondary complications (calcification, ulceration on thrombosis, and intraplate rupture). This leads to that due to narrowing of the lumen and luminal obstruction (39). The basic building element is fibrous tissue, with more than 45% of lesions consisting of lipids, especially cholesterol. This cholesterol is not derived from local synthesis, but is derived entirely from blood (40).

Coronary Artery Disease and the atherosclerosis are a diseases of elastic arteries (aorta, carotid etc.) and medium-sized arteries (coronary, popliteal arteries); rarely small arteries are involved. It forms a group of arterial diseases in which the artery walls thicken , stiff , narrowed and lose their elasticity. It is not known which event or event sequence the atherosclerotic process initiated. Many of theories have been proposed for the pathogenesis of coronary artery disease and atherosclerosis. The injury response theory the modified lipoprotein theory (41).

Many theories contribute of Pathogenesis of coronary artery disease like as theory of retention of low-density lipoproteins (42), the theory hemodynamic disorders, and the immunological theory (43).

The most common acceptance of these theories is the "response to injury" theory. In this theory, initiated by researchers Ross and Glomset in 1976, injury initiate endothelial dysfunction (44). and related ofleads to mechanical, metabolic, immunological, toxic, events and infections that causes endothelial dysfunction. With most known risk factors (smoking, hypertension, diabetes melletismellitus, increase cholesterol and lipid serum level, oxidized LDL) it can lead to endothelial dysfunction. This dysfunction is destroys the selective permeability and antithrombotic structure of arterial wall so that this layer of single cell order can form a barrier between blood and the vessel wall. As a result, the developing inflammatory and proliferative events cause the formation of atherosclerotic plaque (45).

Endothelial dysfunction causes serious cellular interactions due to the development of atherosclerotic lesions. Disruption of endothelial cell and wall balance triggers endothelial permeability, vasoconstriction, coagulation cascade, inflammatory and immunologic reactions (46).

Change of endothelial permeability, together with endothelial dysfunction, causes an increase of endothelium leukocyte adhesion, adhesion molecules (ICAM-1, Cellular adhesion molecule, VCAM-1, Vascular cell adhesion molecule), cytokines (IL-1, Interleukin-1, TNF- (MCP-1, Monocyte chemotactic protein-1, IL-8, Interleukin-8) and growth factors (PDGF, platelet-derived growth factor, bFGF, basic fibroblast growth factor). These cytokines and adhesion molecules regulate the entry of leukocytes from the vessel wall into the intercellular space (47).

Monocytes that migrate to the lesioned area with secreted attractants secrete inflammatory cytokines. Cytokines such as IL-1β and TNF-α give rise to a

prothrombogenic feature besides causing leukocyte and LDL binding to the endothelium (48). One of the most important changes for early lesion formation is LDL oxidation, a consequence of the exposure of vessel cells to oxidative damage. Oxidation of LDL leads to the release of modified lipids such as lysophosphatidylcholine. Some of these lipid species may play a role as signaling molecules that activate endothelial cells. This leads to the expression of VCAM-1, a leukocyte adhesion molecule. VCAM-1 is a receptor for monocytes and T lymphocytes. LDL oxidation occurs in monocytes, macrophages, neutrophils, endothelial cells, fibroblasts and smooth muscle cells (49). Oxidized LDL (Ox-LDL) is not found in normal arteries but only in atherosclerotic lesions in macrophages. The increase of oxidative stress and superoxide anion in vascular cells increases the conversion of LDL to Ox-LDL. Chronic hyperlipidemia begins to accumulate under the endothelium, passing through the circulating LDL formed by endothelial cells. The proteoglycans and glycosaminoglycans present in the matrix interact with LDL and accumulate. Intracellularly, matrix-bound LDL is oxidized by endothelial and smooth muscle cells and macrophages. This is called minimally modified LDL (mmLDL) because it does not change the apo B-100 in the initial LDL structure. Oxides were changed in apo B-100 in LDL and this lipoprotein Figure 2.2 (50).

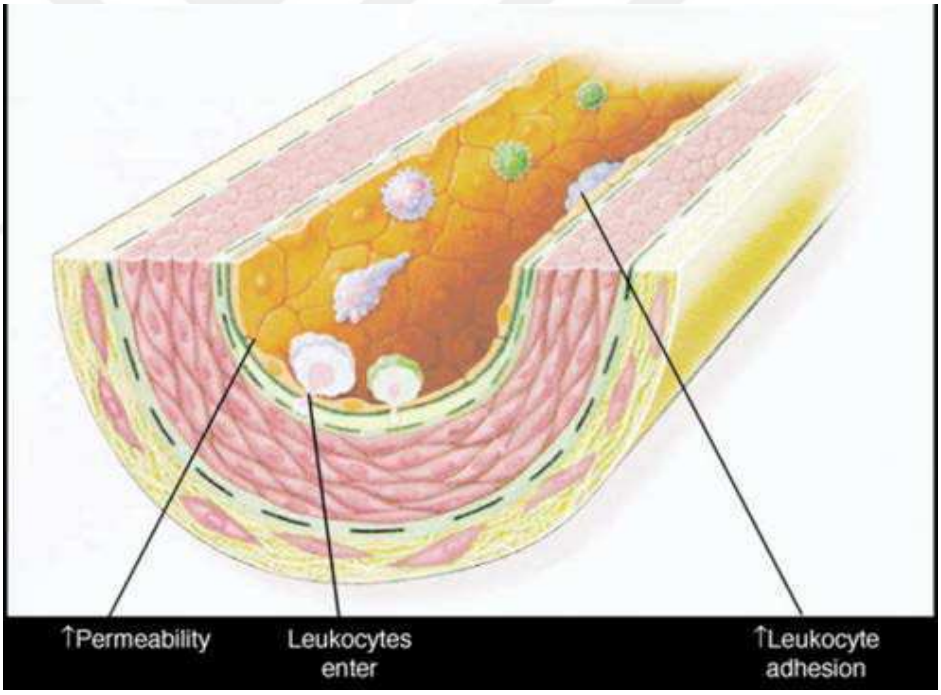


Figure 2.2. Endothelium dysfunction (51)

Stimulates the production of chemotactic substances for monocytes and lymphocytes. Oxidized LDL is phagocytized by macrophages via 'scavenger' receptors. LDL taken in this way transforms into cholesterol esters in the macrophage to form build-up foam cells (52). This lesion is composed from macrophage foam cells and T cells, and it's located under endothelial cells, is the oily line known as the first lesion of atherosclerosis. The T cells in the oily line are active and secrete various cytokines (tumor

necrosis factor-beta, gamma interferon), fibrogenic mediators and also growth factors with other own cells of the vascular wall. They mediate smooth muscle cell migration and proliferation and provide a dense extracellular matrix around them. The smooth muscle cells in the media layer, in response to inflammatory stimuli, cleave elastin and collagen through specialized enzymes. Thus, smooth muscle cells are migrated below the internal elastic lamina and migrate under the intima. At the same time, these smooth muscle cells secrete factors that allow for more monocyte aggregation (53, 54) Figure 2.3.

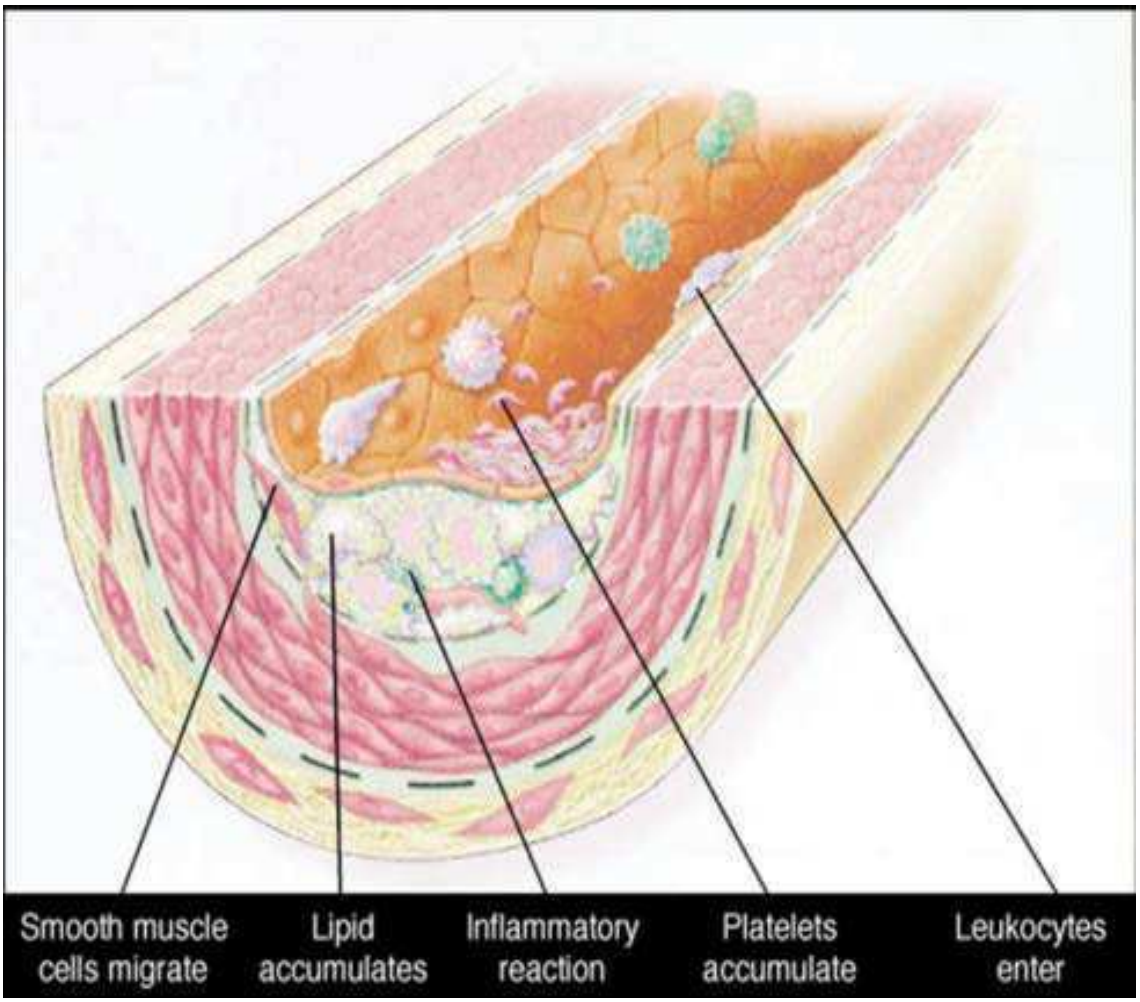


Figure 2.3. The initial events of fatty line lesions (55)

In Figure 2.4. the character of fibrous plaque is composed from a nucleus consisting of lipids and necrotic tissue and a fibrous sheath covering it, the most advanced form of atherosclerosis lesion. The necrotic nucleus composed of macrophages undergo apoptosis and necrosis by releasing the lipid content into the plaque. The fibrous sheath contains smooth muscle cells, collagen fibrils, elastin, proteoglycans and glycosaminoglycan, which migrate from the mediastinum to the intima (Figure 2.4). In atherosclerotic plaques, lesions are complicated by fibrous plaque rupture. Coronary

atherosclerosis-related morbidity and mortality are mainly due to complicated lesions (56).

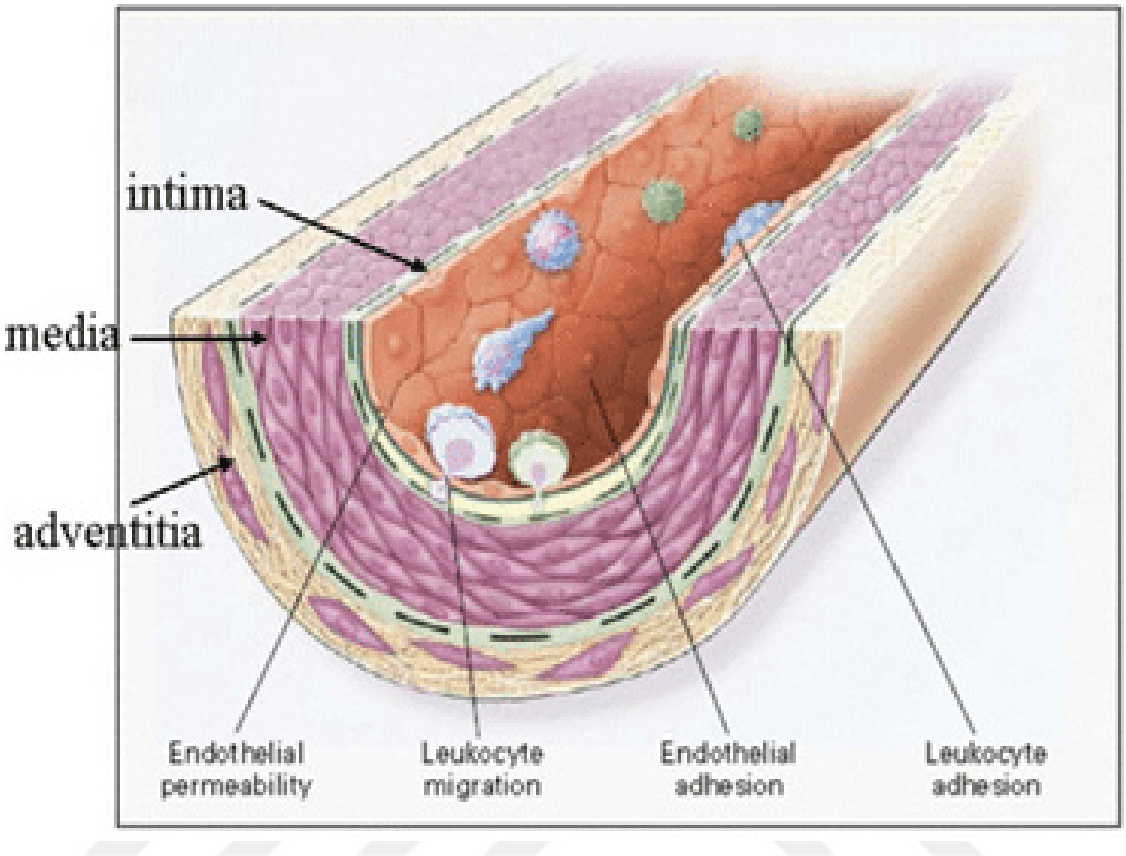


Figure 2.4. The formation of necrotic cores and fibrous sheath (57).

Arterial wall stiffness, the images and structural deficiency in the vessel wall are the changes which develops dynamic and complex interaction (Figure 2.5). These vascular changes affects glucose regulation, extrinsic factors such as salt and hormones, affected by power of hemodynamic. Stiffness is not the same in all parts of the tree veins, peripheral vascular veins occur more from plants (58).

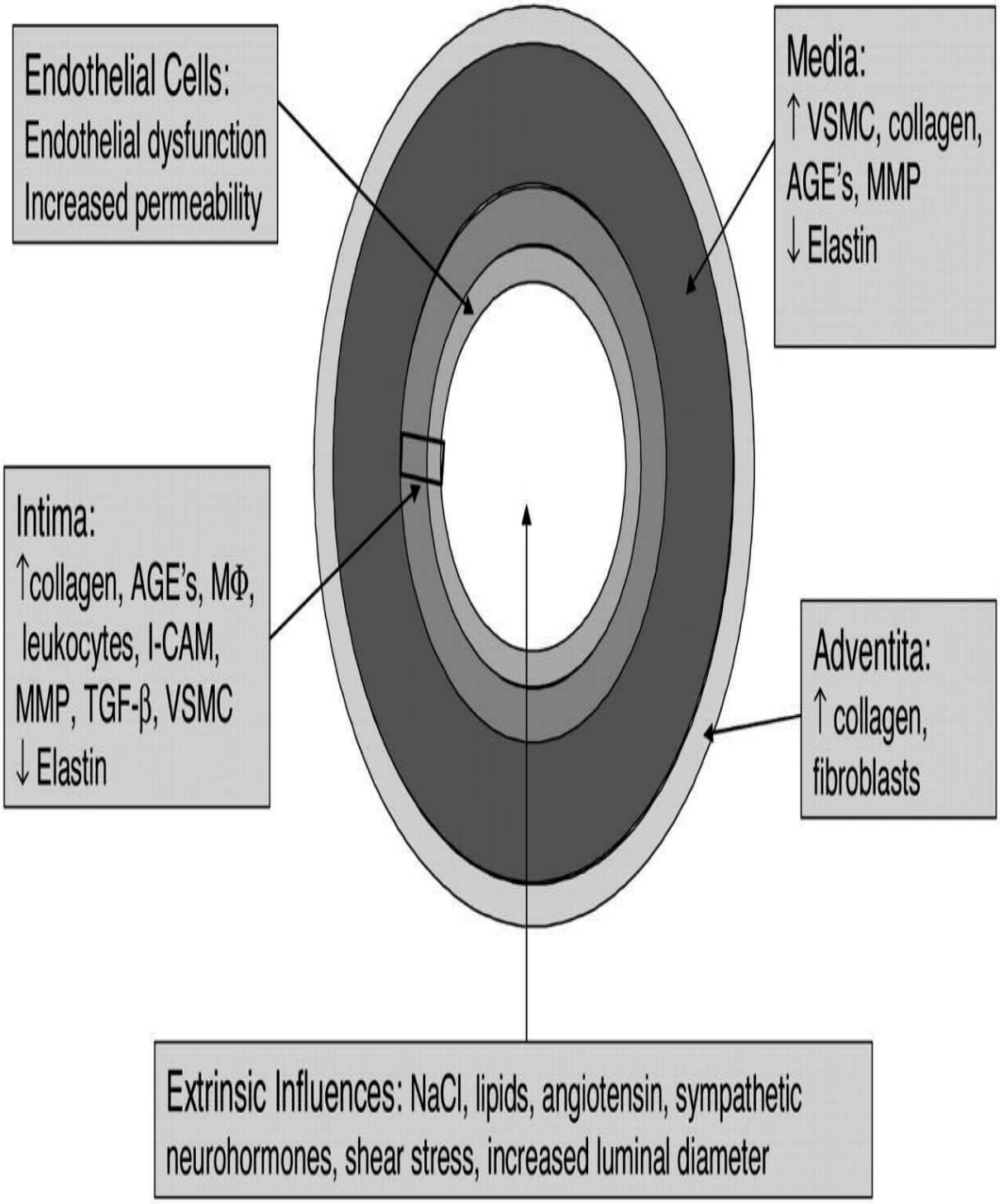


Figure 2.5. Arterial stiffness mechanisms in CAD (59)

2.4. Roles of Tumor Necrosis Factor Alpha (TNFα) and Tumor Necrosis Factor Beta (TNFβ) in Coronary Artery Diseases:

2.4.1. Tumor Necrosis Factor (TNF)

Tumor Necrosis Factor (TNF), is a cytokines which may be formed in the alpha (α) or beta (β), are caused by hemorrhagic necrosis in injury and inflammation cells. TNF-α, ana ubiquitous pleiotropic cytokine by T-hapters, macrophages and other cells, is a lymphotoxin of 157 amino acids, while TNF-β is a lymphotoxin of 171 amino acids. TNF-α, also defined as a cytotoxin, is a macrophage cytotoxic factor, (necrosin, hemorrhagic factor), and TNF-β, is also defined as a lymphotoxin or cytotoxin. They, are both structurally and biologically similar (60, 61).

2.4.1.1. General Characteristics:

The general characteristics of Tumor Necrosis Factor (TNF) is expressed as type II membrane protein containing the transmembrane region of the signal anchor and is intracellularly processed by ADAM-17 (also known as TACE or TACA). TNF-α, is synthesized as a nonglycolytic protein with a weight of approximately 25 kDa, which inverted in the aminoterminal intracellular region and the carboxy terminus region in the extracellular region. When it is synthesized, latent pro-TNF (26 kDa) monocytes or other cells are stored on the cell surface. Pro-TNF- [alpha] was cleaved with ADAM-17, followed by 17 kDa soluble, active form. 17 kDa membrane fragments, including the carboxy terminal end, are degraded by matrix metalloproteinases in the plasma membrane of mononuclear phagocytes, resulting in a stable and biologically active homotrimeric form with a weight of 51-52 kDa. TNF is secreted in this form, and form of a triangular pyramid, and all ends of the pyramid are composed of different monomeric subunits. This pyramid shape is characterized by the edge-to-face organization of the antiparallel sandwich structure of wedge-shaped monomers. The receptor binding site on the bottom of the pyramid allows for the simultaneous binding of more than one receptor (62, 63).

The amino acid sequence homology in human is 79% for TNF-α, 74% for TNF-β. The myelin TNF ligand binds to p55 receptor but not to p75 (64).

2.4.1.2 Chromosome Localization:

Tumor Necrosis Factors Both (TNF-α and TNF-β) are localized in chromosome 6p and contain 4 exons. 80% of the released protein is encoded in the exon expression. Genes are are in MHC class III. TNF-α and TNF-β play roles in the MHC locus in MHC-associated genetic influences (74). In Figure (2.6) TNF is deposited in place. Local_ order TNF is located on chromosome 6 long arm (forward strand), and lies between the PERP (PERP, TP53 apoptosis effector) and OLIG3 (oligodendrocyte transcription factor 3) genes. TNFAIP3 is a gene whose expression is induced by TNF (tumor necrosis factor). TNFAIP3 encodes a cytoplasmic zinc finger protein that inhibits NFKB (nuclear factor of kappa light polypeptide gene enhancer in B-cells) activation and TNF-mediated

apoptosis. Studies in knockout mice show that TNFAIP3 is important for limiting inflammation by terminating TNF-induced NFKB responses (65, 66).

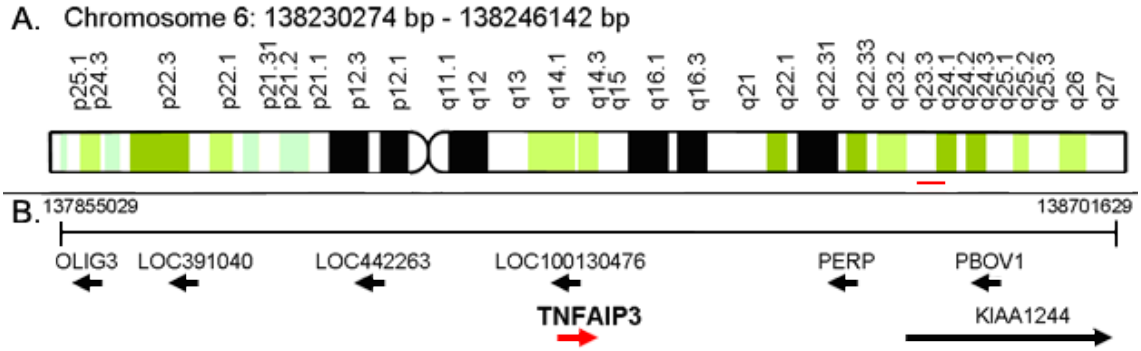


Figure 2.6. A. Chromosomal location of TNFAIP3 gene. .B. Mapping of TNFAIP3 gene and local order on genomic context of the chromosome 6. (67)

Welded cells of Tumor Necrosis Factor formerly known as TNFα or TNF alpha is firstly expressed by activation of monocytes with / or macrophages by activating T-, B-,, NK-, mast-cells, endothelial cells, fibroblasts, keratinocytes, microglia, astrocytes, Kupffer cells, dicotyledons, synovial cells, and basophils blood source CD14 monocytes and / or macrophages. The positive interconnection between IL-6, IL-10 and TNF-α secretions is suggests that cytokines are coordinately expressed (68).

Control of synthesis and release of Tumor Necrosis Factor TNF, in bacteriain bacteria (endotoxins and LPS), viruses, protozoa, cytokines (GM-CSF, IL-1, IL-2, IFNγ and TNF-α itself), the immunocomplexes, complements with the production and inhibited by IL-10, TGF-β, cyclosporin A, PGE2 ,and some drugs like dexamethasone, ibuprofen, methylprednisolone and pentoxifylline; while component C5a is released in response to substance P and reactive oxygen compounds . C3a desArg inhibits cytokine production of systemic proinflammatory cytokines by circulating cells while increasing TNF-a production from monocytes in the local inflammatory region (69). TNF-α production is activated by NO, reactive oxygen compounds, nonsteroidal anti-inflammatory drugs, and hypoxia (70).

Tumor Necrosis Factor (TNF) have two separate receptors (55 and 75 kDa) and encoded by separate genes which are linked to each TNFs. Type II (TNFR-2, type A, p75, CD120b) is released by hematopoietic cells, while erythrocytes and resting T cells excrete Type I receptors (TNFR-1, type B, p55, CD120a). While TNFR-1 is the basic tool for TNF-a activity, TNFR-2 plays an auxiliary role. TNFR-2, TNFR-1 carry a death site in the Fas proteins in its cytoplasmic region. Receptor expression is regulated by vitamin D3, IL-1, IL-2, GM-CSF and TNF itself (71,72).

Cross-linking of TNF signal transduction of the receptor by TNF trimer is important for signal transduction. Activation of TNF by sphingomyelinase results in shedding of the ceramide which will then activate the Mg^{2+} binding protein kinases. These kinases include three types of mitogen-activated protein kinase (ERK (extracellular signal-regulating kinase), JNK / p54 (jun amino-terminal kinase), p38) and β-casein kinase (73,74).

Biological effects of TNF, is a mediator of specific and nonspecific biological effect, and it's has important links between immunological and inflammatory reactions. Uncontrolled or over expression can lead to or be defined as a bipolar sword with cytokine production which can give rise to severe catabolic effects (collapse), tissue damage dysfunction and death. First, TNFs identified by their capacity to form tumor necrosis have also been found to be mediators of antitumor cytotoxicity following severe gram-negative infections. (75)

In many studies of biological effect of TNF in gram-negative bacteria has showen that the main mediators of LPS-mediated host response to TNF-α. Several biological activators (antiviral, antiparasitic, lipolytic, glycogenolytic, osteoclastic) play roles synergistically with a kinIFN-γ and IL-1. Inflammation and healing have a wide range of effects, including granulation, tissue necrosis, fibrosis, overproduction. TNF-a is also an immune-enhancer of IFN-γ, mediators of induction of adhesion molecules, other cytokines (IL-1 and IL-6) and phagocyte activation. The factor for the fibroblasts and the acute phase are to the behavior of multi-cell type with IL-1(76).

In gram-negative bacterial sepsis, TNFs are produced at high concentrations (serum concentrations are above 10-7 mol / L.n) and also in septic shock they becomes a tool with critical prescription in the complex cytokine network. They may have lethal effects when they are in very high concentrations. These effects can be listed as follows: 1. Decreased tissue perfusion by depressing myocardial contractility (inhibition of myocardial contractility by increased production of NO by NO synthase induction), 2. Vascular smooth muscle tone relaxation, reduction in blood pressure and tissue perfusion, 3. Intravascular thrombosis (coagulation of a combination of endothelial and mononuclear phagocyte changes and neutrophil activation diffuse intravascular coagulation) 4. Severe metabolic disorders (low blood glucose levels that are not associated with life) (77). In addition, TNF has many effects on apoptosis, adhesion, cell proliferation, angiogenesis, myocyte proliferation, fibrosis, phagocytosis, cytokine production,cellular activation, leukocyte / macrophage functions, inflammation and tumor control (78).

Generally, IL-1 is not toxic, while TNF-α is a potent cytotoxic factor. Biological effects of TNF are related to its concentration. At low concentrations (~ 1x10-9 mol / L), damage may be treated as a local paracrine and autocrine regulator for immunoinflammation with the release of HLA from damage or infection site (79).

TNF-α, is a neurofilaments, and endothelial cell causes predisposeition to adhesion and leukocyte migration. TNF-α is one of the most prevalent elements of

fibroblast and endothelial cell proliferation. These activities isare enhanced by IL-1 acting synergistically with TNF-α. Local TNF effects can be induced in the oocyte at low concentrations: 1. Expression of adhesion molecules from endothelial cells (leukocyte accumulation at the site where inflammation is the most important physiological local effect of TNF), 2. Activation of inflammatory leukocytes to fight against microorganisms or tumor cells (especially for neutrophils but also for eosinophils and mononuclear phagocytes), 3. The induction of cytokine (IL-1, IL-6, TNF itself) synthesis in mononuclear phagocytes and other cells, 4. Interferon-like protective effects against viruses (80).

These main effects of TNF are critical for any inflammatory response to germs and tumors. If sufficient amount of TNF is not present in the medium, organism infection may be unsuccessful in suppression (81).

At medium concentrations of TNF which is the flux that acts as an endocrine factor,. Tthe effects maybe lead to (82): 1. Behaviors such as an endogenous pyrogen (with IL-1) and mediation of increased PG synthesis, 2. Circulating IL-1 and IL-6 secretion from mononuclear phagocytes and endothelial cells, 3. Increase in the synthesis of acute phase proteins from liver (IL-6 → f), 4. Activation of the coagulation system,, 5. Suppression of bone marrow stem cell division,, 6. Caucasian (by TNF-induced appetite suppression) (83,84, 84).

Clinical Significance of TNF, is clinically indicated for infectious / septic shock, autoimmune diseases, inflammatory / allergic diseases, endometriosis, cerebral ischemia, acute pancreatitis, nephropathies, neurological disorders, alcoholic liver disease, transplant rejection, tumors, inflammatory diseases / trauma and infectious / septic spot therapeutic applications (85).

2.5. Tumor Necrosis Factor Alpha (TNFα) and Tumor Necrosis Factor Beta (TNFβ):

Tumor necrosis factor Alpha (TNF-α) is a multifunctional agent that involves macrophage activation, and it's has strong proinflammatory effects not only for atherosclerosis and coronary artery disease but also for inflammatory diseases such as obesity and insulin resistance, which are risk factors for cardiovascular disease (86).

The role of TNF-α in the pathogenesis of coronary artery disease may be supported by its presence in human atherosclerotic plaques. In addition, circulating TNF-α levels are increase in myocardial infarction (MI), carotid intima-mediated atherosclerotic plaques, and triglyceride and glucose homeostasis disorders, and with age. In addition to these functions, lipoproteins are involved in the formation of hypertriglyceridemia, which inhibits lipase and contributes to the development of . atherosclerosis. Patients with coronary artery disease have been shown to be at increased risk for atherscleroticsis and also anti-TNF-α therapy has been shown to improve cardiovascular prognosis (87). In some study of TNF-α - / - deficient, a rather deleterious

pro-atherogenic role of TNF-α has been shown . The relationship between increased risk of CAD like recurrent myocardial infarction (MI), cardiovascular death and TNF-α has been reported after the first MI. The cardiac index used to predict the level of TNF-α and the severity of coronary artery disease has reflected a correlationes . In healthy middle-aged men, the atherosclerosis is high and , determined by TNF-α levels and carotid ultrasound. Studies have also reported that sTNFR levels for atherosclerotic burden may be a better predictor of TNF-a than for itself (88).

Polymorphisms in the promoter of the tumor necrosis factor alpha (TNF) gene have been reported to affect the transcription rate and the release of this cytokine.

In coronary artery disease (CAD), the TNF-a –308 polymorphism is associated with an increased transcriptional activity and TNF release, whereas the –863 polymorphism is associated with a reduced transcriptional activity. A growing body of evidence indicates that these polymorphisms may affect susceptibility to different diseases (89).

Method to detect the –238, –308, and –863 TNF polymorphisms in coronary artery disease is diescribed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Briefly, DNA is amplified by PCR using mutagenic primers containing a single base-pair mismatch adjacent to the polymorphic site to introduce a restriction site into the wild-type nucleotide sequences after amplification. The PCR products are then digested with specific restriction enzymes and analyzed by agarose gel electrophoresis (90).

Investigators have become increasingly interested in the possible role of genetic polymorphisms in cardiac diseases. Some of polymorphisms have many functional effects on the gene product and may be useful types of polymorphism in disease-association studies, while others with no evidence of functional effects are simply useful markers (91).

Functional effects are most likely to occur: (1) when the polymorphisms are associated with an amino acid substitution in the gene product, (2) when a deletion or insertion results in a frameshift in the coding region, (3) when the polymorphism directly affects gene transcription, RNA splicing, mRNA stability, or mRNA translation (92).

Some cardiac diseases have an autoimmune or inflammatory component involved in their pathogenesis. Individual variation in genes that encode proteins with an involvement in the immune and inflammatory responses is therefore a potential important susceptibility factor. Thus far, one of the cytokine genes that has received much attention is tumor necrosis factor alpha (TNF α). It is possibly because it is located within the MHC gene cluster, raising the possibility that some reported MHC associations might be due to linkage to particular TNF -α alleles . In fact, several studies showed that individual differences in TNF- α production are inherited and could be linked to the MHC ancestral haplotype HLA A1-B8-DR3-DQ2-TNF308A, which is overrepresented in several autoimmune and inflammatory diseases (93).

Several various of polymorphisms different exist in the promoter region of TNF α, of which the most frequent and best studied are those at position –238 and –308 Though it's associations of these polymorphisms with susceptibility to a variety of diseases have been reported, consistent results have not been obtained, possibly due to the lack of a clear-cut functional significance (93). There have been a number of studies on the functional significance of both polymorphisms: Some reported increased transcriptional activity for the variant ,alleles, and others reported no difference compared to the wild type Figure (2.7) (94).

Other polymorphism, the position of –863 in the promoter region, has been studied less extensively. Although the molecular mechanisms by which possibly functional single-nucleotide polymorphism (SNP) influences TNF α expression, are poorly understood, it seems that interactions between nuclear proteins and the SNP sites provide information for the understanding of the allele specific modulation of TNF α expression. However, in the absence of reliable and consistent functional data, it is possible that many of the reported associations between TNF-α alleles and disease susceptibility reflect associations or linkages with MHC genes (95).

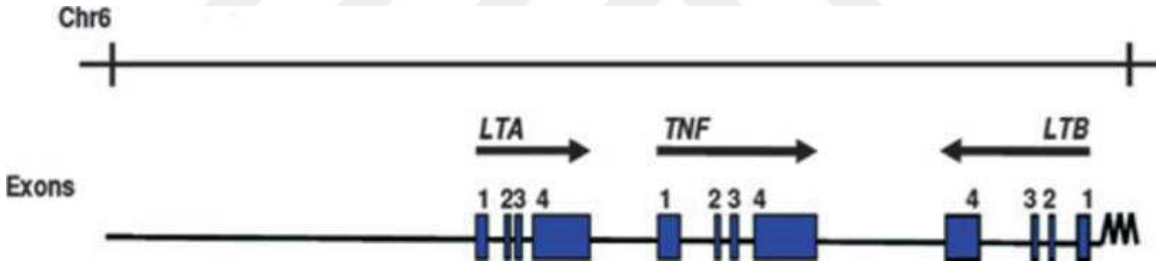


Figure 2.7. Representation of tumor necrosis factor, lymphotoxin alpha and beta gene (96).

In figure 2.8. this stimulation of TNF pathway leads to tyrosine phosphorylation of Ras by phosphotyrosine kinase (PTK); and the protein kinase cascade leads to cytokines' production. Ras activates Raf-1/mitogen-activated protein kinase, followed by the activation of the mitogen-activated protein kinase (MAPK) family of protein kinases, extracellular signal-related kinase (ERKs), stress-activated protein kinase (SAPK), Jun nuclear kinase (JNK) and the P38 MAPK. NFκB is activated by phosphorylation and is translocateds to the nucleus to activate promoter sites. Intracellular signaling pathways

proliferate as downstream events in the regulation of gene expression. A functional genetic variant in the promoter region of the above mentioned inflammatory markers may affect the cytokines' levels via the p38 mitogen-activated protein kinase (p38 MAPK) signaling including nuclear factor kappa B (NFκB) cascade loop (Figure 1). Hence from this aspect, SNPs may lead to elevated gene expression of inflammatory cytokines which could influence clinical outcome in patients with CAD (97).

Molecular mechanisms that reveal and help the role of inflammatory markers have been a focus of immense interest in recent studies. Cytokines' comprising of CAD-susceptible alleles could significantly affect the disease pathophysiology by up-regulating the inflammatory markers expression (98).

TNF is a family of cytokines which are produced mainly from activated macrophages in intracellular signaling pathway and others major extrinsic mediators of apoptosis. Many cells in the human body have two receptors for TNF-alpha: TNFR1 and TNFR2. The binding of TNF-alpha to TNFR1 has been shown to initiate the pathway that leads to caspase activation via the intermediate membrane proteins TNF receptor-associated death domain (TRADD) and Fas-associated death domain protein (FADD). These stimulii lead to tyrosine phosphorylation of Ras by phosphotyrosine kinase (PTK); the protein kinase cascade leads to cytokines' production. Ras activates Raf-1/mitogen-activated protein kinase, followed by the activation of the mitogen-activated protein kinase (MAPK) family of protein kinases,. Binding of this receptor can also indirectly lead to the activation of transcription factors involved in cell survival and inflammatory and also induce apoptosis in a caspase-independent manner. Figure (2.8) (99).

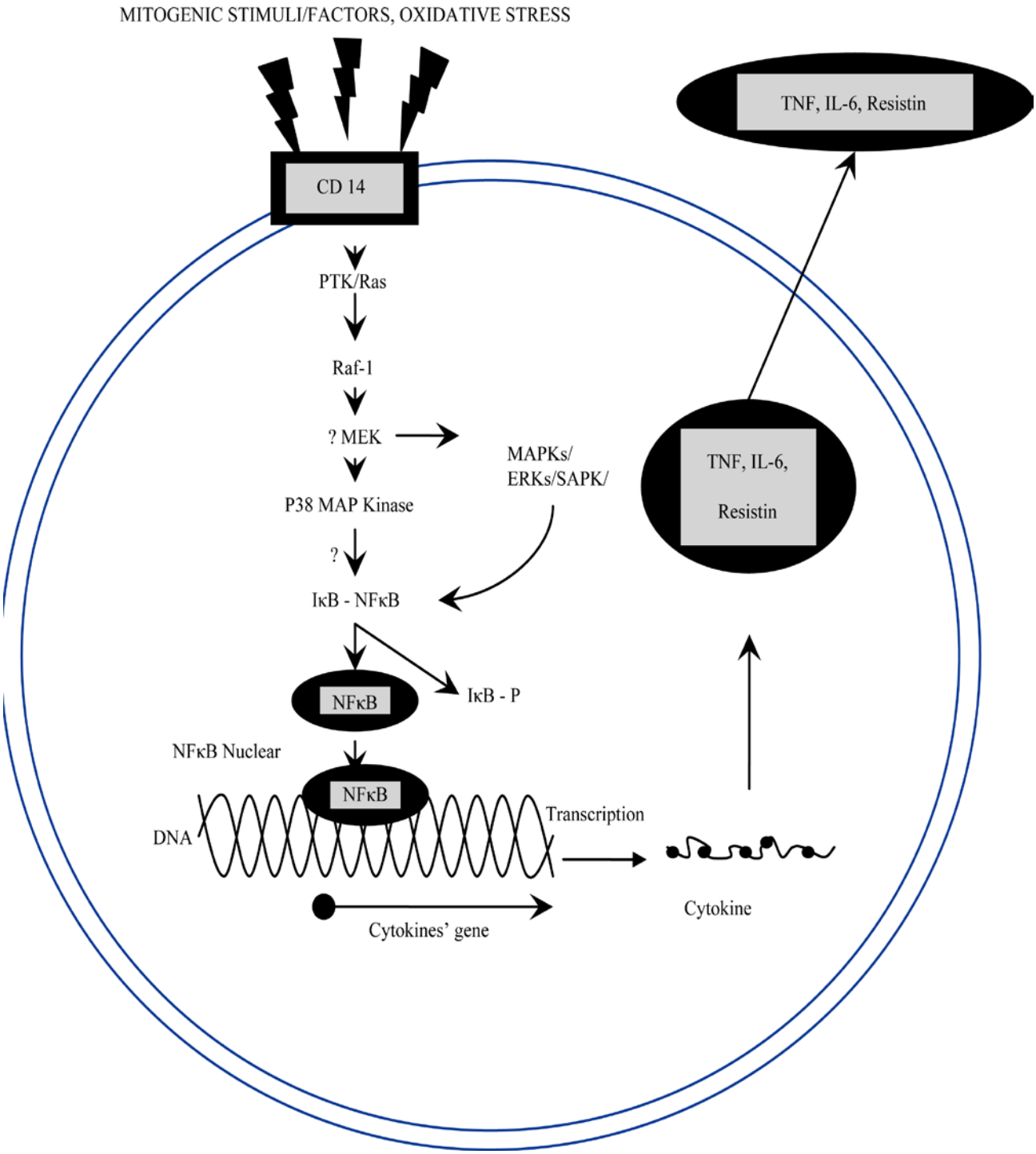


Figure 2.8 TNF single pathway in aged coronary arteries ,(100).

2.5.1. Role of Tumor Necrosis Factor Alpha and Tumor Necrosis Factor Beta Polymorphism in Coronary Artery Diseases :

Coronary artery disease include some diseases like acute myocardial infarction ,stabile and unstable angina and also reperfusion of previously ischemic cardiac tissue. It is associated with a strong inflammatory response that contributes to the acute extension of the initial injury, but that also participates in mechanisms of repair of the myocardium. In coronary artery diseases which are complex sequence of pathophysiological events which be contributed , tumor necrosis factor-α (TNFα) plays a multifaceted role in coronary artery disease . Tumor Necrosis Factor (Alpha (TNFα) affects lipid metabolism and hyper-triglyceridaemia led to decreasing lipoprotein lipase activity in cultured adipocytes and It exerts a specific hypertrophic effect on isolated cardiac myocytes (net synthesis of protein). It increases hepatic fatty acid synthesis and is associated with increased levels of triglycerides and promotes contractile dysfunction mediated by release of cardiac nitric oxide (101).

Furthermore, short-term administration of exogenous TNFα to healthy rats induces a progression but in the reversible depression of left ventricular function and its dilation in cardiac disease, it's possibly to through a control of the subtle balance between proteins that affect collagen turnover (r metalloproteinases and their natural inhibitors (102) .

In coronary artery diseases like Myocardial Infarction, the TNFα protein is produced and gene transcription, and the expression of one of its cellular receptors, TNFR-1, is consistently increased acutely in the myocardium. aAfter myocardial infarction it is induced by permanent ligation of the left anterior descending coronary artery . In contrast, the endogenous TNF system is shown to be the exert a protective effect of TNFα in the infarcted myocardium. Indeed, infarct size and the extent of apoptosis are significantly larger in mice lacking both TNFαreceptors and subjected to acute coronary artery (103).

The TNF α –308 G-A polymorphism, calleed TNF2, is strongly linked to HLA haplotype A1-B8-DR3. This linkage is of interest because it has been reported that individuals with this haplotype produce high levels of TNF α and are more susceptible to a wide spectrum of autoimmune diseases. Studies of the TNF α promoter gene have shown that the TNF2 allele leads to an increased tissue-specific constitutive and inducible expression, both in vitro and in vivo, compared to the wild-type TNF1 allele (104).

Because of the different functions of TNF α, role of the –308 polymorphism in determining the susceptibility and/or the prognosis of coronary artery diseases has extensively been studied. Even if data are controversial, the polymorphism has been associated with different diseases including (105).

TNF polymorphisms may be related to contribution of CAD risk and are reported to be associated with the role of TNF-α in animal models of myocardial infarction (MI).

TNF-α deficiency and TNFR1-deficiency affect infarct size. TNFR2-deficiency did not influence infarct size (106).

TNF promoter polymorphism function was stimulated by several case–control studies of the polymorphism in relation to cardiac disease . The TNF polymorphism has been associated with risk factors such as insulin resistance syndrome, obesity and CAD, and has been associated with various inflammatory and autoimmune diseases. Associations between polymorphism and immune-mediated diseases such as rheumatoid arthritis and Crohn's disease (CD) have been reported (107).

In ischemic heart disease, increased TNF-α levels leads to TNFR1-mediated left ventricular dysfunction and increased MMP-2 activity, resulting in degradation of the matrix, and finally increased apoptosis of cardiomyocytes (108).

Tumor Necrosis Factor (beta (TNF-β), also known as LT-alpha, has been shown to be a mediator of inflammation and immune function. Evidence is also accumulating that TNF-beta is a mediators in the pathogenesis of certain diseases like autoimmune disease and also mmay bealso contribute or be related to coronary artery disease. (TNF-β) has been expressed in atherosclerotic plaques and has been implicated in the pathogenesis of atherosclerosis and Coronary Artery Disease (CAD). (TNF-β) Polymorphisms in the TNF-β gene on Chromosome 6p21 have been associated with susceptibility to CHD,. Some researches has shown the association of seven single nucleotide polymorphisms (SNPs) across the LTA gene, and their related haplotypes, with risk of myocardial infarction (MI) in the International Study of Infarct Survival (ISIS) (109) .

The proinflammatory cytokine of Tumor Necrosis Factor beta or lymphotoxin-α (LTA or tumor necrosis factor-β) was foundwas found in atherosclerotic lesions and may contribute to these processes. Furthermore, (TNF-β) may also induce adhesion molecules and cytokines from vascular endothelial and smooth muscle cells.

A large-scale association study in Japanese Osaka Acute Coronary Insufficiency Study Group has identified functional single nucleotide polymorphisms (SNP's) within the LTA gene that were associated with risk of myocardial infarction (LTA T26N and LTA A252G) (110).

Among these SNPs, the LTA T26N polymorphism induced an increase approximately by two fold higher expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human coronary artery smooth muscle cells and the presence of LTA A252G polymorphism was associated with a 1.5 fold higher transcriptional activity of LTA (111).

Tumor Necrosis Factor beta (TNF-β) and tumor necrosis factor- α (TNF-α) genes are in significant linkage disequilibrium and are situated close to each other within the human leukocyte antigen (HLA) class III cluster on the short arm of chromosome 6. Several other studies have examined the association between LTA gene polymorphisms

and coronary artery disease (CAD), but these studies were based on identification of prevalent cases and results were inconsistent (112)

A prospective longitudinal study is needed to investigate the association between (TNF-β) and (TNF-α) gene polymorphisms and CAD. Furthermore, the association between (TNF-β) and (TNF-α) gene polymorphisms, plasma levels of inflammatory markers and cell adhesion molecules are unknown galectin-2 protein as a regulator of LTA protein secretion and therefore they are also potentially important in modifying the degree of inflammation (113).

3. MATERIALS AND METHODS

3.1. The Patient Population and The Study Protocol:

Research of coronary artery disease (stable and unstable angina pectoris, myocardial infarction) .outpatient examination resulted in complaints and referring diagnosis or with any doubt owed to patients with coronary angiography requested has been created. The research included 50 consecutive patients and 50 control group.

All patients were informed about the scope of the research and approval for participation. Patients were determined to risk factor of CAD . Routine echocardiographic and biochemistry measurements in patients with aortic stenosis by clicking review and then underwent coronary angiography.

The main examples of individuals involved in peripheral venous blood, flesh lendia interested in tubes containing EDTA blood my acid (EDTA) and serum biochemistry of tube for the retrieved content to leave their. EDTA in blood samples taken in the tube,

3.2. The Criteria for Receiving Work:

Patients with Middle or advanced coronary artery disease , Intermediate or advanced patients with valve insufficiency Diagnosed patients, patients with Atrial fibrillation, atrial flutter, other tachyarrhythmia and Bradyarrhythmia are available patients,

Electrocardiographic bundle branch block advanced patients, early acute myocardial infarction or unstable angina pectoris Patients, Patients with congenital heart disease Patients with Symptomatic heart failure, Holds the Aorta systemic diseases , Patients with Aortic aneurysm.

The Volunteers Included The Study Criteria: 1. Age > 18 years. 2. Gender: male and female. 3. Patients diagnosed with cardiac disease (case group).4. Signing the written informed consent form.

3.2.1. Coronary Artery Disease Diagnosis:

Physical Exam: During a physical exam, at health professional may use a stethoscope to check arteries for an abnormal whooshing sound called a bruit, which may indicate poor blood flow due to plaque buildup. also may check to see whether any of pulses (for example, in the leg or foot) are weak or absent, which can be a sign of a blocked artery.

Diagnostic Tests: the healthcare provider may recommend one or more tests to diagnose atherosclerosis. These tests can help define the extent of disease and the best treatment plan.

Blood Tests check the levels of certain fats, cholesterol, sugar, and proteins in your blood. Abnormal levels may put at risk for atherosclerosis.

Ankle/Brachial Index compares the blood pressure in the ankle with the blood pressure in the arm to see how well thr blood is flowing. Used to help diagnose CAD.

ECG (Electrocardiogram) detects and records the heart's electrical activity. It shows how fast the heart is beating and its rhythm (steady or irregular).

Echocardiography uses sound waves to create a moving picture of heart. The test provides information about the size and shape of heart, how well your heart chambers and valves are working, and areas of poor blood flow.

Computed Tomography Scan creates computer-generated pictures and can show hardening and narrowing of large arteries.

Stress Testing: is the exercise (or are given medicine if you are unable to exercise) to make heart work hard and beat fast while heart tests are done. A stress test can show possible signs of CAD.

Angiography: Angiography uses dye and special X-rays to reveal the insides of your arteries. It can show whether plaque is blocking your arteries and how severe the blockage is.

3.3. Materials and Devices:

3.3.1. Preparation and the separators used:

DNA isolation

İsolation mixture:

Ph. 8,8
10.5Mm Tris-CL
10.5 Mm NaCl
10.5 Nm EDTA
8M Guanidine hydrochloride
1.12mg/ml is composed from Proteinase K.
DNA isolatıon system :(IPrep purelink, Invitrogen, Thermo Fisher Scientific Inc)

3.3.2 .The Equipment Used:

DNA isolatıon robot (IPrep purelink, Invitrogen, Thermo Fisher Scientific Inc) Nano drip 2000(Thermo Fisher Scientific Inc) . Real time PCR (LIGHT CYCLE 480 II Instrument, Roche Diagnostics), 7500 fast real time PCR instrument, Apple compuer ,Flurometer (Quibit 3.0, , Invitrogen, Thermo Fisher Scientific Inc) ,plate centrifuge (Hettich), centrifuge (centrifuge 22R,Beckman Coulter), Microplate washing device(WHYM200,Powen Medical co) , +4°C Refrigerator(Haier),-20°C Refrigerator(Haier),Ultra-pure water (Pure lab option Q,Elga), Vortex (V.I. plus Biosan) , pipette Kit (Thermo Fisher Scientific Inc.).

3.4. Methods:

3.4.1. Genomic DNA Isolation from Blood:

All patient and control groups ' tube of EDTA blood DNA taken in isolation have been maintained as regards a + 4 °C refrigerator. IPrep DNA extraction DNA isolation, robot of the examples (Invitrogen) from the blood genomic DNA isolation Kit with iPrep (iPrep performed Blood kit).

All patients and healthy control have been taken blood from peripheral venous, 5 ml EDTA tube. Invitrogen DNA isolation Kit for isolation of DNA blood (iPrep ™ PureLink ™ performed Blood Kit). This system is for each instance, in a study using peripheral blood 350µl 100 example(50 patients and 50 control groups) has the capacity to do DNA isolation. In accordance with the procedure within the kit Invitrogen has been applied to a vent iPrep Insulation. iPrepTM Insulation Device ChargeSwitch ® Technology (CST ®).This system can be changed via the buffer pH, surface load, depending on the magnetic bead-based technology. In the case of low pH CST beads negatively charged nucleic acid skeleton has a positive is connected to the load.

Therefore, proteins and other contaminants are washed with wash buffer solution . Nucleic acid to clean up; beads on the surface of the load, pH low salt washing (EGP) to neutralize pH 8.5 using output buffering is raised. Isolated nucleic acid goes into the wash buffer immediately and would be ready for use in applications. This 45 minute DNA isolation process in closed system took place and in this process the end of the battle, nearly 150 µl DNA. Purify it diluted tube was taken to the aqueous DNA samples at + 4 °C in the fridge.

3.4.2. Measurement of DNA Purity

Tris-EDTA solution with the DNA samples 1/100 sulandırıldı and 260 nm and 280 nm in wavelength Nanodrip in the measurements of DNA purity and concentration. begins\/ml (50ng\/µl) of double-stranded DNA has given 260 nm wavelength absorbance value of the contents of 1 optical density (OD). With this basic information, taking advantage of 260 nm concentration of DNA by applying the following formula in measuring value is evaluated.

dsDNA concentration = 50 µg/mL × OD260 × dilution factor.

3.4.2.1. TNF polymorphısm belonging to Real Tıme PCR Conditions:

TNF alpha & beta genotype code will be analysis by using Real Time PCR method by using ABI 7500 fast real time PCR instrument and TaqMan assay (primer and fluorescent probes) Allelic discrimination will be performed by software of ABI 7500 fast real time PCR instrument automatically or manually.

Genotyping study of Real-time Polymerase chain reaction (Real Time) method with 7500 Fast-Real Time PCR (Applied Biosystems are) using the device. Genotyping made gene regions; group TNF α and TNF-β polymorphism analysis. These regions as primer and probe sets spesfik \"TaqMan Genotyping Assays\" polymorphic region related to the Rs number sequences and probes fluorescence dyes are given .

Real-time PCR , PCR Amplification based on the same Foundation (forward and backward primers Taq polymerase gene region that connects with the DNA polymerase enzyme synthesis with) but additionally depends on DNA replicated zone fluorescence dye probes and probes Taq polymerase by hydrolysis released the result of fluorescence glows genotyping with allow-read is a system that allows.

Real Time fluorescence probes used to Savage (Wildtype, WT) and Mutant alleles at two different alleles at sequence (M) and contains two different wavelength paint and thus alles of distinction.

Place the 96-well plate or 384-well plates on ice for the remainder of the reaction preparation in order to keep the contents of the plate cold. Add 20µL of the prepared reaction mixture to wells that need that particular SNP assay. Add 2µL of PCR water to NTC wells instead of DNA. Add 2µL of 4ng/µL DNA to the appropriate wells. With the plate now sitting on the lab bench, place a cover slip over the plate and use the roller to make sure the seal is tight. The plate must be vortexed and centrifuged (spun-down) before performing a run on the RT PCR machine (see protocol for Allelic Discrimination on the 7500 Fast Real-time PCR.

3.4.2.2. Real Time Protocol:

Real-time PCR reagents and reaction mixture is necessary for RT-PCR showing below in Table 3.1.

Table 3.1. The reaction mixture: Total reaction volume will be per 20µl

THE MATERIAL USED	**QUANTİTY**
Master Mix	10µl
TaqMan Assay	0.5µl
DNAse,RNAse, Free water	8.5µl
Tamplet DNA	1µl

Real Time PCR conditions**:** 95 °C 10-minute Wait at every stage and a for loop (Hold) (Table 3-2);

Table 3.2. Real Time Protocol Condition:

		40 Cycle	
	WAIT	DENATURATION	CONNECTING\/ELONGATION
TEMPERATURE	95 °C	92 °C	60 °C
DURATİON	10 Minute	15 Second	1 Minute

3.4.3. Statistical Analysis

Genotyping or performing statistical analysis of the data obtained with SPSS 13.0 program with Student T-test, Chi-square and Fisher's Exact Tests has been using and expressiveness has been considered as the value of $p < 0.05$. Seen as a risk factor for parameters was evaluated using logistic regression Analysis.

4. RESULTS

4.1. Working Groups Demographic Data

The study was concluded, coronary artery disease Biochemical parameters, and demographic data and, a total of 100 people individuals 50 patient (12 female and 38 male) and 50 control groups (20 female and 30 male) were statistically investigated .

The distribution of risk factors and control groups and identifying information are provided in Table 4.1

Table 4.1. Demographic characterstics of the study population with coronary artery disease

	Control %		Patient %	P Value
Body Mass Index (kg/m2)	26.792±4.421 (n=50)		28.631±6.152 (n=50)	0.870 (NS)
Age , x̄ ± SD (years)	59.87 ±14.793 (n=50)		62.80 ±8.439 (n=50)	0.097 (NS)
Gender	Male %	60.0% (n =30)	76.0% (n =38)	0.086 (NS)
	Female %	40.0% (n =20)	24.0% (n =12)	
History of Diabetics	Yes %	18.0% (n =9)	38.0% (n =19)	0.026* (S)
	No %	82.0% (n =41)	62.0% (n =31)	
Smoking	Yes %	42.0% (n =21)	58.0% (n =29)	0.110 (NS)
	No %	58.0% (n =29)	42.0% (n =12)	

n: number of sample, $\bar{x}$ ± SD**:** mean value ± Standard deviation, , student t test used for comperation of meanage values and chi square and other Demographic characterstics, * =significantly different ($p < 0.05$), NS= non significant ($p > 0.05$).

Advanced control with Chi-squared and student-t test analysis and coronary artery disease patient groups compared in terms of demographic features;

The distribution of Demographic characterstics and descriptive information in the patient and control groups are given in table 4.1. Both groups were found to be homogenous in age (p=0.097), body mass index (p=0.870) , Gender (p=0.086), Smoking(p=110) Presence between groups not showing any difference and not significant. Coronary artery disease was found to be more common in men (76%)than in women (24%).

In terms of the form of individuals people who have coronary artery disease with diabetic diseases is increased risk (p=0.026) and increase about 2 fold (OR=2.79).

4.2. Control and Patient Groups Risk Factor Related to the TNF Alpha Results

Total plasma cholesterol , LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to TNF Alpha genotype findings are given in Table 4.2.

As the result BMI (p=0.893),plasma cholesterol (p=0.052), triglycerides(p=0.531), low density lipoprotien (p=0.144),high density lipoprotien (p=0.192), not showing any significant .

Table 4.2. Total plasma cholesterol , LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to TNF Alpha genotype in all study group

	Genotype of TNF Alpha			
	AA $\bar{x}$ **± SD (n=50)**	**AG** $\bar{x}$ **± SD (n=39)**	**GG** $\bar{x}$ **± SD (n=11)**	**P Value**
Body Mass Index (kg/m2)	**26.869±1.118**	**27.584±1.118**	**31.992±1.78**	**0.893 (NS)**
Plasma Cholesterol (mg/dl)	**197.90±9.130**	**182.15±14.233**	**167.45±14.590**	**0.052 (NS)**
Triglycerides (mg/dl)	**162.86±27.275**	**145.49±17.497**	**136.27±27.960**	**0.531 (NS)**
Low-density lipoprotein (mg/dl)	**127.98±8.254**	**116.38±13.190**	**127.98±12.867**	**0.144 (NS)**
High-density lipoprotein (mg/dl)	**40.62±2.769**	**38.85±1.777**	**34.45±2.839**	**0.192 (NS)**

The result are shwon as **n**: number of individual , **,$\bar{x}$ ± SD:** mean of value ± Standard deviation, , one way ANOVA TEST used for comperation of TNF Alpha genotypes and lipid parmeters ,OR: Odds Ratio for risk, AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant, ***** =significantly different ($p< 0.05$), (NS)= non significant ($p>0.05$).

4.3. Patient Groups Risk Factor Related to the TNF Alpha Results:

The distribution of Total plasma cholesterol , LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to TNF Alpha genotype and descriptive information in the patient groups are given in table 4.3. As the result BMI (p=0.305),plasma cholesterol (p=0.102), triglycerides (p=0.149), low density lipoprotien (p=0.187),high density lipoprotien (p=0.377), not showing any significant.

Table 4.3. Total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters of the study population of Patient Groups Risk Factor related to the TNF α genotype.

	Genotype of TNF Alpha			
	AA $\bar{x}$ ± SD (n=24)	AG $\bar{x}$ ± SD (n=16)	GG $\bar{x}$ ± SD (n=10)	P Value
Body Mass Index (kg/m2)	27.864±2.309	28.151±1.980	31.238±2.473	0.305 (NS)
Plasma Cholesterol (mg/dl)	202.38±17.191	167.06±14.741	169.20±18.412	0.102 (NS)
Triglycerides (mg/dl)	167.88±18.020	128.69±22.507	142.70±21.015	0.149 (NS)
Low-density lipoprotein (mg/dl)	130.46±15.269	103.81±13.093	106.10±16.353	0.187 (NS)
High-density lipoprotein (mg/dl)	38.29±2.797	37.31±2.398	34.60±2.995	0.377 (NS)

The result are shwon as n: number of individual , $\bar{x}$ ± SD: mean of value ± Standard deviation, , one way ANOVA TEST used for comperation of TNF Alpha genotypes and lipid parmeters AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant, (NS)= non significant ($p>0.05$).

4.4. Control Groups Risk Factor Related to the TNF alpha Results:

The distribution of Total plasma cholestero, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to TNF

Alpha genotype and descriptive information in the control groups are given in Table 4.4. As the result BMI (p=0.429), plasma cholesterol (p=0.534), triglycerides (p=0.297), low density lipoprotien (p=0.155), high density lipoprotien (p=0.052), not showing any significant.

Table 4.4. Total plasma cholesterol , LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters of the study population of Control Groups related to the TNF α genotype .

Genotype of TNF Alpha					
		AA	AG	GG	P Value
Body Mass Index (kg/m2)	Male x̄ ± SD	27.704 ±4.05431	26.453 ±5.25957	26.531 ±4.06209	0.429 (NS)
	N	(26)	(27)	(49)	
	Female x̄ ± SD	25.9500 ±4.65356	27.1896 ±3.24656	39.5400± .	
	N	(24)	(23)	(1)	
Plasma Cholesterol (mg/dl)	Male x̄ ± SD	190.88± 36.597	192.15± 41.353	193.24 ±38.647	0.534 (NS)
	N	(24)	(27)	(49)	
	Female x̄ ± SD	193.77 ±41.288	192.65± 36.345	150.00	
	N	26	23	1	
Triglycerides (mg/dl)	Male x̄ ± SD	153.63± 78.328	155.04± 118.279	157.73± 101.113	0.297 (NS)
	N	(26)	(27)	(49)	
	Female x̄ ± SD	158.23± 119.428	157.17± 78.091	72.00	
	N	(24)	(23)	(1)	
Low-density lipoprotein (mg/dl)	Male x̄ ± SD	124.21± 27.214	124.85± 42.427	125.4±3 36.192	0.155 (NS)
	N	(26)	(27)	(49)	
	Female x̄ ± SD	125.69 ±43.037	125.13± 27.440	103.00	
	N	(24)	(23)	(1)	
High-density lipoprotein (mg/dl)	Male x̄ ± SD	39.6±3 6.807	42.41± 10.504	41.43± 9.009	0.052 (NS)
	N	(24)	(27)	(49)	
	Female x̄ ± SD	42.77± 10.539	39.91± 6.809	33.00	
	N	(24)	(23)	(1)	

The result are shwon as n: number of individual , x̄ ± SD: mean of value ± Standard deviation, , one way ANOVA TEST used for comperation of TNF Alpha genotypes and lipid parmeters AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant, (NS)= non significant ($p>0.05$).

4.5. Control and Patient Groups Risk Factor Related to the TNF Beta Results

The distribution of Total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to TNF Alpha genotype and descriptive information in the control groups are given in table 4.5. As the result BMI (p=0.352),plasma cholesterol (p=0.084), triglycerides (p=0.346), low density lipoprotien (p=0.456), high density lipoprotien (p=0.262), not showing any significant.

Table 4.5. Total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters of Control Groups Risk Factor related to the TNF beta genotype

Genotype of TNF Beta				
	AA $\bar{x} \pm SD$ **(n=12)**	**AG** $\bar{x} \pm SD$ **(n=19)**	**GG** $\bar{x} \pm SD$ **(n=69)**	**P Value**
Body Mass Index (BMI) (kg/m2)	**26.432±1.696**	**28.816±2.005**	**27.629±1.696**	**0.352 (NS)**
Plasma Cholesterol (mg/dl)	**174.08±13.511**	**203.58±15.928**	**186.72±13.511**	**0.084 (NS)**
Triglycerides (TG) (mg/dl)	**129.17±30.206**	**165.42±21.224**	**153.96±25.622**	**0.346 (NS)**
Low.density lipoprotein (LDL) (mg/dl)	**121.42±14.371**	**132.58±10.097**	**117.77±12.190**	**0.456 (NS)**
High-density lipoprotein(HDL) (mg/dl)	**40.83±3.107**	**36.63±2.183**	**39.70±2.635**	**0.262 (NS)**

The result are shwon as n: number of individual , , $\bar{x} \pm SD$: mean of value ± Standard deviation, one way ANOVA TEST used for comperation of TNF Beta genotypes and lipid parmeters . AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant, (NS)= non significant ($p>0.05$).

4.6. Assessment of Real Time PCR

Alleles of pateint found in the probe diskriminasyon fluorescence ışımaların 7500 Fast-Real Time PCR device is made in the form of read and interpreted automatically by the software. But diskrimine some examples that cannot be examined and interpreted the glow curves of \"manual\" (Figure 4-1,4-2).

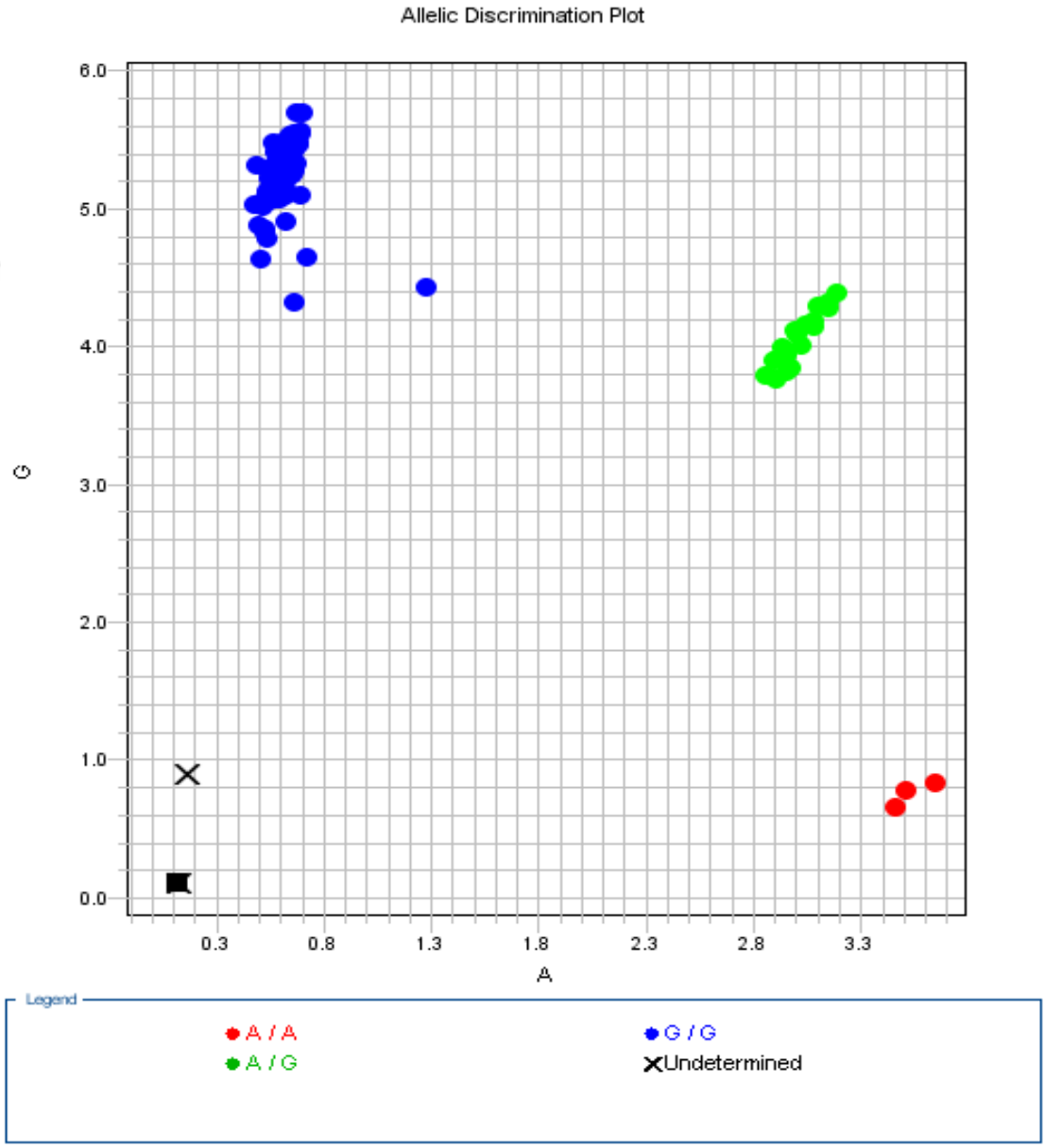


Figure 4-1: Allele Discrimination Display of TNF Alpha genotype

AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant

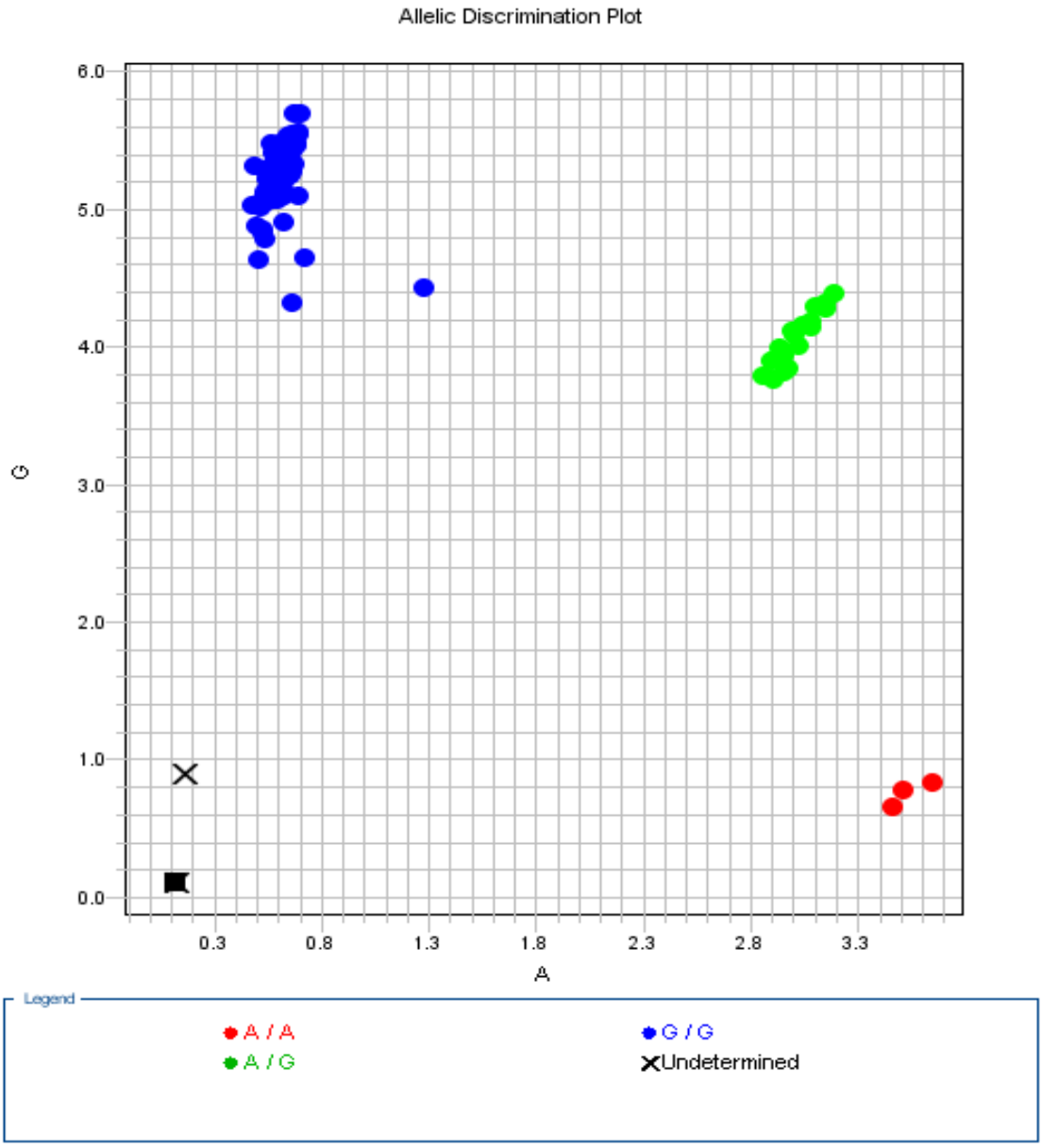


Figure 4-2: Allele Discrimination Display of TNF Beta genotype

AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant

4.7. Evaluation of Real Time PCR Statistical Perspective :

This study worked with 50 patients with coronary artery disease, and 50 healthy controls in this study, TNF Alpha, TNF Beta gene variations and coronary artery disease data distribution were given in Table 4.6 and Table 4.7 .

4.7.1. The Genotype and Allele Distributions for the Gene TNF- α

Table 4.6. we shown There were no significant difference among patient and control groups in AA Homozygote Wild Type Genotype (p=0.689) and also Heterozygote Genotype (p=0.151). The frequency of the Homozygote Mutant Genotype was found to be significantly higher in patients compared to controls (p = 0.004). The cardiovascular risk increased for Homozygote Mutant Genotype was 12.2-times in patients compared with the control group (OR=12.250, 95% CI=1.504-99.798; p=0.004).

Table 4.6. The Genotype and Allele Distributions for the TNF α Genotype .

	Control (n=50)	Patient (n=50)	P value	OR	Confidence Interval 95%
Genotype					
AA	**26(52.0%)**	**24(48.0%)**	**0.689 (NS)**	**0.852**	**1.867-0.389**
AG	**23(46.0%)**	**16(32.0%)**	**0.151 (NS)**	**0.552**	**1.247-0.245**
GG	**1(2.0%)**	**10(20.0%)**	**0.004***	**12.250**	**1.504-99.798**
Alleles					
A	**75(98.0%)**	**64(80.0%)**	**0.004***	**0.082**	**0.665-0 .010**
G	**25(48.0%)**	**36(52.0%)**	**0.689 (NS)**	**1.174**	**2.572- 0.536**

The result are shwon as n: number of individual , $\bar{x} \pm$ SD: mean of value ± Standard deviation, Chi-Square test compare TNF Alpha genotype in study group .,OR: Odds Ratio for risk, AA : Homozygote Wild Type, AG: Heterozygot, GG: Homozygote Mutant, * =significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

4.7.2. The genotype and Allele Distributions for the gene TNF-β

Table 4.7. we shown the AA Homozygote Mutant Genotype ratio (p=0.538), AG Heterozygote Genotype (p= 0.799), while GG: Hemozygot Wild Type ratio is (p=0.829) .(A) allel genotype ratio (p=0.829) and the last (G) allel genotype ratio (p=0.538) (p>0.05) genotype ratios there was no significant difference in the distribution. Table 4.7.

Table 4.7. The Genotype and Allele Distributions for the TNF-β Genotype

	Control (n=50)	Patient (n=50)	P value	OR	Confidence Interval 95%
Genotype					
AA	**5(10.0%)**	**7(14.0%)**	**0.538 (NS)**	**1.465**	**4.969-0.432**
AG	**10(20.0%)**	**9(18.0%)**	**0.799 (NS)**	**0.878**	**2.388- .323**
GG	**35(70.0%)**	**34(68.0%)**	**0.829 (NS)**	**0.911**	**2.126-0.390**
Alleles					
A	**20(30.0%)**	**23(32.0%)**	**0.829 (NS)**	**1.098**	**2.564-0.470**
G	**80(90.0%)**	**77(86.0%)**	**0.538 (NS)**	**0.683**	**2.315-0.201**

The result are shown as n: number of individual , $\bar{x} \pm$ SD: mean of value ± Standard deviation, Chi-Square test compare TNF beta genotype in study group, OR: Odds Ratio for risk, AA : Homozygote Mutant, AG: Heterozygot, GG: Homozygote Wildtype, NS= non significant ($p>0.05$).

5. DISCUSSION AND CONCLUSION

Coronary artery disease is a disease that results in myocardial ischaemia due to reduced blood flow to the heart muscle as a result of constriction or occlusion of the coronary arteries with an atheromatous plaque(114). Atherosclerosis, a major cause of CAD, has been thought of as a passive lipid deposition on the vessel surface for many years, and over time it has been thought that the vessels are completely blocked by accumulation. Today, it is defined as a disease in which both natural and adaptive immune system plays a crucial role in its development, multifactorial, chronic inflammation in every step from initial to advanced stages, and each risk factor contributes to pathogenesis by accelerating the underlying inflammatory process (115,116).

The degenerative atherosclerotic process that progresses progressively with the deterioration of endothelial functions and under the influence of various risk factors ultimately leads to serious clinical events and even death. Coexistence of risk factors significantly increases risk of CAD. Identification and treatment of risk factors in individuals for the prevention of this progressive process is necessary not only for the definition of CAD in asymptomatic individuals but also for the prevention of recurrent events in persons diagnosed with the disease (117).

The main etiological cause of coronary artery narrowing in CAD is atherosclerosis. Atherosclerosis; Is considered to be a multifactorial process involving inflammation, cell proliferation, and impaired lipid metabolism. In the early phase of atherosclerosis, chronic inflammatory response to oxidative modification of LDL is thought to lead to subendothelial accumulation of inflammatory cells such as macrophages and T-lymphocytes (118). Epidemiological studies have demonstrated the role of risk factors such as smoking, cholesterol, hypertension and diabetes mellitus in the development of atherosclerosis. Experimental studies have shown that these risk factors play a widespread body reaction by triggering a general inflammatory response. In response to the risk factors, both systemic acute phase reactors become active and an endothelium signal transduction begins (119).

NCEP published in 2001 III. Factors such as age, gender, family history, smoking, smoking, and hyperlipidemia according to the Guidelines for the Protection and Treatment of Coronary Heart Disease published by the Turkish Society of Cardiology in 2002, as well as the adult treatment panel (ATP III) Are among the factors (120).

Risk factors leading to CAD is a significant economic burden for the treatment and prevention of cardiovascular disease. They are made and installed expenditures that constitute the majority of coronary heart disease (121).

Epidemiological studies proved that smoking, cholesterol, hypertension, diabetes mellitus are risk factors for the development of atherosclerosis.

CAH is the largest cause of mortality and morbidity in Turkey as well as in the United States and European countries. According to the (COHORT) study 2009 report,

3.1 million people are suffering from CAD in the population age 35 and over of 29.5 million people in our country. In addition, raporda has been reported to have a rate of 6.4% increase in CAD since 1990. In Turkey , changes in lifestyle are independent of age, population growth and population, resulting in an average increase of 3% annually. In 2008, 390 thousand cases of coronary cases per year are found in our adults, and when 90 of them are removed immediately, 300 thousand nonfatal CAD patients are left for treatment. While the prevalence of CAD in our country is 6% in the 45-54 age group and 17% in the 55-64 age group, this rate increases to 28% in the individuals aged 65 and over. Prevalences An increase of 80% in individuals aged 50 years and over compared to the results reported in the 1990 report (122).

In this study, we would like to compare in terms of risk factors for coronary artery disease in patient group and control group , There are many risk factors associated with coronary heart disease and ischemic heart diseases. Some of risk factors such as family history, ethnicity and age, cannot be changed and other risk factors that can be treated or changed include tobacco exposure, high blood pressure (hypertension), high cholesterol, obesity, physical inactivity, diabetes, unhealthy diets and harmful use of alcohol(123).

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

In many studies, the incidence of CAD and associated death rates are closely related to age. After 40 years of age, the incidence of atherosclerosis and associated CAD is increasing in parallel with the increase in age. The most common age of CAD is 50-60 for males and 60-70 for females. It is also reported that long-term Framingham is an independent risk factor for older age in prospective cardiac studies (124).

Advanced atherosclerotic lesions occur in males about 20 years earlier than females (125). Men who are exposed to major risk factors for a longer period of time may partly explain the sex difference in CAD. In men, LDL cholesterol and HDL cholesterol decrease with puberty, but this change is not observed in women. This aspect of the gender difference is related to hormonal differences (126).

However, in the (COHORT) study 2009 report, the annual mortality rate associated with CAD in European countries was reported to vary between 2 and 8 in males and 0.6 to 3 in females between the ages of 45-74, while in the same age group this rate was 7.6 in males and 3.8 in females Reported as (127).

According to the Framingham Heart Study results, the incidence of CAD in men was 2 times higher than in women. In a study of 14786 people aged 25-64 years, the incidence of coronary artery disease in men was three times higher and mortality was five times higher in women (128)

In our study the Risk factors that was not be significance from point of view between the gender related to coronary artery disease, and among individuals that are

included in our work overlaps with the literature in a way that no significant difference between men and women ($p > 0.05$).

While the ratio about 76% of the patient group was male, and 24% of the patient group was female and male is suffering to coronary artery disease more than female .

The prevalence of coronary risk factors and the number of cardiovascular deaths show a decline in men, whereas in women it is not mentioned until such a decrease, since the measures we take for cardiovascular diseases during the 30 years we left behind are primarily directed at the male population (129).

According to Turkey Statistics Institution (TSI) heart disease mortality data, the share of total deaths increasingly tends to increase. Heart disease rate in 1989 was 40%, was 45% in 1993 (130), and 40 % in 2009, was 40.4% in 2013 and 39.6% in 2014 Prevalence in our country of 45-54-year-old group and 55-64-year-old group was 6% and 17%; and after 65 years of age this rate rises to 28%. Prevalence in 1990 report compared to these result for individuals over the age of 50, 80% increase is observed The cause of death as of age groups of most the diseases of the circulatory system is more in 75-84 age group (131).

In our study there was not significant difference between smokers and non-smokers in the patients and control groups According to our results, in CAD patient group, smoking rate was about 58 %. However in the control group rate was determined to be 42% it was determined as cigarette usage CAD ($p = 0.110$).

However, according to patient group , cigarette smoking in patients compared with patients who was not smoking the incedice of CAD is increased.

Smoking is the major risk factor in the development of CAD, a large number of studies shows. Some studies compared non-smokers with smokers who smoke 20 or more cigarettes a day. CAD prevalence was 2-3 times more in smokers, the study found. (132).

Smoking, one of the most important preventable risk factors, is of particular importance due to its widespread use in the country as seen in the (COHORT) study follow-up. Again according to COHORT data, smoking habit tends to decrease in males in our country and increase in females. Considering that the rate of CAD mortality in our country is the highest among European countries, the seriousness of this increase in the tendency of smoking in our women is becoming more important(133).

Critchley and Capewell (2003) .showed that the mortality risk reduction is associated with smoking cessation in patients with coronary heart disease systematic review. cigarette consumption, mortality and termination of CAD patients advanced cardiac event is 50% the risk of increase in age, gender, and cardiac event index, They have pointed out that independent of factors such as living area(134).

Nair J. et al. (2007)It has been shown that DNA lesions from lipid peroxidation products are significantly increased in vascular smooth muscle cells isolated from Abdominal Aorta fragments of atherosclerosis patients compared to healthy control group, and when patients are grouped according to smoking cigarette use, There was an increase in the number of patients who did not use cigarettes (patients who quit smoking). It has also been shown that DNA lesions in the patient group with aromatic agents are increased compared to the control group (135).

Nicole Jensky et al and friends (2011) . found in patients with ischemic heart attack and aorta atherosclerosis and regularly smoking , the vascular smooth muscle Cells isolated from the fragment healthy compared to the control group lipid peroxidation products-induced DNA lesions is significantly increased(136).

Epidemiological studies have shown that both active and passive cigarette smoking increase the risk of atherosclerosis. Passive smoking is strongly associated with an increased risk of CAD, and also increased risk is disproportionately high at low levels of exposure. Two immediate effects of smoking on heart and circulation are an increase in your heartbeat rate, a sharp rise in blood pressure (137).

These effects of smoking are caused by nicotine which acts on the nervous system, causing the heart rate to rise and blood vessels to constrict. This narrowing of the blood vessels causes the blood pressure rise and strain is put on the heart(138).

Smoking is the most powerful risk factor that women . smoking tobacco or long-term exposure to second hand smoke raises risk for CAD and heart attack. Smoking exposes carbon monoxide to arterial wall. This chemical robs your blood of oxygen and triggers a buildup of plaque in your arteries. Smoking also increases the risk of blood clots forming in your arteries . Blood clots can block plaque-narrowed arteries and cause a heart attack. The more smoke led to greater risk for a heart attack. Even women who smoke fewer than two cigarettes a day are at increased risk for CAD (139).

Smoking is a major cause of Coronary Artery Disease CAD and causes one of every three deaths from CVD. Smoking can: Raise triglycerides (a type of fat in your blood) (154), lower "good" cholesterol (HDL), make blood sticky and more likely to clot, which can block blood flow to the heart and brain, damage cells that line the blood vessels, increase the buildup of plaque (fat, cholesterol, calcium, and other substances) in blood vessels, cause thickening and narrowing of blood vessels (140,141).

When exposure to high concentrations of carbon monoxide, it is clearly dangerous and can be lethal. Inhaling low levels of carbon monoxide has the potential to produce adverse health effects, especially in people with coronary artery disease (142). Many researchers consider the major mechanism for the toxicity of carbon monoxide is its ability to contend with oxygen for binding to hemoglobin, the protein that transports oxygen through the bloodstream and releases it to cells and tissues. Cells need oxygen to produce adenosine triphosphate (ATP), the energy source for muscle contraction and other body functions. The binding of carbon monoxide to hemoglobin, forming

carboxyhemoglobin, reduces the amount of oxygen available to the heart muscle and other tissues for synthesizing ATP (143,144).

On other study to compare the patient and control groups in terms of CRP levels, non-smokers and smokers does not show a discrepancy between patients, such as smokers and smokers in the control group with smokers and smokers and CRP levels had no significant difference. One of the most important preventable risk factors is smoking, as can be seen in follow-up work COHORT study in our country due to the widespread use of special importance (145).

Still, according to data from the habit of smoking, (COHORT) study , (Heart Disease and Risk Factors in Turkish Adults) in turkey , female smokers and male smokers tend to increase. Mortality of women of turkey from CAD is highest among European countries, considering the seriousness of this increase in the global movement towards our women smoking is becoming even more important (146).

Diabetes Mellitus is an independent risk factor for the CAD. Risk increases twice in diabetic men and the risk in diabetic women increases four times in Framingham Heart Study. Patients with diabetes mellitus have 2 times higher risk of cardiovascular disease (147).

Turkish society of Cardiology by reducing the risk of possible initiatives for the elimination of the factors regarded as CAD, one of the main factors of cardiovascular morbidity and mortality can be increase up to 2 time the of patient with diabetes, due to the increasing importance of community health, the American Heart Association by major risk factors 10 years ago notifiable (148). The prevalence of diabetes is just not in society, as well as such advanced societies in developing countries is increasing rapidly. The number of adults with diabetes in the world in 2025 will be 300 million, the majority of the increase will occur in developing countries has been reported (149,150).

Huxley R, Barzi F, Woodward M. Excessto (2006) studied the risk factors of coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies in meta-analysis examining ensure both 100 diabetes associated with increased risk of coronary mortality in women than men (151).

In our study, the patient groups with diabetes was 38% of CAD while about 62% of the patient without diabetic and in terms of diabetes ($p = 0.026$) have found significant difference between the control group and CAD and increased about two fold odd ratio (OR: 2.79) as shown in (Table 4.1).

Obesity, one of the modifiable risk factors, is known to be a complex multifactorial chronic disease and it is thought that social, behavioral, cultural, physiological, metabolic and genetic factors have developed as an interim result(152).

Beyond just being a problem of my country, it is a serious public health problem in the world. Globally, 1.1 billion adults and 10% of the child population are considered

overweight or obese(153). While obesity and central obesity cause general mortality increase, central obesity is considered as an important risk factor for CAD (154,155).

Prevention of obesity is one of the basic principles of protection from cardiovascular diseases. It will be possible to prevent a significant part of the deaths due to CAD by preventing weight gain from young adulthood, increasing physical activity, raising awareness of eating habits(156,157).

The direct effect of diabetes and hyperinsulinemia to endothelial cells can contribute to the emergence of atherosclerosis, the vascularization of endothelium which has multifunction (regulation of vascular tone, platelet adhesion, coagulation and fibrinolysis) and is a dynamic tissue that is considered to be the link between insulin resistance. Therefore, endothelial dysfunction draws even more attention (158).

The etiology of atherosclerosis in diabetes is most important risk factor. Chronic hyperglycemia, depending on the amino acid residues of non-enzymatic glikolizasyon leads to significant changes in the structure. The degree of hyperglycemia determines the size of the protein in Glycolysis (159,160).

In Type-2 diabetes or uncontrollable type-1 diabetes insulin resistance in accompanying hyperinsulinemia insulin-like growth factor-1 (IGF-1), circulating insulin leads to increase of growth factors, depending on the. Glycolysis of proteins and also of a variety of local growth factors, mature, they can warn the proliferation of atherosclerotic plaque fibromuscular component (161).

In Type 2 diabetes, produced by adipose tissue, TNF-α, IL-6, IL-1β cytokines such as high and low concentrations of adiponectin on glucose homeostasis, chronic hyperinsulinemia and insulin resistance will lead to the development of direction there are harmful effects (162,163).

Coronary Artery Disease CAD is an important risk factor in the development of type 2 diabetes with microvascular complication. Strict blood sugar is control and to be able to prevent it (164). In particular, despite the annual increase rate of 2.7% in the world and in trukish country twice the incidence of new instability in the development of diabetes years speed 360 thousand, to help protect public health strategy in our society in the center of lifestyle changes should be a lot of emphasis on ESDP (164). The two groups with overweight BMI pointed out considering the incidence of type 2 diabetes(165).

Diabetes mellitus DM is included both in the NCEP and International Diabetes Foundation (IDF) definitions of the metabolic syndrome. It is estimated that the majority (~75%) of patients with Type 2 diabetes or impaired glucose tolerance (IGT) have metabolic syndrome. The presence of the metabolic syndrome in these populations relates to a higher prevalence of CVAD compared with patients with Type 2 diabetes or IGT without the syndrome (166)

The approximate prevalence of Diabetic Mellitus in patients with coronary artery disease (CAD) is 50%, with a prevalence of 37% in patients with premature coronary artery disease (≤age 45), particularly in women. With appropriate cardiac rehabilitation and changes in lifestyle (e.g., nutrition, physical activity, weight reduction, and, in some cases, pharmacologic agents), the prevalence of the syndrome can be reduced. (167,168)

An early major contributor to the development of insulin resistance is an overabundance of circulating fatty acids . Plasma albumin-bound free fatty acids (FFAs) are derived predominantly from adipose tissue triglyceride stores released by lipolytic enzymes lipase. Fatty acids are also derived from the lipolysis of triglyceride-rich lipoproteins in tissues by lipoprotein lipase. Insulin mediates both antilipolysis and the stimulation of LPL in adipose tissue. Of note, the inhibition of lipolysis in adipose tissue is the most sensitive pathway of insulin action. Thus, when insulin resistance develops, increased lipolysis produces more fatty acids, which further decrease the antilipolytic effect of insulin. Excessive fatty acids enhance substrate availability and create insulin resistance by modifying downstream signaling. Fatty acids impair insulin-mediated glucose uptake and accumulate as triglycerides in both skeletal and cardiac muscle, whereas increased glucose production and triglyceride accumulation are seen in liver (169,170).

Hyperglycemia may be directly and indirectly led to increasing free fatty acid load cause of oxygen free radicals. This triggers many negative metabolic pathway and increases vascular permeability, fibrinolytic activity, inflammation and then triggers a process that reduces (171).

One of the risk factors of coronary artery diseases is obesity can be modified, complex multifactorial is known to be a chronic disease and social, behavioral, cultural, physiological, metabolic, and genetic factors are thought to develop as a result of the interaction (172). According to the study, obesity (COHORT) study the prevalence in males over 21% 30 pomp type diving, 43% in women (173). In our country and in the world it is a serious public health problem. 1.1 billion all over the world adult child with 10% of the population is overweight or obese is considered excessive (174).

Obesity and abdominal obesity also cause an increase in overall mortality, especially abdominal obesity is considered as an important risk factor in terms of CAD (187,188). In most common definition of obesity BMI criteria of (body weight (kg) divided by square (m^2) is used. Underweight = <18.5, Normal weight = 18.5–24.9, Overweight = 25–29.9, Obesity = BMI of 30 or greater (175).

The BMI in our study was 28,63 ± 6,15 kg / m² in the patient group and 26,79 ± 4,21 kg / m² in the control group and its not significant .

Obesity is considered a risk factor and an independent predictor of coronary artery disease, as observed in the Framingham heart study, Manitoba study and Harvard public health nurses study. In the Framingham cohort, patients aged 28 to 62 years were followed for a mean of 26 years. Among men younger than 50 years, the heaviest group

experienced twice the risk of coronary disease compared with the leanest group. The risk was increased 2.4-fold among obese women of similar age, and this was after adjusting for the influence of other major cardiovascular risk factors. (176)

Nurses Study, the Heart study and (COHORT) study(2013) observed the high risk of cardiovascular diseases and obesity had been shown to be an independent risk factor. Despite this, He, in Framingham 2011 Heart group working and should again be reviewed mortality modeling articulated (177).

Over the last 10 years BMI of women in turkey was1.26 kg\/m2 and in men was 1.29 kg\/m2 it they were increased., BMI relative to risk, in adults CAD < 25 kg\/m². BMI relative to risk, in adults between 25-29.9 kg\/m² increase 1.39 times, and this risk in adult between 30-34.9 kg\/m² increase 1.86 times (178) .

In our study, the mean BMI, this result is in accordance with the patient group, is 28.6310 ± 2.93 kg\/m², while in the control group was found to be 26.7920 ± 3.60 kg\/m². Obesity prevention is one of the basic prevention principles of cardiovascular disease. Weight loss, increasing physical activity, nutrition Habits prevent heart diseases and awareness can limit significant portion of CAD. (179).,

Excess adipose tissue leads to increased levels of proinflammatory activity and consequently reduce anti-inflammatory mechanism of inflammatory activity. Imbalance occurred when inflammatory level generated from adipose tissue the immune cell populations as strong from the fatty tissues are affected. The fat tissue macrophages infiltrating people obese and the number of macrophages to assess adiposity shows the degree of correlation. Peripheral circulating monocytes, MCP-1 and TNF-α, are activated and play role. Pre-differentiable capabilities of macrophages adipocytes. Macrophages activated by insulin resistance effects on adipocytes products and this function can contribute to fatty tissue. This can change the glucose processing (180). Cell culture study by Bassol with macrophages conditioned by adipocytes with and inflammatory cytokine production from increased this hypothesis was supported later. (181).

In the COHORT study 2009 study, prospectively, diabetes mellitus has been shown to increase cardiac events independently of age, blood pressure, and central obesity by about 80% (182).

In Turkey, especially with age people tend to more sedentary lifestyle, have more weight gain and diet habits of change in a negative way, that can result in the disease . Non-traditional cardiovascular risk factors and cardiovascular events in patients exposed to predict cardiovascular risk. The researchers are better directed to research new tokens. (183).

The development of many coronary artery diseases, and high morbidity and mortality rate of CAD lead to increased interest in study to prevent this disease and for early diagnosis and treatment , especially in atherosclerosis and its complications.

Asymptomatic are related to the disease and examinations has become extremely important (184).

The interaction between genetic and environmental factors of CAD is characterized by multifactorial factors. These interactions are largely prone to an individual's degree of CAD (185).

Recently many candidate genes that might be associated with CAD and chromosomal locus has been described and that the vast majority of genes located in the horizons of the process of inflammation. New risk factor like TNFα, and TNF-β polymorphisms single point mutation were studied (186).

The risk of inflammatory and some genetic markers can be used in preventing cardiovascular events in future. Using these tokens in single or combined cardiovascular diseases may be protective. Therefore, genes and inflammatory cytokines can determine CAD risk.(187).

We focused on pro-inflammatory (TNF-α and TNF-β) cytokines in the population. These cytokines have possible roles in atherosclerosis and may led us to understand the disease.

These cytokines (TNF-α and TNF-β) cause vascular endothelial adhesion to the arterial wall damage, wall monocytes molecules expression through various processes, including inflammation and contributes of thermogenesis (189).

Cytokine gene polymorphism (TNF-α and TNF-β) in cytokine signaling pathways leading to a genetic predisposition to diseases which affect or can create the number of studies showing(190).

Cytokines (TNF-α and TNF-β) gene polymorphisms and also the majority of promoter gene polymorphism of receptors, not selectively introns and 3 ' translocation base in the purine gene translasyona-expiring in this array means the gene, though doing the changes and function, transcription regulatory elements or stopping. In addition, a person may be associated with cytokine polymorphisms should only disease etiology of the disease of the polymorphism may affect(191).

The latest study on arterial wall of CAD and atherosclerotic lesion formation showed that progression play an important role of the inflammatory events. The dynamic balance between inflammatory and anti-inflammatory cytokines is regulated by different mechanisms. (192,193)

In our planned was study of (TNF-α and TNF-β) gene polymorphisms in patient group and control group with, metabolic and cardiovascular risk factors were a picture, profile and good as we examine above the TNF polymorphism token in this group had been identified at a high (194).

This studied included Total cholesterol, Triglycerides (TG) , LDL and HDL development of atherosclerosis and coronary artery disease .

In our study, total cholesterol (p = 0.052), TG (p = 0.531), LDL (p = 0.144) and HDL levels (p = 0.192) ,BMI (P=0.893) were not significantly difference between the patient and control groups with coronary artery disease related to TNF-α genotype was shown as in (table 4.2).

In our study, total cholesterol (p = 0.084), TG (p = 0.346), LDL (p = 0.456) and HDL levels (p = 0.262) ,BMI (P=0.352) was not significant difference between the patient and control groups with coronary artery disease related to TNF-β genotype was shown as in Table 4.5.

Blood levels of total cholesterol and LDL was high in patien have coronary artery disease . Coronary artery disease most of the elevation of Cholesterol ,LDL,TG and postprandial lipedema heighrisk factors for developing of cardiovascular diseases (195).

In different populations, the high total serum cholesterol levels are positively associated with CAD. Total serum cholesterol levels can be stimulated with the environment and increase mortality and morbidity rates of coronary artery disease. The relationship between serum cholesterol levels and CAD risk is linear. Total cholesterol levels are also largely associated with LDL cholesterol levels (196).

Kathiresan and his colleagues (2013) research in the Center for Human Genetic Research (CHGR) found a pattern of association between triglycerides and CAD is similar to what they observed for LDL: the effects of triglyceride levels were highly associated with their effect on CAD risk, even after accounting for the potentially confounding effect of each on HDL or LDL levels. The data strongly suggest that triglycerides are a causal factor in CAD (197).

Exactly the mechanism of triglycerides and how they contribute to coronary artery disease is still undetermined. LDL cholesterol is known to cause CAD by contributing to the build-up of “plaque” along the artery walls. As these molecules are deposited, they block blood flow through the arteries, and can lead to ischemic heart attack. One hypothesis is that the lipoprotein particles that carry triglycerides in the blood stream (so-called triglyceride-rich lipoproteins) may deposit in the heart arteries in a manner similar to LDL. (198)

Low-density lipoprotein cholesterol (LDL-C) is a major risk factor for atherosclerosis and coronary artery disease (CAD). The role of LDL-C in CAD has been established through experimental studies, epidemiological and genetic studies and the elucidation of the low density lipoprotein receptor (LDLR) pathway (Michael S. Brown and Joseph L. Goldstein recognized thereby the key role of the receptor for the cholesterol-transport protein LDL) (199).

In fact, for the major risk factors such as total cholesterol and LDL-C in CAD, their levels are different between patient and control group.

In coronary artery disease, HDL-C levels reduces. This reduction is a consequence of changes in HDL composition and metabolism. In case of presence of hypertriglyceridemia, low HDL is a consequence of reduced cholesteryl ester content of the lipoprotein core in combination with cholesteryl ester transfer protein–mediated alterations in triglyceride, making the particle small and dense (200).

This change in composition of HDL lipoprotein also results in increased clearance of HDL from the circulation. The relationships of these changes in HDL to insulin resistance are probably indirect, occurring in accordance with the changes in triglyceride-rich lipoprotein metabolism (201).

Low-density lipoproteins (LDLs) also are modified in composition. With fasting serum triglycerides density more than 2.0 Mm (~180 mg/dL), there is generally a predominance of small dense LDLs. Small dense LDLs are thought to be more atherogenic. This give rise to toxicity of endothelium, and they are able to transit through the endothelial basement membrane and adhere to glycosaminoglycans. They also have increased susceptibility to oxidation and are selectively bound to scavenger receptors on monocytederived macrophages. Subjects with increased small dense LDL levels and hypertriglyceridemia also have increased cholesterol content of both VLDL1 and VLDL2 subtractions. This relatively cholesterol-rich VLDL particles may contribute to the atherogenic risk in patients with metabolic syndrome (202,203)

Mutations in low-density lipoproteins LDL-R in humans form the molecular basis for familial hypercholesterolemia and patients with this disease develop premature CAD . Statins are a class of small molecule compounds that inhibit HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis, thereby reducing LDL-C in humans (The LDL receptor locus in familial hypercholesterolemia. Hobbs et al., 1990) (204)

Physiologically, the circulating LDL-C concentration is determined by the rate of LDL-C production and its clearance. Very low-density lipoprotein (VLDL) particles are assembled and secreted from liver and are converted to LDL particles in circulation through triglyceride lipolysis by lipoprotein lipase (LPL). Proteins involved in this process are intimately related to the rate of VLDL secretion and contribute to circulating LDL-C levels (205)

For instance, the deficiency of apolipoprotein B (apoB) leads to impaired VLDL assembly and secretion, and due to very low levels of plasma cholesterol. LDL particle clearance is primarily mediated through hepatic LDLR, and thus proteins or other agents that affect liver LDLR levels highly impact LDL-C. In the hypercholesterolemic patients, the dysfunctional LDLR results to increased LDL-C in circulation and also to premature coronary artery disease (206).

LDL-C is a major cardiovascular risk factor that has been established through epidemiological, genetic, and pharmacological studies. Statins are small molecule inhibitors of the rate-limiting enzyme HMG-CoA reductase in the cholesterol biosynthetic pathway (207).

In recent years, it has been found that additional reduction of LDL-C has been associated with further reduced coronary artery disease risk, and it's suggesting a strategy of "the lower, the better" for cardiovascular disease prevention and treatment (208) .

Our study has shown that the low levels of HDL present a growing problem and increase risk in patients with CAD as the prevalence of metabolic syndrome and diabetes increases. Low plasma levels of HDL-C are referred to as hyperalphalipoproteinemia. Primary hyperalphalipoproteinemia is defined as a plasma HDL-C level below the tenth percentile in the setting of relatively normal cholesterol and triglyceride levels. No apparent secondary causes of low plasma HDL-C were found. Blood HDL levels vary inversely with those of triglycerides, and the independent role of triglycerides as a cardiovascular risk factor remains unsettled. For these reasons, approaches to raise HDL levels have emerged as a prominent next hurdle in the management of dyslipidemia. Weight loss and physical activity can raise HDL. Nicotinic acid, particularly in combination with statins, can robustly raise HDL (209)

In large number of prospective cohort studies, HDL-c has been shown to have a negative association with the risk of cardiac diseases. For each increase of HDL-c by 1 mg\/dL, the total the risk of cardiovascular disease reduced by 2-3% . In this research, patient group HDL-c levels were significantly lower compared to the control group (210).

HDL has antiatherogenic properties. Lipid-free HDL, or apolipoprotein A-I, mediates this through the adenosine triphosphate (ATP)-cassette binding transporter (ABC) A1. After that, esterification of HDL-C by lecithin-cholesterol acyltransferase (LCAT) generates more mature HDL particles, including small, dense spherical HDL3 and large, less dense spherical HDL2 (22,24). These mature HDL particles may induce further cholesterol release through ABCG1 and ABCG4 (211).

HDL-C includes the cholesterol content of nascent HDL, HDL2, and HDL3 particles and is, therefore, a crude marker of reverse cholesterol transport. The smaller HDL3 particles more efficiently promote cholesterol efflux through the ABCA1 pathway then their larger counterparts, but they are equally as effective through the ABCG1 pathway (212).

In addition to HDL protection against CAD and atherosclerosis through reverse cholesterol transport, antioxidative activity of HDL further protects against atherosclerosis plaque. Apolipoprotein A-I is a major factor in this process. In addition to HDL-C esterification, LCAT can also hydrolyze oxidized phospholipids of LDL (213).

HDL can be also directly inhibit the migration of these monocytes into the subendothelial space. In the endothelium, nitric oxide protects against inflammation and activation. HDL promotes vasoprotection by enhancing nitric oxide synthase and thereby increasing the production of nitric oxide (214).

In addition, HDL may also protect against platelet activation through endothelial protection. HDL inhibits the coagulation cascade through serine protease protein C,

which inactivates factors Va and VIIa. In the literature review conducted for the genes involved in the study; an association between TNF alpha and beta gene variations and atherosclerosis and coronary artery disease was found (215).

For Tumor Necrosis Factor Alpha (TNFα) polymorphism, we examined their genotype distribution between patient and control groups of CAD related to risk factor and not significant has been found . Also for Tumor Necrosis Factor Beta TNF-β polymorphisms, we examined their genotype distribution between patient and control groups of CAD related to risk factor and not significant has been found (216).

Tumor Necrosis Factor Alpha (TNFα) has been considered to have a potent association with recruitment and infiltration of macrophages/monocytes into the sub-endothelium of arteries and its plays an important role in pathophysiology of atherosclerosis. Some researchers consider TNFα as an independent risk factor associated with CAD. It can be a new agent for further atherosclerosis and CAD studies SNPs of Tumor Necrosis Factor Alpha (TNFα) in different populations showed increased the risk for CAD. In the analysis of high quality studies, we found a significant association between the TNFα polymorphism and susceptibility of CAD in total population (217)

Banarje et al (2011) found that study in North India 442 people in studies to find out the efficacy of preventive agents such as anti TNFα agents, antihypertensives and antiplatelets, and study on awareness and attitude of people from various ethnic groups (218).

Francis and et al (2006) found an association between IL-1 α (-889 C\/T) and TNFα (-308G\/A) and CAD in their study with 674 subjects. Also Keso et al reflected an association between TNFα (-308G\/A) and CAD in their study with 700 subjects. (219).

TNFα was reported to be lower in women than men and this lower of presence of cardiovascular risk in women may be associated with genes (220).

The association between Tumor Necrosis Factor Alpha (TNFα) levels and CAD wasn't significant with any of the risk factors (table 4.2). and also The association between Tumor Necrosis Factor Beta (TNF-β) levels and CAD wasn't significant with any of the risk factors (Table 4.5).

In our study of TNF Alpha gene variations and coronary artery disease data distribution . there were no significant difference among patient and control groups in AA Homozygote Wild Type Genotype (p=0.689) and also Heterozygote Genotype (p=0.151). The frequency of the Homozygote Mutant Genotype was found to be significantly higher in patients compared to controls (p=0.004). The cardiovascular risk increased for Homozygote Mutant Genotype was 12.2-times in patients compared with the control group (OR=12.250, 95% CI=1.504-99.798; p=0.004) in Table 4.6.

In our study of TNF Beta gene variations and coronary artery disease data distribution. we shown the AA Homozygote Mutant Genotype ratio (p=0.538), AG Heterozygote Genotype (p=0.799), while GG: Hemozygot Wild Type ratio is (p=0.829) .(A) allel genotype ratio (p=0.829) and the last(G) allel genotype ratio (p=0.538) (p>0.05) genotype ratios there was no significant difference in the distribution (Table 4.7).

TNF-α polymorphism play role in the development of obesity, insulin resistance and type 2 diabetes, in the pathogenesis of this disease, Fluid and insulin have a positive correlation with the resistance (221).

Some studies showed weight loss in obese individuals causes TNF-α reduction. However, TNF-α and anorexia nervosa and the receptor will be decrease in plasma levels. TNF-α secretion increased in Beta adrenergic stimulation (222).

TNF-α, which enables the storage of fat tissue lipoprotein lipase triglycerides, fatty acid transfer protein and Acetyl Coenzyme A syntetazin, will activate the production of prints, lipolysis, fatty tissue non-esterified fatty acid, glucose, adipogenesis and lipogenesis with the transcription of the related genes. TNF-α, adiponectin and insulin production while reducing (223),

Some studies of individuals the TNF-α gene polymorphisms is increased by obesity. Diabetes and the existence of meaningful TNF-α .this finding does not fully explain the potential causal relationships of TNF-α ‧ in beginning of inflammatory and later played in the development of obesity and metabolic diseases supports the key role is expanded(224).

Stec and other friends study (2010) of the purpose of their study 226 of patient with stabil and unstabl angina pectoralis the proinflammatory (TNF-a and IL-2) and Antiinflammatory (IL-10) cytokines concentrations and soluble TNF receptors (sTNFR 1 and 2) is increased (225).

Koch and freinds (2001) studied of the significant coronary stenosis in the study they were not the findings of coronary artery patients (n= 998), old or new or patients with a history of (n= 793) allows to process the grief and the control group with similar characteristics as 340 patients in IL-10 and TNF-a gene polymorphisms significants has been showen(226).

Allen and friends (2009), their study of 58 on a single study, they had been showen of 122 patients with multivessel involvement and proinflammatory cytokines in healthy control 79 of TNF-a gene promoter region-308 gene polymorphism of 93 238 and-vein coronary artery involvement and everything interesting, meaningful has been reported (227)

Mazurek and friends (2006) , their study,of patients with coronary artey disease the , IL-1α, IL-6, TNF-α levels of Inflammatory Cytokines, such as epicardial tissue, acting subcutaneously, Cytokines and inflammation increase in Epicardial tissue (228)

Kamari and his friends study (2012) the oxidized-LDL is activated by IL-1α, TNF-α deficiency, non-deficiency in the macrophage macrophage Ki aghaei. TNF-α and IL-6 in IL-12 Similar heights (229),

All these results shows us that TNF-α and TNF-β has been the subject of long-term researches. Research data evaluated in general showed that TNF-α and TNF-β levels are associated with weight control.

As a result in this study, TNF-α levels patient group was significantly higher compared to the control group, while the TNF-β levels patient group was significantly higher compared to the control group.

For early coronary heart disease detecting, usage of genetic factors in etiology has not been enough . The use of predictive genetic testing and consultancy services to those who need it most often involves the delivery of this approach. So far only a few genetic variations were studied on HDL, LDL, increased traditional risk factors such as blood pressure and diabetes. However recent studies have contributed to the presence of genetic factors of CAD(230).

Genotyping requires technical developments in the near future. Hopefully, cost-benefit ratio threshold will also be passed. Identification of different forms of CAD and individual treatment (pharmacogenetics) will be an important step. Multiloci genotyping of population should be conducted to identify the CAD risk. Most importantly, increasing genetic studies will bring new perspectives to the mechanism of the disease, along with new biochemical approach to new therapeutic concepts and will lead to treatments (231).

Pro and Anti-inflammatory Cytokines contribute to the progress of atherosclerosis. It may also contribute to the prevention of plaque development. More studies of cytokine gene expression will lead to better understanding of the disease and will help to identify individuals at risk (232).

This study certainly included some limitations that should be discussed. Firstly, more SNP genotyping of inflammatory and metabolic markers may be necessary to define the variance as well as the predictive nature of a biomarker, but in support for our findings, several studies previously reported the predictive value of SNPS of these markers. Secondly, this study has relatively short term outcomes and number of stroke patients are insufficient. These factors, together with differences in sample characteristics and study designs, could explain much of the inconsistency between studies (233).

Although the TNF-β polymorphism was not associated with the susceptibility and outcome of ischemic stroke by itself, this genetic variant interact with environmental and other genetic factors by creating a favorable profile that contribute to the development and outcome of stroke. In this post-genomic era, the identification of variant alleles might allow better prediction of risk for stroke as well as the identification of new stroke mechanisms that may be a target of new therapeutic approaches (234).

There is a variety of possible explanations for the failure of anti-TNF therapy: (1) TNF antagonism has untoward effects in the setting of heart failure; (2) the biological agents used in the trials were intrinsically toxic; (3) sex and race may have important implications in the outcome after anticytokine therapy; (4) the TNF-alpha protein contains a polymorphism, and, in fact, genome plays a role in modifying the pharmacologic response to anticytokines; (5) anti-TNF-alpha approaches may have pharmacodynamics interactions with other heart failure medications; and (6) the patients in these trials may have been inappropriately selected(235).

Precisely how TNF-α inhibitors may lower cardiovascular risk is uncertain, but some research has suggested that they may help prevent plaque rupture and improve endothelial function. There are increasing indications that the use of TNF-α inhibitors may decrease the risk of major adverse cardiac events. As more patients receiving TNF-α inhibitor therapy are enrolled in post marketing registries, more long-term data will help elucidate whether these agents may benefit the risk reduction for major adverse cardiac events (236).

Conclusions and Recommendations:

As a result of this study;

•In our study of coronary artery disease in patient group related to smoking , cigarette smoking in patients compared with patients who was not smoking the incedice of CAD is increase but not significant .

•In our study we showed that Type 2 DM was a risk factor between patient and control groups with coronary artery disease. The presence of diabetes is a risk factor for CAD 2 time more sustainable for CAD .

• Tumor Necrosis Factor Beta (TNF-β) genotype distribution in patient group and control group was not significantly differing.

• Tumor Necrosis Factor Alpha (TNFα) genotype was significantly higher in patients group compared to control group. The frequency of the Homozygote Mutant Genotype was found to be significantly higher in patients compared to controls (p=0.004). The cardiovascular risk increased for Homozygote Mutant Genotype was 12.2-times in patients compared with the control group (OR=12.250, 95% CI=1.504-99.798; p=0.004).

Cardiovascular diseases are the main cause of mortality and morbidity around the world. It is a health problem with extremely high-cost treatment and can be executed with primary and secondary prevention efforts.

Although the risk factors leading to the disease have been identified with extensive epidemiological studies, these classic risk factors alone are not sufficient to explain the prevalence of coronary artery disease and to explain the cause of premature coronary artery disease in some patients. This has led scholars to explore new risk factors that can

help clarify and complement the atherosclerosis information, clarify the atherosclerotic risk, and help to take important steps in early detection.

Establishing the tendency of individuals to know their own risk factors has great importance in terms of preventive medicine. In this respect, every new knowledge and genetic studies will contribute to the creation of new approaches in treatment, including early lifestyle changes in individuals with genetic predisposition, as well as the establishment of individual treatment protocols (pharmacogenetics).

New markers to be found and associated with inflammation-atherosclerosis may be used as risk factors, as well as in determining the efficacy of the treatment target or treatment. New studies of genetic predisposition by identifying markers will be enabled early detection of coronary artery dısease for the creation of an early diagnosis and prevent the formation of CAD. Intervention of inflammation at the molecular level by determining the genetic profile may open a new erea in the treatment strategy of atherosclerosis and its associated diseases.

Individual risk factors have great importance in terms of the preventive medicine. New information from genetic studies on this subject added to the literature every day therefore, individuals with genetic predisposition can reach new treatment approaches and individualized treatment protocols in the early stages of their lives. Lifestyle changes and pharmacogenetics will contribute to the creation of individualized medicine.

Genetic predisposition of individuals can be determined with new approaches in the early life stages and thus diseases formation can be prevented with new measures taken against CAD

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7 .APPENDICES

7.1.Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15-1211 **05/05/2016**
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makama (Mohanad Fatlawi)

Yeditepe Üniversitesi Moleküler Tıp Ana Bilim Dalı Prof. Dr. Turgay İsbir'in koordinatör olduğu **"Association of Tumor Necrosis Factor (TNF) Alpha and Beta Polymorphism in Coronary Artery Disease"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1192** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **04.05.2016** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 609**).

Prof. Dr. Turgay ÇELİK
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7.2. FORMS

7.2.1. Biological Materials Transfer form

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER FORMU

Araştırmanın Açık Adı: Association of Tumour Necrosis Factor (TNF) Alpha and Beta polymorphism in Cardiovascular Disease.

Araştırmanın Özeti:

Coronary Artery disease

Coronary artery disease, also called coronary heart disease, or simply, heart disease,
Heart disease is a result of plaque buildup in your arteries, which blocks blood flow and heightens the risk for heart attack and stroke.
coronary heart disease, is a group of diseases that includes: stable angina, unstable angina, myocardial infarction, and sudden coronary death.It is within the group of cardiovascular diseases of which it is the most common type. A common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Occasionally it may feel like heartburn. Usually symptoms occur with exercise or emotional stress, last less than a few minutes, and gets better with rest. Shortness of breath may also occur and sometimes no symptoms are present.The first sign is occasionally a heart attack.
Risk factors include: high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol, among others.Other risks include depression.The underlying mechanism involves atherosclerosis of the arteries of the heart.A number of tests may help with diagnoses including: electrocardiogram, cardiac stress testing, coronary computed tomographic angiography, and coronary angiogram, among others.
Prevention is by eating a healthy diet, regular exercise, maintaining a healthy weight and not smoking.Sometimes medication for diabetes, high cholesterol, or high blood pressure are also used.There is limited evidence for screening people who are at low risk and do not have symptoms.Treatment involves the same measures as prevention. Additional medications such as antiplatelets including aspirin, beta blockers, or nitroglycerin may be recommended. Procedures such as percutaneous coronary intervention (PCI) or coronary artery bypass surgery (CABG) may be used in severe disease. In those with stable CAD it is unclear if PCI or CABG in addition to the other treatments improve life expectancy or decreases heart attack risk.
CAD is caused by plaque buildup in the walls of the arteries that supply blood to the heart (called coronary arteries) and other parts of the body. Plaque is made up of deposits of cholesterol and other substances in the artery. Plaque buildup causes the inside of the arteries to narrow over time, which could partially or totally block the blood flow. This process is called atherosclerosis.
Too much plaque buildup and narrowed artery walls can make it harder for blood to flow through your body. When your heart muscle doesn't get enough blood, you may have chest pain or discomfort, called angina. Angina is the most common symptom of CAD.
Over time, CAD can weaken the heart muscle. This may lead to heart failure, a serious condition where the heart can't pump blood the way that it should. An irregular heartbeat, or arrhythmia, also can develop
To find out your risk for CAD, your health care team may measure your blood pressure, cholesterol, and sugar levels. Being overweight, physical inactivity, unhealthy eating, and smoking tobacco are risk factors for CAD. A family history of heart disease also increases your risk for CAD. If you're at high risk for heart disease or already have symptoms, your doctor can use several tests to diagnose CAD.

steps to help lower your risk for heart attack or worsening heart disease:
Lifestyle changes, such as eating a healthier (lower sodium, lower fat) diet, increasing physical activity, and quitting smoking.

Medications to treat the risk factors for CAD, such as high cholesterol, high blood pressure, an irregular heartbeat, and low blood flow.
Surgical procedures to help restore blood flow to the heart(1).

The tumor necrosis factor (TNF)

superfamily of cytokines represents a multifunctional group of pro-inflammatory cytokines which activate signaling pathways for cell survival, apoptosis, inflammatory responses, and cellular differentiation. Induction of cellular responses to tumor necrosis factor occurs through two receptors(17), TNFR1 (TNF Receptor-1 or CD120a) and TNFR2 (TNF Receptor-2 or CD120b). TNFR1 is activated in most human tissues by the binding of TNFα. TNFR2 is expressed in immune cells and is activated by both TNFα and TNFβ
The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis and viral replication and respond to sepsis via IL1 & IL6 producing cells. Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease, cancer, major depressionand inflammatory bowel disease (IBD).While still controversial, studies of depression and IBD are currently being linked to TNF levels.Recombinant TNF is used as an immunostimulant under the INN tasonermin. TNF can be produced ectopically in the setting of malignancy and parallels parathyroid hormone both in causing secondary hypercalcemia and in the cancers with which excessive production is associated
TNF is primarily produced as a 233-amino acid-long type II transmembrane protein arranged in stable homotrimers. From this membrane-integrated form the soluble homotrimeric cytokine (sTNF) is released via proteolytic cleavage by the metalloprotease TNF alpha converting enzyme (TACE, also called ADAM17). The soluble 51 kDa trimeric sTNF tends to dissociate at concentrations below the nanomolar range, thereby losing its bioactivity. The secreted form of human TNFα takes on a triangular pyramid shape, and weighs around 17-kD. Both the secreted and the membrane bound forms are biologically active, although the specific functions of each is controversial. But, both forms do have overlapping and distinct biology activities.

The common house mouse TNFα and human TNF are structurally different. The 17-kilodalton (kDa) TNF protomers (185-amino acid-long) are composed of two antiparallel β-pleated sheets with antiparallel β-strands, forming a 'jelly roll' β-structure, typical for the TNF family, but also found in viral capsid proteins.

Relationship between CAD and TNF

TNF-α is one of the primary proinflammatory cytokines, mainly produced and secreted by inflammatory cells (i.e., monocytes and macrophages) . Evidence shows that TNF-α is a key contributor in the development, progression, and complications of atherosclerosis . TNF-α is involved in reduced expression of endothelial nitric oxide synthase (eNOS) and thus impaired nitric oxide (NO) production leading to endothelial dysfunction . It has a profound effect on lipid metabolism and has been implicated in insulin resistance which produces changes in lipid and glucose associated with the cardiovascular disease risk .
Genetic variants in the TNF-α promoter region are reported to be associated with the TNF-α serum levels . These promoter polymorphisms regulate the transcriptional activity of TNF-α gene . Based upon these observations, the present study was designed to investigate the association of the −1031T>C (rs1799964) and −863C>A (rs1800630) polymorphisms of the promoter region of TNF-α gene with CHD in a Pakistani population. Prior to this study the said polymorphisms have not been investigated in the study population.

Tissue necrosis factor α (TNFα), an acute phase cytokine, is produced by macrophages and lymphocytes and stimulates the vascular endothelium to produce cellular adhesion molecules. These adhesion molecules, such as platelet–endothelial cell adhesion molecule and endothelial–leucocyte adhesion molecule, cause reactive cells (platelets and leucocytes) to bind to the endothelium and to each other. Platelets also have surface receptors for TNF, and their stimulation theoretically may promote platelet activation, CD40L release, and aggregation. In cardiovascular medicine, elevated concentrations of TNFα have been demonstrated in worsening ischaemic heart disease, AMI, and decompensated heart failure, and persistently elevated values are predictive of recurrent myocardial infarction.

TNF-α therefore may play an important role in the pathophysiology of cardiovascular diseases and . In particular, TNF-α may not only promote the initiation and evolution of atheroma and [but it may also precipitate thrombotic events and plaque ruptures that characterizes CAD and , in part by affecting endothelial function and vascular remodeling and . Thus, TNF-α is believed to be directly involved in the development of atherosclerosis and its progression to CAD

The gene encoding TNF-α is located within the major histocompatibility complex class III region between HLA-B (class I) and HLA-DR (class II). There is a strong relation of these genes and different linkage disequilibrium phenomenon and . Some studies have reported that a specific TNF-α gene polymorphism wherein G is substituted to A in the promoter region at position −308 is associated with several infectious, autoimmune, and immune-mediated diseases and . Moreover, the polymorphism wherein G is substituted to A in the promoter region at position −238 was reported to be associated with chronic hepatitis B and insulin resistance and . Other studies have shown that the TNF-α gene promoter region bears other polymorphisms, including −857 (C/T), −863 (C/A) and −1031 (T/C) and . Several studies have been performed to determine whether these polymorphisms are associated with CAD(2).

Yöntem

Study patients. The patient with sever CAD were documented by angiography

Angiographic inclusion criteria were > 50% stenosis of the least one major coronary vessel because of coronary artery and a vascular event defined as myocardial infaraction percutaneous transluminal coronary angiography or coronary artery by pass grafing .patient were inculded irrespective of concominant risk factor for coronary artery disease such as smoking and arterial hypertention and diabetes mellitus without any lipid lowering medication.

DNA isolation

In order to isolation of DNA strile 5ml EDTA tubes will be used to collect blood samples,then DNA will be exteacted (from 350µl the pheripheral blood) by using the invitrogen-iprep DNA isolation robot. Then ,spectrophotometry will be used to check the puirty of extracted DNA samples by NanoDrop 2000 instrument.

Genotype Analysis

TNF Alpha &Beta genotype will be analysis by using real time PCR method by using ABI 7500 fast real time PCR instrument and TaqMan assays (primer and flouroscent attached probes) . Allelic discrimination will be performed by sowftware of ABI 7500 fast real time PCR instrument automatically or manually.

Statistical Analysis

For the statistical analysis of this study we will use SPSS packet program. P value must be below 0,05 ($p<0,05$) to concider the result as significant . X will be used to compare the allele fractions and genotypes between case and control group .the significance measurment of case/control will be done by student test.

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER FORMU

İş bu anlaşma ile, biyolojik materyali gönderen araştırmacı ve kurum **: 'Association of Tumor Necrosis Factor (TNF) Alpha and Beta polymorphism in Cardiovascular Disease**..isimli araştırmada kullanılmak üzere gönderilecek ....**10ml**.....miktarda ve ....**araştırma** ..... amaçla kullanılacak biyolojik materyali (**periferik kan**) .... ***26 Ağustos Yerleşimi Yeditepe Üniversitesi, Kayışdağı / İstanbul*** **adresindeki .....Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalında'ndaki** merkeze göndermeden önce **ALICI** kurumdan aşağıdaki koşulları kabul etmesi **istenmektedir**;

1. Gönderilen biyolojik materyaller yalnızca yukarıda yazılı amaç için, ya da gönderici kurumun yeniden yazılı iznini almak koşulu ile ikincil amaç için kullanılabilir.
2. **ALICI biyolojik materyali gönderici kuru**mun yazılı izni olmadan üçüncü şahıslara vermeyecektir. ALICI üçüncü şahıslardan gelebilecek istekleri GÖNDERİCİ'ye bildirecektir.
3. Biyolojik materyaller GÖNDERİCİ tarafından bireyin kimlik bilgileri olmaksızın ALICI'ya gönderilecektir.
4. **ALICI biyolojik** materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel Beyannamesine uygun olarak kullanacaktır.
5. Biyolojik materyaller ALICI'ya gönderilmeden önce biyolojik materyalin sağlandığı kişilere ait Sağlık Bakanlığı'nın ve Etik Kurul'un onayladığı bilgilendirilmiş gönüllü olur formunun her bir gönüllüden alınmış olması gerekmektedir.
6. Bu anlaşma ile gönderilecek biyolojik materyalin araştırma için kullanılacak olduğu ve biyolojik materyal kullanımının bazı tehlikeli özelliklerinin var olduğu ALICI tarafından kabul edilmektedir. Biyolojik materyali sağlayan kurum bu konuda sorumlu değildir.
7. GÖNDERİCİ ve ALICI yapılacak ortak bir yayınla ya da doğabilecek patent hakkı ve ticari gelişmelerle ilgili haklarını araştırma başlangıcında karşılıklı olarak belir**leyecektir.**
8. Bu anlaşma aşağıdaki iki maddeden herhangi birinin gerçekleşmesi halinde son bulacaktır.
   a. Araştırmanın sonlanması durumunda,
   b. Taraflardan herhangi birinin diğerine gönderdiği yazılı uyarıyı takiben 30 (otuz) gün içinde Anlaşma kurallarına uymama; patent haklarının ihlali veya sağlık tehdidi oluşturan riskler dışında bu anlaşma 8 (b) koşulunda materyali sağlayan tarafın yazılı uyarısı ile bitirilecek olursa ALICI'nın araştırmasının engellenmemesi için ve ALICI'nın isteği üzerine materyali sağlayan araştırmacı 1 (bir) yıla kadar varan bir süre içinde anlaşmanın sonlanacağı bir tarih belirleyebilir.
9. ALICI bu anlaşmanın bitiminde bütün materyalleri geri vermeyi veya ortadan kaldırmayı ve bunu **belgelemeyi kabul eder.**
10. GÖNDERİCİ biyolojik materyali toplama, hazırlama ve göndermek için bir ücret talep ediyorsa bu ücret **burada belirtilecektir.**
11. Bu anlaşmanın yürümesinde ALICI ve GÖNDERİCİ kurum amirleri ile destekleyici sorumludur. Anlaşmazlık halinde ihtilafın çözümü için her iki ülke mahkemeleri de yetkili**dir.**

BİYOLOJİK MATERYALİ GÖNDEREN ARAŞTIRMACI BİLGİSİ

Adı Soyadı ve Unvanı:	Adı Soyadı: **Selim Isbir**
Uzmanlık Alanı:	Unvan (Dr., ...): **prof. Dr.**
Kurumu:	Uzmanlık alanı: **Kalp Damar Cerrahisi**

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER FORMU

Adresi:	İş adresi: Marmara Üniversitesi Tıp Fakültesi Kalp Damar Cerrahisi ABD
Telefon:	Telefon numarası:
Faks:	-
E-posta:	E-posta adresi: isbir@yahoo.com

BİYOLOJİK MATERYALİ ALAN ALICI BİLGİSİ

Adı Soyadı ve Unvanı:	**M.D. Mohanad Fatlawi**
Uzmanlık Alanı:	
Kurumu:	**Yeditepe üniversitesi Moleküler Tıp Anabilim Dalı**
Adresi:	**26 Ağustos yerleşkesi Kayışdağı /İstanbul**
Telefon:	0537 991 00 62
Faks:	(0216) 578 00 00 (pbx)
E-posta:	**Fatlawi83@hotmail.com**

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

	Gönderen Araştırmacı	**Gönderen Destekleyici Firma Yetkilisi veya Yasal Temsilcisi**	**Klinik Şefi / 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 edilir.

7.2.2. Volunteer Form

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

Hastanın veya yerine onam verecek kişinin okuma, anlama, konuşma, dil sorunu mevcut mu? Evet ☐ Hayır ☐ Cevabınız EVET ise Hasta İlişkileri Sorumlusu ile iletişim kurunuz.	**Tercüman gerektiyse;** Tercümanın adı _____ İmza _____ Tarih _____

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz.** Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da k**arar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz.**
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşürü**nden edinebilirsiniz. Ayrıca Hastanemiz personeli hakkında http://www.yeditepehastanesi.com.tr/ web sayfamızdan daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

TANIMLAMA
Tümör Nekroz Faktörü (TNF) alfa ve beta Koroner Arter Hastalığında polimorfizmi

Araştırma Konusu

TNF polimorfizmi kalp hastalığı risk faktörleri arasında yer almaktadır

Araştırmaya Katılımcı Sayısı

100

Bu araştırmanın

Amacı
Bu araştırmanın amacı, klinik yönetim ve sonuçlarını iyileştirmek için gelecekte kullanılabilecek kalp hastalığı için yeni prediktif ve prognostik faktörlerin bulmak için kalp hastalığı gelişiminde TNF genetik polimorfizminin etkisini tespit etmektir
Süresi
2 yıl

İzlenecek Yöntem / Yöntemler DNA izolasyonu
REAL TIME PCR
Araştırma Sonunda Beklenen Fayda
Tümör Nekroz Faktörü (TNF) alfa ve beta Koroner Arter Hastalığında polimorfizmi
Alternatif Tedavi Veya Girişimler
yok

Araştırma Sırasında Karşılaşılabilecek; yok

Riskleri	***Rahatsızlıklar***
a)	a)
b)	b)
c)	c)
d)	d)
e)	e)
f)	f)
g)	g)

Risk / rahatsızlık durumlarında yapılması gerekenler
yok

Aşağıdaki özel durumlara ait katılımcı var mı?

	EVET*	HAYIR
Çocuk		**X**
Mahkum		**X**
Gebe		**X**
Mental yetersizlik		**X**
Sosyoekonomik eğitim olarak yetersiz		**X**

*Ancak çocuklarda, hamilelik, lohusalık ve emzirme dönemlerinde ve kısıtlılık durumunda; gönüllüler yönünden araştırmadan doğrudan fayda sağlanacağı umuluyor ve araştırma gönüllü sağlığı açısından öngörülebilir ciddi bir risk taşımıyor ise, usulüne uygun bir şekilde alınmış bilgilendirilmiş gönüllü olur formu ile birlikte ilgili etik kurulun onayı ve Bakanlık izni alınmak suretiyle araştırmaya izin verilebilir.

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.

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

Gönüllünün Adı / Soyadı / İmzası / Tarih

Açıklamaları Yapan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Yasal Temsilcinin Adı / Soyadı / İmzası / Tarih

Bilgilendirilmiş Gönüllü Onam Formu asgari olarak yukarıda belirtilen başlıkları içermelidir.

7.3. The raw Data

T-TEST GROUPS

Group Statistics

	tanım	N	Mean	Std. Deviation	Std. Error Mean
yas	kontrol	50	53.70	14.793	2.092
	hasta	50	62.80	8.439	1.194
BMI	kontrol	50	26.7920	4.42132	.62527
	hasta	50	28.6310	6.15280	.87014
kolesterol	kontrol	50	192.38	38.737	5.478
	hasta	50	184.44	48.006	6.789
TG	kontrol	50	156.02	100.808	14.256
	hasta	50	150.30	57.496	8.131
LDL	kontrol	50	124.98	35.961	5.086
	hasta	50	117.06	41.813	5.913
HDL	kontrol	50	41.26	8.996	1.272
	hasta	50	37.24	7.411	1.048

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
yas	Equal variances assumed	12.228	.001	-3.778	98
	Equal variances not assumed			-3.778	77.842
BMI	Equal variances assumed	2.631	.108	-1.716	98
	Equal variances not assumed			-1.716	88.951
kolesterol	Equal variances assumed	3.027	.085	.910	98
	Equal variances not assumed			.910	93.811
TG	Equal variances assumed	6.882	.010	.349	98
	Equal variances not assumed			.349	77.829
LDL	Equal variances assumed	2.266	.135	1.015	98
	Equal variances not assumed			1.015	95.854
HDL	Equal variances assumed	1.106	.296	2.439	98
	Equal variances not assumed			2.439	94.535

TABLES=tanım BY cinsiyet tansiyon diabet sigara

tanım * cinsiyet

Crosstab

			cinsiyet		
			kadın	erkek	Total
tanım	kontrol	Count	20	30	50
		% within tanım	40.0%	60.0%	100.0%
		% within cinsiyet	62.5%	44.1%	50.0%
		% of Total	20.0%	30.0%	50.0%
	hasta	Count	12	38	50
		% within tanım	24.0%	76.0%	100.0%
		% within cinsiyet	37.5%	55.9%	50.0%
		% of Total	12.0%	38.0%	50.0%
Total		Count	32	68	100
		% within tanım	32.0%	68.0%	100.0%
		% within cinsiyet	100.0%	100.0%	100.0%
		% of Total	32.0%	68.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.941[a]	1	.086		
Continuity Correction[b]	2.252	1	.133		
Likelihood Ratio	2.965	1	.085		
Fisher's Exact Test				.133	.066
Linear-by-Linear Association	2.912	1	.088		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	2.111	.892	4.994
For cohort cinsiyet = kadın	1.667	.916	3.033
For cohort cinsiyet = erkek	.789	.600	1.039
N of Valid Cases	100		

tanım * diabet

Crosstab

			diabet		
			yok	var	Total
tanım	kontrol	Count	41	9	50
		% within tanım	82.0%	18.0%	100.0%
		% within diabet	56.9%	32.1%	50.0%
		% of Total	41.0%	9.0%	50.0%
	hasta	Count	31	19	50
		% within tanım	62.0%	38.0%	100.0%
		% within diabet	43.1%	67.9%	50.0%
		% of Total	31.0%	19.0%	50.0%
Total		Count	72	28	100
		% within tanım	72.0%	28.0%	100.0%
		% within diabet	100.0%	100.0%	100.0%
		% of Total	72.0%	28.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.960[a]	1	.026		
Continuity Correction[b]	4.018	1	.045		
Likelihood Ratio	5.045	1	.025		
Fisher's Exact Test				.044	.022
Linear-by-Linear Association	4.911	1	.027		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	2.792	1.113	7.007
For cohort diabet = yok	1.323	1.027	1.703
For cohort diabet = var	.474	.238	.944
N of Valid Cases	100		

tanım * sigara

Crosstab

			sigara		
			yok	var	Total
tanım	kontrol	Count	29	21	50
		% within tanım	58.0%	42.0%	100.0%
		% within sigara	58.0%	42.0%	50.0%
		% of Total	29.0%	21.0%	50.0%
	hasta	Count	21	29	50
		% within tanım	42.0%	58.0%	100.0%
		% within sigara	42.0%	58.0%	50.0%
		% of Total	21.0%	29.0%	50.0%
Total		Count	50	50	100
		% within tanım	50.0%	50.0%	100.0%
		% within sigara	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	2.560[a]	1	.110		
Continuity Correction[b]	1.960	1	.162		
Likelihood Ratio	2.571	1	.109		
Fisher's Exact Test				.161	.081
Linear-by-Linear Association	2.534	1	.111		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	1.907	.862	4.220
For cohort sigara = yok	1.381	.924	2.065
For cohort sigara = var	.724	.484	1.083
N of Valid Cases	100		

ONEWAY BMI kolesterol TG LDL HDL BY TNF_Alfa_rs909253

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BMI	Between Groups	237.741	2	118.871	4.335	.016
	Within Groups	2659.654	97	27.419		
	Total	2897.395	99			
kolesterol	Between Groups	10859.886	2	5429.943	2.973	.056
	Within Groups	177166.304	97	1826.457		
	Total	188026.190	99			
TG	Between Groups	10137.495	2	5068.747	.756	.472
	Within Groups	650615.945	97	6707.381		
	Total	660753.440	99			
LDL	Between Groups	5802.113	2	2901.056	1.943	.149
	Within Groups	144799.847	97	1492.782		
	Total	150601.960	99			
HDL	Between Groups	353.166	2	176.583	2.554	.083
	Within Groups	6707.584	97	69.150		
	Total	7060.750	99			

Post Hoc Tests

Multiple Comparisons

Dependent Variable		(I) TNF_Alfa_rs909253	(J) TNF_Alfa_rs909253	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
BMI	Tukey HSD	AA	AG	-.71510	1.11868	.799	-3.3778	1.9476
			GG	-5.12373*	1.74385	.011	-9.2745	-.9730
		AG	AA	.71510	1.11868	.799	-1.9476	3.3778
			GG	-4.40862*	1.78765	.040	-8.6636	-.1536
		GG	AA	5.12373*	1.74385	.011	.9730	9.2745
			AG	4.40862*	1.78765	.040	.1536	8.6636
kolesterol	Tukey HSD	AA	AG	15.746	9.130	.201	-5.99	37.48
			GG	30.445	14.233	.087	-3.43	64.32
		AG	AA	-15.746	9.130	.201	-37.48	5.99
			GG	14.699	14.590	.574	-20.03	49.43
		GG	AA	-30.445	14.233	.087	-64.32	3.43
			AG	-14.699	14.590	.574	-49.43	20.03
TG	Tukey HSD	AA	AG	17.373	17.497	.583	-24.27	59.02
			GG	26.587	27.275	.594	-38.33	91.51
		AG	AA	-17.373	17.497	.583	-59.02	24.27
			GG	9.214	27.960	.942	-57.34	75.76
		GG	AA	-26.587	27.275	.594	-91.51	38.33
			AG	-9.214	27.960	.942	-75.76	57.34
LDL	Tukey HSD	AA	AG	11.595	8.254	.342	-8.05	31.24
			GG	22.162	12.867	.202	-8.46	52.79
		AG	AA	-11.595	8.254	.342	-31.24	8.05
			GG	10.566	13.190	.703	-20.83	41.96
		GG	AA	-22.162	12.867	.202	-52.79	8.46
			AG	-10.566	13.190	.703	-41.96	20.83
HDL	Tukey HSD	AA	AG	1.774	1.777	.580	-2.45	6.00
			GG	6.165	2.769	.072	-.43	12.76
		AG	AA	-1.774	1.777	.580	-6.00	2.45
			GG	4.392	2.839	.274	-2.37	11.15
		GG	AA	-6.165	2.769	.072	-12.76	.43
			AG	-4.392	2.839	.274	-11.15	2.37

ONEWAY BMI kolesterol TG LDL HDL BY TNF_Beta_rs1800629

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BMI	Between Groups	43.236	2	21.618	.735	.482
	Within Groups	2854.160	97	29.424		
	Total	2897.395	99			
kolesterol	Between Groups	7030.874	2	3515.437	1.884	.157
	Within Groups	180995.316	97	1865.931		
	Total	188026.190	99			
TG	Between Groups	9808.272	2	4904.136	.731	.484
	Within Groups	650945.168	97	6710.775		
	Total	660753.440	99			
LDL	Between Groups	3270.122	2	1635.061	1.076	.345
	Within Groups	147331.838	97	1518.885		
	Total	150601.960	99			
HDL	Between Groups	174.054	2	87.027	1.226	.298
	Within Groups	6886.696	97	70.997		
	Total	7060.750	99			

Post Hoc Tests

Multiple Comparisons

Dependent Variable		(I)TNF_Beta_rs180 0629	(J) TNF_Beta_rs180629	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD		GG	GA	-1.18690	1.4058	.676	-4.5320	2.1582
			AA	1.19525	1.6960	.761	-2.8430	5.2335
		GA	GG	1.18690	1.4058	.676	-2.1582	4.5320
			AA	2.38215	2.0007	.461	-2.3787	7.1430
kolesterol	Tukey HSD	GG	GA	-16.854	11.191	.293	-43.49	9.78
			AA	12.641	13.511	.619	-19.52	44.80
		GA	GG	16.854	11.191	.293	-9.78	43.49
			AA	29.496	15.928	.158	-8.42	67.41
		AA	GG	-12.641	13.511	.619	-44.80	19.52
			GA	-29.496	15.928	.158	-67.41	8.42
TG	Tukey HSD	GG	GA	-11.465	21.224	.852	-61.98	39.05
			AA	24.790	25.622	.599	-36.20	85.78
		GA	GG	11.465	21.224	.852	-39.05	61.98
			AA	36.254	30.206	.456	-35.64	108.15
		AA	GG	-24.790	25.622	.599	-85.78	36.20
			GA	-36.254	30.206	.456	-108.15	35.64
LDL	Tukey HSD	GG	GA	-14.811	10.097	.311	-38.84	9.22
			AA	-3.649	12.190	.952	-32.66	25.37
		GA	GG	14.811	10.097	.311	-9.22	38.84
			AA	11.162	14.371	.718	-23.04	45.37
		AA	GG	3.649	12.190	.952	-25.37	32.66
			GA	-11.162	14.371	.718	-45.37	23.04
HDL	Tukey HSD	GG	GA	3.064	2.183	.343	-2.13	8.26
			AA	-1.138	2.635	.902	-7.41	5.14
		GA	GG	-3.064	2.183	.343	-8.26	2.13
			AA	-4.202	3.107	.370	-11.60	3.19
		AA	GG	1.138	2.635	.902	-5.14	7.41
			GA	4.202	3.107	.370	-3.19	11.60

CROSSTABS

/TABLES=tanım BY rs909253_AA rs909253_AG rs909253_GG rs909253_A rs909253_G

tanım * rs909253_AA

Crosstab

			rs909253_AA		
			Yok	Var	Total
tanım	kontrol	Count	24	26	50
		% within tanım	48.0%	52.0%	100.0%
		% within rs909253_AA	48.0%	52.0%	50.0%
		% of Total	24.0%	26.0%	50.0%
	hasta	Count	26	24	50
		% within tanım	52.0%	48.0%	100.0%
		% within rs909253_AA	52.0%	48.0%	50.0%
		% of Total	26.0%	24.0%	50.0%
Total		Count	50	50	100
		% within tanım	50.0%	50.0%	100.0%
		% within rs909253_AA	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	.160[a]	1	.689		
Continuity Correction[b]	.040	1	.841		
Likelihood Ratio	.160	1	.689		
Fisher's Exact Test				.842	.421
Linear-by-Linear Association	.158	1	.691		
N of Valid Cases	100				

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.852	.389	1.867
For cohort rs909253_AA = Yok	.923	.623	1.367
For cohort rs909253_AA = Var	1.083	.732	1.604
N of Valid Cases	100		

tanım * rs909253_AG

Crosstab

			rs909253_AG		
			Yok	Var	Total
tanım	kontrol	Count	27	23	50
		% within tanım	54.0%	46.0%	100.0%
		% within rs909253_AG	44.3%	59.0%	50.0%
		% of Total	27.0%	23.0%	50.0%
	hasta	Count	34	16	50
		% within tanım	68.0%	32.0%	100.0%
		% within rs909253_AG	55.7%	41.0%	50.0%
		% of Total	34.0%	16.0%	50.0%
Total		Count	61	39	100
		% within tanım	61.0%	39.0%	100.0%
		% within rs909253_AG	100.0%	100.0%	100.0%
		% of Total	61.0%	39.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.060[a]	1	.151		
Continuity Correction[b]	1.513	1	.219		
Likelihood Ratio	2.068	1	.150		
Fisher's Exact Test				.218	.109
Linear-by-Linear Association	2.039	1	.153		
N of Valid Cases	100				

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.552	.245	1.247
For cohort rs909253_AG = Yok	.794	.577	1.092
For cohort rs909253_AG = Var	1.438	.869	2.378
N of Valid Cases	100		

tanım * rs909253_GG

Crosstab

			rs909253_GG		
			Yok	Var	Total
tanım	kontrol	Count	49	1	50
		% within tanım	98.0%	2.0%	100.0%
		% within rs909253_GG	55.1%	9.1%	50.0%
		% of Total	49.0%	1.0%	50.0%
	hasta	Count	40	10	50
		% within tanım	80.0%	20.0%	100.0%
		% within rs909253_GG	44.9%	90.9%	50.0%
		% of Total	40.0%	10.0%	50.0%
Total		Count	89	11	100
		% within tanım	89.0%	11.0%	100.0%
		% within rs909253_GG	100.0%	100.0%	100.0%
		% of Total	89.0%	11.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.274[a]	1	.004		
Continuity Correction[b]	6.537	1	.011		
Likelihood Ratio	9.459	1	.002		
Fisher's Exact Test				.008	.004
Linear-by-Linear Association	8.191	1	.004		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	12.250	1.504	99.798
For cohort rs909253_GG = Yok	1.225	1.061	1.415
For cohort rs909253_GG = Var	.100	.013	.752
N of Valid Cases	100		

tanım * rs909253_A

Crosstab

			rs909253_A		
			Yok	Var	Total
tanım	kontrol	Count	1	49	50
		% within tanım	2.0%	98.0%	100.0%
		% within rs909253_A	9.1%	55.1%	50.0%
		% of Total	1.0%	49.0%	50.0%
	hasta	Count	10	40	50
		% within tanım	20.0%	80.0%	100.0%
		% within rs909253_A	90.9%	44.9%	50.0%
		% of Total	10.0%	40.0%	50.0%
Total		Count	11	89	100
		% within tanım	11.0%	89.0%	100.0%
		% within rs909253_A	100.0%	100.0%	100.0%
		% of Total	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.274[a]	1	.004		
Continuity Correction[b]	6.537	1	.011		
Likelihood Ratio	9.459	1	.002		
Fisher's Exact Test				.008	.004
Linear-by-Linear Association	8.191	1	.004		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.082	.010	.665
For cohort rs909253_A = Yok	.100	.013	.752
For cohort rs909253_A = Var	1.225	1.061	1.415
N of Valid Cases	100		

tanım * rs909253_G

Crosstab

			rs909253_G		
			Yok	Var	Total
tanım	kontrol	Count	26	24	50
		% within tanım	52.0%	48.0%	100.0%
		% within rs909253_G	52.0%	48.0%	50.0%
		% of Total	26.0%	24.0%	50.0%
	hasta	Count	24	26	50
		% within tanım	48.0%	52.0%	100.0%
		% within rs909253_G	48.0%	52.0%	50.0%
		% of Total	24.0%	26.0%	50.0%
Total		Count	50	50	100
		% within tanım	50.0%	50.0%	100.0%
		% within rs909253_G	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	.160[a]	1	.689		
Continuity Correction[b]	.040	1	.841		
Likelihood Ratio	.160	1	.689		
Fisher's Exact Test				.842	.421
Linear-by-Linear Association	.158	1	.691		
N of Valid Cases	100				

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	1.174	.536	2.572
For cohort rs909253_G = Yok	1.083	.732	1.604
For cohort rs909253_G = Var	.923	.623	1.367
N of Valid Cases	100		

CROSSTABS

/TABLES=tanım BY rs1800629_GG rs1800629_GA rs1800629_AA rs1800629_G rs1800629_A

tanım * rs1800629_GG

Crosstab

			rs1800629_GG		
			Yok	Var	Total
tanım	kontrol	Count	15	35	50
		% within tanım	30.0%	70.0%	100.0%
		% within rs1800629_GG	48.4%	50.7%	50.0%
		% of Total	15.0%	35.0%	50.0%
	hasta	Count	16	34	50
		% within tanım	32.0%	68.0%	100.0%
		% within rs1800629_GG	51.6%	49.3%	50.0%
		% of Total	16.0%	34.0%	50.0%
Total		Count	31	69	100
		% within tanım	31.0%	69.0%	100.0%
		% within rs1800629_GG	100.0%	100.0%	100.0%
		% of Total	31.0%	69.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.047[a]	1	.829		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.047	1	.829		
Fisher's Exact Test				1.000	.500
Linear-by-Linear Association	.046	1	.830		
N of Valid Cases	100				

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.911	.390	2.126
For cohort rs1800629_GG = Yok	.938	.522	1.683
For cohort rs1800629_GG = Var	1.029	.791	1.339
N of Valid Cases	100		

tanım * rs1800629_GA

Crosstab

			rs1800629_GA		
			Yok	Var	Total
tanım	kontrol	Count	40	10	50
		% within tanım	80.0%	20.0%	100.0%
		% within rs1800629_GA	49.4%	52.6%	50.0%
		% of Total	40.0%	10.0%	50.0%
	hasta	Count	41	9	50
		% within tanım	82.0%	18.0%	100.0%
		% within rs1800629_GA	50.6%	47.4%	50.0%
		% of Total	41.0%	9.0%	50.0%
Total		Count	81	19	100
		% within tanım	81.0%	19.0%	100.0%
		% within rs1800629_GA	100.0%	100.0%	100.0%
		% of Total	81.0%	19.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.065[a]	1	.799		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.065	1	.799		
Fisher's Exact Test				1.000	.500
Linear-by-Linear Association	.064	1	.800		
N of Valid Cases	100				

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.878	.323	2.388
For cohort rs1800629_GA = Yok	.976	.807	1.180
For cohort rs1800629_GA = Var	1.111	.494	2.500
N of Valid Cases	100		

tanım * rs1800629_AA

Crosstab

			rs1800629_AA		
			Yok	Var	Total
tanım	kontrol	Count	45	5	50
		% within tanım	90.0%	10.0%	100.0%
		% within rs1800629_AA	51.1%	41.7%	50.0%
		% of Total	45.0%	5.0%	50.0%
	hasta	Count	43	7	50
		% within tanım	86.0%	14.0%	100.0%
		% within rs1800629_AA	48.9%	58.3%	50.0%
		% of Total	43.0%	7.0%	50.0%
Total		Count	88	12	100
		% within tanım	88.0%	12.0%	100.0%
		% within rs1800629_AA	100.0%	100.0%	100.0%
		% of Total	88.0%	12.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.379[a]	1	.538		
Continuity Correction[b]	.095	1	.758		
Likelihood Ratio	.380	1	.537		
Fisher's Exact Test				.760	.380
Linear-by-Linear Association	.375	1	.540		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	1.465	.432	4.969
For cohort rs1800629_AA = Yok	1.047	.905	1.210
For cohort rs1800629_AA = Var	.714	.243	2.100
N of Valid Cases	100		

tanım * rs1800629_G

Crosstab

			rs1800629_G		
			Yok	Var	Total
tanım	kontrol	Count	5	45	50
		% within tanım	10.0%	90.0%	100.0%
		% within rs1800629_G	41.7%	51.1%	50.0%
		% of Total	5.0%	45.0%	50.0%
	hasta	Count	7	43	50
		% within tanım	14.0%	86.0%	100.0%
		% within rs1800629_G	58.3%	48.9%	50.0%
		% of Total	7.0%	43.0%	50.0%
Total		Count	12	88	100
		% within tanım	12.0%	88.0%	100.0%
		% within rs1800629_G	100.0%	100.0%	100.0%
		% of Total	12.0%	88.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.379[a]	1	.538		
Continuity Correction[b]	.095	1	.758		
Likelihood Ratio	.380	1	.537		
Fisher's Exact Test				.760	.380
Linear-by-Linear Association	.375	1	.540		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	.683	.201	2.315
For cohort rs1800629_G = Yok	.714	.243	2.100
For cohort rs1800629_G = Var	1.047	.905	1.210
N of Valid Cases	100		

tanım * rs1800629_A

Crosstab

			rs1800629_A		
			Yok	Var	Total
tanım	kontrol	Count	35	15	50
		% within tanım	70.0%	30.0%	100.0%
		% within rs1800629_A	50.7%	48.4%	50.0%
		% of Total	35.0%	15.0%	50.0%
	hasta	Count	34	16	50
		% within tanım	68.0%	32.0%	100.0%
		% within rs1800629_A	49.3%	51.6%	50.0%
		% of Total	34.0%	16.0%	50.0%
Total		Count	69	31	100
		% within tanım	69.0%	31.0%	100.0%
		% within rs1800629_A	100.0%	100.0%	100.0%
		% of Total	69.0%	31.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.047[a]	1	.829		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.047	1	.829		
Fisher's Exact Test				1.000	.500
Linear-by-Linear Association	.046	1	.830		
N of Valid Cases	100				

Risk Estimate

		95% Confidence Interval	
	Value	Lower	Upper
Odds Ratio for tanım (kontrol / hasta)	1.098	.470	2.564
For cohort rs1800629_A = Yok	1.029	.791	1.339
For cohort rs1800629_A = Var	.938	.522	1.683
N of Valid Cases	100		

8. CURRICULUM VITAE

Personal Details

- Surname : Fatlawi
- First names : Mohanad Adnan
- Nationality : Iraqi
- Marital status: Single
- Date of Birth : 19 November 1983
- Place of Birth: Baghdad / Iraq
- Gender : Male
- Religion : Muslim
- Address : istanbul/ beşiktaş
  Örtaköy mehellası/ dereboyu caddesi/
  Müsahip sok 24.3
- Email : fatlawi83@hotmail.com
  Muhanned_adnan83@yahoo.com

- Telephone (mobile) : +9 05379910062
- Telephone (home) : +9 02122593962

Education

- 2002 – 2005 AL Qadisiyah University / School of Medicine
- 2006 – 2007 Baghdad University / School of Medicine

Educational Qualifications

- Bachelor of Medicine and General Surgery(M.B.Ch.B) in 2007 from the University of baghdad.

Academic years : 6 years

CERTIFICATIONS

- Certification of medical graduation
- Certification of registration (iraqi medical association)

WORK EXPERIENCE

- 2007 - 2008 Gynecological and Obstetrical Hospital / Karbala

  Worked three months in gynecological emergency unit

- 2008 – 2009 Karbala hospital for Children / Karbala

  Worked three months in pediatric emergency unit

  Worked three months in neonatal care unit

- 2009 - 2012 Al Hussein Teaching Hospital/ Karbala

  Worked six months in medicine department

  Worked six months in general surgical department

  Worked three months in urological department

  Worked three months in orthopedic department

  Worked six month in coronary care unit

- 2012 – 2013 AL Hussein Hospital Education / Karbala

  Worked one year in emergency unit

RESEARCH and thesis

- 2014 - 2015 Association of Tumour Necrosis Factor (TNF) Alpha andBeta polymorphism in Cardiovascular Diseases yeditepe university (department of molucular biology)

Other Medical Activities

- 2003 anatomy I was teaching anatomy to 2nd year medical students

  AL Qadisiyah University / School of Medicine

- 2007 medical aid i taken one month course of first aid at AL Hussein Hospital Education / Karbala

- 2009 ECG i taken one month dvanced ECG course at coronary care unit in aL Hussein Hospital Education / Karbala

- 2014 doctor thesis department of molucular biology yeditepe university (see research)

Knowledge of languages:

- Arabic Language : Native “Mother Language”
- English Language : Fluently “write, speak& read”

  Toefl exam : 89 score
- Turkish Language : Fluently “write, speak& read”

  istanbul university/ language center/ level C

Technical skills:

- Computer skills MS: Word-Power point - Internet Explorer
- Full adaptation to work within the team.
- The ability to perform under pressure at work.
- Enjoy the ability to build good relations, constructive social
- Other special skills