Arab American University

Faculty of Graduate Studies

Associations Among Fibrinogen Polymorphisms -455G/A and -148C/T, Plasma Levels of The Protein and Coronary Disease in Northern Palestine

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Thesis Approval

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Declaration

I certify that this dissertation is primarily my own work and has not been submitted for consideration for any other degree at Arab American University or any other institution, with the exception of instances in which the contributions of others are specifically acknowledged.

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Dedication

I dedicate this thesis to my parents in appreciation of their unwavering love, encouragement, and support throughout my life. Your principles and direction have helped me become the person I am today, To my brothers and sisters, the constant support in all circumstances. I'm grateful to my beautiful kids for being my inspiration and brightness, even when things were difficult, your laughter and love gave me the strength to keep going.

I want to thank my spouse in particular because his love, support, patience, and understanding helped me succeed and inspired me to pursue my goals. This success was made possible by your continuous emotional and practical support. I will always be appreciative of your support and encouragement through this journey.

I am so grateful to the person who conceived and realized this dream in me, as well as to everyone who helped and encouraged me along the way.

This thesis is a representation of my family's love and support along with the outcome of my own efforts. This work is dedicated to all of you.

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Abstract

Background: The primary cause of death for those over 35 in developed nations is coronary disease (CD). Avoiding CD and the mortality associated with it has been viewed as a concern in every country. Fibrinogen (Factor I) is a glycoprotein, which participates in coagulation cascade. According to large sample prospective research, the amount of plasma fibrinogen can independently predict the development of coronary disease. Two single nucleotide polymorphisms in the beta-fibrinogen gene's promoter region (G/A-455, C/T-148) have been linked to elevated concentrations of this protein, and a number of articles have revealed their links with acute cardiovascular events.

Objectives: The current research aims to investigate the associations among 455G/A, 148C/T fibrinogen polymorphisms and coronary disease (CD) including unstable coronary disease (UCD) and stable coronary disease (SCD), as well as their relationship to major adverse cardiovascular events (MACE) in a group of Northern Palestine.

Methodology: This is a multi-center, prospective control design with a follow-up study of six months. A total of 100 patients with coronary disease (CD), including 41 UCD and 59 SCD were recruited in order to ascertain the relationships between major cardiovascular adverse events (MACE), levels of plasma fibrinogen, and fibrinogen gene polymorphisms in a population of Northern Palestine. Sedentarism, gender, age, and the body mass index were all matched for the groups, which included 70 control subjects. The level of plasma fibrinogen was determined by the von Clauss method. DNA was extracted out of buffy coat and the β-fibrinogen gene polymorphisms (G/A-455, C/T-148) were analyzed by means of PCR-RFLP.

Results: The concentrations of plasma fibrinogen, polymorphisms of the fibrinogen gene (455G/A and -148C/T) were compared in all groups. Patients with coronary disease had substantial amounts of plasma fibrinogen (>465 mg/dl). An increase in fibrinogen plasma concentration above 450 mg/dl was linked to cardiovascular death within the group with unstable coronary disease during follow-up study ($p = 0.03$). Plasma high levels of fibrinogen proteins were associated with the allelic loads of -455G/A genotype and -148C/T ($p < 0.005$ and $p = 0.041$, respectively) and with coronary disease ($p = 0.021$ and $p < 0.004$, respectively). The -148T allele's genetic load was linked to significant unfavorable cardiovascular events, according to the investigation of subsequent events following an acute coronary event (RR = 1.6, 95% CI = 1.13 - 3.42, $p = 0.03$).

Conclusion: The results of this study provide crucial insight into the connections among plasma fibrinogen levels, fibrinogen polymorphisms (-455G/A, -148C/T), and cardiovascular disease in Northern Palestine. MACE and cardiovascular disease are associated with plasmatic fibrinogen levels above 450 mg/dl and fibrinogen gene polymorphisms (-455G/A and 148C/T). According to this study, cardiovascular risk is linked to these gene variations.

Keywords: Fibrinogen, -455G/A, -148C/T, Polymorphism, Palestine.

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List of Definitions of Abbreviations

Abbreviations	Title
ACD	Acute Coronary Disease
ADP	Adenosine Diphosphate
APC	Activating protein C
BMI	Body Mass Index
CAD	Coronary Artery Disease
CCS	Chronic Coronary Syndrome
CD 36	Cluster of Differentiation 36
CHD	Coronary Heart Disease
CRP	C-reactive protein
DAPT	Dual antiplatelet treatment
DIC	Disseminated Intravascular Coagulopathy
DNA	Deoxyribonucleic acid
EBP	Enhancer-binding protein
EDTA	Ethylene Diamine Tetraacetic Acid
EF	Ejection fraction
FDP	Fibrin Degradation Product
Fg	Fibrinogen
FGA	Alpha fibrinogen gene
FGB	Beta fibrinogen gene

FGG	Gamma fibrinogen gene
FPA	Fibrinopeptide A
FPB	Fibrinopeptide B
GP	Glycoprotein
HDL	High-density lipoprotein
HNF3	Hepatocyte nuclear factor-3
IL6	Interleukin 6
LDL	Low density lipoprotein
Lp(a)	Lipoprotein(a)
MI	Myocardial infarction
NF-kappa B	Nuclear Factor kappa B
NO	Nitric Oxide
NSTEMI	Non-ST-elevation myocardial infarction
OMT	Optimal medical treatment
OR	Odds Ratio
PAI	Plasminogen activator inhibitor
PAR	Protease-activated receptor
PAR1	Protease-activated receptors-1
PAR2	Protease-activated receptors-2
PCI	Percutaneous Coronary Intervention
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Polymorphism

PTM	post-translational modification
ROS	Reactive Oxygen Species
SCD	Stable Coronary Disease
SMCs	Smooth muscle cells
SNP	Single Nucleotide Polymorphism
SR-A	Scavenger receptor A
STEMI	ST-elevation myocardial infarction
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TAFI	Thrombin-activatable fibrinolysis inhibitor
TF	Tissue Factor
TFPI	TF pathway inhibitor
t-PA	Tissue plasminogen activator
T-PA	Tissue Plasminogen Activator
UCD	Unstable Coronary Disease
vWF	von Willebrand factor
WHO	World Health Organization
MACE	Major adverse cardiovascular events

Chapter One: Introduction

1.Introduction

1.1 Background

Coronary disease (CD) is a multifactorial disease in which the pathophysiology of illness that is characterized by atherosclerotic plaques within the small coronary arteries of the heart may be influenced by both acquired and hereditary risk factors (Munger & Hawkins, 2004).This process can be controlled by invasive procedures, medication treatments, and lifestyle modifications intended to stabilize or reverse the condition (Winzer et al., 2018). Long, stable periods of the illness are possible, but it can also suddenly worsen at any moment, usually as a result of an acute atherothrombotic event brought on by plaque erosion or rupture (Knuuti, 2020).

In the last several decades, the prevalence of CD has continued to decline in developed countries, which may be attributable to both better primary and secondary preventative strategies in addition to effective acute phase treatment. (Galindo et al., 2023). The prevalence of CD varies significantly among developing nations (Ralapanawa & Sivakanesan, 2021). In Palestine, coronary disease (CD) is a serious public health issue. According to population-based studies, 8.3% of people in the Gaza Strip have CD. The World Heart Federation reports that 6,461 people died from CD in the Palestinian Territories in 2021, equivalent to a crude mortality rate of 126 per 100,000 people (World Heart Observatory, 2021).

The range of coronary disease (CD) is broad, ranging from individuals who do not exhibit any symptoms to those who exhibit acute coronary disease (ACD) and unexpected cardiac deaths. Since CD is a dynamic process, it often begins with a lengthy stable phase that can suddenly turn unstable and result in a sudden cardiac attack. Therefore, the concept of "stable CD" might be deceptive because there is still a chance of acute episodes. Numerous developments in coronary healthcare have occurred, and worldwide associations' recommendations have

been updated. The Taiwan Society of Cardiology's 2023 CD recommendations include a novel idea that classifies the disease category based on whether it manifests clinically as acute coronary syndrome (ACS) or chronic coronary syndrome (CCS) (Kwo-Chang Ueng et al., 2023).

Hemostatic variables, particularly fibrinogen, have been linked to the development of atherosclerosis and its subsequent complications (Bembde, 2012). Actually, thrombus formation starts by exposing the damaged artery's endothelium surface to different blood components that cause coagulation. This will cause thrombin to be produced, platelets to be activated, and fibrin to be deposited when the clotting cascade is activated (Smith et al., 2015). increased plasma fibrinogen (Hyperfibrinogenemia), is a potential risk factor for CD (Stec et al., 2000). Increased plasma fibrinogen slows blood flow, improves blood coagulation, renders blood more viscous, and promotes platelet adhesion and aggregation to endothelial cells. Thus, it has an impact on blood rheology and contributes significantly to the development of atherosclerotic lesions, which cause CD. (Imran et al., 2015a).

Fibrinogen, also known as coagulation factor I, is a glycoprotein essential for several physiological and metabolic activities. It is mostly detected in plasma at concentrations of 1.5-3.5 g/L and has a half-life (T1/2) of 3 to 5 days (Wolberg, 2023).
Fibrinogen is predominantly expressed in hepatocytes, and the production begins with the expression of three genes, FGA, FGB, and FGG, which are found in a 50 kb region of human chromosome 4 (long arm; 4q31.3-4q32.1)(Surma, n.d.) .The end of transcription of mRNA produces three homologous polypeptide chains: Bβ, Aα, and γγ that are linked by disulfide bonds (Wolberg, 2023).Fibrinogen production is regulated by and glucocorticosteroids (GC), and acute-phase proteins, including IL-6, which is generated by vascular endothelial cells, monocytes, and macrophages, which promote synthesis in the liver. While IL-1β and TNF-α inhibit its production. Hence, because it is produced in greater quantities during inflammation, fibrinogen is an acute phase protein. Plasma fibrinogen levels during the acute stage of inflammation can go close to 7 g/L (Kryczka et al., 2021).

It is well known that Numerous genetic and physiological/environmental variables have been demonstrated to affect fibrinogen. Physiological factors that might raise the amounts of fibrinogen in the blood include gender, age, BMI, smoking, pregnancy, high blood pressure, diabetes, high lipid, cholesterol, and certain medications, such as oral contraceptive pills. On the other hand, strenuous exercise and alcohol consumption lead to decreased amounts of fibrinogen (Damluji et al., 2023).

A number of SNPs were found in potential fibrinogen gene areas, which are thought to be involved in controlling the quantity of plasma fibrinogen and the structure of fibrin clots, and so affecting the risk of CD (Albert et al., 2009).Some genetic variations of the fibrinogen gene have been shown to have a role in the increase in plasma fibrinogen levels (Ken-Dror et al., 2012). According to several reports, a proportion of CD complications can be explained by variations in the fibrinogen genes, particularly the beta chain gene, whose synthesis is directly opposite to that of the alpha and gamma chains in the direction of transcription. Therefore, the rate-limiting step in the synthesis of the whole FG molecule is the synthesis of the fibrinogen beta-polypeptide chain (Han et al., 2021).

It has been demonstrated that the beta-455 G/A polymorphism, which is particularly engaged in the rate-limiting steps of beta-chain synthesis, may be linked to increased plasma fibrinogen concentration. according to some research, G/A may cause alterations in fibrinogen activity. An earlier study found that the fibrinogen level was significantly higher in the GA + AA genotype compared with the level in the GG genotype group (Hu et al., 2017).

Furthermore, the 148C/T SNP might affect fibrinogen levels since they are in complete linkage disequilibrium with 455G/A in Caucasians (Verschuur et al., 2005). According to previous studies, individuals with mutant T alleles (CT and TT) in 148C/T had noticeably greater levels of fibrinogen than those who carried the normal C allele (Imran et al., 2015b).

Coagulation is part of a larger process called hemostasis. Hemostasis is the ability of the circulatory system to hold blood as a fluid in the vessels and prevent excessive bleeding in the event of an injury. The word "hemostasis" is derived from the Greek word "hem," which means blood, and "stasis" (stagnation) (Rodrigues et al., 2019). Hemostasis involves a

complex reaction between three compartments: the blood vessels, platelets, and one group of soluble coagulation factors. Blood coagulation (“clotting”) is the mechanism that transforms fibrinogen, a soluble protein, into fibrin, an insoluble form. This makes fluid plasma convert into a stable gel mesh (Smith et al., 2015b).

The clotting process consists of three steps: primary hemostasis, secondary hemostasis, and fibrinolysis. In the first stage, platelets stick to one another through damaged vessels. This sticking leads to the formation of a platelet aggregate called "the primary hemostatic plug", which can stop the bleeding for just a short time and at a weak strength that can be easily dissociated from the wall of the blood vessel. Then, after this weak clot is formed from platelets, it needs support and stabilization, and here comes the role of the second stage, which prepares clotting factors in order to work and convert fibrinogen into fibrin through thrombin (Sang et al., 2021).

Fibrin is formed by a sequence of complicated biochemical reactions that include soluble plasma proteins (coagulation factors) and the platelet plug around of injured blood vessels. The plug, or thrombus, is then called the secondary hemostatic plug. In this stage, the blood at the site of injury has turned into a semisolid gel. After the wound has healed, other factors are involved in order to remove and break up the clot. This is what happens in the fibrinolytic stage (Van Herrewegen et al., 2012).

In summary, hemostasis occurs because of the interaction of the blood vessels, platelets, and certain plasma proteins. Proteins of hemostasis include those that form fibrin, those that are involved with fibrinolysis, and those that inhibit all stages of the process (Gale, 2011).
To the greatest extent of our knowledge, no prior studies regarding potential links between fibrinogen gene polymorphisms and CD have been reported in Palestine. In this study we aim to investigate the association between FGB polymorphisms and CD. One hundred and seventy members were involved in the study. Forty-one patients with UCD, fifty-nine SCD patients, and 70 subjects as a control group got involved to determine the associations between fibrinogen polymorphisms, level of plasma fibrinogen, and MCAE in a sample from Northern Palestine/West Bank. The control subjects were composed of apparently healthy subjects,

matched based on sedentarism, body mass index, age, and sex. Plasma fibrinogen levels and the polymorphisms, 455G/A and -148C/T, of the fibrinogen gene were examined in both CD patients and controls.

1.2 Importance of the study:

This study will be the first report to investigate potential associations of fibrinogen gene polymorphisms and hemostatic fibrinogen levels with CD patients in Palestine. The elevated fibrinogen levels are reported to be correlated with CD. Although the -455G/A and -148C/T polymorphisms of the β-fibrinogen gene have been thoroughly explored in other communities, the present study presents the first population-specific data from Northern Palestine. This research clarifies the contrasting worldwide findings by illustrating how these genetic variations act within a community that has a particular genetic makeup and environmental background. By linking the SNPs with real plasma fibrinogen levels, CAD presence, and clinical outcomes, the study gives better biological and clinical evidence for their significance in disease risk. Moreover, the detection of a substantial combined effect of the -455A and -148T alleles gives fresh insight into possible genetic linkage and its influence on cardiovascular risk. All things taken into account , this study addresses a significant information gap in the region and offers data that might enable early genetic screening, risk assessment, and preventative measures specific to the Palestinian community also ,This study gives crucial information on the inflammatory and genetic risk profiles in CD patients in Palestine and most likely serves as the initial research in our region.

While molecular medicine promises "prevention before the disease onset," modern medical practices primarily concentrate on "treatment after the disease onset." The study will show how fibrinogen gene polymorphism and CD risk are related. This will assist in determining which alleles may be at risk for CD, By identifying vulnerable groups based on their DNA polymorphisms, it may be possible to clarify the causes of illness and create the most effective preventative measures(Hunter, 2005).

1.3 Study problem:

Coronary disease is highly prevalent in Palestine, yet the genetic factors contributing to its risk remain unclear. Although the -455G/A and -148C/T fibrinogen polymorphisms have been studied internationally, their effects are unknown in the Palestinian population, which has a distinct genetic and environmental profile. This lack of local data creates a gap in

understanding how these variants influence fibrinogen levels and CD risk in Northern Palestine, In which this Genetic variations in the fibrinogen genes (455G/A, -148C/T) may increase the risk of CD.

1.4 Aim of the study

1.4.1 General objective

The principal goal of the study is to examine the associations between fibrinogen polymorphisms 455G/A and -148C/T and coronary disease (CD), including UCD and SCD, and their associations with MACE in a sample of Northern Palestine.

1.4.2 Specific objectives

1. To measure the levels of plasma fibrinogen in all the enrolled CD patients and in controls.
2. to examine the role of plasma fibrinogen levels to the variations of CD risk.
3. To determine the fibrinogen gene's (455G/A, -148C/T) genetic variations that may influence plasma fibrinogen levels, and therefore to the risk of CD.
4. To investigate the relationship between hyperlipidemia, smoking, diet, and physical activity on CD complications using a well-designed questionnaire.
5. To study the correlation between fibrinogen levels, (455G/A, -148C/T) gene polymorphism and their association with CD and MACE.

.

1.5 Study questions:

1. When comparing CD patients to healthy controls, what are the genotype and allele rates of these polymorphisms?
2. Do people with the genotypes -148C/T and -455G/A have differing amounts of plasma fibrinogen?
3. Do Northern Palestinian CD patients differ in their plasma levels or fibrinogen polymorphisms according to their age, sex, or other demographic characteristics?

1.6 Study hypothesis:

1. The minor alleles (-455A, -148T) will be associated with greater levels of fibrinogen in both cases and controls.
2. individuals carrying the -148C/T or −455G/A genotypes exhibit significantly different plasma fibrinogen levels compared to individuals without these genotypic variants.
3. Plasma fibrinogen levels will be more elevated in CD patients compared to controls.
4. The minor alleles (-455A, -148T) will be more frequent in CD cases compared to controls.
5. The fibrinogen polymorphisms 148C/T and 455G/A, as well as elevated fibrinogen plasmatic levels, are linked to coronary disease and MACE.

Chapter Two: Literature Review

2- Literature Review:

This chapter provides a comprehensive literature review that explains the coronary disease, epidemiology of CD and classification. On other hand, give an extensive explain about hemostasis, phase of hemostasis, fibrinogen, association between fibrinogen and CD,

variations in fibrinogen structure, molecular basis of fibrinogen abnormality and prevention of Atherothrombosis.

2.1 Coronary disease (CD)

Coronary artery disease (CD) represents the leading cause of mortality worldwide. Crucially, it is the most frequent cause of sudden death. CD is a prevalent type of heart disease characterized by a continuous, dynamic process of plaque buildup and coronary circulation functional changes that lower cardiac blood flow. This process can be modified through lifestyle changes, therapeutic medications, and invasive interventions to achieve disease stabilization or regression (Knuuti, 2020).

The widespread nature of this disease indicates that there are several causes and various factors that influence it. Identifying these factors will help us develop methods for accurate and appropriate diagnosis, as well as prevent any modifiable risk factors that contribute to the disease (Coronary Artery Disease - A Guide for Patients and Families, n.d.).This disease remains stable for a long time but may suddenly increase in symptom intensity and become unstable. This change may be due to an acute atherothrombotic event. Therefore, the disease is dangerous, chronic, and progressive, especially during periods when the symptoms are dormant (Munger & Hawkins, 2004). The distinctive dynamic nature of this disease and the apparent variation in symptoms have led to its classification into two distinct categories: acute coronary syndromes (ACS) and chronic coronary syndromes (CCS) (Knuuti, 2020; Kwo-Chang Ueng et al., 2023).

2.2 Epidemiology of CAD

According to the WHO, coronary artery disease, cerebrovascular illness, rheumatic heart disease, and other conditions are all classified as cardiovascular diseases (CVDs). More than 250 million people are estimated to have coronary disease(Murray, 2022). An estimated 17.9 million deaths globally were attributed to CAD in 2019, representing 32% of total mortality, these deaths occurring prematurely in adults under the age of 70. More than 75% of CD-related deaths occur in low- and middle-income countries (Ralapanawa & Sivakanesan, 2021).

The most prevalent cardiac condition identified globally is ischemic heart disease. Approximately 110 million women and 145 million men worldwide suffer from this

condition. Coronary heart disease has been the world's leading cause of mortality for at least 30 years prior to the coronavirus epidemic (Murray, 2022).

2.3 Etiology of CD

Atherosclerosis, also known as coronary artery atherosclerotic occlusion, It is the main cause of coronary disease (CD), a cardiovascular condition. It develops when the endothelial function of the arterial wall is compromised. Depending on their size and quantity, lipoprotein particles along with plasma molecules, they are infiltrate the compromised and permeable endothelium into the subendothelial compartment, where there is a potential for atherogenic lipoproteins to be sequestered and undergo modification, (oxidized, for example) to become proatherogenic. This results in the accumulation of lipoprotein droplets in the intima of the coronary arteries (Ghose, 2024).

Ionic interactions with matrix proteins such as collagen, fibronectin, and proteoglycans can trap lipoproteins once they are in the subendothelial area. They can then undergo chemical alteration, most often oxidation, glycation, and enzymatic modification. Because of these changes, the lipoproteins become even more "proatherogenic," which means they trigger an inflammatory response and encourage the formation of atherosclerotic plaques. This stage is essential for the production of foam cells and the recruitment of immune cells (like monocytes) that underlie atherosclerosis (Buckley & Ramji, 2015) .

Both macrophages and endothelial cells produce nitric oxide, a strong oxidant that appears to have both beneficial and atherogenic impact based on its source. Endothelial nitric oxide synthase (eNOS) generates nitric oxide, which has vasodilatory properties and may exert atheroprotective effects. In contrast, nitric oxide produced by macrophages through the more potent inducible nitric oxide synthase (NOS) serves antibacterial functions due to its strong oxidative properties but may also contribute to atherogenesis (Podrez et al., n.d.). Monocytes within the intima differentiate into macrophages which recognize &engulf modified, atherogenic lipoproteins through well-characterized scavenger receptors. Among these, SR-A and CD36 have been shown to be physiologically significant in experimental atherosclerosis (Manning-Tobin et al., 2009). These macrophages are now characterized by Foam cells which are lipid-loaded macrophages that contain high levels of cholesterol esters

in both the early and late phases of atherosclerosis (Galindo et al., 2023). Unlike native LDL receptors, scavenger receptors are not controlled by the buildup of cholesterol within cells. As a result, macrophages continue to ingest lipoproteins until they die, provided there is a constant supply of atherogenic lipoproteins (Falk, 2006).

These foamy cells, characterized by their lipid-laden, foamy appearance, proliferate and form lesions referred to fatty streaks. Fatty streaks represent the earliest type of atherosclerotic lesion. Smooth muscle cells (SMCs) are recruited to the location of fatty streak in response to signals triggered by lesion development. Subsequently, the SMCs generate extracellular matrix components, mostly proteoglycans and collagen. As the fibrous plaque accumulates on the coronary artery lumen, tiny blood vessels proliferate, which may later lead to plaque calcification. The final lesion is complex and advanced, featuring a fibrous cap that covers a lipid-rich core containing necrotic material and exhibiting high thrombogenic potential (Malakar et al., 2019).

Atherosclerotic plaque buildup in the coronary arteries restricts blood flow, resulting in an imbalance between the heart's oxygen supply and demand. The symptoms of CD include heaviness, pressure-like feeling, and substernal discomfort that may spread to the arm, shoulder, back, or jaw. Usually lasting a few minutes, these sensations are brought on by a heavy meal, mental stress, physical exertion, or exposure to cold. They can usually be relieved within minutes by nitroglycerin or rest (Podrezet al., n.d.).

2.4 Classification of CD

2.4.1 chronic coronary syndrome

The phrase "stable coronary artery disease" or "stable angina" which suggests that these patients are low risk and that there is little urgency to begin optimal medical treatment (OMT) or lifestyle modification (LSM), has been replaced with the term "chronic coronary syndrome" (CCS) to reflect and highlight the dynamic nature of this syndrome (Damluji et al., 2023). It is important to know that many organizations like the European Society of Cardiology have chosen the more modern name CCS to highlight the fact that the illness is dynamic and progressing even in "stable" cases. The more general, underlying issue of coronary artery

atherosclerosis is known as CD. CCS is a more specific phrase for the clinical picture, covering all the ways this progressive illness might appear, including symptoms like angina, or a total lack of symptoms (silent ischemia)(Hussain et al., 2021).

Moreover, this disease can progress to an unstable form, primarily because of plaque rupture that result in an acute atherothrombotic event at any time. In particular, CCS encompasses six clinical scenarios in individuals with suspected or established diseases. First, stable angina: individuals who have persistent chest pain, regardless of whether or not they have dyspnea. Second, suspected coronary artery disease or new-onset heart failure with or without reduced ejection fraction (EF); this occurs when the heart muscle cannot pump an adequate amount of blood to meet the body's demands, thereby impairing its function. Third, patients with stable symptoms after ACS or after percutaneous coronary intervention (PCI), which is used to allow sufficient blood flow to the heart by opening constricted arteries. Fourth, vasospastic angina (variant angina), which occurs when sudden transient spasms in the coronary arteries reduce blood flow to the heart. Fifth, microvascular angina, resulting from endothelial dysfunction in small arterioles and capillaries. Finally, silent CAD detected on screening (evidence of myocardial infarction [MI] in the absence of typical angina symptoms). All of these scenarios are classified as CCS but vary in their severity and risk for future cardiovascular events such as death or MI, and this risk may change over time (Kwo-Chang Ueng et al., 2023).

2.4.2 Acute coronary syndrome

Acute coronary syndrome (ACS) is used to refer to a group of diseases that are characterized by the sudden decrease in the supply of blood to the heart. These diseases involve heart attacks and unstable angina or chest pains. When blood flow in the heart reduces, the cardiac muscle is not supplied with sufficient oxygen. A heart attack, also known as a myocardial infarction, occurs when heart tissue cells are damaged or die due to this oxygen deprivation. ACS encompasses unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI), all of which are forms of cardiac ischemia. Diagnosis and classification of ACS are based on a comprehensive analysis of clinical features, including electrocardiogram (ECG) findings and biochemical markers of myocardial necrosis (Bergmark et al., 2022; Smith et al., 2015).

Any patient presenting with symptoms of ischemia should be evaluated for an acute ACS diagnosis. Clinical manifestations of ischemia include various presentations of pain in the upper extremities, mandible, or epigastric region, as well as syncope, nausea, dizziness, dyspnea, and chest pain. An ACS episode can cause pain and discomfort that is diffuse rather than localized and may occur at rest or during physical activity. Discomfort accompanied by diaphoresis and pain radiating to the left arm, right shoulder, or both arms is more strongly associated with myocardial infarction (MI) (J. N. Smith et al., 2015; Costello & Younis, 2020). Endogenous estrogen is believed to protect women against CD, specifically MI . It exerts various effects on the circulatory system, including raising high-density lipoprotein (HDL) levels and lowering low-density lipoprotein (LDL) levels, thereby enhancing the cholesterol profile. Additionally, estrogen helps lower blood pressure by relaxing smooth muscle cells and is thought to aid the body in eliminating cellular free radicals, which can otherwise exacerbate inflammatory processes and promote cardiovascular disease (Costello & Younis, 2020).

2.5. Prevention of CD

Stabilizing vulnerable plaques and preventing rupture and/or subsequent thrombosis through a number of significant lifestyle changes, especially lowering the seven primary modifiable cardiovascular risk factors, which include diabetes (fasting glucose < 130 mg/dL, Hb1C < 7%), hypertension (<140/90 mmHg), and hypercholesterolemia (the target: LDL-C < 130 mg/dL; total cholesterol < 200 mg/dL), Obesity (BMI < 30 kg/m2), smoking (non-smoking), lack of physical activity (frequent exercise), and poor nutrition (good diet),all of this can affect the atherothrombotic process and prevent coronary artery disease and cardiovascular events(Kryczka et al., 2021).

2.5. 1. Lifestyle modifications and managing risk factors

2.5.1.1 Smoking Cessation

Smoking cigarettes is a notable, independent risk factor for peripheral artery disease, stroke, and CD. It is likely that smoking cigarettes destabilizes arterial plaques and stimulates thrombosis and plaque rupture. Stopping cigarettes can quickly and significantly lowers the risk of CAD (Gallucci et al., 2020). Clinicians should adhere to the "Five A's" while

interacting with smokers in the clinic: ask about smoking, offer advice to quit, assess readiness to quit, and assist with stopping smoking (pharmacological assistance and referral) and arrang a follow-up (Knuuti, 2020).

2.5.1.2 Diet

The primary reason for CD is atherosclerosis, which is caused by the buildup of fatty plaques in the arteries as a result of unhealthy diets, especially those heavy in saturated, trans, and cholesterol fats. Poor eating habits are a major cause of CD and its progression, and individuals who adopt healthier eating habits have shown a decrease in mortality and cardiovascular events (Debelo, 2025). A diet rich in fruits, vegetables, legumes, fiber, nuts, and fish and low in refined carbohydrates, red meat, dairy, and saturated fat is selected (Diab et al., 2023).

2.5.1.3 Weight management

According to a population-based study, those who were overweight had a greater overall risk of incident CD, as well as cardiovascular morbidity and death, than people with a normal BMI (20–25 kg/m2) (Powell-Wiley et al., 2021).

Increasing physical activity and eating a nutritious diet with energy consumption restricted according to what is suggested for managing weight required to achieve and maintain a healthy weight (BMI<25 kg/m2) (Knuuti, 2020).

2.5.1.4 Physical activity

Due to its various beneficial impacts on cardiovascular system physiology The term "polypill" has been used to describe exercise. Exercise increases oxygen delivery to the myocardium, which improves angina(Powell-Wiley et al., 2021).

Patients with CD are advised to exercise aerobically at an appropriate frequency for 30 to 60 minutes five days a week. Regular time spent in physical activity lowers mortality (Mi et al., 2025).

2.5.1.5. Psychosocial factors

Compared to those without heart disease, patients with heart disease are twice as likely to experience anxiety and mood problems. It seem that anxiety, depression, and psychosocial

stress are linked to poorer results and make it challenging for patients to follow a treatment plan and make beneficial lifestyle adjustments(Religioni et al., 2025).

2.5.2 Pharmacological management

2.5.2.1 Antihypertensive and Anti-glycemic Therapy

Since both diabetes and hypertension are known to promote coronary disease, an elevated risk of CD is associated with elevated blood pressure and blood glucose levels in a particular individual. The risk of cardiovascular events will be significantly decreased by preventing or managing diabetes and hypertension (Basile, 2009). It has been demonstrated that among hypertension individuals with a blood pressure of ≥140/90 mmHg, statin users had a higher chance of achieving regulated blood pressure than non-users(Besekar et al., 2025). there is strong evidence that statins dramatically reduce blood pressure by enhancing endothelial function, encouraging vasodilation, lowering oxidative stress, minimizing arterial system inflammation, and downregulating the angiotensin II type 1 receptor (Ruszkowski et al., 2019).

2.5.2.2 Lipid-Lowering Therapy

While HDL cholesterol has a positive, anti-atherogenic impact, high cholesterol causes increased synthesis of LDL cholesterol, which in turn promotes endothelial dysfunction and tissue factor expression that promote atherothrombosis. Lipid-modifying medication treatment, which lowers LDL and triglycerides while raising HDL, has been demonstrated in several clinical trials to postpone or minimize sudden appearance of CD. Gemfibrozil and cholestyramine were found to lower the incidence of both fatal and nonfatal myocardial infarctions. Even in individuals with moderately high LDL-cholesterol levels (i.e., 150 mg/dL or less), the risk of fatal and nonfatal myocardial infarction was significantly decreased in follow-up trials that used statins as the primary preventative medication(Munger & Hawkins, 2004).

2.5.2.3 Antiplatelet Therapy

Apart from the well-known risk factors for CD, such as smoking, high blood pressure, and a sedentary lifestyle, platelet activity and inflammation are additionally linked to the risk of CD morbidity and death. Several pharmaceutical methods can be used to alter platelet function. For CD patients, combining aspirin with an oral P2Y12 receptor inhibitors, such as ticagrelor, prasugrel, or clopidogrel, continue to be the core of treatment. Following either an acute coronary syndrome (ACS) or a scheduled percutaneous coronary intervention (PCI), this dual antiplatelet treatment (DAPT) is still recommended for secondary coronary prevention (Sabouret et al., 2021).

2.6 Hemostasis

The capacity of the circulatory system to keep blood in the blood vessels as a fluid and to stop excessive blood loss in the event of an injury is known as hemostasis. The term hemostasis is originating from the Greek word's "stasis", meaning to stop and "heme", meaning blood. The hemostatic plug, also called a blood clot or thrombus, is the barrier that forms to prevent blood loss. Three components must work together to maintain hemostasis: platelets, blood vessels, and a group of soluble plasma proteins known as coagulation factors (De Vrij et al., 2023).

Primary hemostasis, secondary hemostasis, and fibrinolysis are the three stages of hemostasis. Hemostasis results from the interaction of platelets, specific plasma proteins, and blood vessels. The proteins involved include those that promote fibrinolysis, generate fibrin, and inhibit the overall process (Kuijpers et al., 2022).

The platelet is the crucial cell in this process, while fibrinogen is the essential matrix element. When the endothelial cell monolayer is healthy, platelets are protected from premature activation. In healthy skin, platelets neither clump together nor adhere to the vessel wall. Hepatocytes produce fibrinogen (factor I), which is then transported by the blood. (Kattula et al., 2017).

The control of hemostasis is similar to that of a number of other body functions. Activators and inhibitors, cellular and humoral systems, and positive and negative feedback mechanisms are all involved in this regulation process. Hemostatic disequilibrium may be caused by defects in the reactants involved or by deregulation brought on by the activation or

inhibition of the regulatory systems, which would then lead to either hemorrhage (bleeding) or thrombosis (formation of a blood clot) (Figure2.1). So effective use of both hemostatic compartments can compensate for defects in one; pathologic hemostasis and bleeding often result from abnormalities in two of the three compartments (Mackenzie Clinical Hematology, n.d.). There are three phases of hemostasis: primary hemostasis, secondary hemostasis, and fibrinolysis (De Vrij et al., 2023).

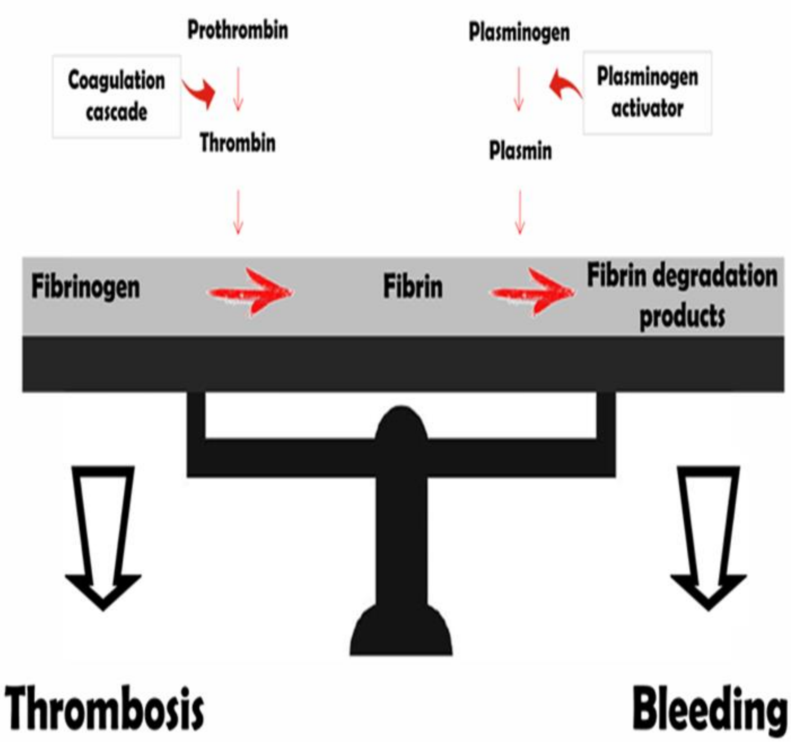


Figure 2.1: Schematic representation of the balance between the fibrin clot formation and fibrinolysis processes. (J. E. Hall & M. E. Hall, 2021)

2.6.1 Vasoconstriction

To stop bleeding from damaged blood vessels, arteries rapidly contract following injury. The damaged endothelium produces vasoconstrictors like endothelin, which cause the vascular smooth muscle to spontaneously contract to achieve this effect. Circulating catecholamines, such as norepinephrine and epinephrine, also control vasoconstriction, as well as prostaglandins secreted by damaged cells. Additionally, Platelets produce a substance called platelet-derived growth factor (PDGF), which specifically activates mesenchymal cells,

especially the smooth muscle cells in artery walls, causing contraction. However, this initial reflexive constriction only temporarily halts bleeding (Bowen-Pope & Raines, 2011).

To further regulate vasoconstriction through mediators such as bradykinin, fibrinopeptides, serotonin, and thromboxane A2, and to permanently halt bleeding, the coagulation cascade must be activated (Hofman et al., 2016).

2.6.2 primary hemostasis (Formation of the platelet plug)

Megakaryocytes give rise to anucleate cells called platelets, which contain secretory granules that are essential for platelet function. These cells migrate in close proximity to endothelial cells during homeostasis. However, the intact endothelial cell monolayer exhibits anti-thrombotic properties by producing prostacyclin, nitric oxide, and negatively charged heparin-like glycosaminoglycans, which inhibit platelet attachment, activation, and aggregation (Rodrigues et al., 2019).

Following blood vessel rupture, the thrombogenic subendothelial matrix is revealed. In order to promote platelet adhesion to other platelets and activate integrin, platelets bind to this matrix through G protein-coupled receptors on their surface, starting an inside-out signaling cascade, (figure2.2).

The most prevalent platelet integrin is αIIbβ3, mediates adherence to von Willebrand factor (vWF), fibrinogen, and fibronectin via RGD sequences. This interaction subsequently activates the outside-in signaling pathway, which remodels the actin cytoskeleton and amplifies platelet activity. Additionally, the combining of the plasma membrane and intracellular granule increases the activated platelet's surface area. These granules release over 300 active substances, including ADP, serotonin, calcium, and histamine, all essential for platelet activation. Both integrins and vWF play critical roles in primary and secondary hemostasis (Sang et al., 2021)

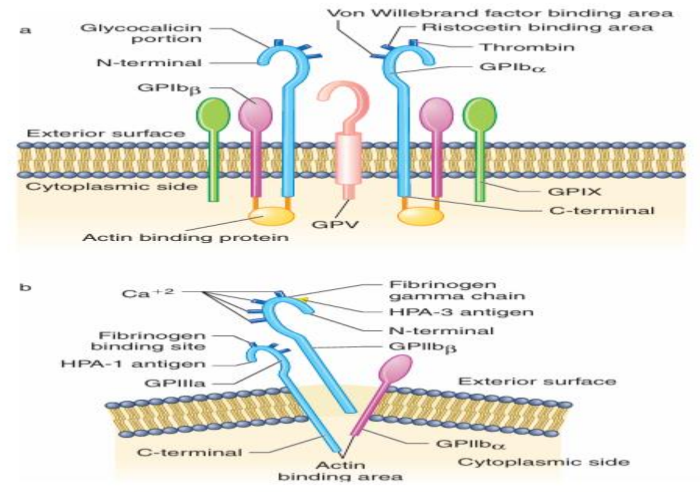
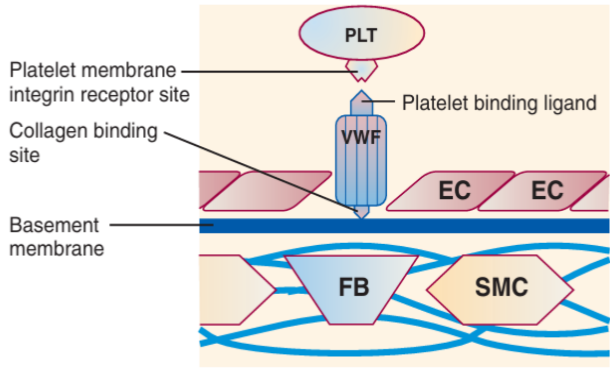


Figure 2.2: Structure of platelet membrane glycoproteins.

a. Glycoprotein Ib is composed of an alpha and a beta chain. Both span the phospholipid bilayer. The alpha chain is larger and contains the binding sites for thrombin, ristocetin, and VWF. Actin-binding protein is attached to the cytoplasmic side. Glycoprotein IX and glycoprotein V associate with glycoprotein Ib in the platelet membrane. **b.** Glycoproteins IIb and IIIa associate in a complex after platelet activation. Binding sites for fibrinogen as well as platelet-specific antigens are present. Cytoplasmic portions of each component have binding areas for actin, adabted from (McKenzie, S. B. (2010). Clinical laboratory hematology (2nd ed.). Prentice Hall).

The platelet aggregation process involves integrins as well as several other cell surface receptors. For example, platelet glycoproteins Ib, IX-V, and VI bind collagen and immobilized vWF in the subendothelial matrix, respectively, inducing platelet attachment and aggregation. (figure 2.3 represent Interaction of platelets, von Willebrand factor, and collagen).

Additionally, substances such as thromboxane A2 are released by activated platelets, promoting further platelet aggregation. These interactions collectively generate the "platelet plug." The first hour of platelet activation corresponds to the most intense release of platelet factors; however, activated platelets can continue to release these factors for up to seven days (Varga-Szabo et al., 2008).



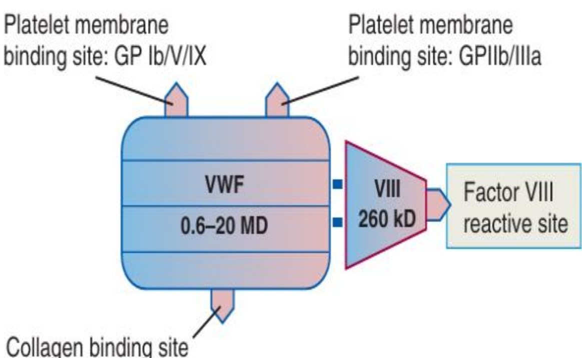


Figure 2.3: Interaction of platelets, von Willebrand factor, and collagen.
(From Rodaak, B. F., Fritsma, G. A., & Keohane, E. M. (Eds.). (n.d).

2.6.3 Secondary hemostasis (Platelet plug reinforcement)

The production of fibrin through the coagulation cascade is known as secondary hemostasis. The cascade of coagulation. is typically split into three pathways: common, extrinsic pathway, and intrinsic pathway. The common pathway is where the intrinsic and extrinsic pathways converge; it involves the generation of thrombin from prothrombin by FXa and the production of fibrin from fibrinogen, (figure 2.4) (Palta et al., 2014).

Blood-clotting factors are a group of distinct plasma proteins that are important in both the intrinsic and extrinsic routes. The majority of these proteins are proteolytic enzymes in inactive form. Their enzymatic activities trigger the sequential, cascading events of the clotting process when they are transformed into active forms. The majority of clotting factors are denoted by Roman numerals. A tiny letter "a" is appended to the Roman number to denote the active version of the factor; for example, Factor VIIIa denotes the activated state of Factor VIII(Palta et al., 2014a).

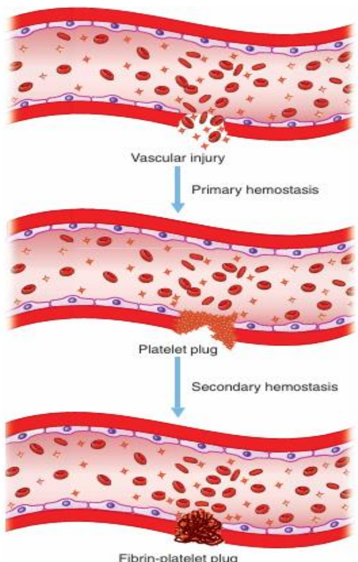


Figure 2.4: Stages of hemostasis after vascular injury.
Bleeding occurs after an injury to a blood vessel, (from McKenzie, S. B. (2010). *Clinical laboratory hematology* (2nd ed.). Prentice Hall)

2.6.3.1 Extrinsic pathway

It is regarded as the initial phase of plasma-mediated hemostasis, according to this phase of coagulation, tissue factor (TF) exposure following endothelial damage initiates the coagulation process (Van Herrewegen et al., 2012). During the first stage, The TF-FVIIa complex is created when TF binds to activated factor VII (FVIIa), which is found in trace concentrations in the circulation. Factors X (FX) and IX are activated by this complex (FIX). cascading reactions shown in Figure 2.5. The concentration of TF determines the activation efficiency, although both FIX and FX are activated by the tissue factor (TF) complex, FX activation is promoted by high TF concentrations, whereas FIX activation is promoted by low TF concentrations. However, activated FX (FXa) is the direct precursor to the thrombin-generating prothrombinase complex, and the TF/FVIIa complex is the primary driver of Factor IX activation during the initiation phase(Schuijt et al., 2013).

This initiation phase produces a relatively small amount of thrombin, which is insufficient to generate enough fibrin to stabilize the platelet plug (S. A. Smith et al., 2015).

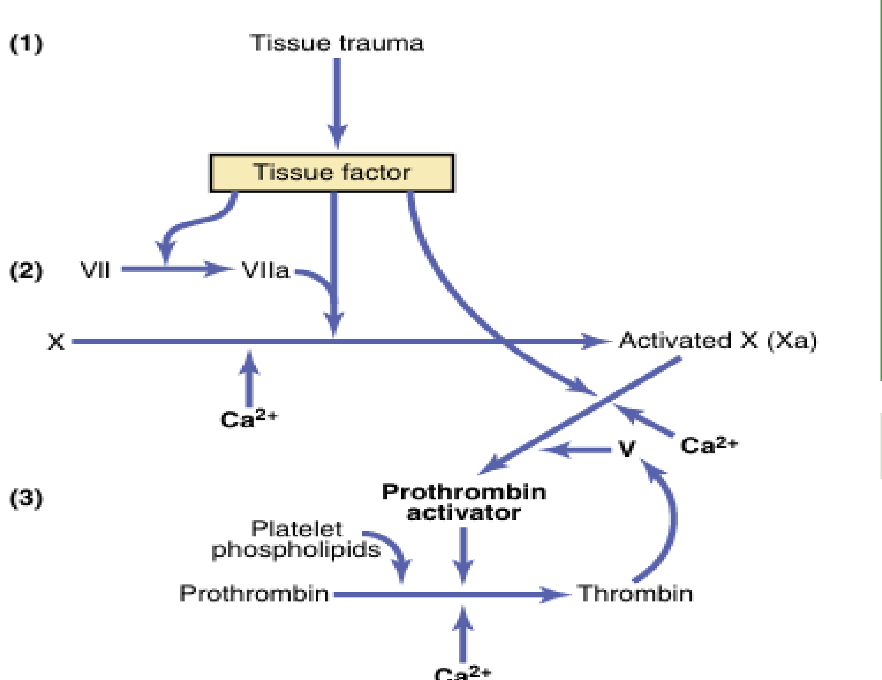


Figure 2.5: Extrinsic pathway for initiating blood clotting
From (Hall, J. E., & Hall, M. E. (2020).

2.6.3.2 Intrinsic pathway

The intrinsic pathway of blood coagulation, which starts clotting when a blood artery wall has trauma or exposure vascular wall to collagen, when a foreign surface like collagen or glass comes into contact with blood, it activates Factor XII, a protein that initiates the clotting cascade, and simultaneously damages platelets.

This damage releases platelet factor 3, which contains platelet phospholipids and contributes to the subsequent coagulation reactions that form a blood clot. Platelet factor 3 also contributes to subsequent clotting reactions (Rodak's Hematology: Clinical Principles and Applications, 6th Edition).

So, when blood is exposed to collagen due to trauma, Factor XII (FXII), a crucial initiator, is activated. This triggers up a series of events that eventually result in the production of prothrombin activator and thrombin, which forms the fibrin clot (Park & Park, 2024) figure 2.6.

The activated Factor XII also activates Factor XI by acting enzymatically on it. Prekallikrein speeds up this process, which also needs high molecular weight kininogen(Pathak et al., 2018).

Then, Factor IX is also activated by the enzymatic action of the active Factor XI also, Factor X is triggered by active Factor IX, which works in conjunction with activated Factor VIII, the platelet phospholipids, and Factor III from the injured platelets. It is evident that this step is inadequate when there is a shortage of either platelets or Factor VIII (Hultin & Nemerson, 1978). Factor VIII, also known as antihemophilic factor, is the component that is absent in individuals with classic hemophilia (Chavin, 1984).

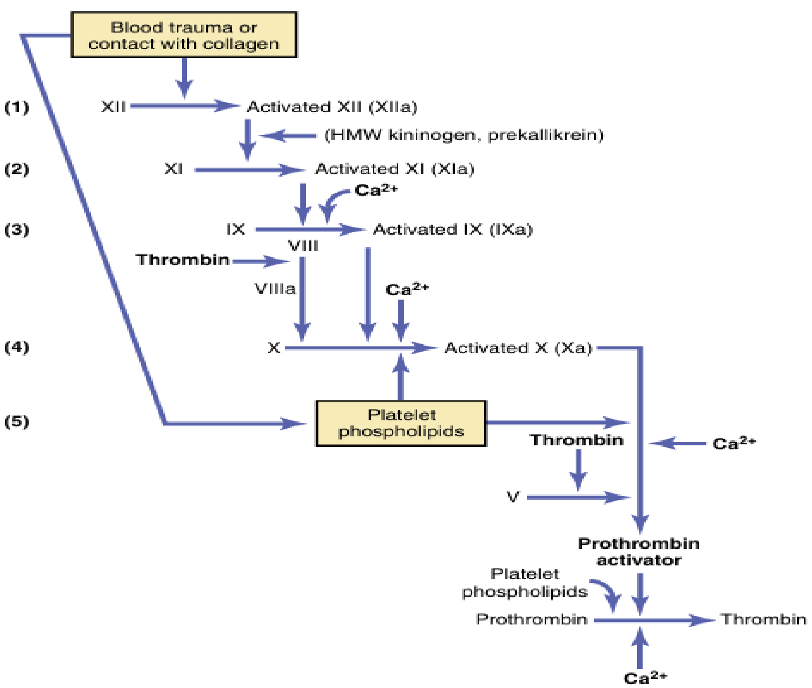


Figure 2.6 Intrinsic pathway for initiating blood clotting.
HMW, High- molecular weight.
(From Hall, J. E., & Hall, M. E. (2020).

2.6.3.3 Common pathway

The prothrombinase complex's formation is an important stage in the common pathway, the complex known as prothrombin activator is created when activated factor X combines with tissue or platelet phospholipids and Factor V. The prothrombin activator then starts the

ultimate clotting process by causing prothrombin to cleave into thrombin in a matter of seconds (Palta et al., 2014b). After that, fibrinogen is broken down by thrombin to produce fibrin, which creates the insoluble mesh that holds the platelet plug in place and forms a permanent blood clot(I. Lee & Marchant, 2003).

However, thrombin also activates platelets during the amplification phase (Wolberg & Campbell, 2008). Thrombin, the primary serine protease enzyme in the blood coagulation cascade, performs numerous important functions. First, Insoluble fibrin is produced when fibrinogen is broken down. Additionally, thrombin cleaves protease-activated receptors 1 and 4 (PAR1 and PAR4) to activate platelets. furthermore, thrombin is in responsible of the positive feedback activation of coagulation process, which is necessary for the development of clots, Cofactors VIII, V, and factor XI are all activated by thrombin, which also activates factor IX. Historically, this has been referred to as the intrinsic pathway of coagulation; however, describing it as a positive feedback loop is more accurate (Adams & Huntington, 2006).

Once initiated, the extrinsic route may be explosive, and the only factors limiting its speed to the final clot are the amounts of tissue factor that is expelled from the injured tissues and the blood levels of Factors X, VII, and V. This is an especially significant distinction between the extrinsic and intrinsic pathways. Clotting can happen in as little as 15 seconds when there is substantial tissue damage. The intrinsic route moves considerably more slowly; it typically takes one to six minutes to induce clotting (Palta et al., 2014b).

Finally, by binding to endothelial cell thrombomodulin and subsequently activating protein C (APC), thrombin significantly contributes to the down regulation of the coagulation cascade. The inhibition of this process depends on the active protein C anticoagulant system, the procoagulant cofactors are cleaved and rendered inactive by APC. Additionally, APC and protein S use factor V's anticoagulant properties as a cofactor to inactivate factors VIIIa and Va. Activated platelets offer negatively charged membrane surfaces for these complexes, which are made up of proteases and cofactors (both anticoagulant and procoagulant) (Gale, 2011).

2.6.4 Fibrinolysis

The transformation of the inactive proenzyme plasminogen into the active enzyme plasmin is the crucial step in the fibrinolytic system. Plasmin breaks down fibrin to produce soluble fibrin degradation products. Tissue-type and urokinase-type plasminogen activators (t-PA and u-PA, respectively) are two physiological plasminogen activators that catalyze this conversion(Claire S Whyte & Nicola J Mutch, 2020). Among them, t-PA is likely the the primary plasminogen activator involved in the breakdown of fibrin. Although u-PA may contribute to the complementary activity alongside t-PA, t-PA alone is an inefficient plasminogen activator when fibrin is absent. However, because Plasminogen and t-PA both attach to fibrin and create a cyclic ternary structure, the plasminogen activator activity of t-PA increases by two orders of magnitude in the presence of fibrin. Consequently, t-PA is considered a fibrin-specific activator. On the other hand, serpins found in the blood downregulate all three of these serine proteases. Plasminogen activator inhibitors 1 and 2 suppress t-PA and u-PA, whereas alpha-2-antiplasmin inhibits plasmin look figure 2.7 (Rijken & Uitte de Willige, 2017).

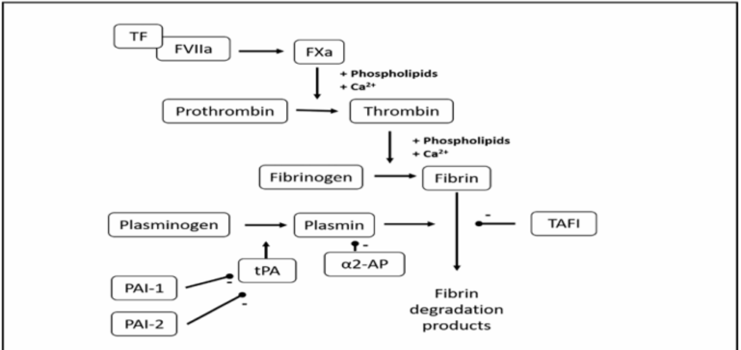


Figure 2.7 Overview of the fibrinolytic process.
α2-AP, α2-antiplasmin; F, coagulation factor; PAI, plasminogen activator inhibitor; PL, phospholipids; TAFI, thrombin-activatable fibrinolysis inhibitor; TF, tissue plasminogen activator. Adapted from (Larsen & Hvas, 2020).

2.7 Fibrinogen:

Fibrinogen is a hexameric homodimer protein with a of 340,000 Da molecular weight. it has a significant role in hemostasis and homeostasis. Fibrinogen is produced in the liver, and Its production is regulated to enable the precise control of protein levels through transcriptional regulation to determine the initial mRNA supply and translational regulation

which modifies the rate of mRNA translated into a protein. The plasma concentration of fibrinogen ranges from 150 to 350 mg/dL. It is encoded by three gene clusters located on the long arm of human chromosome 4 (Pieters & Wolberg, 2019). This molecule might have restricted permeability due to their large size.
only a small amount of fibrinogen typically escapes from the circulatory into the interstitial space. However, when permeability of capillaries is pathologically increased, fibrinogen can infiltrate tissue fluids in sufficient quantities to allow clotting, similar to that observed in plasma and whole blood (Saravi et al., 2023).

2.7.1 Structure and synthesis

The elongated 45 nm fibrinogen molecule is made up of three pairs of polypeptide chains: two copies each of the α, β, and γ chains (Mosesson, 2005). These polypeptide chains are held together by disulfide bonds, forming two symmetrically arranged halves with a central E domain linked by coiled-coil segments to the outer D domains (Center for Vascular and Inflammatory Diseases and Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, USA et al., 2020). The fibrinopeptides A and B (FPA and FPB), which are cleaved by thrombin in the last stage of the coagulation cascade, are found in the amino-terminal sequences of the fibrinogen Aα and Bβ chains, which arise from the central E domain. The carboxyl-terminal portions of the fibrinogen Aα chains, known as the αC domains, stretch from the outer D domains and are non-covalently joined to the central E domain (Hanss et al., 2011). The overall structure of fibrinogen is designed to efficiently form long fibrin threads that can crosslink, creating a stable and strong blood clot (Anderson et al., 1993). figure 2.8

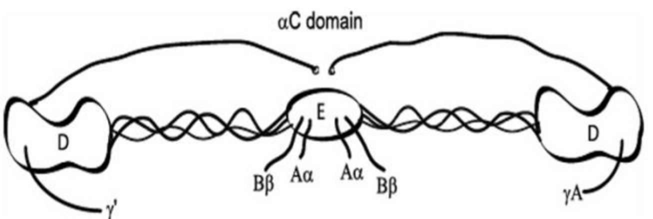


Figure 2.8: Simplified illustration of the fibrinogen molecule.

The presented structure is a heterodimer (AαBβγ) (AαBβγ’). The central E domain is linked by coiled-coil segments to the outer D domains from where the αC domains emerge (Adapted from Mosesson 2003).

2.7.2 association between fibrinogen and CD

Elevated levels of plasma fibrinogen are a significant potential risk element for both myocardial infarction and stroke. Fibrinogen has been identified as an independent predictor of CD, including stroke and myocardial infarction , particularly due to the consequences of elevated fibrinogen, (Heinrich & Assmann, n.d.). This association affects both African and Caucasian populations. Numerous possible explanations have been stated to explain fibrinogen’s role in the pathophysiology of CD; however, it remains unclear whether this involvement is causative or consequential. The primary proposed mechanisms linking elevated fibrinogen levels to increased CD risk include enhanced platelet aggregation, increased plasma viscosity and red blood cell aggregation, impaired vascular and endothelial function, participation in inflammatory processes, and alterations in fibrin network structure (Lominadze et al., 2010).

2.7.2.1 Increasing platelet aggregation

Fibrinogen plays an important part in hemostasis by facilitating Aggregation of platelets. Binding of platelets to the damaged vascular wall occurs when fibrinogen binds to the glycoprotein IIb/IIIa (αIIbβ3) receptor sites on activated platelets (Rumbaut & Thiagarajan, 2010). Repair of blood vessels requires the endothelium platelet aggregation and attachment to one another, and thrombus formation. However, under pathological conditions such as inflammation or the development of fatty streaks associated with CD, platelet activity including enhanced adhesion and aggregation increases, potentially leading to thrombus formation and ischemic events (Winzer et al., 2018).

It has been demonstrated that pathological conditions characterized by elevated fibrinogen levels exhibit increased platelet activity, suggesting a potential mechanism by which elevated fibrinogen may raise the risk of (CD) (Schneider et al., 1999).

2.7.2.2 Raising blood/plasma viscosity

Fibrinogen is the primary factor influencing erythrocyte aggregation and plasma viscosity, both of which are linked to a higher chance of developing CD. Elevated plasma fibrinogen adversely affects the rheological properties of blood. For instance, increased viscosity can reduce microcirculatory flow, cause endothelial damage, and elevate the risk of thrombosis (Liu et al., 2022).

Following endothelial cell (EC) activation induced by disturbed blood flow, several events occur, including the release or activation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and the fibrinogen receptor αvβ3.(Gong et al., 2025).

When fibrinogen binds to ICAM-1, it triggers reactions that lead to inflammation and the formation of atherosclerotic plaques. Consequently, elevated fibrinogen levels can alter blood rheology by reducing blood flow, increasing the risk of thrombosis, and promoting atherogenesis, thereby elevating the risk of CD (Pulani & Rudan, 2005).

2.7.2.3 Increase red blood cell aggregation

A significant factor influencing blood flow in resistance arteries at low shear rates is red blood cell (RBC) aggregation, which increases blood viscosity and causes microcirculatory sludging and stagnation in small capillaries (Lominadze et al., 2010).

Compared to other plasma proteins, fibrinogen has a more pronounced effect on erythrocyte aggregation. The prevailing consensus is that the nonspecific binding of fibrinogen to the erythrocyte membrane causes this process, so any Increases of Adhesive proteins in plasma, like fibrinogen, cause the red blood cells to clump together by binding to them. In contrast, platelets have a fibrinogen integrin receptor on their membrane surface, which is essential for blood coagulation and is represented by the membrane glycoprotein complex αIIbβ3, this receptor is crucial for binding fibrinogen and is activated upon platelet stimulation(C. K. Lee et al., 2007).

Specifically, the observed increases in red blood cell (RBC) aggregation are closely correlated with elevated fibrinogen concentrations especially during inflammation and acute phase response. Abnormalities in RBC aggregation also occur in metabolic disorders, including diabetes mellitus, and cardiovascular diseases such as arterial hypertension, atherosclerosis, myocardial infarction, and ischemia. (Semenov et al., 2020).

Erythrocyte Aggregation (EA), the temporary aggregation discussed, is caused by macromolecules such as fibrinogen bridging across red blood cells (RBCs), which causes the cells to adhere to one another to form rouleaux. By raising blood viscosity and causing microcirculatory dysfunction, this rouleaux creation might worsen coronary illnesses, especially in acute coronary syndromes. Elevated EA and decreased cardiac blood flow can result from abnormal amounts of these macromolecules, which are frequently caused by inflammatory conditions or metabolic diseases (Weber-Fishkin et al., 2022).

Certain physicochemical properties such as the ligand's molecular mass, charge, and the concentration of interacting molecules are essential for erythrocyte assembly(Pajic-Lijakovic et al., 2025).

2.7.2.4 Involvement in the inflammatory process

Atherosclerosis and other arterial diseases are significantly influenced by inflammatory processes. Fibrinogen has been shown to regulate NF-kappa B activation and the production of inflammatory chemokines in endothelial cells; therefore, it may play a role in mediating inflammation(Liu et al., 2017).

Certain integrin receptors on leukocytes, including αMβ2 (Mac-1) on neutrophils and macrophages, bind to fibrinogen By binding to its integrin receptor on the surface of leukocytes, fibrinogen serves additional role in the inflammatory process by promoting phagocytosis of debris, apoptotic cells, and pathogens at the site of inflammation; this is a crucial step in eliminating infections and repairing tissue damage, enhancing antibody-mediated leukocyte toxicity, delaying apoptosis, and increasing chemotactic responsiveness (Flick et al., 2004).

2.7.2.5 Endothelial layer integrity

There is a complicated set of tight junctional proteins (TJPs) that includes adherens, gap, and tight junctions that hold endothelial cells together. The primary TJPs include occludin, claudins, and molecules of junctional adhesion. These proteins provide endothelial cells with a paracellular barrier, serving as their first line of defense against blood(Naser et al., 2023).

Confirmed the idea that certain agonists may cause fibrinogen (Fg) leakage through the vascular wall by increasing the amount of Fg that passes through the endothelial cell (EC)

layer (Tyagi et al., 2007). It was proposed that the Fg induced increase in EC layer permeability results from Fg binding to its receptors (integrins and ICAM-1) on the surface of microvascular ECs to interact with them (Patibandla et al., 2009).

The subendothelial matrix may accumulate Fg due to increased microvascular permeability. There is a significant connection between the progression of atherosclerotic plaques and increased Fg levels. Consequently, increased Fg concentration in the blood leads to greater Fg deposition on the vascular endothelium and/or the subendothelial matrix, especially when the plasminogen system is less active or remains unchanged(Lominadze et al., 2010). Because the The breakdown system is currently not effective to regulate the overproduction of fibrin, immobilized Fg is subsequently converted into fibrin by thrombin. This increased fibrin deposition exacerbates the formation of atherosclerotic plaques and other circulatory complications associated with CD (Lominadze et al., 2010).

2.8 Variations in Fibrinogen Structure:

2.8.1 alternative mRNA splicing (e.g. the fibrinogen γ' chain)

The blood contains several forms of fibrinogen due to a number of inherent alternating basic molecules such as the different forms of γ chains that result from a different polyadenylation signal in intron 9 of the FGG gene The fibrinogen gamma chain's major form is the γA chain, whereas the γ′ chain is a minor(Lovely et al., 2012).

The γ′ chain makes up 8–15% of total fibrinogen, with the majority of this in the heterodimeric γA/γ′ form and just 1% in the homodimeric γ ′/γ′ form. γ′ chains have been demonstrated to modify fibrin production and structure and slow down the protofibril lateral aggregation. A higher plasma fibrinogen γ′ concentration is linked to an increased risk of myocardial infarction and other thrombotic conditions (Weisel & Litvinov, 2017a).

2.8.2 Genetic determinants

According to information from studies, between 20 and 50 percent of the variance in plasma fibrinogen concentration has been attributed to the inheritance of genes, The noncoding sections of the fibrinogen genes include the other most common polymorphisms, which can alter the amount of plasma fibrinogen (Williams, 2022).

Multiple additional situations exist where fibrinogen polymorphisms, clot characteristics ,

structure, and disease are strongly correlated (Ząbczyk et al., 2023).figure 2.9 show common fibrinogen polymorphism .

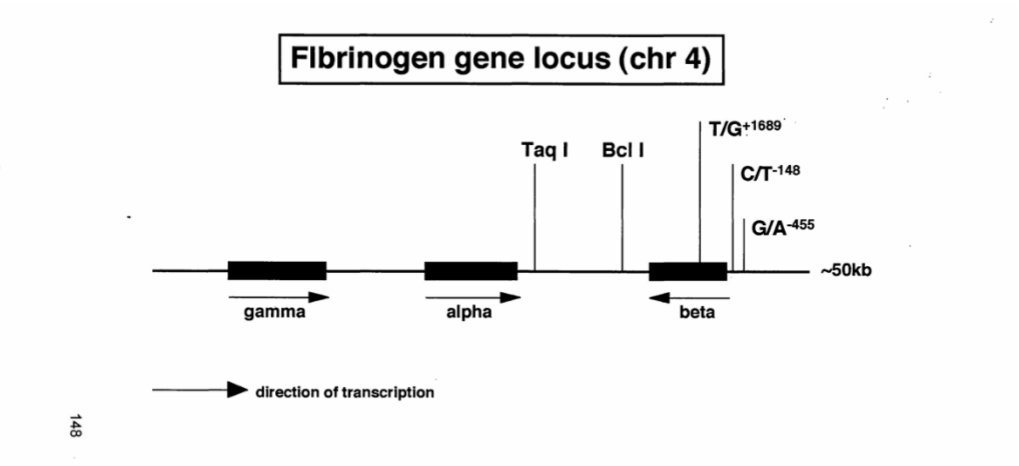


Figure 2.9: fibrinogen polymorphism ,from (Thomas, n.d.).

2.8.2.1 FGB -455G/A (rs1800790)

Currently, the majority of SNPs linked to FGB have been identified in their promoter (Luo et al., 2025). The 455G/A (rs1800790) polymorphism which can be detected by HaeIII restriction enzyme) in the non-coding region of the β gene is the most extensively investigated polymorphism (Ken-Dror et al., 2012). this polymorphism affects fibrinogen levels and has been linked to inflammatory and thrombotic disorders. It is known that the –455G>A polymorphism's minor allele was exclusively linked to higher fibrinogen levels in Asians and Whites. According to several findings, white populations with the A/A genotype had higher amounts of fibrinogen (Albert et al., 2009). A thrombus development on surfaces may be triggered by the presence of defective fibrinogen, which can alter blood viscosity. Therefore, it seems obvious that the beta-fibrinogene 455G/A polymorphism might contribute to stasis development, which would predispose thrombus formation (Bozdemir et al., 2010).

2.8.2.2 FGB -148C/T (rs1800787)

Often referred to HindIII polymorphism, it results from a C to T mutation at the FGB gene's 148 position. It appears that white people are more likely than black or Asian people to have FGB 148C/T. Higher fibrinogen levels have been linked to the minor Heterogeneous T allele than to homozygotes for the C allele, The effect of FGB 148C/T on fibrinogen levels

might be explained by its proximity to an IL-6 highly responsive portion, which is the main mediator of acute-phase-induced fibrinogen production that can affect fibrinogen synthesis in response to an acute-phase reaction. as well as its position between binding sites for CCAAT box/enhancer-binding protein (C/EBP), which is also crucial for the IL-6 response, and hepatocyte nuclear factor-3 (HNF-3), which is required for the IL-6 response of fibrinogen β-promoter (Imran et al., 2015).

2.8.2.3 1689 T/G (The Avall Polymorphism)

The nucleotide change that resulted in the removal of the Avall restriction site at position 1689,which results in replacement of T to G base change in the fibrinogen gene , The +1689 T/G variation has not been as well studied for its impact on fibrinogen levels and correlation with CD as some other fibrinogen polymorphisms.

2.8.2.4 BclI polymorphisms

This is a betta chain gene polymorphism that is located downstream of the alpha -chain gene in the 3' flanking region (Sell et al., 2001) . Although its characteristics are unclear, this area may include mRNA synthesis regulatory sequences. However, it could only serve as a marker for functional variations in the promoter or codifying regions that alter the protein's production or sequence (Zito et al., 1997) .The common allele was coded as B1 and the rare allele as B2 ,In individuals with coronary artery disease, the significant effect of this polymorphism on fibrinogen levels was reported (Božič et al., 2003). Both elevated plasma levels of fibrinogen and a higher risk for AMI are independently linked to the presence of the allele B2 of the fibrinogen gene (Zito et al., 1997).

2.8.3. Post-translational modifications

It has been demonstrated that post-translational changes of the fibrinogen molecule, such as oxidation, glycation, or homocysteinylation, impact fibrin clot structure as well as clot formation and lysis; as a result, they can lead to thrombotic illnesses (Nencini et al., 2024a).

2.8.3.1 Effects of oxidation

Protein oxidation, the change of amino acids and the creation of carbonyl groups are the outcomes of increased reactive oxygen species (ROS) generation. Increased density, reduced both vulnerability to fibrinolysis and permeability are all characteristics of fibrin clots that are are linked to higher oxidative stress levels. These alterations raise the risk of thrombotic events and are seen in people with inflammatory and thrombotic conditions such as diabetes and coronary disease.

Also ,Oxidative stress can result from environmental factors such as pollution, medications, and radiation that raise ROS levels (Nencini et al., 2025).

Reactive compounds of protein carbonyl or thiobarbituric acid, as well as common ROS sources like NADPH oxidase activity, are examples of multiple indicators of oxidative stress measured in plasma. Among patients who have systemic inflammation and those recovering from AMI, fibrinogen carbonyl content is particularly linked to altered fibrinogen polymerization and lysis (Weisel & Litvinov, 2017b).

2.8.3.2 A consequence of nitration

Tyrosine and cysteine residues are the main targets of nitration, another important PTM, the specific covalent attachment of a nitro group (-NO2) to the carbon atom at position 3 of the tyrosine residues' & cysteine residues phenolic ring, creating 3-nitrotyrosine and 3-Nitrocysteine , The reactive nitrogen species that can result in tyrosine nitration are produced by neutrophils and monocytes, which are important elements of the inflammatory response. Myeloperoxidase is an important enzyme that contributes to this process by converting hydrogen peroxide and nitrite into nitrating intermediates (Colling et al., 2021).

Numerous human illnesses, such as inflammatory, cardiovascular, ischemic stroke, and metabolic disorders, have been linked to nitrated proteins. coronary disease Patients may have greater quantities of circulating antibodies that block 3-nitrotyrosine, an antigenic neoepitope present on nitrated proteins in atherosclerotic plaques that may trigger up an immunological reaction. Peroxynitrite-mediated oxidative damage in the artery walls is indicated by this phenomena, which implies that these antibodies might be a potential therapeutic target as well as a biomarker for the existence and activity of CD (Thomson et al., 2012).

In contrast to the action of oxidation, which slowed clot formation and preserved normal clot structure, fibrinogen nitration accelerated fibrin clot formation and increased clot permeability, resulting in a pro-thrombotic condition. This implies that fibrinogen nitration is a strong pro-thrombotic agent, which may explain why it is linked to vascular diseases such as coronary artery disease (Tenopoulou, 2025).

2.8.3.3 Acetylation

The process by which an acetyl group is attached to amino acids is known as acetylation. In vivo, Nt-acetyl transferases acetylate polypeptide chains at their N-termini, while lysine acetyl transferases acetylate lysine residues at their ε-amino group. Important physiological roles are played by both processes. Aspirin, which is known to help prevent heart disease via acetylating serine residues in the platelet cyclooxygenase enzyme. Aspirin can acetylate lysine residues in fibrinogen and other coagulation proteins, altering their biological function (De Vries et al., 2020).

2.8.3.4 glycosylation and glycation

An enzyme-mediated post-translational modification known as glycosylation adds a specific carbohydrate molecule to a predefined protein region. During biosynthesis, glycosylation, the covalent bonding of carbohydrates to proteins by N-glycosidic or O-glycosidic bonds, includes sialylation, in which sialic acid serves as the terminal monosaccharide. It causes reduced fibrin fiber diameter, stiffness, permeability and density. Conversely, it was discovered that hypersialylation resulted in clots with thinner fibers, more rigidity, and higher density.

The non-enzymatic molecule glycation represents the interaction between the aldehyde group of a sugar molecule and the ε-amino group of a lysine residue, is frequently seen in diabetes because of hyperglycemia. After glycation, clot stiffness and fibrin fiber diameter either remained the same or reduced, whereas permeability dropped and density typically increase (Nencini et al., 2024b).

2.9 Molecular basis of fibrinogen abnormality

2.9.1 Congenital Deficiency

According to plasma concentration, Traditionally, there are two types of congenital abnormalities of fibrinogen: qualitative or Quantitative deficits. type II or qualitative deficits, which include hypo-dysfibrinogenemia and dysfibrinogenemia, indicating that their fibrinogen levels are either normal or decreased and that their functional activity is aberrant (Mohsenian et al., 2025).

dysfibrinogenemia usually inherited as an autosomal dominant characteristic, that resulting from mutations in any one of the three fibrinogen genes that impact any one of the fibrinogen's functional characteristics, such as the delayed or absent release of FPA and FPB, the delayed or accelerated polymerization, the impaired crosslinking, the diminished thrombin binding, and the delayed degradation of plasmin (Asselta et al., 2006).

Afibrinogenemia and hypofibrinogenemia are examples of quantitative or type I deficiencies, which are defined by the total absence or decreased levels of fibrinogen (Asselta et al., 2006).

2.9.1.1 Afibrinogenemia, and Hypofibrinogenemia

Mutations in the homozygous or compound heterozygous condition in one of the three genes producing fibrinogen chains cause Afibrinogenemia, which is an autosomal recessive disease. Although hypofibrinogenemia has historically been seen as a separate clinical entity from afibrinogenemia, it is essentially the phenotypic manifestation of the heterozygous state for a single mutation that takes place within the fibrinogen gene cluster. Causative mutations in afibrinogenemia and hypofibrinogenemia might impact the hexameric fibrinogen's production, assembly, intracellular processing, stability, or secretion, resulting in reduced amounts of circulating fibrinogen (Simurda et al., 2021).

2.9.1.2 Dysfibrinogenemia

It's unclear exactly how dysfibrinogenemia increases the risk of thrombosis, however it probably varies according on the kind of fibrinogen abnormality identified. Theoretically, a mutant fibrinogen might cause excessive thrombosis by either increasing the production of clots or impairing their breakdown (fibrinolysis). Alternatively, the abnormal fibrinogen molecule could cause thrombosis by failing to bind thrombin.

As a result, there would be more circulating thrombin available to promote platelet activation. Several of the different dysfibrinogens have been shown to have defective thrombin binding. There have also been observations regarding impaired fibrinolysis, which includes tissue-type plasminogen activator binding abnormally and resistance to plasmin lysis (Hayes, n.d.-a).

2.9.2 Acquired disorders

In contrast to hypofibrinogenemia and dysfibrinogenemia, which are congenital conditions, acquired fibrinogemia is a later-life disease characterized by unusually low or incorrectly functioning fibrinogen in the blood. Acquired fibrinogen abnormalities can result from trauma-induced coagulopathies, such as hemodilution after blood loss with volume replenishment, or consumptive coagulopathies, such as disseminated intravascular coagulopathy (DIC).The use of albumin as a replacement fluid in plasma exchange, cancer (like multiple myeloma), drugs (like L-asparaginase), and autoimmune diseases that produce anti-fibrinogen antibodies (like rheumatoid arthritis and systemic lupus erythematosus) are another factor contributing to acquired fibrinogen problems (Hayes, n.d.-c).

2.9.2.1 Acquired hypofibrinogenemia

Hemodilution and consumption of coagulation factors are the most common causes of acquired hypofibrinogenemia. Nowadays, one of the main aspects of treating severe bleeding is the intensive replacement of fibrinogen. A prolonged PT and PTT may raise suspicions of acquired fibrinogen problems, which can then be verified through a test that reveals a low amount of fibrinogen (Besser & MacDonald, 2016). Nevertheless, since fibrinogen is an acute phase protein, levels of the protein may be within the normal range in acquired fibrinogen disorders, in clinical settings when a normal range fibrinogen level truly indicates a clinically

significant acquired "Relative fibrinogen deficiency" may be a more accurate term to describe hypofibrinogenemia (May et al., 2021).

2.9.2.2 acquired dysfibrinogenemia

The most frequent cause of acquired dysfibrinogenemias is liver illness. From cirrhosis to liver failure, dysfibrinogenemia is seen in 80–90% of individuals with different types of liver disease. Sialic acid concentration is elevated in the majority of dysfibrinogens linked to liver disease. Fibrin polymerization is impeded by this higher carbohydrate content, which may be caused by an increase in negative charge making it more difficult for it to properly polymerize and form a functioning clot. The abnormal fibrinogen activity and clotting times in these patients can be corrected by the enzymatic elimination of this excess sialic acid (Martinez et al., 1983).

This altered fibrinogen is similar to Fetal fibrinogen which has a comparable rise in sialic acid concentration compared to adult. A prolonged thrombin time is the most prevalent indicator of this abnormal fibrinogen function. Additionally, acquired dysfibrinogenemia has been associated with allogeneic bone marrow transplantation, renal cell cancer, and the usage of drugs such as L-asparaginase, mithramycin, and isoretinoin (Hayes, n.d.-b).

The existing literature has established a clear role for the 455GA SNP and its impact on level of blood fibrinogen and the increased of coronary disease risk. However, the role played by the -148C/T SNP remains inconclusive. Importantly, the vast majority of this evidence comes from European populations, leaving the impact of these genetic variants in Middle Eastern populations, such as Palestinians, largely unexplored. Consequently, this research aims to fill up the following information gaps by investigating the associations between the 455G/A and 148C/T polymorphisms, plasma fibrinogen levels, and coronary disease in a sample of the northern Palestinian population. The results are expected to provide useful information about the genetic basis of cardiovascular disease in this unique population and contribute to personalized prevention strategies.

Chapter Three: Materials and methods

3. Materials and methods:

In this chapter, the research methodology is given with details of the study design, study setting, terms and definitions, ethical issues that were considered, sampling strategies and sample size, inclusion and exclusion criteria, data gathering procedures, blood sample collection, fibrinogen plasma determination Using von Clauss technique, extraction of DNA, quantification of DNA, amplification method using the polymerase chain reaction (PCR), following Restriction Fragment Length polymorphism (RFLP) technique using specific restriction enzymes for the Human Fibrinogen Gene polymorphisms HaeIII and Hind IIII and finally statistical methods.

3.1. Study design:

The research method is a multi-center, prospective controlled research design with a follow-up study of six months.

3.2. Study Participants and setting:

It was a multi-center research study conducted from three hospitals in the north of Palestine, i.e. Jenin Governmental Hospital (Jenin), Thabet Thabet Governmental Hospital (Toulkarm) and National Government Hospital - Al-Watani (Nablus). The aim and the study's objectives were presented to patients. It was guaranteed that no one would know their identity except the researcher. The data would be securely encrypted, used only to

research, and kept in password-protected file folder that would be unrestricted to view by any other person rather than the authorized researcher. Also, they were informed that it was completely voluntary and they could refuse. The participants were allowed to withdraw themselves out of the research without any form of consequences behind them. An Arabic copy of signed informed consent form was given to the patients who gave consent to participate. They were subsequently requested to engage in study in a person interview to answer a questionnaire.

A total of 100 individuals who have been diagnosed with CD were enrolled in the study, including UCD and SCD, and their associations with MACE in a sample of northern West Bank of Palestine. The control group, 70 subjects, is from apparently healthy people.

3.3. Definitions:

The terms that are used in the study have the definitions indicated as follows:

- *Unstable coronary disease (UCD)*: is characterized by Chest discomfort that is acute and ischemic lasting longer than 20 minutes while at rest, ST (elevation or depression), and necrosis.
- *Stable coronary disease (SCD)*: is defined as prior ischemic heart disease caused by atherosclerosis as determined by coronary angiography and without a history of a coronary acute episode during the previous 12 months.
- *Healthy subject*: is one who has never had a history of chronic metabolic disease or cardiovascular disease. Every participant underwent laboratory testing required in the study.
- *Clinical stability*: Patients with coronary heart disease and without visible signs of left ventricular failure and with normal blood pressure.
- *Major adverse cardiovascular events (MACE)*: include recurrent ischemia, a new chest pain episode lasting longer than five minutes, ST abnormalities, and enzymatic increase.
- *Acute reinfarction*: Chemotherapy-induced chest discomfort that persists longer than 20 minutes at rest and is followed by ST (elevation or depression) and enzymatic increase.

- *Cardiogenic shock*: indicated by clinical signs of left ventricular dysfunction/weakening, renal output below 20 milliliters per minute, ejection fraction less than 40% as indicated by echocardiography and low systolic blood pressure below 100 mmHg.
- *Follow-up*: Every three months for six months, all patients received follow-up calls or in-office visits.
- *Cardiovascular mortality*: includes infarction, acute ischemic event, cardiogenic shock, subsequent ventricular fibrillation or arrhythmia, and death.

3.4. Ethical Consideration:

The study was carried out in compliance with the Helsinki Declaration principles. The permission granted by the Institutional Review Board (IRB) of Arab American University (AAUP), given on October 29, 2023, to conduct the current study can be found in archive number 2023/B/157/N (Appendix 1). One more, the legal authorization was obtained through the letter of facilitation issued by the Palestinian Ministry of Health on December 17, 2023, with the purpose to conduct laboratory work on the basis of providing blood samples and patient laboratory data access in its registration (Approval Number: 2023/2725/162; Appendix 2). The research tool also used a structured questionnaire, English and Arabic, to obtain documentation in relation to specific data in medical history and demographic information (Appendices 3-4). The patient samples were analyzed at the research laboratory of the Arab American University in Palestine, Allied Medical Sciences Faculty, 2nd floor room AMS-B 101.

3.5. Sampling and Sample Size Determination:

In 2023, the population of Palestinians living in the West Bank region was 3,256,906 in June, and the aged (> 60 years) contributed 6.3% of the total population with a recent reportedly high prevalence (> 30%) of CD among the Palestinians of the West Bank (Palestinian Central Bureau of Statistics [PCBS], 2023). Estimation of the sample size required in the study was performed using Cochran Formula whose parameters were: the

p-value that was set as a two-sided, < 0.05, 95% confidence interval and 80% of study power.

$$n = \frac{Z^2 \cdot p\ (1-p)}{e^2}$$

Where: n = the size of the sample, Z = Z-score with the desired confidence level (e.g. 1.96 for 95% confidence interval), p = the estimated rate of population of the characteristic under study (prevalence). When this is unknown, 0.5 is usually used as the maximum sample size. e = desired margin of error (as a decimal).

Given: * Z = 1.96 (for 95% confidence) * p = 0.30. * e = 0.05. * N = 500,000. = 323

It should be mentioned that it is merely an estimation and the real size should be changed with regard to numerous factors such as expected response rate and perquisite level subgroup analysis. Include probability of non-response (e.g. 15 percent non-response rate).

$$n\,final = 323/1\text{-}0.15 = 380.$$

Thus, a sample size of about 380 adults is needed to estimate the prevalence in 95% confidence interval and 5% margin of error considering a non-responding rate of 15%. According to the limited budget and financial issues, a total of 100 patients with CD were included in the study, including SCD and UCD, and their correlations with major MACE in a sample of northern West Bank of Palestine. The control group, 70 subjects, is from Obviously a healthy state people. Each groups were age, sex and body mass index, and sedentary-matched ,as well as all samples extracted under identical circumstances. Due to the delicate sensitivity of the plasma markers used in in vitro maneuver, blood samples were extracted with utmost carefulness, a venous puncture was conducted using a vacuum test tube by well-trained individuals.

3.6. Study Population: Inclusion and Exclusion Criteria:

A total of 100 patients with CD were participated in the study, including unstable UCD SCD. The control group, 70 subjects, is from apparently healthy people. All groups were age, sex and body mass index, and sedentary-matched and all samples extracted under identical conditions. To be eligible, patient has to be age 40-65, experience UCD or SCD with secondary atherothrombosis and be clinically stable. Heart failure, anemia,

thyrotoxicosis, acute or chronic pulmonary arterial hypertension, aortic stenosis, excessive vasodilation and age greater than 65 or younger than 40 were among the exclusion criteria. Additionally, patients with hematological or hepatic or neoplastic disease; acute coronary event within the previous three months, ejection fraction < 35% and acute or chronic inflammatory activity were not included in the research.

3.7. Data Collection and Consent:

CD patients admitted in the 3 major Palestinian Ministry of Health public hospitals that fulfilled the inclusion requirements criteria were enrolled in the study. Medical history and records for each patient were retrieved from patients' files to ensure he/she met the inclusion criteria. Before beginning the study, each participant signed an informed consent form (Appendix 5). Medical and demographic information was gathered via a unique questionnaire, which included the patient's age, sex, history of diabetes, hypertension, lipid profile, smoking, ST-elevation, non-ST elevation, and sedentary life.

3.8. Blood Sample Collection:

- After every participant filled out the questionnaire, venous blood samples were extracted with utmost carefulness, by well-trained individuals. Two vacutainer tubes were used to collect the blood: About 3 milliliters tube containing Ethylene Diamine Tetraacetic Acid was used to collect blood (EDTA) and 3 milliliters in a tube that contained Acid Sodium Citrate (ACD). In contrast to EDTA, which can interfere with the measurement of fibrinogen, we utilize acid sodium citrate to detect plasma fibrinogen since it chelates calcium, preventing blood from clotting while maintaining fibrinogen in a quantifiable condition. While EDTA permanently binds to calcium, it can introduce artifacts into fibrinogen tests, resulting in erroneous findings. Citrate is the favored option for researching coagulation components like fibrinogen, which are crucial for the coagulation cascade because of its capacity to chelate calcium reversibly (Skeppholm, M., Wallén, N. H., Blombäck, M., & Kallner, A. (2008)). An area in the arm without folds, cracks, or skin breaks was punctured with a 20-gauge needle. Prior to testing, plasma and EDTA whole Blood specimens were kept at -40 °C and 4 °C, respectively.

The blood sample was taken, prior to any invasive procedure, including insertion of intravenous lines, or fibrinolytic, antithrombotic or antiplatelet medicines. The level of plasma fibrinogen in the anticoagulated samples using ACD was determined by the use of the von Clauss method after performing centrifugation of the samples instantly at 4 °C. The anticoagulated EDTA tube was used in isolating DNA to assess the gene for β-fibrinogen (G/A-455, C/T-148) polymorphisms.

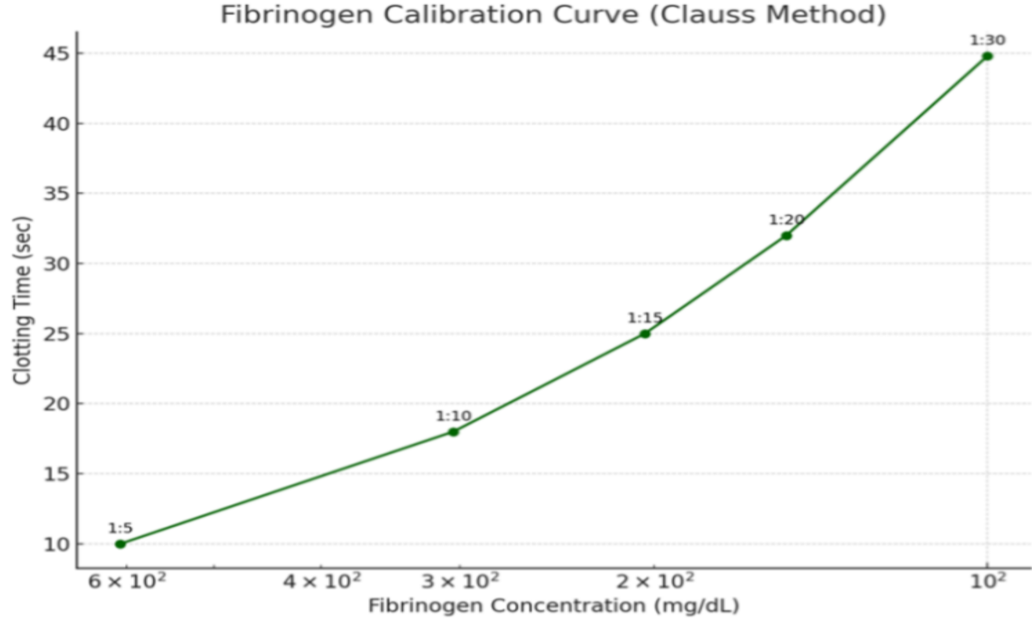
3.9. Fibrinogen assay determination by the von Clauss method:

The level of plasma fibrinogen was determined by the von Clauss method, which is a functional assay based on the measured time required to have a fibrin clot arise due to the excess human thrombin added to the sample using a semiautomated coagulation analyzer (Thrombotimer, Behnk Elektronik, Germany) (A. Clauss, 1957). The DIAGON (Dia-FIB, DIAGON, Hungary) Kit was used to measure the amount of fibrinogen in both the CD patients and controls following manufacturer instructions. Briefly, a standard curve is made by using plasma with a known fibrinogen content (e.g. Normal Coagulation Reference Plasma) at 1:7, 1:10, 1:20 and 1:30 and a standard curve is obtained. The clotting time that results from the Thrombin's addition is negatively correlated with the amount of fibrinogen. After that, thrombin is added to patient plasma at a dilution of 1:10, and the clotting time that results is used in order to extract the fibrinogen level based on the standard curve. The test was run in duplicates as recommended by manufacturer. Normal and abnormal Coagulation Reference Plasma were used after reconstitution according to manufacturer instructions. Three groups were made according to concentration level in plasma fibrinogen: low (<350 mg/dl), intermediate (350- 450 mg/dl), and high (>450 mg/dl).

Table 3.1: Fibrinogen Assay Determination by the von Clauss Method Summary

Step	Description	Notes / Tools

Principle	Functional assay measuring clotting time after adding excess human thrombin to plasma.	Clotting time → **inversely proportional** to fibrinogen concentration
Instrument Used	Semiautomated coagulation analyzer	DIAGON (DIA-FIB, Hungary) kit
Preparation of Standard Curve	Plasma with **known fibrinogen concentrations** diluted at 1:7, 1:10, 1:20, 1:30	Creat **standard curve**(Fibrinogen vs. Clotting Time)
Measurement	Add thrombin to patient plasma (dilution 1:10) and **measure clotting time.**	**Compare** with standard curve to determine fibrinogen level
Interpretation	**Shorter CT → higher fibrinogen concentration**	(A .Clauss ,1957)



3.10. DNA Extraction and Quantification:

Based on instructions of the manufacturer, genomic DNA was extracted out of buffy coat via use of the QIAamp mini kit (QIAGEN, Heldin, Germany). This was done by mixing 25µL of the proteinase K and 200µL of the blood. Exact 200µL of AL lysis buffer was carefully added to the microtube. The mixture was incubated at 56°C in a 15 sec span. This was followed by vortexing the mixture in the presence of 250µL of absolute ethanol. Subsequently, incubation of the lysate was carried out at 15-25°C under ambient temperature (5 min). Further, the lysate was spun down a QIAamp Min Elute column in a minute with a rpm rate of 8000 rpm. Thereafter, the Min Elute column is washed using the AW1 and AW2 washing buffers twice. Subsequently, 96-100% ethanol, in the volume of 500mL, was added to the Min Elute column, and Centrifugation of the column at 8000 rpm for one minute. A sterile microtube was subsequently filled with 70µL of the AVE buffer contained in the kit which was then used to elute DNA. The isolated DNA was stored in -20 °C until used.

DNA purity and concentration were assessed using a NanoDrop spectrophotometer. (IMPLEN, Germany). Briefly, the extracted DNA sample (1µL) was used following a blank sample of 1 µL AVE solution. The ratio OD260/OD280 was computed to determine the approximate purity of the DNA, acceptable ranges are 1.8-2.2 meaning low degree of protein contamination and suitability to molecular analysis later on.

3.11. Genotyping:

Two polymorphisms of the β-fibrinogen gene (G/A^{-455}, C/T^{-148}) located in the promoter region were analyzed, by means of the polymerase chain reaction (PCR), as described previously (Thomas, Angela Eleine; 1996), followed by splicing with restriction enzymes (PCR-RFLP). The specificity of primer sequences was examined using UCSC In-Silico PCR (http://www.genome.ucsc.edu) and NCBI Primer-BLAST. PCR primers (hylabs, Ltd., Israel) for G/A^{-455} (HaeIII) and C/T^{-148} (HindIII) are shown in table 3.1:

Table 3.2 Polymerase chain reaction conditions and primer sequences.

Gene	Primer sequence (5'-3')	Reaction Conditions *				
		Denaturing	Annealing	Extension	Cycle #	Fragment Size (bp)
(β-fibrinogen)	F-5′AAG AAT TTG GGA ATG CAA TCT CTG CTA CCT-3′ R-5′CTC CTC ATT GTC GTT GAC ACC TTG GGA C-3′	95°C (1 min)	59°C (1 min)	72°C (1 min)	35	1301
* an initial denaturing step at 96°C for 7 min						

To guarantee that the PCR product was free of contamination, a PCR mixture devoid of a DNA sample was utilized as a negative control. The PCR was performed in a total volume of 50µl using 100 ng of DNA, 1x reaction buffer, 1.5 µM MgCl2, 0.5 µM dNTPs and 0.5 µM Taq polymerase (Promega, Madison, USA).

DNA was amplified using a Biometra Tadvanced Thermal Cycler (analytikjena, Fullerton, USA). The PCR was amplified under the following conditions: a 7-minute initial denaturing phase at 96°C, followed by 35 denaturation cycles at 95°C for 1 min, one minute of annealing at 59°C, one minute of extension at 72°C, and ten minutes of final extension at 72°C. The amplified Products from PCR were analyzed in 2% agarose gels (Sigma, Deisenhofen, Germany) containing ethidium bromide in 1x TBA buffer (Promega, Madison, USA) and visualized using ultraviolet transilluminator documentation system (Uvitec, Cambridge, UK).

3.11.1. Detection of the HaeIII G/A^{-455} polymorphism (NC_000007.14.) – (rs1800795)

The β-fibrinogen gene 5'-flanking region has the HaeIII G/A-455 polymorphism, which can be detected by PCR-RFLP and digestion using the Haelll restriction enzyme. Figure 3.1 displays the diameters of the Haelll digestion products as well as the oligonucleotide primers utilized for the PCR. It was later discovered that the G to A base mutation at -455 bp from the beginning of transcription was the cause of the Haelll polymorphism.

The HaeIII restriction enzyme was used to digest the PCR product before it was examined on a 3% agarose gel. Three bands (343, 383, and 575 bp) were found to be present in the normal G allele, whereas two fragments (343 and 958 bp) were found to be present in the mutant A allele.

Using an ultraviolet transilluminator documentation system (Uvitec, Cambridge, UK), the digested PCR products were examined in 3% agarose gels (Sigma, Deisenhofen, Germany) with ethidium bromide in 1x TBA buffer (Promega, Madison, USA).

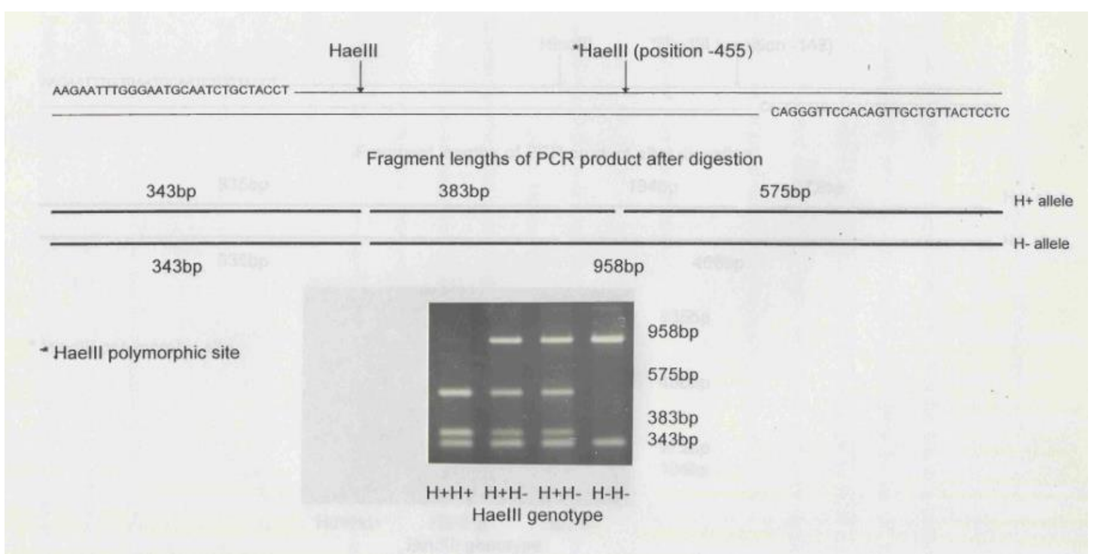


Figure 3.1. Human β-Fibrinogen Gene: HaeIII Polymorphism (Thomas, Angela Eleine; 1996)

3.11.2. Detection of the Hind III C/T $^{-148}$ polymorphism (NC_000004.12.) – (rs1800787)

The Hind III C/T-148 polymorphism in the β-fibrinogen gene 5'-flanking region can be identified by PCR-RFLP and digestion using the restriction enzyme Hind III. The diameters of the Hind III digestion products and the oligonucleotide primers used for the PCR are

shown in Figure 3.2. The Hind III polymorphism was eventually found to be caused by a C to T base mutation at -148 bp from the start of transcription.

Prior to examination on a 3% agarose gel, the PCR product was digested using the Hind III restriction enzyme. The normal C allele was found to contain three bands (194, 272, and 835 bp), while the mutant T allele was found to contain two fragments (466 and 835 bp).

The digested PCR products were examined using a UV transilluminator documentation system (Uvitec, Cambridge, UK) and 3% agarose gels (Sigma, Deisenhofen, Germany) with ethidium bromide in 1x TBA buffer (Promega, Madison, USA).

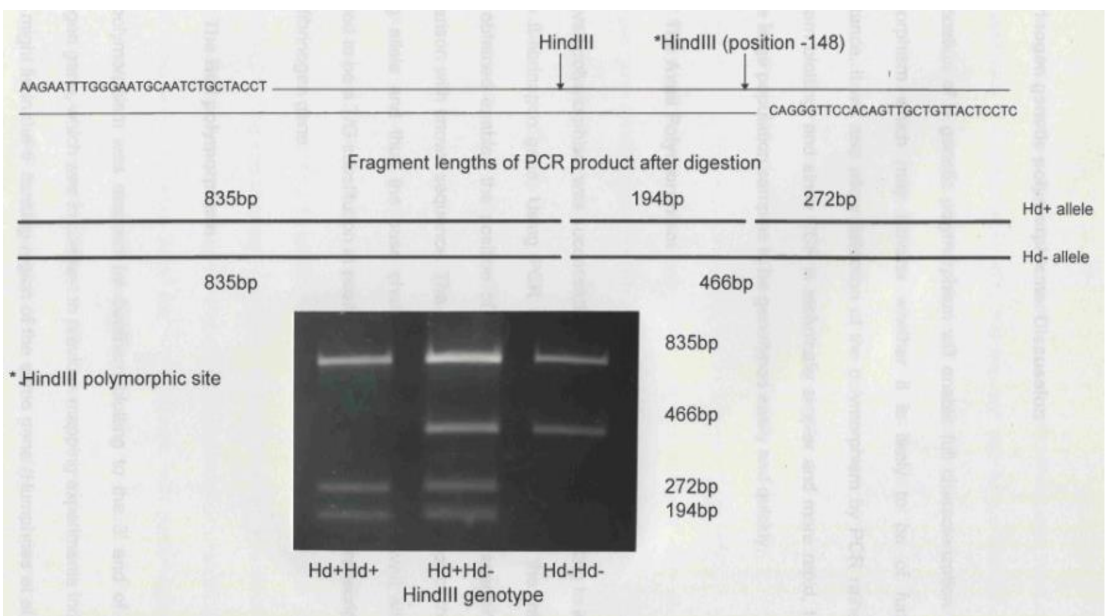


Figure 3.2. Human β-Fibrinogen Gene: Hind III Polymorphism
(Thomas, Angela Eleine; 1996)

3.12. Statistical analysis

The continuous variables and the difference between the group averages were evaluated by Wilcoxon test. Using the Yates correction, Chi square analysis was performed on the discrete variables. The variability of the fibrinogen level among UCD, SCD and healthy participants was tested by a two-way analysis of variance (ANOVA) for repeated measures. The effect of the level of fibrinogen in the plasma and its polymorphisms on the result of the MACE at the acute phase and at the follow-up was estimated on the basis of logistic and multiple

regression analysis models. Kaplan-Meier survival curves were taken into account, as well as relative risk (RR) and odds ratio (OR) with the 95% confidence interval (CI). Results were deemed statistically significant if $p < 0.05$. The information was presented as OR, CI, standard deviations, averages, and percentages. The statistical analysis in this study was carried out using GraphPad Prism 5.0, a program created by the La Jolla, California-based company GraphPad.

Chapter Four: Results

4.1. Sociodemographic and General Characteristics

In this study, one hundred and seventy members were involved in the study between January 2024 and April 2025. Forty-one patients with UCD and fifty-nine patients with SCD were recruited into three major governmental hospitals, respectively, out of the 100 patients, and 70 people as a control group. Demographic characteristics of the groups are shown in Table 4.1.

The average age of SCD patients was 54.8 ± 9.3 years, those with UCD was 56.1 ± 8.1, while the average age of the control group was 54.4 ± 8.7. Within the different groups, around 30% of participants were females and 60% were males. No age and gender differences were observed between patients and controls. The UCD group had more patients with diabetes, hypertension, smoking, lipids abnormalities, and the history of a sudden coronary event. Obesity was noted in every group, and more than 50% of them did not engage in physical activity. Compared to controls, individuals with coronary disease had noticeably higher levels of fibrinogen (p=0.001). After six months of follow-up for patients with UCD, the value of moderate plasmatic fibrinogen was related to cardiovascular mortality (p=0.03) (Figure1).

Table 4.1: Sociodemographic and Clinical Features of the study participants.

Variable	**Control group**	**SCD group**	**UCD group**	***p* value**

	N = 70 (%)	N = 59 (%)	N = 41 (%)	
Age (yr. ± SD)	54.4 ± 8.7	54.8 ± 9.3	56.1 ± 8.1	NS
Gender				
• Female	24 (34.3)	21 (35.6)	13 (31.7)	NS
• Male	46 (65.7)	38 (64.4)	28 (68.2)	
Known history				
• Hypertension	19 (27.1)	25 (42.4)	19 (46.3)	NS
• Diabetes	23 (32.8)	22 (37.3)	29 (49.2)	0.0396
• CD	11 (15.7)	30 (50.8)	13 (31.9)	<0.00010
Predisposing factors				
• Diabetes	14 (20)	32 (54.2)	22 (53.6)	<0.00001
• Hypertension	23 (32.9)	37 (62.7)	25 (61.0)	<0.00010
• Smoking	20 (28.6)	27 (45.7)	22 (53.7)	<0.00010
• Dyslipidemia	2 (2.6)	13 (22)	9 (21.9)	<0.00010
• Stationary life	37 (52.9)	31 (52.5)	23 (56.1)	NS
• BMI	26.4 ± 3.9	27.6 ± 4.1	29.3 ± 3.4	NS
Trigger Event				
• ST elevation	—	—	24 (58.5)	
• Non-ST elevation	—	—	18 (43.9)	
Biochemical marker				
• Fibrinogen(mg/dl)	411.7 ± 58.6	479 ± 113.4	562.3 ± 184.2	0.001

NS = not significant ($p > 0.05$).

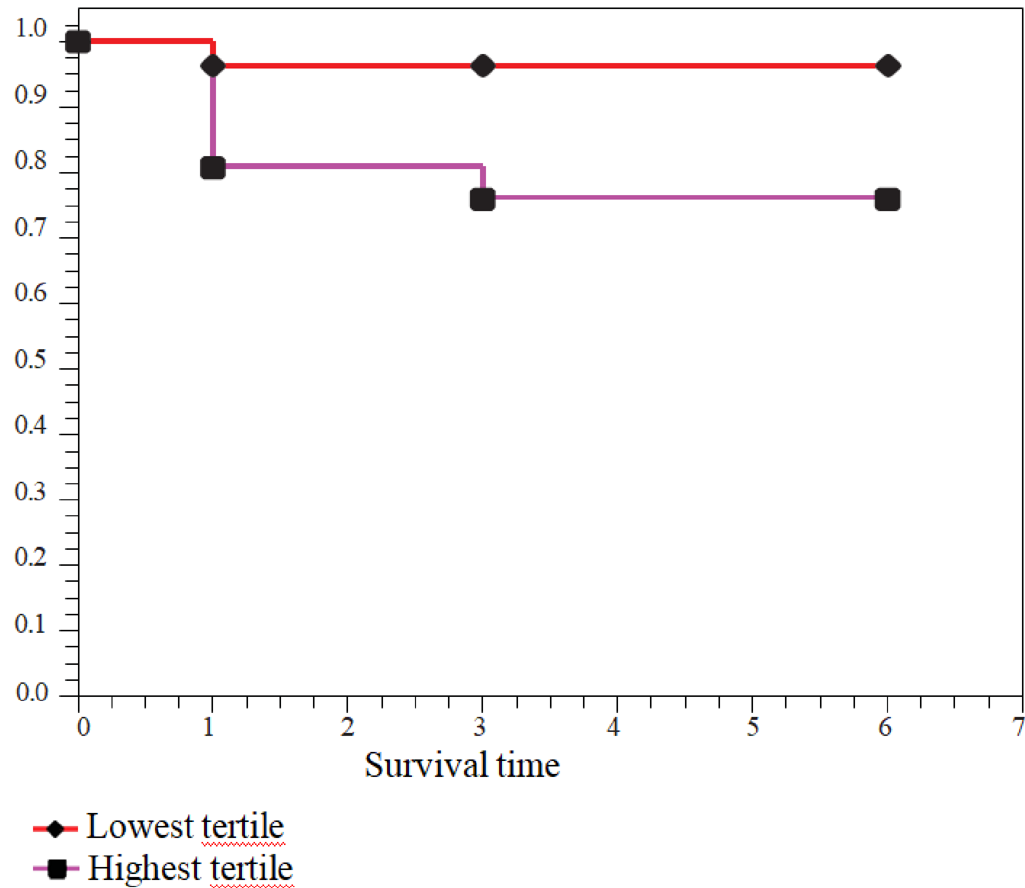


Figure 4.1: Highest tertile fibrinogen versus the lowest tertile in mortality to one year (p =0.03).

4.2. β-Fibrinogen Genotype and Allele Frequencies:

Two polymorphisms of the β-fibrinogen gene (G/A-455, C/T-148) located in the promoter region were analyzed, by means of PCR-RFLP. A representative gel image for PCR examination which yield a DNA fragment of 1301 bp is shown in Figure 4.2.

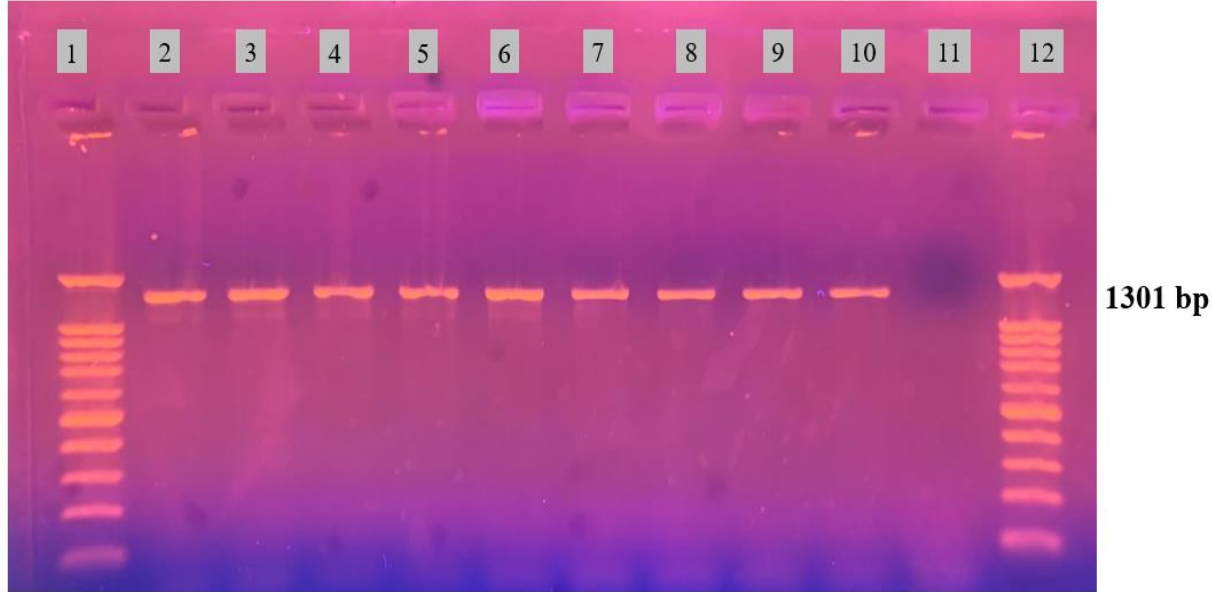


Figure 4.2: A representative gel photograph for PCR analysis of the 5' flanking promotor region of the β-fibrinogen gene, product size 1301 bp. Lane1 & 12 are DNA ladder 100bp. Lane 11 is a negative control. Lanes 2-10 are the PCR product 1301 bp.

The PCR-RFLP results and the two alleles of Haelll and Hindlll digestion product sizes are appear in figures 4.3 and 4.4 respectively. It was subsequently emerged that the Haelll polymorphism resulted from a G/A base change at -455 bp from the transcription start and a Hindlll caused by a C/T base substitution at -148 bp from the transcription start.

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Figure 4.3. A representative gel photograph for the HaeIII G/A^{-455} polymorphism. Lane 1 is 100 bp DNA ladder. Lanes 2, 5 and 7 are normal wild type GG. Lanes 3 and 4 homozygous AA mutants. Lanes 6 and 8 are heterozygous GA genotypes.

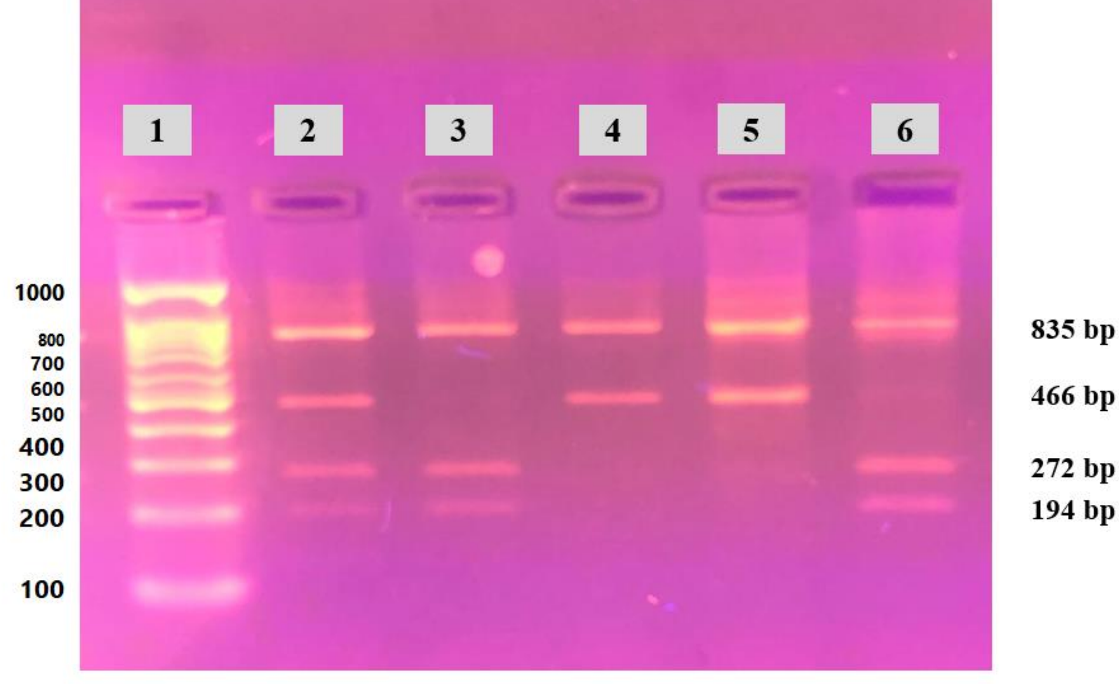

Figure 4.4. A representative gel photograph for the Hind III C/T $^{-148}$ polymorphism. Lane 1 is 100 bp DNA ladder. Lane 2 is a heterozygous CT. Lanes 3 and 6 are wildtype homozygous CC. Lanes 4 and 5 are homozygous TT mutants.

Table 4.2 represents the genotype and allele distribution among the three studied groups. Both polymorphism in the fibrinogen B gene that were examined was in Hardy-Weinberg equilibrium ($p > 0.05$). Patients who have coronary disease had significantly increased frequencies of the genotypes -148C/T and -455 G/A (p value 0.004 and 0.021), respectively. However, when allele frequencies were examined, patients with UCD and SCD had considerably higher frequencies of the polymorphisms -455 and -148T than controls (p value 0.021 and 0.0004, respectively).

Table 4.2: The β-fibrinogen gene (G/A^{-455}, C/T^{-148}) genotype/allele frequencies in CD patients and controls.

Genotype	**Control group** [a] **N = 70 (%)**	**SCD group** [b] **N = 59 (%)**	**UCD group** [c] **N = 41 (%)**	***p* value**
HaeIII G/A^{-455}				
G/G	57 (81.4)	31 (52.5)	24 (58.5)	**0.021***
G/A	10 (14.3)	24 (40.7)	16 (39.0)	
A/A	3 (4.3)	4 (6.8)	1 (2.4)	
Hind III C/T $^{-148}$				
C/C	41 (58.6)	23 (38.9)	13 (31.7)	**0.004***
C/T	26 (37.1)	30 (50.8)	20 (48.8)	
T/T	3 (4.3)	6 (10.2)	8 (19.5)	
Allele Frequency				
G/A^{-455}				
G	124 (0.8857)	86 (0.7288)	64 (0.7805)	**0.021**
A	16 (0.1143)	32 (0.2712)	18 (0.2195)	
C/T $^{-148}$				
C	108 (0.7714)	76 (0.6440)	46 (0.5609)	**0.0004**
T	32 (0.2286)	42 (0.3559)	36 (0.4390)	

Stable coronary disease (SCD), unstable coronary disease (UCD). *Yates' p-value (the star indicates a statistically significant difference ($p<0.05$)).

Among the potential genotypes under investigation, the effects of the -455A and -148T genetic variations on an individual allele's allelic burden revealed a higher OR for coronary disease. The

effects of genetic variations in the -455A allele on an individual allelic burden were attributed to SCD (OR = 3.20, 95%CI = 1.5 - 6.9, $p < 0.005$) and UCD (OR = 2.67, 95%CI = 1.2 - 7.0, $p = 0.019$). Also, both SCD (OR = 2.03, 95%CI = 1.0 - 5.1, $p = 0.041$) and UCD (OR = 2.90, 95%CI = 1.4 - 6.2, $p < 0.003$) were associated with the -148T allelic burden (Table 4.3).

Table 4.3: The impact of the -455A and -148T genetic variants on the allelic load.

Genotype	**Control group** [a] **N = 70 (%)**	**SCD group** [b] **N = 59 (%)**	**UCD group** [c] **N = 41 (%)**	***p* value**	**OD (95%CI)**
G/A $^{-455}$					
GG	57	31	24	0.005 [ab]	3.20 (1.5 - 6.9)
G/A+A/A	13	28	17	0.019 [ac]	2.67 (1.2 - 7.0)
				0.411 [bc]	0.95 (0.3- 2.2)
C/T $^{-148}$					
C/C	41	23	13	0.041 [ab]	2.03 (1.0 - 5.1)
C/T+T/T	29	36	28	0.003 [ac]	2.90 (1.4 - 6.2)
				0.967 [bc]	1.20 (0.7 - 3.3)

ab: stands for controls against SCD, **ac**: for controls against UCD, and **bc**: for SCD against UCD.

4.3. Levels of Fibrinogen based on genotype:

An influence of the genotypes variations on plasma fibrinogen levels is presented in Table 4.4. The two studied polymorphisms, -455G/A and -148C/T were associated with fibrinogen concentrations that were above 450 mg/dl. Plasma high levels of fibrinogen proteins were linked to the -455G/A genotype and -148C/T ($p <0.005$ and $p = 0.041$).

Table 4.4: Correlation between the polymorphism and plasma fibrinogen levels.

Fibrinogen N = 170	**< 350 mg/dl N = 51**	**350-450 mg/dl N = 66**	**> 450 mg/dl N = 53**	**p value**
G/A $^{-455}$				
GG	39 (76.5)	45 (68.2)	27 (50.9)	**0.005**
G/A	11 (21.6)	20 (30.3)	26 (49)	
A/A	1 (2)	1 (1.5)	0 (0)	

C/T -148				
C/C	26 (50.9)	31 (46.9)	19 (35.8)	**0.041**
C/T	18 (35.3)	32 (48.5)	24 (45.3)	
T/T	7 (13.7)	3 (4.5)	10 (18.9)	

4.4. Multivariable Regression Models:

The 148T and 455A alleles' genetic burden is a predictive factor on its own of elevated fibrinogen ($r = 0.4$, $p = 0.007$) in a multiple regression model with additional risk factors such as diabetes, smoking, age, sex, BMI, dyslipidemia, and stationary life.

4.5. Clinical follow up and Major adverse cardiac events (MACE):

The effects of the -148T genetic mutation of a person allelic burden were demonstrated in a predictive nature of MACE in acute and recurring ischemia in UCD group with a six months follow-up (Table 4.5). There was a logistic multiple regression model which showed that -148T allele was an independent predictor of 6-month follow-up of MACE (RR = 1.6, 95%CI = 1.13 - 3.42, p = 0.03), in-hospital recurrent ischemia (RR = 1.8, 95 % CI = 1.19 - 3.51, p = 0.02). Plasma fibrinogen was associated with survival in Kaplan-Meier study in UCD, wherein, the highest intensity of fibrinogen plasma was associated with the lowest chance of survival with those subjects having the lowest (Figure 4.1).

Table 4.5: Cardiovascular adverse events for 6 months period.

UCD polymorphism (N = 41)	**Nonevent (N = 14)**	**Adverse event (N = 27)**	**MACE type (6-months)**			
			Angina (N = 27)	**Reinfarction (N = 4)**	**Shock (N = 4)**	**Death (N = 6)**
G/A -455						
GG	8	15	14	2	3	4
G/A+A/A	6	12	13	2	1	2
C/T -148						
C/C	9	6	5	1	0	2
C/T+T/T	5	21*	22#	3	4	4

* RR = 1.6, 95%CI = 1.13 - 3.42, p = 0.03; # RR = 1.8, 95 % CI = 1.19 - 3.51, p = 0.02.

Chapter Five: Discussion

This chapter presents an analysis of the study's results to offer comprehensive insights and highlight key findings.

5. Discussion:

This was a multi-center case control investigation that was carried out on an entire of 100 patients diagnosed with coronary disease (CD), including Forty-one patients with UCD and fifty-nine with SCD and 70 apparently healthy people as a control group, It was carried out at three tertiary care facilities in northern Palestine: National Government Hospital-Al-Watani (Nablus), Jenin Governmental Hospital (Jenin), and Thabet Thabet Governmental Hospital (Toulkarm) Throughout the period in January 2024 and April 2025. Genotypes 455 G/A and 148C/T were identified using polymerase chain reaction and restriction fragment length polymorphism with restrictive enzymes HaeIII and HindIII. The measurement of fibrinogen was done by the von Clauss method using a semi-automated coagulometer.

This study investigated the relationship between polymorphisms in fibrinogen -455G/A and -148C/T, concentrations of protein in the blood, and coronary disease in Northern Palestine. The results are relevant to the knowledge of genetic and biochemical factors contributing to coronary artery disease in this particular group of people. The discussion of this chapter will compare the findings with what has been written in other literature in terms of similarities and difference that are noted in regional and global research backgrounds.

5.1.The Role of Fibrinogen in Coronary Artery Disease:

Fibrinogen, which is a soluble plasma glycoprotein product of the liver, is central to both hemostasis and thrombosis. Besides its preeminent role in clot formation, higher levels of plasma fibrinogen have persistently been reported as a causative risk factor of cardiovascular diseases, and coronary disease (CD) in particular (Yarnella J.et al., 2004, Yano K. et al., 2001, Doggen C. et al., 2001). Its implications in the atherosclerosis process range across different phases, such as endothelial dysfunction and inflammatory levels as well as the formation and rupture of the plaque. Fibrinogen possesses a pro-inflammatory and pro-thrombotic effect, which adds to its atherogenicity, so it is one of the most important biomarkers and therapeutic

targets in cardiovascular medicine (Tousoulis D. et al., 2011, Canseco-Avila LM. Et al., 2019). Interestingly, when fibrinogen is measured using the von Clauss method, the average plasma levels of this protein in people with coronary disease (479 mg/dl) and controls (411 mg/dl) are higher than those observed in other populations (Stec J. et al., 2001, Heinrich J., et al., 1994). Other evidence reports establish similar values (Koenig W. et al., 2003) even though they are inside reference levels (Yarnell. J. et al., 1991). The UCD group's six-month follow-up revealed that fibrinogen levels were additionally predictive of cardiovascular mortality. The values (473.2 ± 191.5 mg/dl) found in UCD individuals who passed away during the study are comparable to those seen in Spanish (Arnau Vives M. A. et al., 2002), American (M. Acevedo et al., 2002), Japanese American (Yano K. et al., 2001), and Israeli populations (Benderly M. et al., 1996).

5.2. Fibrinogen (-455G/A, -148C/T) Polymorphisms and Coronary Disease:

The correlation between levels of plasma fibrinogen and CAD risk has been thoroughly investigated in relation to the -455G/A polymorphism (rs1800795) in the β-fibrinogen gene promoter region. The β-fibrinogen gene's transcriptional activity is known to be impacted by this polymorphism, a G to A substitution at position -455, which in turn affects the levels of fibrinogen in circulation. Research on its direct relationship to the risk of CAD has produced mixed findings; some have found a positive correlation, while others have found no association, and some have even suggested a protective benefit (Rallidis L.S. et al., 2010, Leander K. et al., 2002). The correlations between coronary disease and the -455G/A polymorphism, as well as its effect on levels of plasma fibrinogen, are important in the context of the current investigation in Northern Palestine. To comprehend the possible impact of genetic background and environmental circumstances on the manifestation of this polymorphism's effects, these data should be compared with those from various ethnic and geographic populations. The G-455A polymorphism of the beta-fibrinogen gene, for example, may have a "protective effect" against the development of non-fatal acute myocardial infarction, according to a study conducted in a Greek population (Rallidis L.S. et al., 2010). On the other hand, additional research has linked the -455A allele to greater fibrinogen levels and, as a result, a higher risk of cardiovascular disease (Leander K. et al., 2002).

Another well researched genetic variation that has been connected to plasma fibrinogen levels and CAD susceptibility is the -148C/T polymorphism (rs1800787) in the β-fibrinogen gene promoter. Additionally, the C to T mutation at position -148 may affect gene expression and, in turn, fibrinogen levels. Like the -455G/A polymorphism, the -148C/T polymorphism has been linked to CAD, although the results have varied throughout populations, indicating complicated interactions with additional environmental and genetic factors. The Northern Palestine study's results on the -148C/T polymorphism should be compared to those from other areas. According to certain research, for instance, the -148T allele is linked to higher fibrinogen levels, which may raise the risk of CAD (Surma S. et al., 2021). However, some studies have not discovered any meaningful correlation or even a protective effect (Lam K. et al., 1999). These disparities demonstrate the importance of conducting population-specific research due to the possibility of a vast difference in environmental conditions and genetical predispositions among populations.

Depending on lifestyle, environmental factors, and genetic variation, the prevalence and effect of fibrinogen polymorphisms on coronary disease might vary greatly among groups. The results from Northern Palestine shed important light on the genetic makeup of the area with regard to the risk of CD. The function of fibrinogen polymorphisms in cardiovascular health has also been investigated regionally in the Middle East and North Africa (MENA) area. For example, studies on the relationship between the beta fibrinogen gene's G-455A and C-148T polymorphisms and coronary artery disease in the Iranian population can offer a pertinent comparison (Motavas M. et al., 2018).

There are many studies all around the globe that have been carried out on these polymorphisms. As an example, the ECTIM study which was carried on European populations has provided an early evidence of the existence of a relationship between polymorphisms and the β-fibrinogen gene and that of the plasma fibrinogen and the coronary artery disease among patients with myocardial infarction (Behague I. et al., 1996). Additional research in Asian populations has also investigated the polymorphism in the β-fibrinogen gene G-455A and its correlation with the risk of coronary heart disease (Ashavaid T.F. et al., 2011). Variations in study design, sample size, CAD diagnostic criteria, and the existence of confounding variables including diabetes, hypertension, and smoking can all lead to disparities in results between studies. Additionally, gene-environment interactions are also important, as food or lifestyle

choices that are common in a certain area may influence the impact of a given polymorphism. Thus, the findings from Northern Palestine add to a more thorough comprehension of the intricate relationship between genetics, level of fibrinogen, and coronary disease when considered in the context of regional and international literature.

According to the current study's examination of the polymorphisms' allelic frequencies under investigation, just the 455A and 148T alleles are linked to coronary disease in general (UCD and SCD combined), but the genotype analysis reveals that the 455G/A and 148C/T and T/T genotypes are linked to coronary disease. Most ethnic groups worldwide have observed a correlation between cardiovascular disease and the -455 polymorphism (Pegoraro R. J. et al., 2005, Iacoviello L. et al., 2001), however other studies found no correlation (Doggen C. et al., 2002, Tybjaerg-Hansen A. et al., 1997). While there is no data supporting the -148 variation as a cardiovascular risk factor, it has been linked to myocardial infarction in Asian individuals (Lam K. et al., 1999) and is thought to be a predictor of carotid atherosclerosis (Schmidt H. et al., 1998).

The -455A and -148T alleles' genetic loading provided pertinent ORs for SCD and UCD, supporting their clinical impact. Fibrinogen levels more than 450 mg/dl were linked to these polymorphisms, with genotype -455G/A being the most significant (p=0.005), followed by -148C/T (p=0.041). It is crucial to stress that these findings are based on a comparison of fibrinogen levels and polymorphisms, not on a distinction between specific clinical situations. The work's most pertinent conclusion is that UCD patients with the -148T allele had nearly twice the probability of MACE and recurrent ischemia throughout the 6-month follow-up. According to our research, the -148T genotype is strongly linked to elevated plasmatic fibrinogen levels, coronary heart disease, and unfavorable outcomes following a UCD incident. Furthermore, cardiovascular disease and elevated plasmatic fibrinogen levels are linked to the -455A allele. The alleles -455A and -148T's simultaneous relationship supports the theory of a genetic linkage, which has been documented in Caucasian populations (M. A. Laffan et al., 2001). The -455A and -148T gene variants that are pertinent to this investigation are found in the fibrinogen B gene's promoter region, which is quite near to the HNF1 and HNF3 components as well as the elements of response to IL-6 and C/EBP (Doggen C. et al. 2000, Iacoviello L. et al. 2001, Gervois P. et al., 2001). They might alter how the promoter interacts with IL-6, a

cytokine that triggers the inflammatory response during the acute phase of a cardiac event, which would account for the correlations found in our study.

5.3. Conclusions:

The results of this investigation provide crucial insight into the connections between coronary disease in Northern Palestine, levels of plasma fibrinogen, and fibrinogen polymorphisms (455G/A, 148C/T). Our sample showed plasmatic fibrinogen levels greater than 450 mg/dl and polymorphisms 148C/T and -455G/A of fibrinogen gene linked to MACE and coronary disease. The results highlight the intricate and multifaceted nature of CD when seen in the context of regional and international research. Although some genetic variations may make people more probable to have greater fibrinogen levels and a higher chance for development of CD, the exact effect can vary greatly among groups because of differences in genetic backgrounds and environmental factors. To further understand the complex mechanisms driving CD susceptibility in the Palestinian population and beyond, future research should concentrate on bigger cohort studies, look into gene-environment interactions, and investigate additional potential genetic markers.

5.4. Limitations

1. *Potential Confounding*: It is impossible to completely rule out residual confounding caused by unmeasured or inadequately measured variables (such as a meticulous diet, psychological stress, or subclinical inflammatory indicators other than CRP).
2. *SNP Scope Restrictions*: A focused but insufficient picture of the genetic variation impacting fibrinogen and CAD may be obtained by concentrating just on two FGB promoter SNPs. More comprehensive genetic studies might be beneficial, such as haplotype, additional fibrinogen SNPs, and GWAS.
3. *Measurement of fibrinogen*: Long-term exposure levels might not be accurately reflected by a single plasma fibrinogen assay. Direct comparisons may become more difficult if the test methodology differs from those of other investigations.
4. *Budgetary Restrictions*: Insufficient funds made it difficult to get the first estimated sample size (n=380), which would have diminished the reliability of the findings.

5.5. Recommendations:

1. Identify further polymorphisms within the coding and non-coding areas of the fibrinogen gene locus and replicate the most promising of association studies like Bcl-1 and 1689 T/G.
2. Determining the β-fibrinogen and α-fibrinogen genotypes as well as the plasma fibrinogen level in a broader population would be interesting in an attempt to clarify which variation of the fibrinogen gene is most impactful.
3. Coronary disease patients should be monitored for their fibrinogen levels for early detection of complications.
4. Determining fibrinogen level and genotype in a younger-aged sample, before the development of CD, may be beneficial in an effort to avoid selection bias, which could mask gene-environment interactions.

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Appendices

Appendix 1. Institutional Review Board (IRB) of Arab American University

Arab American University- Palestine
Deanship of Scientific Research
IRB committee
Tel: 04-241-8888, ext 1196
E-mail: irb.aaup@aaup.edu

الجامعة العربية الامريكية- فلسطين
عمادة البحث العلمي
لجنة أخلاقيات البحث العلمي
تلفون: 04-241-8888 ext 1196
البريد الالكتروني: irb.aaup@aaup.edu

IRB Approval Letter

Study Title: The associations among fibrinogen polymorphisms -455G/A and -148C/T, plasma levels of the protein, and coronary disease in Northern Palestine

Submitted by: Ansam Ezzat Mahmoud Zakarna

Date received: 19th August 2023

Date reviewed: 20th September 2023

Date approved: 29th October 2023

Your Study titled "**The associations among fibrinogen polymorphisms -455G/A and -148C/T, plasma levels of the protein, and coronary disease in Northern Palestine**" With archived number 2023/B/157/N was reviewed by the Arab American University IRB committee and was approved on 29th October 2023.

Reham Khalaf-Nazzal, MD, PhD
IRB committee chairman
Arab American University of Palestine

General Conditions:
1. Valid for 1 year from date of approval.
2. It is important to inform the committee with any modification of the approved study protocol.
3. The committee appreciates a copy of the research when accomplished.

لجنة أخلاقيات البحث العلمي في الجامعة العربية الامريكية

Appendix 2. Approval to allow the collection of patient samples and the results of their previous tests registered with the Palestinian Ministry of Health.

State of Palestine
Ministry of Health
Education in Health and Scientific Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي والبحث العلمي

Ref.: ....
Date:..................

الرقم: ٢٠٢٢/٤٧٢٥/١٢٦
التاريخ: ٢٠٢٢/١٢/٢٧

الوكيل المساعد لشؤون المستشفيات والطوارئ المحترم،،،

تحية واحترام...

<u>الموضـوع: تسهيل مهمة بحث</u>

يرجى تسهيل مهمة الطالبة: **أنسام زكارنـه – ماجستير علم الدم والمناعة** – الجامعة العربية الامريكية، بعنوان:

"The associations among fibrinogen polymorphisms -455G/A and -148C/T, plasma levels of the protein and coronary disease in Northern Palestine"

حيث ستقوم الطالبة بجمع معلومات عن طريق تعبئة استبانة الدراسة، وذلك في:

- **مستشفى جنين – مستشفى طولكرم – مستشفى الوطني – مستشفى رفيديا**

مع العلم ان مشرف الدراسة: د. فكري سماره.

على ان يتم الالتزام بالمحافظة على اخلاقيات البحث العلمي وسرية المعلومات، وعدم التعرض للمعلومات التعريفية للمشاركين.

على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة وزارة الصحة.

مع الاحترام...

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: عميد كلية الدراسات العليا المحترم/ الجامعة العربية الامريكية

Appendix 3 English

Appendix 3. Coronary Artery Disease Questionnaire – English format

Coronary Artery Disease Questionnaire

Name (Last, 1st, M.I.)		☐**Male** ☐**Female**	**DOB:**	
Phone		**Height:**	**Weight:**	
Address			**Institute**	

Health History

1. Name and address of cardiologist or other physician seen most recently for your heart condition.
______________________________ Date of last consultation
_______/_______/_______

2. Age when diagnosed: ____________________

3. Have you ever had or been diagnosed with the following?

I. Angina pectoris or coronary artery disease ☐ Yes ☐ No

II. Coronary artery surgery (angioplasty, stent, or coronary bypass) ☐ Yes ☐ No

III. Stroke (TIA or major stroke) or coronary artery obstruction ☐ Yes ☐ No

IV. Diabetes mellitus ☐ Yes ☐ No

4. Do you currently smoke cigarettes? ☐ Yes ☐ No

I. If "Yes", How often or many per day? ____________________

II. If "No", when did you stop? ____________________

5. Are you currently taking medications for any of the following conditions?

I. High blood sugar ☐ Yes ☐ No

II. Elevated blood triglycerides ☐ Yes ☐ No

III. Low level of "good" cholesterol (HDL-cholesterol) ☐ Yes ☐ No

IV. High level of "bad" cholesterol (LDL-cholesterol) ☐ Yes ☐ No

6. Do you participate in a cardiac rehab or other exercise program? ☐ Yes ☐ No

Family History

7. Is there a history of diabetes, stroke, heart disease, high blood pressure or kidney disease among your parents, brothers, or sisters? ☐ Yes ☐ No

8. If "Yes", give the following information:

	Age. If Living	**Health**	**Age at Death**	**Cause of Death**
Father				
Mother				
Brothers & sisters				

Diet Program:

9. Do you check your weight periodically to detect any change? ☐ Yes ☐ No

10. Do you make any planned or supervised adjustments in your eating habits to maintain what you consider to be a desirable weight? ☐ Yes ☐ No

11. Have you, within the past 3 years, followed a controlled diet? ☐ Yes ☐ No

I. If "Yes" was it controlled with respect to:

☐ Total calories ☐ Cholesterol
☐ Fats ☐ Salt

II. Was information obtained from?

☐ Nutritionist ☐ Dietician
☐ Physician ☐ Your reading

12. Laboratory Findings:

☐FBS ________________
☐ Cholesterol ________________
☐ Triglycerides ________________
☐ HDL-c ________________
☐ LDL-c ________________

Physical activity

13. Do you currently regularly participate in exercise classes? ☐ Yes ☐ No

14. Indicate your current type of activity:

____

I represent that all statements and answers to the questions are complete and true to the best on my knowledge and belief.

Patient signature ____________________________ **Date** ________/________/________

Appendix 4. Coronary Artery Disease Questionnaire – Arabic format

استبيان مرض الشريان التاجي

الاسم		☐ ذكر ☐ انثى	تاريخ الميلاد	
الوزن		الطول	الهاتف	
المستوى العلمي			العنوان	

التاريخ الصحي:

١. اسم وعنوان طبيب القلب او طبيب آخر تمت رؤيته مؤخرًا بخصوص حالة قلبك.

____________________ تاريخ آخر استشارة ______/______/______

٢. العمر عند التشخيص ________________

٣. هل سبق أن عانيت أو تم تشخيصك بما يلي؟

I. ذبحة صدرية أو مرض الشريان التاجي — نعم ☐ لا ☐
II. جراحة الشريان التاجي (رأب الوعاء أو الدعامة أو المجازة التاجية) — نعم ☐ لا ☐
III. السكتة الدماغية)أو السكتة الدماغية الكبرى) أو انسداد الشريان التاجي — نعم ☐ لا ☐
IV. داء السكري — نعم ☐ لا ☐

٤. هل تدخن السجائر حاليا؟ — نعم ☐ لا ☐

I. إذا كانت الإجابة "نعم"، فكم مرة باليوم؟____________________
II. إذا كانت الإجابة "لا"، فمتى أقلعت وتركت التدخين؟____________________

٥. هل تتناول حاليًا أدوية لأي من الحالات التالية؟

I. ارتفاع نسبة السكر في الدم — نعم ☐ لا ☐
II. ارتفاع نسبة الدهون الثلاثية في الدم — نعم ☐ لا ☐
III. انخفاض مستوى الكوليسترول "الجيد" (HDL –cholesterol) — نعم ☐ لا ☐
IV. ارتفاع مستوى الكوليسترول "الضار" (LDL-cholesterol) — نعم ☐ لا ☐

٦. هل تشارك في إعادة تأهيل القلب أو برنامج تمارين أخرى؟ — نعم ☐ لا ☐

تاريخ العائلة:

٧. هل هناك تاريخ مرضي لمرض السكري او السكتة الدماغية او امراض القلب او ارتفاع ضغط الدم او امراض الكلى بين والديك أو إخوتك أو أخواتك؟ — نعم ☐ لا ☐

٨. إذا كانت الإجابة "نعم"، قدّم المعلومات التالية:

	العمر/ ان كان حيا	الحالة الصحية	العمر/ عند الوفاة	سبب الوفاة
الاب				
الام				
الاخوة والاخوات				

Appendix 5. English informed consent

الجامعـــة العربيـــة الأمريكيـــة
عمادة البحث العلمي
لجنة اخلاقيات البحث العلمي

INFORMED CONSENT

AAUP-IRB Code No.: ....
AAUP-IRB Date: ....

I, ...am Ansam ezzat zakarni .... *(Name of Participant / optional)* hereby agree to take part in the clinical research (clinical study/questionnaire study/drug trial) specified below:

<u>Title of Study:</u> . The associations among fibrinogen polymorphisms -455G/A and -148C/T, plasma levels of the protein, and coronary disease in Northern Palestine
....
....
....,
Fulfillment of ......master .... degree, in
......immunohematology..., in AAUP.
(Name of program)

The nature and purpose of which has been explained to me by ...., and interpreted by .... to the best of his/her ability in English.

I have been told about the nature of the research in terms of methodology, possible adverse effects and complications (as per Participant Information Sheet).
After knowing and understanding all the possible advantages and disadvantages of this research, I voluntarily consent of my own free will to participate in the clinical research specified above.
I understand that I can withdraw from this research at any time without assigning any reason whatsoever.

Date: .... **Signature:**
....
(Participant)

<u>IN THE PRESENCE OF:</u>

Name: ....

Designation: . ... **Signature:** ....

Appendix 6 Arabic informed consent

الجامعـــة العربيـــة الأمريكيـــة
عمادة البحث العلمي
لجنة اخلاقيات البحث العلمي

الجامعة العربية الأمريكية
عمادة البحث العلمي
لجنة المراجعة الأخلاقية

موافقة مسبقة

رقم كود IRB-AAUP: ....

تاريخ IRB-AAUP: ....

انا اسلام عزات محمود زكارنة اوافق بموجبه على المشاركة في البحث السريري (الدراسة السريرية / دراسة الاستبيان / تجربة الدواء) المحددة أدناه

عنوان الدراسة: "الارتباطات بين تعدد الأشكال الفبرينوجين -٤٥٥ G / A و -١٤٨ C / T، ومستويات البروتين في البلازما، وأمراض الشريان التاجي في شمال فلسطين"
كمتطلب لإتمام دراسة الماجستير في علم الدم المناعي بالجامعة العربية الأمريكية.

تم شرح طبيعتها والغرض منها من خلال ....، وتفسيرها بواسطة .... على أفضل وجه في اللغة الإنجليزية.

لقد تم إخباري عن طبيعة البحث من حيث المنهجية والآثار السلبية المحتملة والمضاعفات (حسب ورقة معلومات المشارك).
بعد معرفة وفهم جميع المزايا والعيوب المحتملة لهذا البحث، أوافق طواعية بمحض إرادتي على المشاركة في البحث السريري المحدد أعلاه.
أفهم أنه يمكنني الانسحاب من هذا البحث في أي وقت دون إبداء أي سبب على الإطلاق.

التاريخ: .... **التوقيع:** ....
(المشارك)

في حضور :

الاسم: ....

التسمية : .... **التوقيع:** ....

(شاهد على توقيع المشارك)

أؤكد أنني أوضحت للمريض طبيعة وهدف البحث المذكور أعلاه.

التاريخ: .... **التوقيع:** ....

(بحضور الباحث)

الملخص باللغة العربية:

يعد مرض القلب والأوعية الدموية السبب الرئيسي للوفاة لدى الأفراد الذين تزيد أعمارهم عن 35 عامًا في البلدان المتقدمة. ان تجنب مرض القلب والأوعية الدموية والوفيات المرتبطة به يشكل معضلة في كل دولة. الفيبرينوجين (العامل الأول) هو جليكوبروتين، والذي يلعب دورا مهما في عملية التخثر. أشارت الدراسات المستقبلية التي أجريت على عينات كبيرة إلى أن مستوى الفيبرينوجين في البلازما يعد مؤشرا مستقلا لأمراض القلب والأوعية الدموية. تم ربط تعدد أشكال النوكليوتيدات المفردة في منطقة المحفز لجين بيتا فيبرينوجين (G/A-455، C/T-148) بتركيزات هذا البروتين، كما أفاد عدد من المقالات بارتباطهما بالأحداث القلبية الوعائية الحادة.

الأهداف: تهدف الدراسة الحالية إلى التحقيق في الارتباطات بين تعدد أشكال الفيبرينوجين G/A455، -148C/T وأمراض الشريان التاجي (CD) بما في ذلك أمراض الشريان التاجي غير المستقرة (UCD) وأمراض الشريان التاجي المستقرة (SCD)، وارتباطاتها بالأحداث القلبية الوعائية الضارة الرئيسية (MACE) في عينة من شمال فلسطين.

المنهجية : هذا تصميم بحثي مستقبلي خاضع للرقابة ومتعدد المراكز مع دراسة متابعة لمدة ستة أشهر. تمت الدراسة على ما مجموعه 100 مريض مصاب بأمراض الشريان التاجي (CD)، بما في ذلك 41 مريضا مصابا بأمراض الشريان التاجي غير المستقرة (UCD) و 59 مريضا مصابا بأمراض الشريان التاجي المستقرة (SCD) من أجل تحديد العلاقات بين الأحداث السلبية القلبية الوعائية الرئيسية (MACE)، ومستويات الفيبرينوجين في البلازما، وتعدد أشكال جين الفيبرينوجين في مجموعة سكانية من شمال فلسطين. تم مطابقة العمر والجنس ومؤشر كتلة الجسم و نمط حياة يتميز بقلة النشاط البدني للمجموعات التي شملت 70 من المشاركين الاصحاء و الغير مصابين بامراض القلب و الاوعية الدموية كمجموعة تحكم. تم تحديد مستوى الفيبرينوجين في البلازما بطريقة فون كلاوس. تم استخراج الحمض النووي من الغلاف البافي وتم تحليل تعدد أشكال جين بيتا فيبرينوجين (G/A-455، C/T-148) عن طريق PCR-RFLP.

النتائج: تمت مقارنة تركيزات الفيبرينوجين البلازمي، تعدد أشكال جين الفيبرينوجين (G/A455 و-C/T148) في جميع المجموعات. كان لدى المرضى الذين يعانون من أمراض الشريان التاجي كميات كبيرة من الفيبرينوجين في البلازما (أعلى من 465 mg/dl). ارتبطت الزيادة في تركيز الفيبرينوجين في البلازما فوق 450 mg/dl بالوفاة بسبب أمراض القلب والأوعية الدموية داخل المجموعة التي تعاني من مرض الشريان التاجي غير المستقر أثناء دراسة المتابعة ($p = 0.03$). ارتبطت المستويات العالية من بروتينات الفيبرينوجين في البلازما بالأحمال الأليلية للنمط الجيني -G/A455 و-C/T148 ($p < 0.005$ و$p = 0.041$، على التوالي) ومرض الشريان التاجي ($p = 0.021$ و$p < 0.004$، على التوالي). ارتبط الحمل الجيني للأليل -T148 بأحداث قلبية وعائية غير مواتية كبيرة، وفقًا لمتابعة الأحداث الخلفية بعد حدث تاجي حاد (RR = 1.6، 95% CI = 1.13 - 3.42، $p = 0.03$).

الخلاصة: توفر نتائج هذه الدراسة رؤى حاسمة حول الارتباط بين مستويات الفيبرينوجين في البلازما، وتعدد أشكال الفيبرينوجين (-G/A455، 148C/T-)، وأمراض القلب والأوعية الدموية في شمال فلسطين. ترتبط الأحداث القلبية الوعائية الضارة الرئيسية (MACE) وأمراض القلب والأوعية الدموية بمستويات الفيبرينوجين البلازمية التي تزيد عن 450 mg/dl وتعدد أشكال جين الفيبرينوجين (-G 455 A / و C / T148). ووفقا لهذه الدراسة، فإن خطر الإصابة بأمراض القلب والأوعية الدموية يرتبط بهذه الاختلافات الجينية.