Arab American University

Faculty of Graduate Studies

The Association between Genetic Variations in the PAI-1 Promoter Sequence and the Susceptibility to Acute Cardiac Infarction and Stable Angina.

By

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Supervisor

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This thesis was submitted in partial fulfillment of the requirements for the Master's degree in Immunohematology

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Thesis Approval

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This thesis was defended successfully on 12/2/2026 and approved by:

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Declaration

I declare that my dissertation, entitled “The Association between Genetic Variations in the PAI-1 Promoter Sequence and the Susceptibility to Acute Cardiac Infarction and Stable Angina” submitted by me under the supervision of Dr. Bilal Ghareeb for the partial fulfillment of the requirements for the award of a Master’s degree in Immunohematology, is original and has been written independently, except where explicit reference is made to the contributions of others.

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Dedication

I dedicate this thesis to Palestine, the wellspring of my strength and resilience, to my supervisor Dr. Bilal Ghareeb for his guidance and above all, to my family—my parents, my husband Mohannad Yaqub, and my sons, Qais and Al-Yaman—whose unwavering love and support made this work possible.

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Firstly, I dedicated this study to Almightily Allah that guides me in each step during my life and throughout my whole Master's journey and gives me strength to continue hard working. In addition, I thank my homeland, Palestine, which has taught me many lessons in determination.

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Furthermore, I thank the Laboratory at the Palestinian Ministry of Health for their generous support and collaboration and to the entire team (especially Ms. Mais Nofal) for their timely assistance and shared expertise. In addition, I am deeply thankful to the late Mr. Osama Al-Najjar-Head of the Medical Laboratory Association, for granting permission and facilitating access to the essential resources within the Ministry of Health laboratories.

I sincerely thank my family; my supportive parents and my beloved husband Mohannad, my lovely sons Qais and Al-Yaman, my understanding family-in-law, my helpful sisters, my sister-in-law Shahd, for their enduring support and belief in me, which have been vital to completing this journey.

Abstract

Cardiovascular diseases, such as Acute Cardiac Infarction and Chronic Stable Angina, cause illness and death globally, including in Palestine. Plasminogen activator inhibitor-1 (PAI-1) contributes to regulating the process of breaking down blood clots. Elevated levels of PAI-1 are linked with an increased risk of cardiovascular disease due to blood clots. Genetic variations in the promoter region of the PAI-1 gene, specifically the -675 4G/5G and -844 G/A polymorphisms, may affect the production of PAI-1 and an individual's risk of cardiovascular disease. Despite this, the effect of these genetic variations in the Palestinians remains poorly understood.

This study evaluates the presence of the -675 4G/5G (rs1799889) and the -844G/A (rs2227631) variants in the PAI-1 gene and their possible association with Acute Cardiac Infarction or Chronic Stable Angina in patients from Northern Palestine.

A case-control study design was employed; each of the three study groups—Acute Cardiac Infarction, Chronic Stable Angina, and healthy controls matched for age and sex—had 50 participants. Clinical and demographic data were collected using structured questionnaires over a one-year period from the Palestinian National Health System, An-Najah National Hospital, and Al-Razi Hospital. Genomic DNA was isolated from whole blood, allele-specific polymerase chain reaction (AS-PCR) was used for -675 4G/5G genotyping, and PCR followed by restriction fragment length polymorphism (RFLP) analysis was used to assess -844G/A.

Patients with Acute Cardiac Infarction and Chronic Stable Angina were significantly more likely than healthy controls to have the -675 4G and -844 A alleles. Acute cardiac infarction was more common in carriers of at least one 4G or A allele. These genetic differences increase vulnerability to cardiac disease in Palestine, along with typical risk factors including diabetes, hypertension, smoking, and family history of coronary artery disease.

In conclusion, the PAI-1 -675 4G/5G and -844 G/A promoter polymorphisms are connected to a higher incidence of coronary artery disease in Palestinians. To establish their clinical importance, larger investigations using plasma PAI-1 values are advised.

Keywords: PAI-1, polymorphisms, -675 4G/5G, -844 G/A, Palestinians.

Table of Contents

Thesis Approval i

Declaration ii

Dedication iii

Acknowledgements iv

Abstract v

List of Tables ix

List of Figures x

List of Abbreviations xi

Chapter 1: Introduction 1

1.1 Study Problem 1

1.2 Justification 2

1.3 Specific Objective 2

1.4 Study Hypothesis 2

Chapter 2: Literature Review 3

2.1 Fibrinolysis System 3

2.2 Plasminogen Activator Inhibitor Type-1 5

2.3 PAI-1 and Coronary Artery Disease 7

2.4 PAI-1 Gene Polymorphisms 9

2.4.1 -675 4G/5G Polymorphism 10

2.4.2 -844G/A Polymorphism 11

2.5 Previous Studies 12

Chapter 3: Material and Methods 15

3.1 Study Design 15

3.2 Study Area 15

3.3 Sampling Method and Sampling Size 15

3.4 Inclusion and Exclusion Criteria 16

3.4.1 Inclusion Criteria 16

3.4.2 Exclusion Criteria 16

3.5 DNA Extraction and Blood Collection 17

3.6 Genotype Analysis 18

3.6.1 -675 4G/5G Polymorphism 18

3.6.2 -844G/A Polymorphism 21

3.6.3 Allele-Based and Dominant Model Analysis 25

3.6.4 DNA Sequencing 26

3.7 Statistical Analysis 26

Chapter 4: Results 27

4.1 Demographic and Clinical Features of the Studied Subject 27

4.2 Molecular Analysis 31

4.2.1 Molecular Analysis of the PAI-1 4G/5G Polymorphism 31

4.2.2 Molecular Analysis of the PAI-1 −844 G/A Polymorphism 33

4.3 Genotypic Analysis 36

4.3.1 Genotype Analysis of 4G/5G Variant among Study Groups 36

4.3.2 Genotype Analysis of -844G/A Variant among Study Groups 39

Chapter 5: Discussion 43

5.1 Demographic and Clinical Characteristics 44

5.2 PAI-1 Promoter Polymorphisms 45

5.2.1 Association between 4G/5G and -844G/A 45

5.2.2-675 4G/5G Polymorphism and Coronary Artery Diseases 46

5.2.3 -844 G/A Polymorphism and Coronary Artery Diseases 47

5.3 Perspective 48

5.4 Conclusion and Recommendations 49

References 51

Appendices 56

Appendix 1: Questionnaire at English 57

Appendix 2: Questionnaire at Arabic 61

Appendix 3: Reagents and Laboratory Equipment 64

الملخص 66

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List of Tables

No.	Title	Page
3.1	Primer sequences, product sizes, and PCR methods for -675 4G/5G PAI-1 Polymorphism.	20
3.2	Primer sequences, product sizes, and PCR methods for -844G/A PAI-1 Polymorphism.	22
3.3	RFLP set-up for a 25 µL XhoI digestion (New England Biolabs, 2024).	23
3.4	Band sizes resulting from XhoI digestion according to PAI-1 −844 G/A genotypes (Torres-Carrillo *et al.,* 2008; García-González *et al.,* 2015).	24
4.1	Age and gender distribution among study groups.	28
4.2	Comparison of cardiovascular risk factors among study groups.	30
4.3	Genotype distribution of -675 4G/5G variant among study groups.	36
4.4	Allele frequency of -675 4G/5G genotype among study groups.	37
4.5	Dominant model of -675 4G/5G genotype among study groups.	39
4.6	Genotype distribution of -844G/A variant among study groups.	40
4.7	Allele frequency of -844G/A genotype among study groups.	41
4.8	Dominant model of -844G/A genotype among study groups.	42

List of Figures

No.	Title	Page
2.1	The fibrinolysis pathway and the role of PAI-1 in regulating plasmin activity (adopted from Kohler & Grant, 2000).	4
2.2	Mechanism of PAI-1 interaction with proteases (Altalhi *et al*., 2021).	7
2.3	Progression of atherosclerosis to clinical coronary artery disease (Abrams, 2005).	9
2.4	Influence of the -675 4G/5G variant on PAI-1 gene expression. (Kohler & Grant, 2000).	11
3.1	In silico analysis of the -675 4G/5G polymorphism showing primer sequences and positions.	19
3.2	In silico analysis of the -844 G/A polymorphism showing primer sequences and positions.	22
4.1	Geographic representation of participants in the study.	29
4.2	Visualization of -675 4G/5G genotypes using allele-specific PCR on a 2% agarose gel.	31
4.3	Sanger sequencing chromatograms of the -675 4G/5G polymorphism for three representative samples.	32
4.4	Visualization of -844 G/A genotypes using PCR-RFLP on a 2% agarose gel.	34
4.5	Sanger sequencing chromatograms of -844 G/A polymorphism for three representative samples.	35

List of Abbreviations

A	Adenine
ANOVA	Analysis of variance
APE	A Plasmid Editor
AS-PCR	Allele Specific Polymerase Chain Reaction
bp	base pair
C	Cytosine
CI	Confidence Interval
DNA	deoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
Elf-1	E74-Like factor 1
Elk-1	ETS-like protein 1
Fig	Figure
G	Guanine
IL-1	Interleukin-1
Ind	Individual
LD	linkage disequilibrium
NF- κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
OD	Optical Density
PAI-1	Plasminogen activator inhibitor type-1
PAI-1 gene -675 4G/5G	Plasminogen Activator inhibitor type -1 gene 4guanine/5 guanine polymorphism at position 675 (rs1799889)
PAI-1 gene -844A/G	Plasminogen activator inhibitor type-1 Adenosine/Guanine at position 844 (rs2227631)
P-value	Probability value
RCL	reactive center loop
RFLP	Restriction fragment Length Polymorphism
Rpm	revolutions per minute
SERPINE1	Serine Protease Inhibitor, Clade E, Member 1

SNPs	single-nucleotide polymorphisms
STEMI	ST-segment elevation myocardial infarction
TAE	Tris-acetate-EDTA
TGF-β	transforming growth factor-beta
TNF-α	tumor necrosis factor-alpha
tPA	Tissue plasminogen activator
UCSC	University of California Santa Cruz
uPA	Urokinase plasminogen activator
wt/vol	Weight/volume

Chapter 1: Introduction

Atherothrombotic changes are a key factor in disease and death within the cardiovascular system. Coronary Artery Disease (CAD) comprises Acute Cardiac Infarction, Unstable Angina, and Chronic Stable Angina, and there are multiple recognized genetic and environmental risk factors for CAD. Smoking, excessive alcohol consumption, dyslipidemia, type two diabetes mellitus, hypertension, and a positive family history of the disease are all known to increase the likelihood of CAD. These risk factors are mostly connected to genetic influences. Genes involved in blood coagulation and fibrinolysis processes have been implicated as genetic risk factors for CAD (Al-Wakeel *et al.,* 2018).

The plasminogen activator inhibitor type-1 (PAI-1) gene is notably significant in the development of CAD. Variations in its promoter region influence transcription and consequently, circulating PAI-1 protein levels. Increased PAI-1 concentration impairs fibrinolysis, contributing to thrombosis and vascular dysfunction (Jung *et al.,* 2018). Among these genetic variants, the -675 4G/5G polymorphism at position -675 in the regulatory region of the PAI-1 gene, a deletion or insertion resulting in either four or five guanine bases (-675 4G/5G). Furthermore, at position -844 in the promoter region, a single nucleotides substitution replaces guanine with adenine that is frequently investigated for associations with CAD.

This study aims to find out which of these gene variants (-675 4G/5G and -844 G/A) is associated with a higher chance of developing Acute Cardiac Infarction and Chronic Stable Angina, which are both caused by CAD, in people from Northern Palestine.

1.1 Study Problem

International research investigates how genes affect PAI-1. However, only a small number of studies were conducted in the Palestinian population. The only study conducted in Gaza, focusing on coronary heart disease in general, tested only -675 4G/5G polymorphisms with PAI-1. (Ayesh, n.d.) In comparison, the current study was conducted in Northern West Bank

and only included Acute Cardiac Infarction and Chronic Stable Angina, examining the PAI-1 -844G/A and -675 4G/5G variants.

Studying single-nucleotide polymorphisms (SNPs) provides insight into how genetic variability influences PAI-1 expression and thrombotic risk in Acute Cardiac Infarction and Chronic Stable Angina. The expected results identify specific risk factors for various groups and help in understanding the probability of developing Acute Cardiac Infarction and Chronic Stable Angina in the Palestinian population.

1.2 Justification

This research is important due to the limited genetic information about PAI-1 variants in Palestine and their possible association with coronary artery disease. Current knowledge is based on a single study conducted in Gaza that included only 86 individuals. Determining such associations may aid in early detection of genetic susceptibility, inform preventive strategies, and enhance cardiovascular health management (Ayesh, n.d.).

1.3 Specific Objective

The aim of the study was to investigate the correlation among PAI-1 variations (-675 4G/5G and -844 G/A) and the risk of cardiac diseases, namely Acute Cardiac Infarction and Stable Angina.

1.4 Study Hypothesis

It is hypothesized that certain variations in the PAI-1 gene promoter, specifically the -675 4G/5G and -844 G/A polymorphisms, are associated with a higher or lower odds of blood clotting problems in people who have CAD.

Chapter 2: Literature Review

2.1 Fibrinolysis System

Fibrinolysis is the process where the main acting enzyme, plasmin, breaks down fibrin into smaller, soluble pieces. This process, which is carried out by proteolytic enzymes, is important for several body functions like making new blood vessels, healing wounds, reproduction, and even for the growth of cancer. Plasmin arises after the activation of plasminogen by two enzymes tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA).

The activities of tPA and uPA are controlled by a group of inhibitors known as plasminogen activator inhibitors (PAIs). Among them, PAI-1 (plasminogen activator inhibitor type 1) is the main thing in the body that controls fibrinolysis. Additionally, Alpha **(α)** 2-antiplasmin plays a vital role in inhibiting plasmin activity, particularly when plasmin is bound to fibrin that has been stabilized by factor XIII. This helps keep the fibrin from breaking down and involve in controlling the process of fibrinolysis and also fibrin manages how it breaks down by controlling which parts can be accessed.

When a clot forms, it shows certain areas that help start the process of breaking down the clot and making more plasmin. However, when factor XIII links the fibrin strands together, it covers up these areas, which slows down the breakdown of the clot, as shown in the Fig (2.1) that shows how the fibrinolysis system works and how it is controlled (Kohler&Grant, 2000).

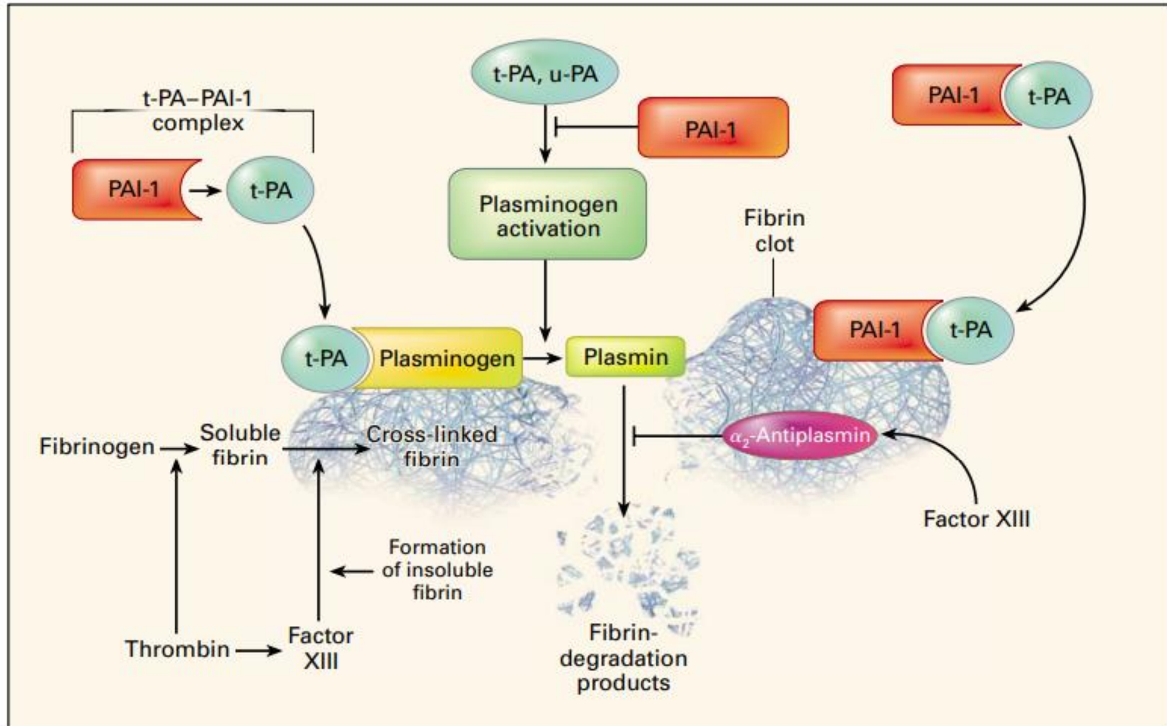


Figure 2.1: The fibrinolysis pathway and the role of PAI-1 in regulating plasmin activity (adopted from Kohler & Grant, 2000).

PAI-1 binds with equal affinity to both t-PA and u-PA, forming stable complexes that are rapidly cleared from circulation through hepatic uptake. Its active form is short-lived, but interaction with vitronectin and release from activated platelets helps extend its stability and prevents clots from breaking down too soon. This rapid local release increases PAI-1 levels during clot formation. Evidence also shows that lowering or blocking PAI-1 activity can make thrombolysis more effective by allowing clots to dissolve more easily (Kohler & Grant, 2000).

Reduced levels of PAI-1 can boost fibrinolytic activity, which may lead to a higher risk of excessive bleeding. Alternately, increased PAI-1 concentrations may advance a prothrombotic state and cause vascular changes by repressing the action of tissue-type and urokinase-type plasminogen (Zorio *et al.*, 2008).

In healthy people, the amount of PAI-1 in the blood is usually more than four times higher than the amount of tPA. The liver is considered the main place that removes PAI-1 from the body; however, in certain disease states, PAI-1 levels in the body increase significantly due to certain signals such as tumor necrosis factor-alpha (TNF-α), insulin, and transforming growth factor beta (TGF-β).This increase makes it more difficult for the body to break down blood clots, which can cause serious issues like stroke and heart disease. Elevated levels of PAI-1 in circulation are also observed in patients with type two diabetes, high insulin levels, and insulin resistance. This observation highlights the strong relationship between issues of both the body's metabolism and clot breakdown (Jung et al., 2018).

2.2 Plasminogen Activator Inhibitor Type-1

PAI-1 is a linear glycoprotein with a weight of approximately 50 kilo Daltons and being a member of the group of serine protease inhibitors (SERPIN), produced by several specific cell types, including endothelial cells, liver cells, smooth muscle cells, and platelets. PAI-1, categorized as SERPINE1 which belongs to clade E within the serpin and functions by uniquely inhibiting serine proteinases. Although serpins are classified into clades A through I based on sequence similarity, they display limited overall sequence conservation, apart from a few conserved residues in the core 'shutter region' of the protein (Wilkinson, 2021).

Its gene is situated on chromosome seven, within the region spanning q21.3 to q22. This gene is made up of nine exons and eight introns, covering a DNA segment of about 12.3 kilobases. PAI-1 is influenced by various agents like cytokines, thrombin, and glucocorticoids, which act at the transcriptional level through the gene's promoter in response to regulatory signals (Ye et al., 1995).

PAI-1 is composed of either 379 or 381 amino acids. This variation results from cleavage at different sites through two different N-terminal signal peptidases. In general, the signal peptide is cleaved by the signal peptidase, enabling the mature protein to fold correctly. In one form, the exons code a 23– amino acids signal sequence, followed by the mature PAI-1 protein of 379 amino acids. In another form, the mature protein includes 381 amino acids due to two additional N-terminal residues. In this case, the exons code a 21–amino acid signal

sequence. This form is likely the outcome of signal peptidase cleaving at a substitute site (Mathews *et al.*, 2024).

The PAI-1 structure consists of two domains: the reactive center loop (RCL), important for protease attachment, and a flexible joint region that includes binding sites near helices D, E, and F (hD, hE, hF). The RCL has a function in PAI-1's activity, which is vital for its interaction with t-PA and u-PA. Notably, PAI-1 does not contain cysteine, which blocks disulfide bond formation and helps explain its natural instability in solution. Also, its sequence contains many methionine, making it more susceptible to irreversible deactivation by oxidants (Altalhi *et al.*, 2021).

Structural configurations of PAI-1 are active, latent, and cleaved forms. The active structure has an exposed RCL that causes inhibition of proteases. In the latent state, the RCL folds into the β-sheet, causing PAI-1 inactive. In the cleaved form, the RCL is cut by a protease, producing irreversible loss of inhibitory activity. Binding of PAI-1 to a particular region of vitronectin causes a conformational change and stabilize its active form (Altalhi *et al.*, 2021).

PAI-1 controls the breakdown of blood clots by attaching to t-PA or u-PA and inactivating them. This interaction first occurs loosely, then progresses to a temporary intermediate, and finally results in a permanent bond that inactivates the enzyme. Through this process, PAI-1 inhibits plasmin formation and slows the breakdown of blood clot. In the inhibitory pathway, PAI-1 traps and inactivates the protease, while in the substrate pathway; the protease cleaves PAI-1 but remains active as shown in Fig (2.2).

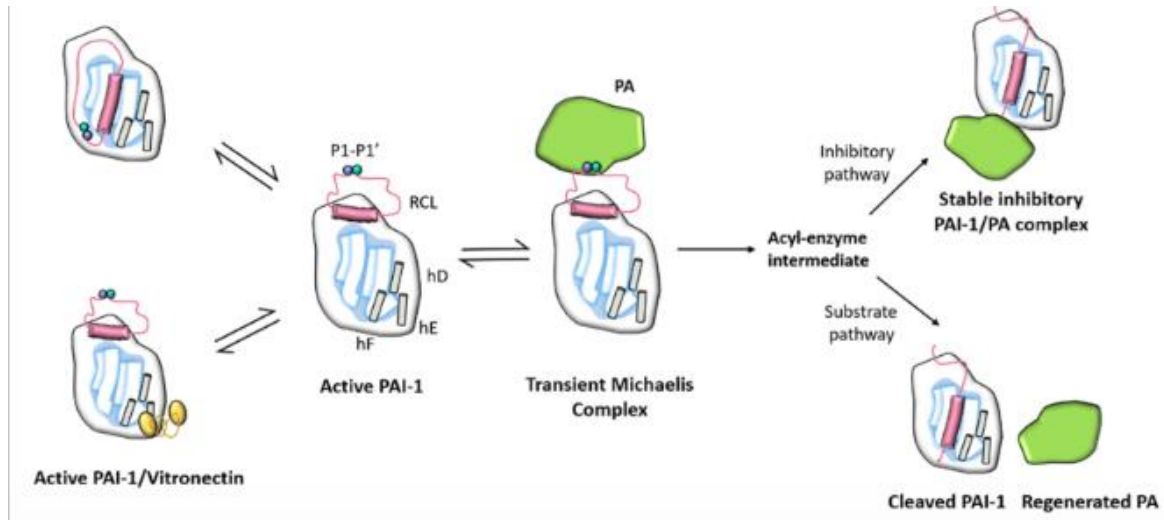


Figure (2.2): Mechanism of PAI-1 interaction with proteases (Altalhi *et al*., 2021): Active PAI-1 engages with plasminogen activators (PA) to create a transient Michaelis complex. This contributes to either the inhibitory pathway, producing a stable PAI-1/PA complex, or the substrate pathway, resulting in cleaved PAI-1 and regenerated PA.

2.3 PAI-1 and Coronary Artery Disease

Coronary artery disease (CAD) is affected by both genetic and modifiable variables. While some rare gene changes can increase the risk, more common gene variations usually work along with lifestyle choices and other traditional risk factors to make someone more likely to develop the disease. Genes coding for proteins involved in controlling blood vessels and metabolism, like PAI-1, play a key role because they influence blood clotting and how the body breaks down clots.

PAI-1 levels fluctuate daily, with lower concentrations during the day and higher at night, which can decrease the body's ability to dissolve blood clots at night and may contribute to the higher occurrence of heart attacks in the morning hours. Overall, increased PAI-1 levels can lead to decreased fibrinolysis, greater fibrin accumulation, and thrombus formation, thereby potentially increasing CAD risk (Kohler & Grant, 2000).

CAD has different forms of symptoms that can vary in seriousness. It often begins with Stable Angina, which is chest pain that happens in a regular way when someone is doing physical

activity, because there's less blood flowing to the heart. It can then progress to Unstable Angina, where a blood clot is present but doesn't fully block the artery. In more severe cases, it can lead to ST-segment (a part of Electrocardiogram) elevation myocardial infarction (STEMI), a significant heart attack due to total blockage of the coronary artery, leading to serious lack of blood flow to the cardiac muscle (Al-Wakeel *et al*., 2018).

Acute Cardiac Infarction occurs when some of the heart muscle gets injured or stops working. Doctors check high or changing levels of a protein in the blood called troponin, as well as signs like chest pain, changes on an electrocardiogram (ECG) test, or strange heartbeats (significant alterations in the ST-segment or T-wave on an ECG, the appearance of new left bundle branch block, or the presence of pathological Q waves on ECG). Additional diagnostic evidence may include imaging that reveals new areas of nonviable myocardium or abnormal movement of the heart wall, as well as the identification of a coronary artery thrombus through angiography or post-mortem examination (Reddy *et al***.,** 2015).

Chronic Stable Angina is having chest pain or discomfort that keeps happening, usually on the left side of the chest. This kind of discomfort usually happens when someone is exercising, feeling stressed, or going through both at the same time. It can get more intense in cold weather or after a meal. The discomfort typically subsides with rest or by taking sublingual nitroglycerin. It is most often linked to significant narrowing of the coronary arteries, usually greater than 70%, which limits blood flow to the cardiac muscle (Abrams, 2005).

As shown in Fig (2.3), coronary atherosclerosis develops slowly over time. It starts with damage to the inside of the blood vessels, which leads to the formation of lipid streaks and plaque accumulation. As these plaques grow larger, they constrict the arteries and decrease the blood supply to the cardiac muscle, leading to chest pain during physical activity. Later on, the plaques might rupture, causing blood clots that can partly or completely block the artery. These states can result in unstable chest pain, Acute Cardiac Infarction, or sudden cardiac death, as characterized by Abrams in 2005.

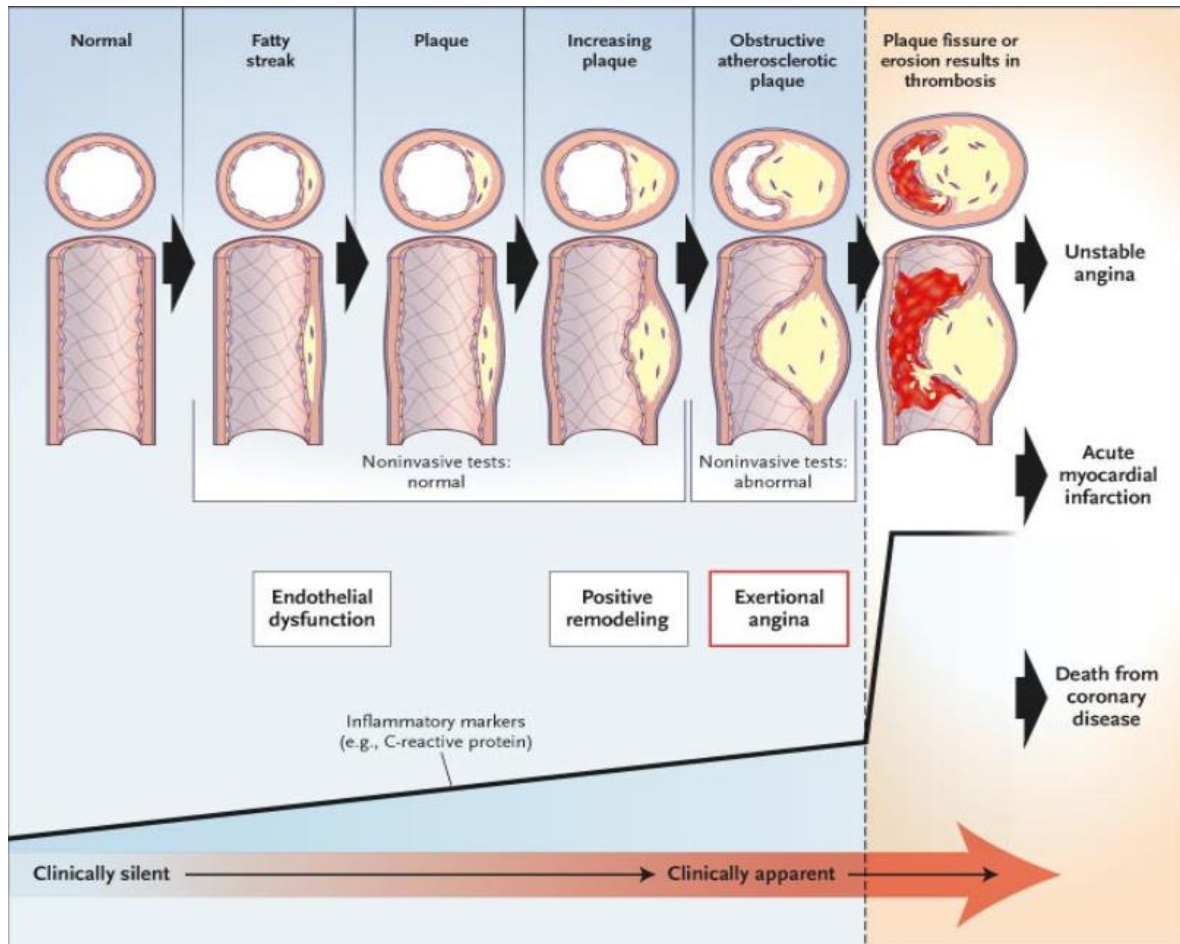


Figure (2.3): Progression of atherosclerosis to clinical coronary artery disease (Abrams, 2005).

2.4 PAI-1 Gene Polymorphisms

Although numerous polymorphic variants of the PAI-1 gene have been detected, only a few variants affect the levels of circulating PAI-1. Among these, the -675 4G/5G and –844 G/A variants have been well studied because they affect how the gene functions and the body's capability to dissolve clots (Abboud *et al*., 2010).

Elevated PAI-1 levels lead to impaired fibrinolysis, thereby promoting thrombus persistence and arterial occlusion. This indicates that those with these variants may show an elevated

predisposition to CAD progression and its clinical manifestations, particularly when coupled with cardiovascular risk factors.

2.4.1 -675 4G/5G Polymorphism

Within the promoter of the PAI-1 gene, positioned at 675 base pairing upstream, a polymorphism involving the insertion or deletion of single guanosine (-675 4G/5G) exists. The presence of the 5G allele introduces an extra binding spot for repressive molecules, which causes decreased transcription processes and reduced PAI-1 activation. The 4G allele, on the other hand, has been related to somewhat higher levels of plasma PAI-1.

The 4G allele is a contributing factor to CAD, as it enhances PAI-1 gene expression at the transcriptional stage by lacking a repressor recognition site. In contrast, the 5G allele retains this site, enabling partial suppression of gene activity and is therefore regarded as the protective variant as follows: The transcription factor Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) helps control how much of the PAI-1 gene is made by interacting with a specific part of the gene called the 5G allele. This part of the gene has a spot where NF-κB can attach, which stops the gene from being active. This stopping effect doesn’t happen in the 4G allele. So, when there's inflammation, like from Interleukin-1 (IL-1), people with the 4G version make more PAI-1, which might increase their risk of blood clots and heart problems as shown in Fig (2.4) (Cho *et al.*, 2001).

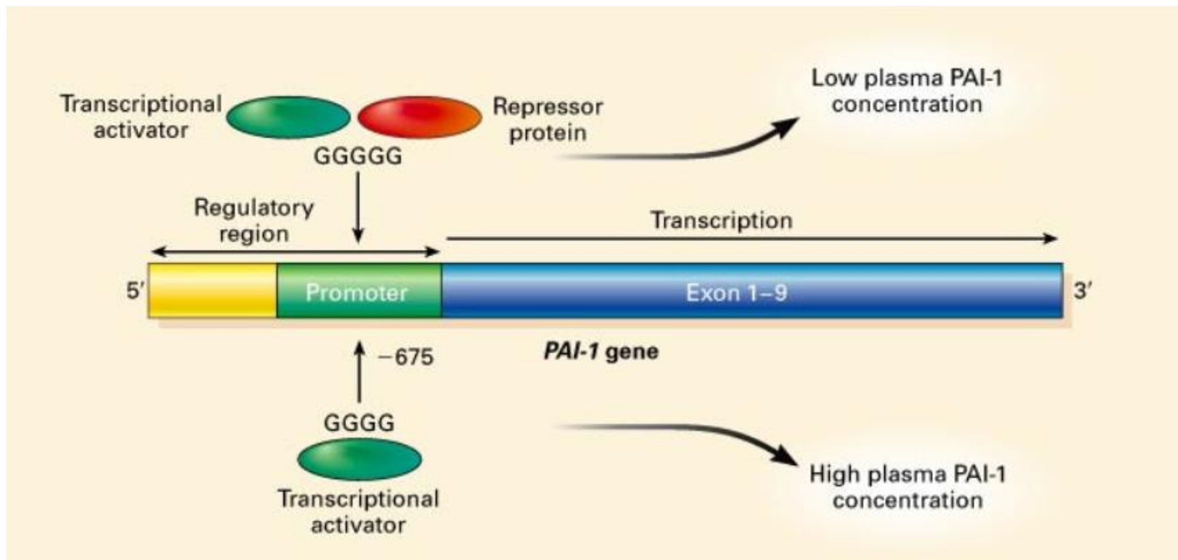


Figure (2.4): Influence of the –675 4G/5G variant on PAI-1 gene expression.
(Kohler & Grant 2000).

2.4.2 -844G/A Polymorphism

Another polymorphism influences both PAI-1 gene expression and also plasma concentration. It involves the replacement of guanine with adenine at location -844. This polymorphism may influence the nuclear protein's interaction with the PAI-1 promoter region. Both of the G and A variants are specifically bound by the nuclear proteins of human endothelial cells, according to electrophoretic mobility analyses, and promoter constructs with the -844 A allele demonstrated more transcriptional activity than those with the -844 G allele. This observation shows that the -844 G/A polymorphism may play a role in controlling PAI-1 quality expression (Hultman et al., 2010).

The -844 G/A polymorphism impacts how strongly the PAI-1 gene is transcribed. The -844 A Variant is a contributing factor to CAD and modulates PAI-1 transcription by lacking a repressor binding site. On the other hand, -844 G allele preserves this region and leads to partial suppression of gene expression, resulting in a protective variant as follows: The G allele has been shown to interact with transcriptional repressors such as E74-Like factor 1 (Elf-1) and ETS-like protein 1 (Elk-1), which suppress promoter activity and decrease PAI-

1 expression. In contrast, the A allele exhibits reduced binding affinity for these repressors, resulting in diminished inhibitory control. People who have the AA type of this gene have more activity in the promoter region, which leads to more PAI-1 being made due to the absence of strong repressor binding. This elevated expression of PAI-1 can impair fibrinolysis, thereby promoting thrombus formation and increasing susceptibility to cardiovascular disease (Hultman *et al*., 2010).

2.5 Previous Studies

In previous studies, the results varied from one study to another. The Italian Study Group (2003) did not find any proof of nine genetic variants have been studied in genes connected to blood clotting, like fibrinogen, factor VII, t-PA, and PAI-1. Among these variants, the PAI-1 -675 4G/5G change has also been studied and Acute Cardiac Infarction in individuals younger than 45 years who didn't have any known heart disease risks. (Atherosclerosis,Thrombosis and Vascular Biology Italian Study Group, 2003).

Fu Lu et al. (2001) demonstrated that the PAI-1 -675 4G/5G variant is correlated with Acute Cardiac Infarction, with the 4G/4G genotype representing an important hereditary risk factor due to its effect in elevating PAI-1 levels and reducing fibrinolysis.

This finding is reinforced in a cumulative analysis including 72 studies involving 45,083 people by Zhang et al (2014), which confirmed that the -675 4G/5G variation is linked to coronary artery. Furthermore, the data discovered that those with the 4G allele were more likely to suffer an Acute Cardiac Infarction, especially in Asian and Caucasian populations.

Hoekstra and colleagues (2003) observed an increased Acute Cardiac Infarction risk among individuals who have the 4G allele, although this link was not statistically significant. Those individuals with elevated plasma PAI-1 activity were more likely to die from cardiovascular disease.

According to Morange and colleagues in 2007, the incidence of Acute Cardiac Infarction in those who do not smoke and the levels of PAI-1in the bloodstream are weakly correlated with single nucleotide polymorphisms that capture common variant of the PAI-1 gene haplotypes and one of these SNPs -844G/A.

In 2006, Su and their team found that among Chinese people who do not smoke, those with the -675 4G variant as well as the -844 A variant of the PAI-1 gene are more likely to get coronary heart disease.

Similarly, Abboud et al (2010) revealed the same result, people with these gene versions are more susceptible to heart attack and show increased PAI-1concentration in their blood and have less tissue plasminogen.

In 2014**,** Nikolopoulos and colleagues showed that Acute Cardiac Infarction is recognized as a complex cardiac disease influenced by multiple factors and a variety of genes. There is evidence that the 4G allele might increase the risk of Acute Cardiac Infarction by raising PAI-1 levels. Furthermore, the risk associated with the 4G allele in Acute Cardiac Infarction is also associated with cholesterol and triglyceride levels.

According to Parpugga and colleagues (2015), individuals with the heterozygous PAI-1 -675 4G/5G genotype and Acute Cardiac Infarction had 1.6 times higher odds of developing coronary artery blockage compared to homozygotes (4G/4G or 5G/5G).

In order to investigate Egyptian population sample, Al-wakeel and others (2018) demonstrated no connection was observed between the PAI-1 -675 4G/5G variants and the susceptibility to CAD or the degree of PAI-1 activities.

Kumar *et al*. (2021) initially observed that there is a strong link between a certain genetic variation in the -675 4G/5G allele and a higher risk of acute heart attacks in people from India. This finding can help identify genetic factors that contribute in coronary artery disease, which could lead to better ways of diagnosing and treating the condition.

In the meta-analysis, it was observed that those with elevated PAI-1 levels exhibited a higher likelihood of coronary heart disease relative to individuals with the lowest levels (Song *et al*, 2017).

This research concentrates on a research gap that has not yet been investigated in Palestine. Moreover, this research gives a chance for the researcher to enhance practical and technical skills including DNA extraction and characterization of genetic variants using approaches like allele-specific PCR and RFLP.

Chapter 3: Material and Methods

3.1 Study Design

A case-control study was employed to assess the connection between genetic variations in the regulatory region of PAI-1 and cardiovascular conditions.

3.2 Study Area

The research was carried out at three major clinical centers: Palestinian Health Centers, An-Najah National University Hospital, and Al-Razi Hospital during the period from January 2025 to September 2025.

3.3 Sampling Method and Sampling Size

The research includes three groups: patients diagnosed with Acute Cardiac Infarction, patients with Chronic Stable Angina, and healthy control participants, with 50 individuals in each group.

A 1:1 case-to-control ratio was used to select 50 individuals for each group using OpenEpi software and standard equations. This sample size had a power of about 80% to identify moderate-to-large SNP effects (OR $\geq$ 2.8). The gnomAD database (v4.1.0) provided the allele frequencies for the -844 G/A (A $\approx$ 0.42) and -675 4G/5G (4G $\approx$ 0.35) variants. The expected frequencies were assumed to represent the general population controls (Kent et al., 2002). Overall, calculations for sample size were conducted using Kelsey, Fleiss, and Fleiss with continuity correction (Sullivan & Soe, 2007)

Approvals for the study outline and consent form came from the Ethics Committee at the Arab American University (approval number R-2024/A/50/N). Because the consent is normally only valid for six months, it was later renewed and approved again with a new approval number (approval number R-2025/A/2/CR). In addition, permission was obtained

from the Clinical Scientific Research Center at An-Najah National University Hospital to collect samples from patients diagnosed with Acute Cardiac Infarction or Chronic Stable Angina. Similarly, the hospital and medical directors of Al-Razi Hospital granted the approval. The numbers of diagnosed cases were obtained from hospital records, and many patients who had previously undergone catheterization were available during data collection.

Every participant provided their informed consent prior to joining the study. Participants recruited from Palestinian health centers, Al-Razi Hospital and An-Najah National University Hospital provided consent in person. The diagnosis for each individual was established by cardiologists and catheterization experts and confirmed through examination of their medical records. Data collection involved a structured medical questionnaire answered by study participants that covered personal information, medical history, lifestyle factors, and clinical status.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Age: Participants aged 40 years and above.

Groups: Healthy Controls: 50 individuals without a history of a cardiovascular disease.

Stable Angina Group: 50 individuals who have been diagnosed with chronic stable angina.

Acute Cardiac Infarction Group: 50 individuals who have been diagnosed with acute cardiac infarction.

Consent: Participants provided permission and informed consent for taking blood samples and performing tests including genetic analysis for the PAI-1 promoter variants -844 G/A and -675 4G/5G.

3.4.2 Exclusion Criteria

Unstable Angina: Patients with unstable angina were omitted due to their acute, variable nature linked to transient inflammatory and endothelial activation, which might obscure stable Genotype–disease relationships. (Biasucci *et al*., 1996; Kumar *et al*., 2021).

Other Comorbidities: individuals with other health problems that might affect evaluating the Cardiovascular risks were excluded (e.g., severe liver or kidney problems, cancer, or certain inflammatory Diseases).

3.5 DNA Extraction and Blood Collection

Blood specimens were collected from participants. Genomic deoxyribonucleic acid (DNA) was extracted from venous blood collected in ethylenediaminetetraacetic acid (EDTA) tubes using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), as per the manufacturer's protocols. First, 200 microliters of blood sample, 200 microliters of lysis buffer called AL, and 20 microliters of Proteinase K were combined in a microcentrifuge tube. The mixture was left to sit at 56°C for 15 minutes to break down the cells. 200 microliters of 96% ethanol were added to the mixture, and it was spun in a centrifuge.

The sample was placed onto a QIAamp Mini spin column where the DNA would stick to the column. It was spun again at 8000 revolutions per minute (rpm) for one minute. The column was positioned into a new Eppendorf tube, and the liquid left in the old tube was discarded. The MinElute column was washed two times—first with AW1 buffer and followed by - AW2 buffer. 50 to 100 microliters of preheated elution buffer (AE) from the kit were used to elute the DNA. DNA was placed into a sterile microtube and preserved at -20°C degrees until use (QIAGEN, 2016) DNA quantification.

The agarose gel showed clear DNA bands after DNA extraction, indicating that the DNA quality was acceptable and the extraction was successful.

NanoDrop spectrophotometer (IMPLEN) was used to assess the DNA concentration, with the elution buffer as a blank, and the optical density (OD260/OD280) ratio was used to

evaluate sample purity. A ratio between 1.7 and 2.0 showed that the extracted DNA was suitable for subsequent analyses (Smith et al., 2022).

3.6 Genotype Analysis

-675 4G/5G and -844 G/A variants were determined from DNA samples. The -675 4G/5G polymorphism was genotyped by Allele-Specific Polymerase Chain Reaction (AS-PCR), while the -844 G/A polymorphism was identified through Restriction Fragment Length Polymorphism (RFLP) analysis.

3.6.1 -675 4G/5G Polymorphism

The 4G/5G polymorphism (rs1799889), involving the insertion or deletion of one guanosine positioned 675 bp before the transcription start site within the PAI-1 promoter region, was described by Morange et al. (2007). The -675 4G/ 5G polymorphism was examined by AS-PCR technique. This approach depends on primers with a mismatched base at their 3′ end binding less efficiently, enabling the selective amplification of specific alleles. Primers designed to match each variant at the 3′ end allow identification of both homozygous and heterozygous genotypes (Newton et al. 1989).

The method used to detect the -675 4G/5G variant in the PAI-1 gene involved primers obtained from Hy-Labs that targeted specific alleles of the gene. Following Abboud *et al.,*2010; Cho *et al*., 2012; Fu *et al.,* 2001; and Kumar *et al.,* 2021,the primers included a control primer (5′-AAGCTTTTACCATGGTAACCCCTGGT-3′), a common reverse primer (5′-TGCAGCCAGCCACGTGATTGTCTA-3′), a 4G-specific forward primer (5′-GTCTGGACACGTGGGGA-3′), and a 5G-specific forward primer (5′-GTCTGGACACGTGGGGG-3′).

Primers were checked successfully by using University of California Santa Cruz (UCSC) Genome Browser and A Plasmid Editor (APE) program, and the primer sequences matched

exactly as expected (Kent et al., 2002; Davis, 2017; Fig (3.1)).

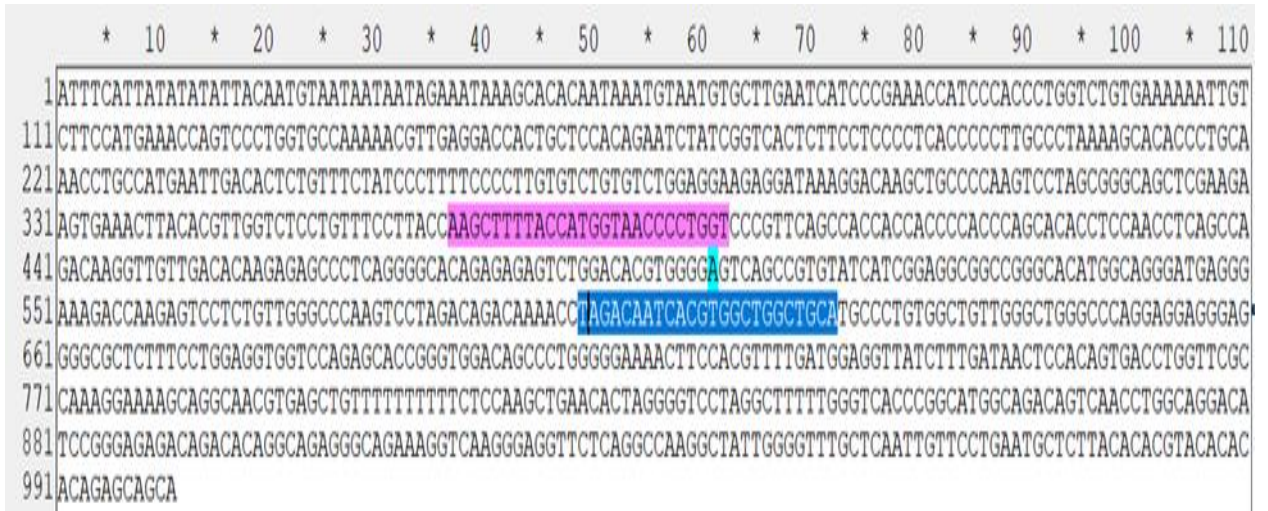


Figure 3.1: *In silico* analysis of the −675 4G/5G polymorphism showing primer sequences and positions: The polymorphic site is colored light blue, with the forward primer in pink and the reverse primer in dark blue (Kent *et al.,* 2002; Davis, 2017).

These primers were created to specifically enhance the particular alleles to clearly differentiate between homozygous and heterozygous genotypes. According to Kollabathula *et al*. (2022), the internal control primer yielded a 256 bp result (pink-dark blue fragment), while the allele-specific forward and reverse primers generated 138 bp DNA fragments (light blue-dark blue fragment). In this study, the expected sizes of the PCR amplification products were verified using the UCSC In-Silico PCR (computational analysis) tool by inputting the forward and reverse primer sequences. The observed fragment sizes on the agarose gel matched the predicted sizes, confirming the successful amplification of the target regions.

The PCR protocol used to check for the -675 4G/5G variation had a volume of 15µL. PCR mixture made up of 7.5 µL of GoTaq® Green Master Mix, 1 µL each of forward and reverse primers diluted ten-fold, 3.5 µL of nuclease-free water, and 2 µL of DNA. This composition is comparable to previously published procedure for allele-specific PCR assays intended for efficient and cost-effective genetic testing (Ravichandran *et al*., 2022).

For each sample, three PCR tests were done at the same time. The first test utilized a common primer (dark blue) along with a PAI-1 primer (Pink) as an internal control. The second test employed the same common primer paired with a 4G allele - specific primer, while the third

test combined the common primer with a 5G allele - specific primer. (FU *et al,* 2001) The primers used in each PCR, along with their sequences and the corresponding product sizes, are presented in Table (3.1).

Table 3.1: Primer sequences, product sizes, and PCR methods for -675 4G/5G PAI-1 Polymorphism.

Primer	Sequence	Product size	Method used
Forward (PAI-Control) Primer in pink	3'AAGCTTTTACCATGGTAACCCCTGGT 5'	256	Conventional PCR
Reverse (Common) Primer complementary to the dark blue	3' TGCAGCCAGCCACGTGATTGTCTA 5'		
4G-Forward Primer (light blue)	3' GTCTGGACACGTGGGGA 5'	138	AS-PCR
Reverse (Common) Primer complementary to the dark blue	3' TGCAGCCAGCCACGTGATTGTCTA 5'		
5G-Forward-Primer (light blue)	3' GTCTGGACACGTGGGGG 5'	138	AS-PCR
Reverse-(Common) Primer Complementary to the dark blue	3' TGCAGCCAGCCACGTGATTGTCTA 5'		

PCR amplification was carried out using a Bio-Rad CFX Opus 96 thermal cycler through repeated trials and optimization, in the following order: DNA was initially denatured for five minutes at 95°C followed by 35 cycles (95°C for 30 seconds to denature the DNA, 61°C for 30 seconds to allow the primers to bind to the DNA, 72°C for 40 seconds to extend the DNA

strands. Final extension step at 72°C was performed for five minutes and terminated by holding temperature at 4°C.

After PCR amplification, gel electrophoresis was performed in 2% agarose gels (weight/volume (wt/vol)) to enable visualization of the results under UV light gel. The agarose gels were prepared using 1% Tris-Acetate-EDTA (TAE) buffer. The agarose mixture was heated until totally dissolved. After cooling slightly, ethidium bromide (Hy-Labs) was carefully added as the staining agent from a stock solution containing 1 mg/mL. Once the solution reached a safe handling temperature, it was poured into gel molds. After the gel had solidified, the PCR samples were loaded into the wells, and electrophoresis was performed at 90 volts for 45 minutes (Fu *et al*., 2001).

3.6.2 -844G/A Polymorphism

The -844G/A polymorphism (rs2227631) features a G/A substitution located 844 bp before the transcription start site within PAI-1 promoter region (Morange *et al*., 2007).

Detection of -844G/A polymorphism was performed using PCR-RFLP. The target region is first amplified in a single PCR reaction with gene-specific primers. In this study, the -844 G/A polymorphism was amplified using a forward primer (5′-CAGGCTCCCACTGATTCTAC-3′) and a reverse primer (5′-GAGGGCTCTCTTGTGTCAAC-3′). Primers purchased from Hy-Labs matched those utilized in earlier research, generating a PCR product of 510 bp (Fu *et al.,* 2001; Abboud *et al.,* 2010).

The verification of the product size was carried out with the UCSC Genome Browser by aligning the primers to the target region. The results were confirmed using APE software to ensure that the primers were specific and correctly aligned with the −844 G/A variant site (Kent et al., 2002; Davis, 2017; Fig (3.2)).

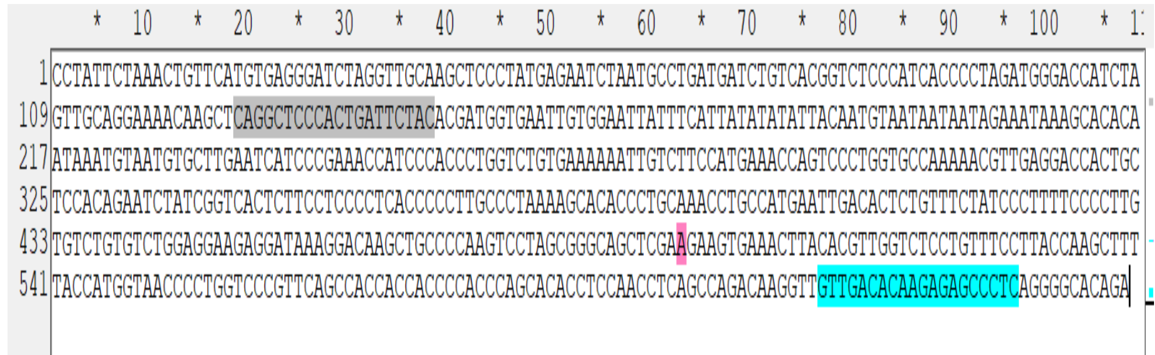


Figure 3.2: *In silico* analysis of the -844 G/A polymorphism showing primer sequences and positions: The polymorphic site is shown in pink, with the forward primer in gray and the reverse primer in blue. (Kent *et al.,* 2002; Davis, 2017)

The primers used in the PCR reactions, along with their sequences and expected product sizes, are listed in Table (3.2).

Table 3.2: Primer sequences, product sizes, and PCR methods for -844G/A PAI-1 Polymorphism.

Primer	Sequence	Product size	Method used
844G/A- forward Primer	3' CAGGCTCCCACTGATTCTAC5'	510	RFLP
844G/A-reverse Primer	3' GAGGGCTCTCTTGTGTCAAC5'		

A 25 µL PCR reaction was constructed to identify the -844 G/A polymorphism in the samples, consisting of of 12.5 µL of GoTaq® Green Master Mix, 1 µL each of forward and reverse primers, 4 µL of DNA template, and 6.5 µL of nuclease-free water (Promega Corporation, n.d.).

Gradient PCR was carried out to determine the annealing temperature that yields the maximum specificity and band clarity. The PCR conditions were successfully refined as

follows: an initial denaturation step at 95°C for three minutes to complete separation of the DNA strands. This was followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 61°C for 30 seconds to allow primer binding, and extension at 72°C for 45 seconds for DNA synthesis. The reaction was concluded with a final extension at 72°C for five minutes to accomplish amplification of all products, after which the samples were held at 4°C until further analysis.

Amplification products were treated with the XhoI restriction enzyme (New England Biolabs, Catalog #R0146S, 20,000 units/ mL). Digestion was done for one hour at 37°C in Biotech heating block. The enzyme was then deactivated at 65 °C for 20 minutes, as per the manufacturer's guidelines, to avoid excessive digestion (New England Biolabs, 2024).

To ensure completing the digestion in a 25 µL reaction, the volumes of PCR product, buffer, XhoI enzyme, and nuclease-free water were carefully calculated. All components were gently well-mixed by gentle pipetting to avoid DNA fragmentation, with the restriction enzyme added at last to maintain its enzymatic activity. The detailed setup of the RFLP reaction is in Table (3.3).

Table 3.3: RFLP set-up for a 25 µL XhoI digestion (New England Biolabs, 2024).

Stock Components	**Stock Concentration**	**25 µL Reaction**	**Final Concentration in 25 µL**
PCR Product		15	<100 ng/µL
NEB Buffer	10X	2.5	1X
Restriction Enzyme Xhol	20,000U/ml	0.5	(5-10) units
Nuclease free water		7	To bring total volume to 25µL

XhoI digests between the first C and T on the top strand (5'–C | T C G A G–3') and at the corresponding location on the bottom strand (3'–G A G C T | C–5') to produce two fragments (364 bp and 146 bp) when it detects a sequence that is unique to the G allele. The sequence

in the A allele, due to single nucleotide substitution (G → A) changes to 5'–C T C G A A–3' and 3'–G A G C T T–5', which eliminates the XhoI recognition site and stops cleavage, resulting in a single uncut fragment (510 bp). For the G/A genotype, XhoI cuts the G allele but not the A allele, resulting in three bands on the gel (510 bp, 364 bp and 146 bp). The different band patterns serve, therefore, to distinguish between genotypes (García-González et al., 2015)

After digestion, the fragments were separated on a gel composed of 2% agarose that had been subjected to migration for 45 minutes at 90 volt and visualized with ethidium bromide. The sizes of the observed bands in base pairs for each genotype are summarized in Table (3.4).

Table 3.4: Band sizes resulting from XhoI digestion according to PAI-1 −844 G/A genotypes (Torres-Carrillo *et al.,* 2008; García-González *et al.,* 2015).

Genotype	**Band Size (bp)**	**Description**
A/A	510	Single undigested fragment
G/G	364,146	Two fragments
G/A	510,364,146	Three fragments representing heterozygous genotype

3.6.3 Allele-Based and Dominant Model Analysis

Allele-based and dominant model analyses were performed for −675 4G/5G and −844 G/A polymorphisms in order to assess the relationship between PAI-1 promoter polymorphisms and Acute Cardiac Infarction and Chronic Stable Angina. In the allele-based analysis, each group's allele frequencies (4G and 5G for −675; G and A for −844) were determined and compared between patients and controls. This method makes it possible to identify genetic relationships that might not be visible at the genotype level (Abboud *et al*., 2010 ; Li, 2012).

In a dominant genetic model, dominant signifies 'a single copy of a gene can produce an effect.' A risk allele is 'a variant of a gene that may raise the likelihood of a disease.' Having one risk allele is enough to heighten disease susceptibility; therefore, individuals who are either homozygous or heterozygous for the risk allele are grouped together and compared against those homozygous for the non-risk allele (Madsen & Browning, 2009; Setu & Basak, 2021). This model was utilized specifically to enhance the statistical power of the research.

For the -675 4G/5G polymorphism, individuals with the homozygous 4G/4G genotype along with those possessing the heterozygous 4G/5G genotype are examined collectively and compared against 5G/5G homozygous. In the same way, for the -844 G/A polymorphism, individuals with the homozygous A/A genotype and those with the heterozygous G/A genotype and compared to those possessing the G/G genotype.

The -675 5G/5G and -844G/G genotypes were considered good, no-risk reference variants, as previous study indicates they do not correlate with higher PAI-1 expression or increased cardiovascular risk, while the 4G and A alleles are linked to raised PAI-1 levels and negative cardiovascular effects (Kumar *et al*., 2021; Park *et al*., 2020).

3.6.4 DNA Sequencing

Sanger sequencing technique was used to verify the PCR results. It provides accurate genetic sequence data and enables the detection of specific nucleotide changes, making it notably useful for confirming the presence of relevant polymorphisms (Chang et al., 2017).

Six representative samples were selected for Sanger sequencing to confirm the existence of the -675 4G/5G or -844 G/A polymorphism that had already been amplified using PCR.

The sequencing outcomes were aligned with those obtained from agarose gel electrophoresis. The sequencing generated two files for each PCR product: one with the text-based sequence and another in ab1 format called a chromatogram. The data from these files were then analyzed using ApE software.

3.7 Statistical Analysis

The acquired data were input and statistically analyzed by the Statistical Package for Social Science (SPSS) version 20. Measurable data were presented as mean ± SD, whereas genotype and allele frequencies were shown in percentage terms. A dominant model was used, comparing individuals having at least one risk allele to those without the risk allele. Associations were displayed as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was defined at $P \leq 0.05$. Every analysis was carried out under the direction of a statistician and adhered to conventional procedures (Charan & Saxena, 2012).

Chapter 4: Results

4.1 Demographic and Clinical Features of the Studied Subject

The Research groups, including healthy controls, Acute Cardiac Infarction, and Chronic Stable Angina, consist of 50 individuals. Age differences among the three groups were analyzed through one-way Analysis of Variance (ANOVA). The average age of individuals in the healthy controls was 54.66 ± 4.92 years (mean ± standard deviation (SD)), while the Acute Cardiac Infarction patients had a mean age of 56.84 ± 5.49 years, and the Chronic Stable Angina patient's mean age was 56.82 ± 5.94 years.

The ANOVA test revealed that the groups' mean ages did not differ (F=2.808, P=0.064) as shown in Table (4.1). The F-test (ANOVA test) compares the means of a continuous variable between groups relative to the variation within the groups. Statistical significance determined by the P-value. P-value (probability value): $P \leq 0.05$ indicates that the observed difference is considered significant.

Of the 150 participants in the study, 56 (37.3%) were females and 94 (62.7%) were males. The healthy group's gender distribution was about equal (23 females, 46%; 27 males, 54%). In contrast to the Chronic Stable Angina group, which had a modest male majority (21 females, 42%; 29 males, 58%), the Acute Cardiac Infarction group was primarily male (12 females, 24%; 38 males, 76%). The gender distribution across the groups did not differ significantly (χ^2= 5.870, P = 0.053) as presented in Table (4.1) χ^2: Chi-square test analyzes the association between categorical variables; significance is indicated by $P \leq 0.05$.

Table 4.1: Age and Gender Distribution among Study Groups.

Variable	**Healthy controls (n =50)**	**Acute Cardiac Infarction (n = 50)**	**Chronic Stable Angina (n = 50)**	***P*-value**
Age (year) (Mean ± SD)	54.66 ± 4.92	56.84 ± 5.49	56.82 ± 5.94	0.064[1]
Sex n (%)				
Female	23 (46.0%)	12 (24.0%)	21 (42.0%	
Male	27 (54.0%)	38 (76.0%)	29 (58.0%)	0.053[2]

[1] One-way ANOVA test (F = 2.808): mean age did not differ significantly among the three groups.

[2] Chi-square test (χ^2)= 5.870: sex distribution did not differ significantly among the three groups.

Among the study participants, 58% were from Tulkarm, gathered during standard check-ups at the Palestinian Ministry of Health, 30.7% were from Jenin through Al-Razi Hospital, and 11.3% were from Nablus via An-Najah National University Hospital, as presented in Fig (4.1).

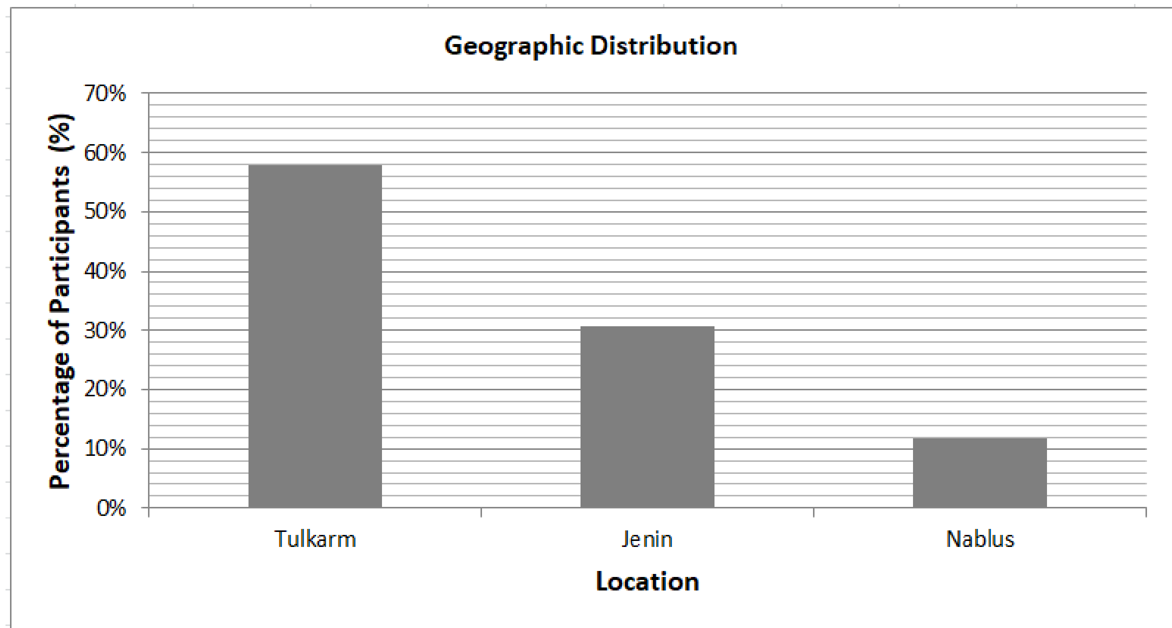


Figure 4.1: Geographic representation of participants in the study.

The various study groups showed distinct trends in cardiovascular risk variables. Patients with Acute Cardiac Infarction and Chronic Stable Angina had greater rates of smoking and a family history of Coronary Artery Disease (CAD) than those without. That clearly indicated a higher risk in these cohorts ($P < 0.05$). Patients with chronic Stable Angina had the highest incidence of diabetes, and the incidence was less in Acute Cardiac Infarction patients; healthy controls had the lowest incidence. The variations in diabetes incidence across the three groups were highly significant (P=0.001), revealing the strong correlation between healthy controls and lower incidence of diabetes.

Similar trends were seen in hypertension, which was most common in Chronic Stable Angina group, followed by Acute Cardiac Infarction group, and least common in the healthy controls ($P < 0.001$). Hyperlipidemia was characterized by elevated cholesterol levels (>200 mg/dL) and/or triglyceride levels (>150 mg/dL), according to self-report or medical diagnosis. The Acute Cardiac Infarction and Chronic Stable Angina groups had higher rates of hyperlipidemia. However, no significant effect was observed ($P > 0.05$).

In terms of lifestyle characteristics, individuals with Acute Cardiac Infarction and Chronic Stable Angina were marginally less physically active than healthy controls; however, this difference showed no significant effect (P = 0.600). Participants characterized their typical

diet as “mostly unhealthy,” “balanced,” or “mostly healthy,” and those who chose “mostly healthy” were categorized as having a healthy diet. Similarly, healthy controls consumed more healthful food than the sick groups, although this difference did not achieve statistical significance (P = 0.197). Table (4.2) displays the percentages for each variable in three study groups.

Table (4.2): Comparison of cardiovascular risk factors among study groups.

Variable	**Healthy controls(n=50)**	**Acute Cardiac infarction(n=50)**	**Chronic Stable Angina(n=50)**	***P*-Value**
Smoking	18(36.0%)	34(68.0%)	25(50.0%)	0.019*
Family History of CAD	11(22.0%)	25(50.0%)	24(48.0%)	0.006*
Diabetes	4(8.0%)	14(28.0%)	20(40.0%)	0.001*
Hypertension	8(16.0%)	23(46.0%)	38(76.0%)	<0.001*
Hyperlipidemia	9(18.0%)	19(37.0%)	21(42.0%)	0.069
Physical activity (Active)	26(52.0%)	21(42.0%)	23(46.0%)	0.600
Health food (healthy)	44(88.0%)	37(74.0%)	39(78.0%)	0.197

* Statistically significant

Smoking, family history of CAD, diabetes and hypertension were significantly lower in the healthy controls and highest in cardiac patients, particularly those with Chronic Stable Angina, while the three groups showed no significant differences in hyperlipidemia, physical activity, or consumption of healthful foods.

4.2. Molecular Analysis

4.2.1. Molecular Analysis of the PAI-1 -675 4G/5G Polymorphism

Three PCR runs were performed for every sample. The PAI-1 internal-control primer (pink) and the common primer (dark blue) were employed in the initial reaction as an internal control. The common primer and the 4 G-specific primers were used in the second reaction, and the common primer and the 5G-specific primer were used in the third. This process was previously explained in Chapter 3 Fig (3.1).

According to the electrophoresis results, homozygous genotypes (4G/4G or 5G/5G) produced two bands- control band (256 bp) and the allele-specific band (138 bp), whereas heterozygous (4G/5G) samples produced three bands (both allele-specific bands in addition to the control band) as shown in Fig (4.2).

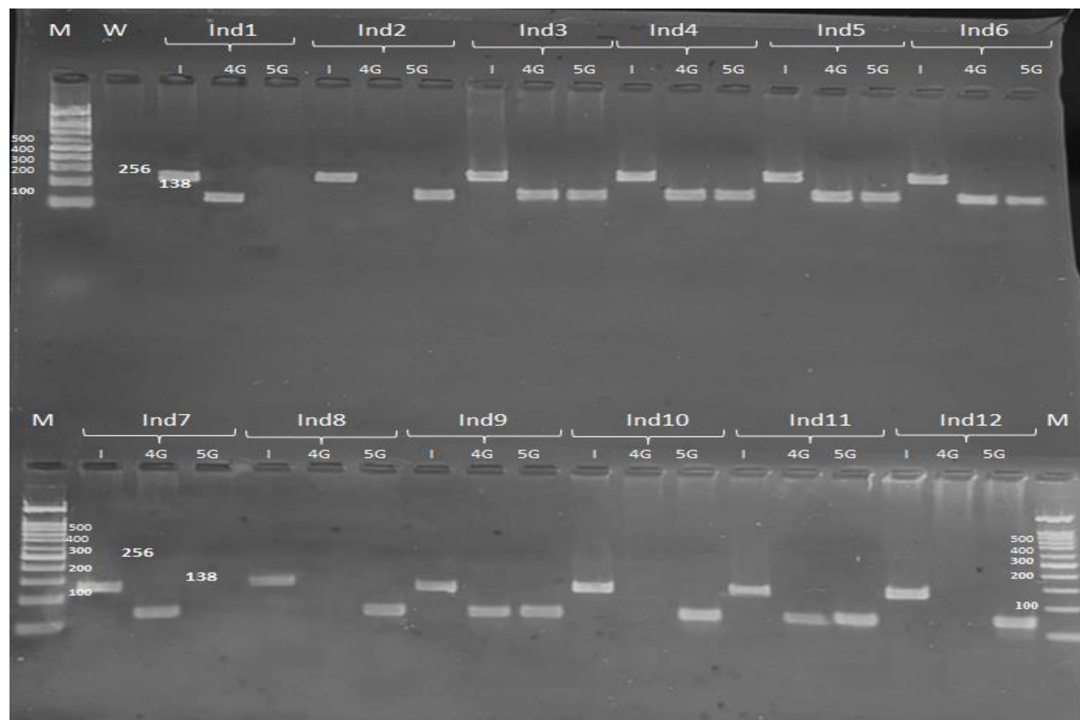


Figure (4.2): Visualization of -675 4G/5G genotypes using allele-specific PCR on a 2% agarose gel: Lane M contains the 100-bp molecular weight marker, and lane W contains the

water control (no DNA template) to ensure the absence of contamination. Each individual (Ind) was evaluated using three lanes: an internal control, a 4G-specific primer, and a 5G-specific primer respectively. The result for each sample along with genotype distribution as follow: 4G/4G was displayed by Ind1, Ind7, 5G/5G by Ind2, Ind8, Ind10, and Ind12, and heterozygous 4G/5G by Ind3, Ind4, Ind5, Ind6, Ind9, and Ind11. To confirm their genotypes, sequencing was done for Ind1, Ind2, and Ind3.

To verify the -675 4G/5G polymorphism detections made by agarose gel electrophoresis, Sanger sequencing was performed on three randomly selected samples from each genotype group. The sequencing data matched the gel electrophoresis findings mainly to prove that the genotyping method was accurate. These sequenced samples were used as references in subsequent experiments to validate that the analysis was reliable and consistent. The absence of mixed peaks showed that samples were homozygous. Overlapping peaks, showing both Guanine (G) and adenine (A) signals, represented by the letter R confirms a heterozygous genotype as shown in Fig (4.3).

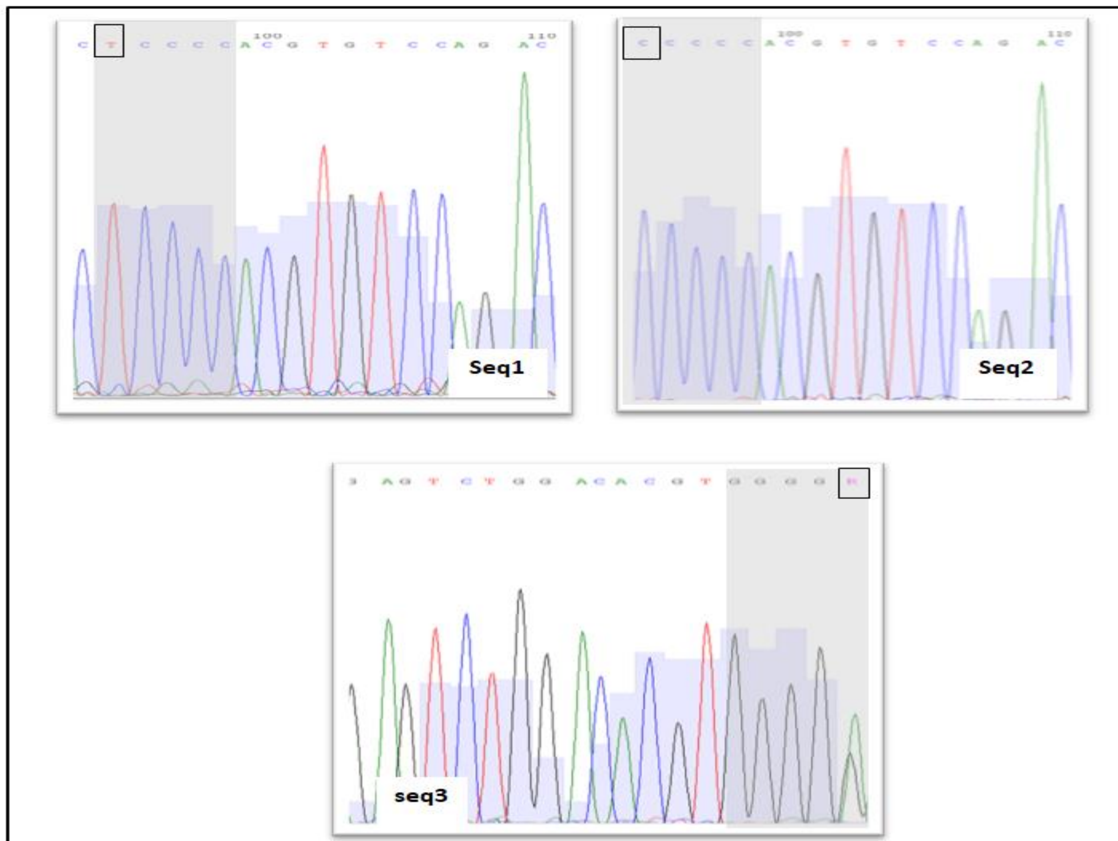


Figure (4.3): Sanger sequencing chromatograms of the −675 4G/5G polymorphism for three representative samples: Seq 1 shows four consecutive Cytosine (C) peaks, indicating a homozygous −675 4G/4G genotype, seq 2 shows five consecutive C peaks, indicating a homozygous −675 5G/5G genotype (cytosine peaks are observed due to sequencing with the reverse primer), while seq 3 shows four consecutive Guanine (G) peaks followed by an overlapping G/A peak (R), indicating a heterozygous −675 4G/5G genotype (Guanine peaks are observed due to sequencing with the forward primer). Sequencing results confirmed the genotypes shown in Figure 3.2: seq1 = ind1, seq2 = ind2 and seq3 = ind3.

4.2.2. Molecular Analysis of the PAI-1 −844 G/A Polymorphism

PCR-RFLP was used to examine the −844 G/A polymorphism using XhoI. Each sample displayed a 510 bp band before digestion. Band patterns were used to identify genotypes after digestion: A/A homozygotes displayed 510 bp; G/G homozygotes displayed 364 and 146 bp; and G/A heterozygotes displayed 510, 364, and 146 bp bands as shown in Fig (4.4).

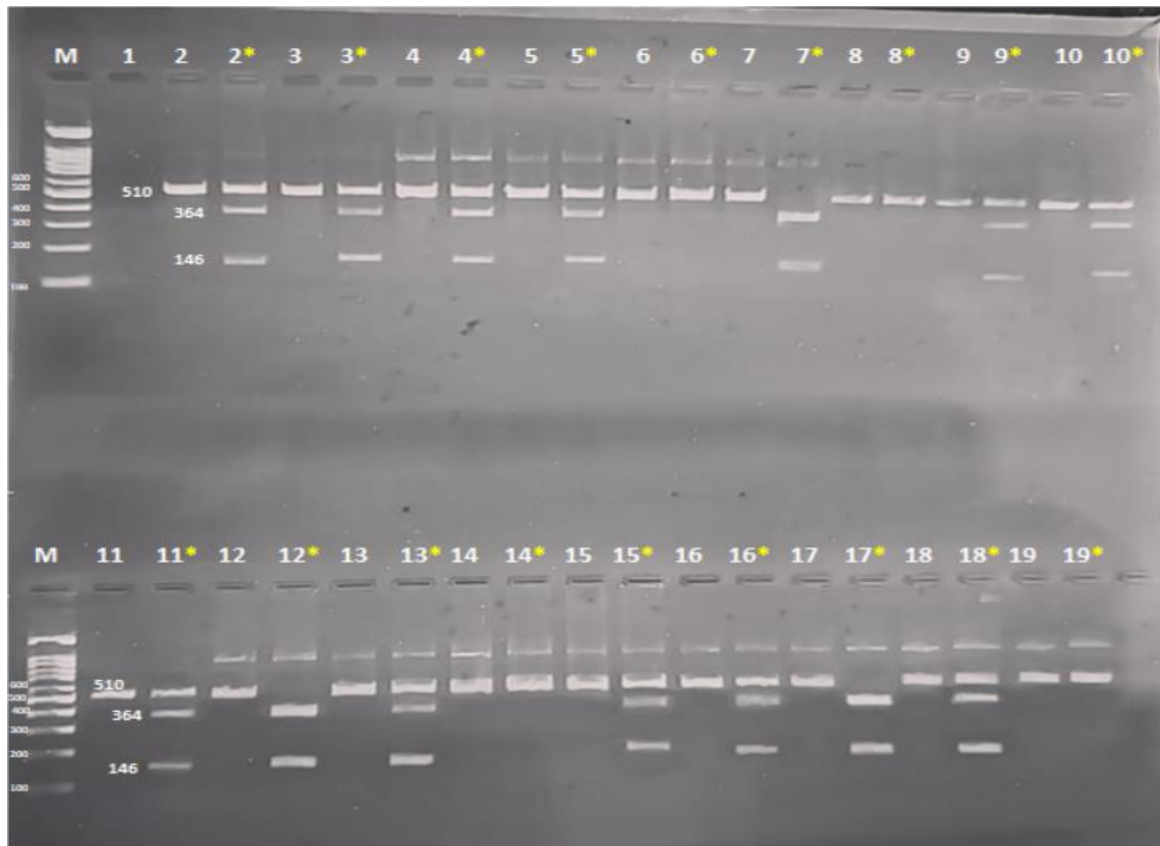
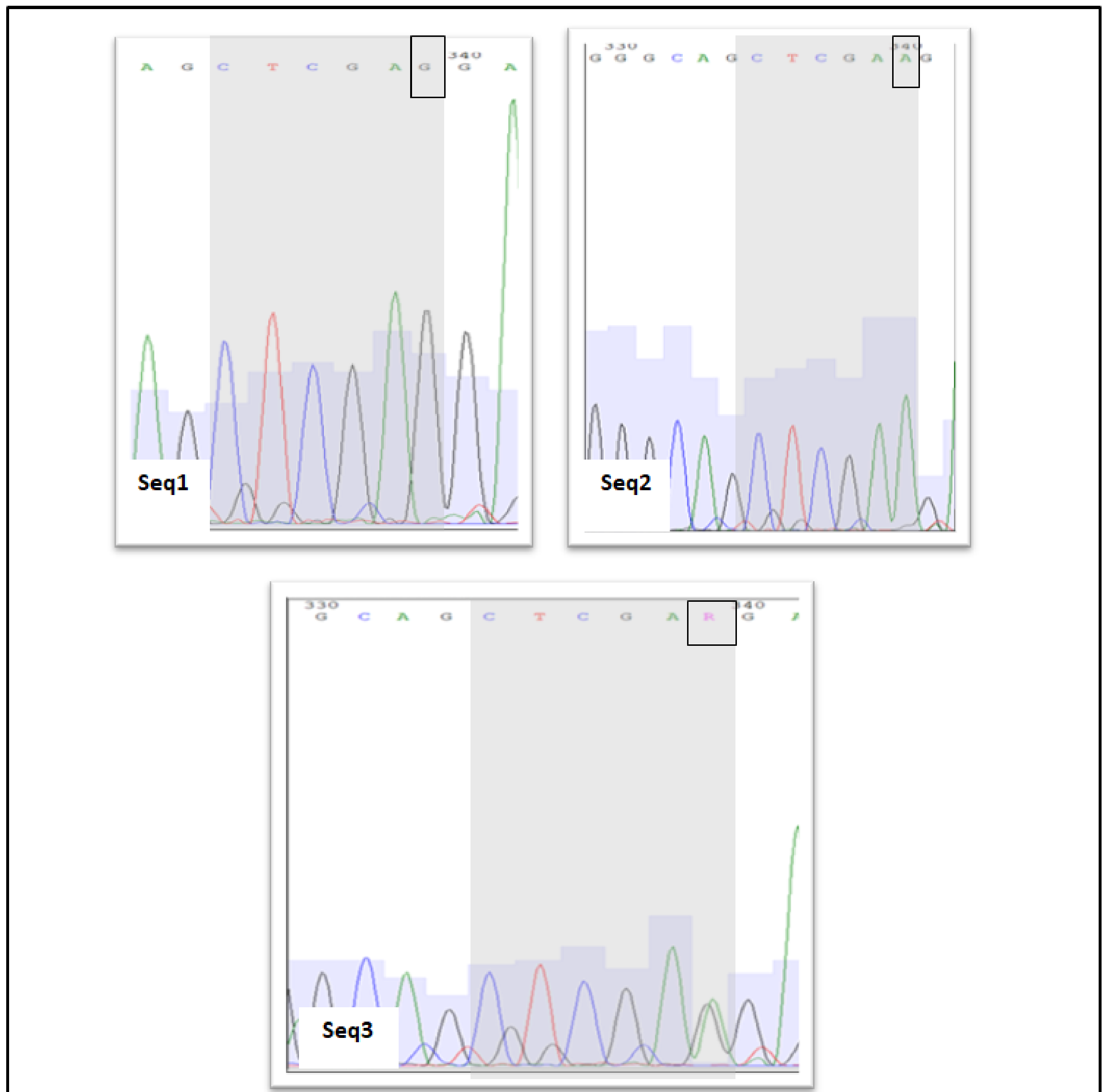


Figure (4.4): Visualization of -844 G/A genotypes using PCR-RFLP on a 2% agarose gel: Each sample was run before and after digestion with the XhoI enzyme; undigested samples are shown without an asterisk, and digested samples are marked with an asterisk. In the digested lanes, distinct banding patterns correspond to various genotypes. Sanger sequencing was used to further validate samples (17, 18, and 19). M: 100 bp DNA molecular marker. Lane 1: water control (no-DNA template) .The G/G homozygous genotype was represented by lanes 7*, 12*, and 17*; the A/A homozygous genotype was represented by lanes 6*, 8*, 14*, and 19*; and the G/A heterozygous genotype was represented by lanes 2*, 3*, 4*, 5*, 9*, 10*, 11*, 13*, 15*, 16*, and 18*.

Three random samples had been decided on to verify the genotyping outcomes of -844G/A polymorphism found via way of means of agarose gel electrophoresis. These samples were sequenced using Sanger sequencing. The sequencing data perfectly matched the gel electrophoresis pattern. This confirmed the PCR-RFLP technique used. These sequenced samples were then used as controls in subsequent analysis to ensure consistency and reliability of the analysis. The Sanger sequencing chromatogram showed a single distinct

signal for homozygous variations. Whereas the heterozygous genotype was indicated by the letter R, which showed overlapping G and A peaks at the −844 location as shown in Fig (4.5).



Figure(4.5): Sanger sequencing chromatograms of -844 G/A polymorphism for three representative samples: Seq 1 displays a single G peak, consistent with a homozygous -844G/G genotype, seq 2 displays a single A peak consistent with an -844A/A homozygous, while seq 3 displays overlapping A and G peaks (R), consistent with -844G/A heterozygous genotype.

4.3 Genotypic Analysis

4.3.1 Genotype Analysis of -675 4G/5G Variant among Study Groups

Genotype Distribution: The 4G/4G, 4G/5G, and 5G/5G genotypes were 4.0%, 50.0%, and 46.0% among healthy controls, 14.0%, 60.0%, and 26.0% in patients, who had an Acute Cardiac Infarction, and 18.0%, 52.0%, and 30.0% in patients with Chronic Stable Angina. Table (4.3) lists the percentage of each genotype in each of the three groups. Since the healthy group's 4G/4G count was less than five, Fisher's exact test was utilized to compare genotype differences .Genotype distribution of -675 4G/5G variants differ significantly for Chronic Stable Angina (F=6.075, P = 0.047) but not significant difference in Acute Cardiac Infarction (F=5.862, P = 0.062) when compared to healthy controls.

Table 4.3: Genotype distribution of -675 4G/5G variant among study groups.

Groups	4G/4G (%)	4G/5G (%)	5G/5G (%)	*P*-value
Healthy Controls N=50	4.0%	50.0%	46.0%	---
Acute Cardiac Infarction N=50	14.0%	60.0%	26.0%	0.062
Chronic Stable Angina N=50	18.0%	52.0%	30.0%	0.047*

*Statistically differences (P-value ≤ 0.05)

Allele Frequency

The frequencies of 4G and 5G alleles are summarized in table (4.4). In the healthy controls, the 4G allele was 29.0% and the 5G allele was 71.0%. The 4G and 5G alleles displayed identical rates of 44% and 56% in the groups with Acute Cardiac Infarction and Chronic Stable Angina, respectively.

A significant variation was observed in the allele frequencies of -675 4G/5G genotype between the healthy controls and the sick groups (χ^2=4.854, P-value =0.028). the odds ratio and confidence interval (OR = 0.520, 95% CI: 0.290–0.933), and because OR < 1 this indicates that the 4G allele is more frequent in patients, whereas the 5G allele is more frequent and protective in healthy controls.

OR (Odd Ratio) calculates the odds of a particular result in one group compared to a reference group, increased risk is indicated by OR > 1, reduced risk or protection is shown by OR < 1, and no effect is indicated by OR = 1. The 95% CI (Confidence Interval) provides the range of the true OR, and significance is shown if it excludes 1.

Table 4.4: Allele frequency of -675 4G/5G genotype among study groups.

Groups	4G allele	5G allele	OR (95%CI)	*P*-value
Healthy Controls	29.0%	71.0%	---	---
Acute Cardiac Infarction	44.0%	56.0%	0.520 (0.290-0.933)	0.028*
Chronic Stable Angina	44.0%	56.0%	0.520 (0.290-0.933)	0.028*

*Statistically Significant (P-value ≤ 0.05)

Note: Allele frequencies were calculated per 100 alleles: homozygous contributes two alleles; heterozygous contributes one allele.

Healthy controls: 4G = (4+ 50/2)/100=29%, 5G= (46 +50/2)/100=71%

Acute Cardiac infraction: 4G = (14 +60/2)/100=44%, 5G= (26+60/2)/100=56%

Chronic Stable Angina: 4G= (18 +52/2)/100=44%, 5G= (30+52/2)/100=56%

Dominant model

According to the dominant model, patients were more likely than healthy controls to have at least one 4G allele. Among healthy controls, 54% had the 4G allele with 4% displaying the 4G/4G genotype and 50% the 4G/5G genotype. The frequency of the 4G/4G and 4G/5G genotypes rose to 14% and 60%, respectively, in individuals with Acute Cardiac Infarction, resulting in an overall 74% of individuals having one or two 4G alleles. Similarly, 70% of individuals with Chronic Stable Angina had the 4G allele, with 18% having the 4G/4G genotype and 52% having the 4G/5G genotype as shown in Table (4.5).

The chi-square analysis revealed a difference in individuals possessing one or more 4G alleles between Acute Cardiac Infarction patients and healthy controls ($\chi^2 = 4.340$, $P = 0.037$; OR = 0.412, 95% CI: 0.178–0.957),and because of OR < 1, this suggests that patients tend to have the 4G allele, whereas those with the 5G/5G genotype seem to be more protected, but no statistically significant in the Chronic Stable Angina ($\chi^2 = 2.716$, $P = 0.099$; OR = 0.503, 95% CI: 0.221–1.144) when compared to healthy controls as presented in Table (4.5).

Table 4.5: Dominant model of -675 4G/5G genotype among study groups.

Groups	(4G/4G%+4G/5G %)	5G/5G (%)	OR (95%CI)	*P*-value
Healthy Controls	54.0% **(4.0%+50.0%)**	46.0%	---	---
Acute Cardiac infarction	74.0% **(14.0%+60.0%)**	26.0%	0.412 (0.178-0.957)	0.037*
Chronic Stable Angina	70.0% **(18.0%+52.0%)**	30.0%	0.503 (0.221-1.144)	0.099

*Statistically significant (P-value ≤ 0.05)

4.3.2 Genotype Analysis of -844G/A Variant among Study Groups

Genotype Distribution

The frequency of A/A, G/A, and G/G genotypes were 10.0%, 44.0%, and 46.0% among healthy controls and 20.0%, 56.0%, and 24.0% in patients with Acute Cardiac Infarction but among patients with Chronic Stable Angina, they were 20.0%, 52.0%, and 28.0% as shown in the Table 4.6. Genotype distribution between healthy controls and Acute Cardiac Infarction (χ^2 = 5.844, P = 0.054) or Chronic Stable Angina (χ^2 = 4.189, P = 0.123) was not significantly different, according to the chi square test.

Table 4.6: Genotype distribution of -844G/A variant among three groups.

Groups	A/A (%)	G/A (%)	G/G (%)	*P*-value
Healthy Controls	10.0%	44.0%	46.0%	---
Acute Cardiac Infarction	20.0%	56.0%	24.0%	0.054
Chronic Stable Angina	20.0%	52.0%	28.0%	0.123

Allele Frequency

The A allele was more common in patients with Acute Cardiac Infarction (48.0%) and Chronic Stable Angina (46.0%) compared to the healthy controls (32.0%), but the G allele was more common in healthy controls (68.0%) than in Acute Cardiac Infarction group (52.0%) and Chronic Stable Angina group (54.0%). The number and percentage of each allele in the study groups are displayed in Table (4.7). Allele distribution differed significantly for both Acute Cardiac Infarction ($\chi^2 = 5.333$, P = 0.021; OR = 0.510, 95% CI: 0.287–0.906) and Chronic Stable Angina ($\chi^2 = 4.119$, P = 0.042; OR = 0.522, 95% CI: 0.311–0.982), and because OR<1, this indicates that the 5G allele was more protective in the healthy controls, whereas the 4G allele was more common in patients.

Table 4.7: Allele frequency of -844G/A genotype among study groups.

Groups	A allele	G allele	OR(95%CI)	*P*-value
Healthy Controls	32.0%	68.0%	---	---
Acute Cardiac Infarction	48.0%	52.0%	0.510 (0.287-0.906)	0.021*
Chronic Stable Angina	46.0%	54.0%	0.522 (0.311-0.982)	0.042*

*Statistically differences (P-value ≤ 0.05)

Note: Allele frequencies were calculated per 100 alleles: Homozygous contributes two alleles; heterozygous contributes one allele.

Healthy controls: A :(10+44/2)/100 =32.0% G :(46+44/2) =68.0%

Acute Cardiac Infarction: A :(20 +56/2)/100 = 48.0% G :(24 +56/2) = 52.0%

Chronic Stable Angina: A :(20 +52/2)/100 = 46.0% G :(28+52/2) = 54.0

Dominant Model

Patients were more likely than healthy controls to have at least one A allele under the dominant model. Among healthy controls, 54.0% have the A allele, with 10.0% having the A/A genotype and 44.0% having the G/A genotype. In patients with Acute Cardiac Infarction, the frequencies of the A/A and G/A genotypes increased to 20.0% and 56.0%, respectively, (i.e., 76.0% for having at least one A allele). Similarly, among patients with Chronic Stable Angina, 72.0% have the A allele, including 20.0% with the A/A genotype and 52.0% with the G/A genotype, as shown in Table (4.8).

The Chi-square analysis revealed a difference in individuals possessing one or more A alleles between Acute Cardiac patients and healthy controls (χ^2 = 5.319, P = 0.021; OR = 1.705, 95% CI: 1.032–2.818), and because OR > 1, this indicates that A allele have greater odds and an increased likelihood of disease. No significant differences for Chronic Stable Angina (χ^2 = 3.475, P = 0.062; OR = 0.457, 95% CI: 0.199–1.048) when compared to healthy controls.

Table 4.8: Dominant model of -844G/A genotype among study groups.

Groups	**(A/A% + G/A%)**	**G/G (%)**	**OR(95%CI)**	***P*-value**
Healthy controls	54.0% **(10.0%+44.0%)**	46.0%	---	---
Acute Cardiac Infarction	76.0% **(20.0%+56.0%)**	24.0%	1.705 (1.032-2.818)	0.021*
Chronic Stable Angina	72.0% **(20.0%+52.0%)**	28.0%	0.457 (0.199-1.048)	0.062

*Statistically differences (P-value ≤ 0.05)

Chapter 5: Discussion

This is the initial study that looks into the correlation between the likelihood of developing coronary artery disease and these PAI-1 (-675 4G/5G and -844G/A) genetic variations in the West Bank–Palestine. The study's hypothesis is that these PAI-1 polymorphisms could be associated with both Chronic Stable Angina and Acute Cardiac Infarction vulnerability.

Along with genotype analysis, dominant-model and allele-based analyses were applied to increase statistical power by combining rare genotypes, in line with previous evidence showing that having at least one risk allele (-675 4G or −844A) affects PAI-1 function and disease susceptibility (Abboud *et al*., 2010; Grubic *et al*., 1996). In this study, significant genotypic associations were observed for the -675 4G/5G variants in Chronic Stable Angina, whereas 4G and A alleles showed higher frequencies in Acute Cardiac Infarction and Chronic Stable Angina. Dominant-model analysis, which compares individuals who have at least one risk allele with those who do not have the risk allele to assess disease risk, demonstrated a significant association between individuals who have 4G or A allele and Acute Cardiac Infarction.

In addition to genetic analysis, healthy controls and patients with Acute Cardiac Infarction or Chronic Stable Angina were compared for demographics, clinical characteristics, and cardiovascular risk factors. Traditional risk factor -smoking, diabetes mellitus, hypertension, and a family history of coronary artery disease- differed significantly among groups, whereas physical activity, healthy eating habits, and hyperlipidemia did not differ significantly.

5.1 Demographic and Clinical Characteristics

Age and sex demographics were similar among the healthy controls, Acute Cardiac Infarction, and Chronic Stable Angina groups' (age: P = 0.064; sex: P = 0.053), suggesting that these variables were unlikely to bias subsequent association results. Compared to the healthy controls (54.66 ± 4.92 years), Patients with Acute Cardiac Infarction (56.84 ± 5.49 years) and Chronic Stable Angina (56.82 ± 5.94years) were slightly older, and displayed a slightly higher age variation.

The female-to-male ratios were 46%/54% in the healthy controls, 24%/76% in Acute Cardiac Infarction, and 42%/58% in Chronic Stable Angina. It is true that there is a tendency for more males to be found, among patients with Acute Cardiac Infarction, and this is because males are more susceptible to the disease, as mentioned in previous studies (Liang *et al*., 2015).

The majority of study participants were from Tulkarm (58%) followed by Jenin (30.7%) and Nablus (11.3%). Travel and patient outreach in Jenin and Nablus were challenging due to movement limitations under occupation or the prevailing circumstances. It was also easier to obtain medical records and confirm diagnoses in Tulkarm compared to the other regions. The validity of the genetic association findings is unlikely to have been impacted by this geographical imbalance because as it is a very small-scale geographical discrepancy and more importantly diagnostic accuracy was given priority despite this small-scale unequal distribution.

Hypertension, diabetes, family history and smoking—were strongly correlated with both Acute Cardiac Infarction and Chronic Stable Angina. Hypertension and diabetes mellitus showed the highest correlation (P ≤ 0.001), followed by, family history (P = 0.006) and smoking (P = 0.019). Hyperlipidemia was more often seen in people with Acute Cardiac Infarction and Chronic Stable Angina, but the link wasn't strong enough to be statistically significant (P = 0.069), most likely due to the study's insufficient sample size. Even so, hyperlipidemia is still known to contribute to atherosclerosis and making coronary artery disease worse, as shown in the research by Ference *et al*. (2017).

Healthy dietary choices (P = 0.197) and physical activity (P = 0.600) did not differ substantially between groups; nevertheless, healthy controls continued to exhibit somewhat healthier lifestyle patterns compared with the patient groups. These results are consistent with

earlier studies that demonstrate that while dietary patterns and physical activity may provide cardio protection, they are not necessarily reliable indicators of cardiovascular outcomes (Martinez-Gomez *et al.*, 2019). Instead, conventional risk factors, such as diabetes, smoking, and high blood pressure seem to be more important in the progression of disease, as supported by previous works (Liang *et al*., 2015; Yusuf *et al*., 2004).

5.2 PAI-1 Promoter Polymorphisms

5.2.1 Association between -675 4G/5G and -844G/A

Our results show that, though not exclusively, the PAI-1 promoter polymorphisms frequently co-occur in specific association groups. Specifically, most -675 4G/4G carriers had the −844 A/A genotype, most -675 4G/5G carriers had the −844 G/A genotype, and most -675 5G/5G carriers had the −844 G/G genotype.

This pattern suggests that the −675 4G/5G and −844 G/A variants are not randomly associated. Supporting this, Henry *et al*. (1997) reported strong linkage disequilibrium between these two polymorphisms ($D' = 0.98$, $P < 0.001$), D' close to 1 indicating that the alleles are almost always inherited together due to their close proximity in the promoter region, $P < 0.001$ confirming that this link is statistically significant rather than due to chance. These findings align with subsequent research linking PAI-1 promoter haplotypes to the incidence of Acute Cardiac Infarction (Abboud *et al.,* 2010; Morange *et al.,* 2000).

Linkage disequilibrium statistics between the −675 4G/5G and −844 G/A variants were not used in this study because the haplotype frequency at equilibrium can be biased to one side and produce LD by chance when the minor allele frequencies of one variant are very low relative to the sample size (Park, 2019).

5.2.2 -675 4G/5G Polymorphism and Coronary Artery Diseases

The -675 4G/5G polymorphism in this study demonstrated a distinct pattern of association with both Acute Cardiac Infarction and Chronic Stable Angina. In the healthy controls, the frequencies of the 4G/4G, 4G/5G, and 5G/5G genotypes were 4.0%, 50.0%, and 46.0%, respectively. Among patients with Acute Cardiac Infarction, the frequencies shifted to 14.0%, 60.0%, and 26.0%, indicating a noticeable change; however, this difference was not significant at the genotype level (p = 0.062). In contrast, the allele-based analysis, which compared allele frequencies between groups and the dominant model, which assesses the effect of possessing one or more risk alleles (as detailed in the Methodology part and presented in the Results), demonstrated significant correlations.

The 4G allele was more frequent in patients; whereas the 5G allele was more frequent and protective in healthy controls. Odds ratios were used to quantify the strength of these connections, with 95% confidence intervals indicating estimate precision. The chi-square (χ^2) test was used to determine statistical significance. (OR = 0.520, 95% CI: 0.290–0.933, P= 0.028; $\chi^2 = 4.854$). Patients were more likely to have at least one 4G allele, and that the 5G/5G genotype appeared protective (OR = 0.412, 95% CI: 0.178–0.957, p = 0.037; $\chi^2 = 4.340$).

In Chronic Stable Angina patients, genotype frequencies were 18.0% (4G/4G), 52.0% (4G/5G), and 30.0% (5G/5G). A statistically significant difference was detected between patients and healthy controls (p = 0.047). Similarly, allele-based analysis revealed a protective trend for the 5G allele and a higher frequency of the 4G allele in Chronic Stable Angina (OR=0.520, CI:0.290-0.933,P=0.028; $\chi^2 = 4.854$). Although the dominant model did not show significance in Chronic Stable Angina (OR = 0.503, 95% CI: 0.221–1.144, p = 0.099; $\chi^2 = 2.716$), the association tendency continued to be consistent in Acute Cardiac Infarction, with patients more likely to have at least one 4G allele, whereas healthy controls more frequently had the -675 5G/5G genotype.

Taken together, our results indicate that the 4G allele contributes to increased vulnerability to both Acute Cardiac Infarction and Chronic Stable Angina, whereas the 5G allele may be associated with a protective effect; these trends are consistent with the findings of Kumar *et al*. (2021) and established data from meta-analyses (Zhang *et al.,* 2014). The stronger

statistical signals reported in allele analyses likely reflect higher sensitivity at the allele level (Li, 2012), especially when genotype subgroup sizes are relatively small (Babaev & Zul'fugarova, 2017).

5.2.3 -844 G/A Polymorphism and Coronary Artery Diseases

Like the -675 4G/5G polymorphism, the −844 G/A polymorphism was analyzed using genotyping, allele frequency analysis, and dominant model evaluations between healthy controls and cardiac patients. The findings also indicated associations with Acute Cardiac Infarction and Chronic Stable Angina. In the healthy controls, the genotype frequencies of A/A, G/A, and G/G were 10.0%, 44.0%, and 46.0%, respectively, and for Acute Cardiac Infarction patients, the corresponding frequencies were 20.0%, 56.0%, and 24.0%.

Despite the genotype distribution revealing a borderline association ($p = 0.054$), the allele-based and dominant model analyses highlighted a significant effect, suggest that risk alleles found in at least one case or allele frequencies are stronger indicators of the risk. Acute Cardiac Infarction patients had a higher A-allele frequency ($\chi^2 = 5.333$, $P = 0.021$; OR = 0.510, 95% CI: 0.287–0.906), and individuals possessing at least one A allele had a greater risk than G/G individuals ($\chi^2 = 5.319$, $P = 0.021$; OR = 1.705, 95% CI: 1.032–2.818), indicating a 1.7-fold increased susceptibility for individuals with the A allele.

These outcomes are matched with earlier research suggesting that the A allele may increase the risk of Acute Cardiac Infarction by increasing PAI-1 production and reducing fibrinolysis (Abboud *et al*., 2010).

Conversely, a different pattern was observed in Chronic Stable Angina. The genotype distribution 20.0% (G/G), 52.0% (G/A), 28.0% (A/A) did not reach a significant association ($p=0.123$); however, allele level analysis still revealed a significant difference between groups ($\chi^2 = 4.119$, $P = 0.042$; OR = 0.522, 95% CI: 0.311–0.982), suggesting a potential role of A allele in Chronic Stable Angina susceptibility.

In Chronic Stable Angina, individuals having at least one copy of the A allele ($\chi^2 = 3.475$, $P = 0.062$; OR = 0.457, 95% CI: 0.199–1.048) did not reach statistical significance; this

marginal finding suggests a possible, yet inconclusive effect of the prevalent A-allele pattern. While Kumar *et al.* (2021) showed no significant association in allele, genotype, or dominant models, Park *et al.* (2020) did not perform allele-level analysis and found no significant genotype or dominant model associations in Chronic Stable Angina.

These results highlight the power of our allele-level findings regarding associations. Allele-based approaches may be able to identify minor genetic effects, the −844 A allele has been associated with disease risk at the allele level in general coronary artery disease (Su *et al.*, 2006).

The findings indicate that although the -844 A allele might serve as a risk-increasing variant for Acute Cardiac Infarction; it shows a non-significant (inconclusive), though potentially protective effect in Chronic Stable Angina. They also support the idea that the genetic association of the -844 G/A polymorphism may vary across different clinical presentations of coronary artery disease, which is consistent with earlier studies that have reported heterogeneity in PAI-1–related cardiometabolic profiles across disease subtypes (Basurto *et al.*, 2019).

5.3 Perspectives

The findings in the current study offer significant insights for further research on PAI-1 genetic variation regarding cardiovascular disease in Palestine, despite the imposed limits (e.g., difficult circumstances and relatively small sample size). The results emphasize the need for additional research to validate the involvement of the −844 G/A and -675 4G/5G polymorphisms in cardiac risk in various groups. In this context, the present work can be regarded as pilot study that determines local allele and genotype distributions and gives useful guidance for addressing the encountered challenges (e.g. choosing suitable sample sizes and analytical techniques) for subsequent, more definitive investigations.

This study examined the distribution of these polymorphisms in connection to significant cardiovascular risk factors, but it did not evaluate whether the variants are linked to conventional risk factors such as smoking, diabetes, hypertension, or a family history of

CAD. Even though descriptive patterns indicated a degree of linkage disequilibrium (LD) between the −675 4G/5G and −844 G/A promoter polymorphisms, formal examination of the association between the two promoter polymorphisms was not performed. Subsequent studies should investigate possible haplotype arrangements and their functional impact, as LD among these variants may affect PAI-1 expression and potentially alter disease risk.

Future studies should also include functional testing, such as measuring PAI-1 levels, alongside genotyping, that clarify the molecular pathways linking these genetic variants to disease causation. These variants can be studied along with other genes associated with inflammation and coagulation (blood clotting) to generate more precise genetic risk models. To determine how these variants influence the course of the disease, larger and more diverse study populations are required, along with long-term studies that follow patients over time to evaluate disease progression, risk, treatment responses, and outcomes. Overall, these directions may contribute to creating more individualized strategies for cardiovascular disease prevention and early detection.

5.4 Conclusions and Recommendations

This research suggests that coronary artery diseases in Palestinians population are significantly associated with the PAI-1 -675 4G/5G and −844 G/A promoter variants. Patients with Acute Cardiac Infarction and Chronic Stable Angina exhibited a higher frequency of the 4G and A alleles (respectively) compared to healthy controls, indicating that inheritance of these alleles may increase genetic susceptibility to CAD.

Dominant-model studies supported this conclusion by showing that individuals with at least one 4G or A allele (respectively) had a markedly increased risk of Acute Cardiac Infarction, suggesting their potential function in the disease progression. Conversely, the -675 5G/5G and -844 G/G genotypes appeared to be associated with protection. The patient groups also demonstrated a greater burden of cardiovascular risk factors like a family history of CAD, hypertension, diabetes mellitus and smoking. Genetic predisposition and modifiable factors interact extensively in the development of CAD. When genetic screening and conventional

risk assessment are combined, early detection and individualized prevention can be improved, thus decreasing the prevalence of CAD in genetically susceptible groups.

These results pave the way for gene profiling of PAI-1 -675 4G/5G and −844 G/A for individuals at high risk of cardiovascular disease. The purpose is to support early preventive strategies, including the modification of lifestyle habits, monitoring clinical status, and more individualized therapeutic strategies.

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Appendices

Appendix 1

Questionnaire at English

Arab American University _ Jenin

MSc Hematology and Immunology

Questionnaire Form

Dear all,

This questionnaire aims to collect some related information about this study entitled: "The association between genetic variations in the PAI-1 promoter sequence and the susceptibility to Acute cardiac Infarction and Stable Angina"

Out of our concern of the safety of Palestinian community, please answer the following questions in an objective way by clicking the appropriate choice.

N.B: Your answers shall be confidential; the collected information will solely be for scientific research purposes.

Thanks in advance.

The researchers:

The supervisor:

Personal Data

Name : …………………

Age : …………………

Sex : ☐ Male ☐ Female

Weight: ………………..

Social Status: ☐ Single ☐ Married ☐ Otherwise

Place of Residence: …………………

The Phone Number: ……………………

History with illness

1. Have you ever been diagnosed with heart disease such as acute myocardial infarction or stable angina)? ☐ Yes ☐ No
2. If your answer is "yes" what is the date of Myocardial Infarction or Stable Angina diagnosis? ______
3. Have you experienced previous heart-related issues before the diagnosis?

☐ Yes ☐ No ☐ Not sure

4. Do you have a family history of cardiovascular disease?
☐ Yes ☐ No ☐ Not sure

Lifestyle

1. How often do you engage in physical activity?

☐ Rarely or never ☐ Occasionally (1-2 times per week) ☐ Regularly (3 or more times per week)

2. Describe your typical diet:

☐ Mostly health ☐ Balance ☐ Mostly unhealthy

3. Are you a current or former smoker? ☐ Yes ☐ No

4. Do you suffer from stress and anger? ☐ Yes ☐ No

Medical Record

1. Have you ever been diagnosed with any of the following conditions? (Check all that apply)

☐ Hypertension (High Blood Pressure) If yes, please indicate your blood pressure ………...

☐ Diabetes

☐ Hyperlipidemia (High Cholesterol)

☐ Coronary Artery Disease (CAD)

☐ Peripheral Artery Disease (PAD)

☐ Stroke

☐ Cancer

☐ Bleeding disorder

☐ Thrombocytopenia

☐ Cardiogenic shock

☐ Chronic Kidney Disease, If yes, please provide the results of Creatinin ,BUN: -----------------------

☐ Chronic Liver Disease, If yes, please provide the results of ALT, AST and ALP : ----------

2. Did you undergo a surgical procedure, severe trauma, and fracture or organ biopsy in the last Ninety days? ☐ Yes ☐ No

Clinical status of the Patient

1. Which of these symptoms did you sense when you were diagnosed with a heart attack ? (Check all that apply)

☐ Pain in the chest ☐ Pain in jaw, neck, back or arms

☐ Weakness or dizziness ☐ Disturbance of vision in one or both eyes

☐ Shortness of breath ☐ Others

2. Have you undergone thrombolytic therapy in the last 24 hours or taken oral anticoagulants in the past 7 days? ☐ Yes ☐ No

   If yes, please specify the name of the medication ----------------------

Appendix 2

Questionnaire at Arabic

الجامعة العربية الامريكية – جنين

ماجستير علم الدم والمناعة

استمارة استبيان

السلام عليكم ورحمة الله وبركاته:

عزيزي المشترك اضع بين يديك هذا الاستبيان الذي أعد بهدف الحصول على البيانات المتعلقه بدراسة عنوانها : الارتباط بين الاختلافات الجينيه في تسلسل مثبطات منشط البلازمينوجين وقابلية الاصابة بإحتشاء عضلة القلب الحاد والذبحة الصدرية المستقرة وذلك لنيل درجة الماجستير في مجال علم الدم والمناعة .

وحرصا منا على سلامة المجتمع الفلسطيني ونظرا لاهمية ارائكم لذا يرجى قراءة العبارات بعناية وتعبئة الاستبيان بموضوعية علما بأن المعلومات التي ستدلي بها ستظل سرية وتستخدم فقط لأغراض البحث العلمي.

شاكرين لكم حسن تعاونكم

الباحث :

المشرف:

البيانات شخصية

الاسم :.............

العمر: ....

الجنس : ☐ ذكر ☐ أنثى

الوزن : ....

الحالة الاجتماعية: ☐ أعزب ☐ متزوج ☐ غير ذلك

مكان الإقامة: ....

رقم الهاتف : ....

التاريخ المرضي

هل تم تشخيصك من قبل باحدى أمراض القلب مثل احتشاء عضلة القلب الحاد أو الذبحة الصدرية المستقرة؟1.

☐ نعم ☐ لا 2. اذا كان اجابتك بنعم فما هو تاريخ تشخيصك باحتشاء عضلة القلب أو الذبحة الصدرية المستقرة؟ ----------------

هل واجهت مشاكل سابقة متعلقة بالقلب قبل التشخيص؟.3

☐ نعم ☐ لا ☐ غير متأكد

4.هل لديك تاريخ عائلي للإصابة بأمراض القلب والأوعية الدموية؟ ☐ نعم ☐ لا ☐ غير متأكد

نمط الحياة

1.كم مرة تمارس النشاط البدني؟1

☐ نادرًا أو أبدًا ☐ أحيانًا (1-2 مرات في الأسبوع) ☐ بانتظام (3 مرات أو أكثر في الأسبوع)

2.قم بوصف نظامك الغذائي؟ 2

☐ غير صحي في الغالب ☐ بين الصحي والغير صحي ☐ صحي في الغالب

3.هل أنت مدخن حالي أو سابق؟ ☐ نعم ☐ لا

4.هل تعاني من التوتر والغضب ؟ ☐ نعم ☐ لا

السجل الطبى

هل سبق أن تم تشخيص إصابتك بأي من الحالات التالية؟ (تحقق من كل ما ينطبق

☐ ارتفاع ضغط الدم إذا كانت الإجابة بنعم، يرجى الإشارة إلى ضغط دمك

☐ السكري

☐ فرط شحميات الدم (ارتفاع نسبة الكوليسترول)

☐ مرض الشريان التاجي

☐ مرض الشريان المحيطي

☐ سكتة دماغيه

☐ سرطان

☐ اضطراب النزيف

☐ قلة الصفيحات

☐ صدمة قلبية

☐ مرض الكلى المزمن، إذا كانت الإجابة بنعم، يرجى الادء بنتائج الكرياتينين واليوريا :............

☐ مرض الكبد المزمن، إذا كانت الإجابة بنعم، يرجى الادء بنتائج انزيمات الكبد
:...............

هل خضعت لعملية جراحية أو صدمة شديدة أو كسر أو خزعة عضو خلال التسعين يومًا الماضية؟ ☐ نعم
لا ☐

الحالة السريرية

أي من هذه الأعراض شعرت بها عندما تم تشخيص إصابتك بنوبة قلبية؟ (تحقق من كل ما ينطبق)1.

☐ ألم في الصدر ☐ ألم في الفك أو الرقبة أو الظهر أو الذراعين

☐ ضعف أو دوخة ☐ اضطراب الرؤية في إحدى العينين أو كلتيهما

☐ ضيق في التنفس ☐ اعراض أخرى

2.هل خضعت لعلاج التخثر خلال الاربع والعشرين ساعة الماضية أو تناولت مضادات التخثر الفموية خلال السبعة أيام الماضية؟ ☐ نعم ☐ لا إذا كانت الإجابة بنعم، يرجى تحديد اسم الدواء ----------------------
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Appendix 3

Reagents and Laboratory Equipment

Table: Chemicals and reagents used in this study:

Reagents	Provider
Agarose powder	Invitrogen
Ethidium Bromide	Hy-labs
Ladder 100 bp	Hy-labs
GoTaq® Green Master Mix	Promega
NEBuffer	Biolabs
Nuclease free water	Promega
QIAamp DNA Mini kit	Qiagen/Germany
TAE	Biolobical Industries
XhoI	New England Biolabs

Table: Materials and consumables

Materials	Provider
Analytical Balance	ADAM equipment
Gel Electrophoresis Chamber	Cleaver Scientific UV Transilluminator
Gel Documentation System	BIORAD
Flasks	SCHOTT
Gel Tray	BIORAD
Heating block	Biotech
Microcentrifuge	SIGMA
Micropipettes	CAPP ApS
Microwave	Mega
Nanodrop Analyzer	IMPLEN
Needles	ZHEJANG INI MEDICAL DEVICES CO., LTD

Pipets tips	LIFEGENE
Sterile EDTA Vacationer tubes	Al-Marwa Medical Supplies and General Trading
Syringes	JCMD Co., Ltd
Thermo cycler	Medipharm
Vortex	AHN Biotechnologie GmbH

الملخص

تُعدّ أمراض القلب والأوعية الدموية، مثل احتشاء عضلة القلب الحاد والذبحة الصدرية المستقرة المزمنة، من أبرز أسباب المرض والوفاة على مستوى العالم، بما في ذلك فلسطين. ويساهم مثبط المنشّط البلازميني-1 (PAI-1) في تنظيم عملية تكسير الجلطات الدموية، وقد ارتبطت المستويات المرتفعة من هذا المثبط بزيادة خطر الإصابة بأمراض القلب والأوعية الدموية.

قد تؤثر الاختلافات الجينية في منطقة المحفّز لجين مثبط المنشّط البلازميني-1 (PAI-1)، وتحديدًا تعدد الأشكال الجينية (675 4G/5G- و844G/A-)، في إنتاج هذا المثبط. ومع ذلك، لا يزال تأثير هذه الاختلافات الجينية غير مدروس بشكل كافٍ لدى الفلسطينيين.

هدفت هذه الدراسة إلى تقييم وجود المتغيرين الجينيين 675 4G/5G− (rs1799889) و844 G/A− (rs2227631) في جين مثبط المنشّط البلازميني-1 (PAI-1)، ودراسة ارتباطهما المحتمل لدى المرضى الذين تم تشخيصهم باحتشاء عضلة القلب الحاد أو الذبحة الصدرية المستقرة في شمال فلسطين.

تم استخدام تصميم دراسة الحالات والشواهد، حيث ضمّت ثلاث مجموعات: مرضى احتشاء عضلة القلب الحاد، ومرضى الذبحة الصدرية المستقرة المزمنة، ومجموعة الشواهد السليمة المطابقة في العمر والجنس، بواقع خمسين مشاركًا في كل مجموعة. جُمعت البيانات السريرية والديموغرافية عبر استبيانات مُنظَّمة على مدار عام من النظام الصحي الفلسطيني، شاملا مستشفيات حكومية... ومستشفى النجاح الوطني، ومستشفى الرازي.

تم عزل الحمض النووي الجينومي من الدم الكامل، وأُستخدم تفاعل البوليميراز المتسلسل الخاص بالأليل (Allele-Specific PCR) لتحديد النمط الجيني 675 4G/5G- ، كما استُخدم تفاعل البوليميراز المتسلسل متبوعًا بتحليل تباين اطوال الحمض النووي بالقطع الإنزيمي (RFLP) لتقييم النمط الجيني 844G/A-.

أظهرت النتائج أن المرضى المصابين باحتشاء عضلة القلب الحاد والذبحة الصدرية المستقرة المزمنة لديهم أكثر من مجموعة الأشخاص السليمين الأليلان (4G و844A-)، كما كان احتشاء عضلة القلب الحاد أكثر شيوعًا لدى من عندهم أليل واحد على الأقل من الأليلين (4G أو A). وعلى ما يبدوا فإن هذه الاشكال الجينية مرتبطة بقابلية الإصابة بأمراض الشرايين التاجية في فلسطين، إلى جانب عوامل الخطر الشائعة مثل داء السكري، وارتفاع ضغط الدم، والتدخين، والتاريخ العائلي لأمراض الشريان التاجي.

وفي الختام، تظهر هذه الدراسة ارتباطا بين الأشكال الجينية (675 4G/5G- و844G/A-) في محفّز جين مثبط المنشّط البلازميني-1 (PAI-1) وارتفاع معدل الإصابة بأمراض الشرايين التاجية لدى الفلسطينيين. ويوصى بإجراء دراسات أوسع نطاقًا تتضمن قياس مستويات مثبط المنشّط البلازميني-1 (PAI-1) في البلازما، وذلك لتحديد أهميته السريرية.

الكلمات المفتاحية: PAI-1، تعدد الأشكال الجينية، 675 4G5G- ، 844 G/A- ، الفلسطينيون.