Arab American University

Faculty of Graduate Studies

Department of Health Sciences

Master Program in Immunohematology

Association Between P582S HIF-1A Gene Polymorphism And Hematological Parameters Among Healthy People In The Northern Districts Of The West Bank-Palestine.

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Thesis Approval

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

This work is lovingly dedicated to my family mother, father**,** sisters and brothers especially my sister Doaa whose unwavering support, patience, and encouragement have been my greatest source of strength throughout this journey. Your belief in me made all of this possible.

I also dedicate this achievement to my entire family, who have stood by me every step of the way, sharing in my challenges and celebrating my successes. This is as much yours as it is mine.

I sincerely thank my dear friend Shahlaa Omer for her unwavering motivation and heartfelt encouragement, which lifted me during the most challenging moments and inspired me to keep going.

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Association Between P582S HIF-1A Gene Polymorphism and Hematological Parameters Among Healthy People In The Northern Districts Of The West Bank-Palestine.

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Abstract

Background: More than 60 genes responded to Hypoxia-inducible factor 1-alpha (HIF-1α) as a vital regulator of transcription for a huge number of cellular processes, such as angiogenesis, erythropoiesis, iron metabolism, and oxygen balancing. Variations in the HIF-1α gene, primarily the P582S (rs11549465) polymorphism, have been associated with alterations in gene expression, protein stability, and an increased risk for various disorders, including different types of cancers and hematological diseases (Haase, 2013; Weidemann & Johnson, 2008a).

Objectives: The purpose of this study was to investigate whether there was any association between the P582S HIF-1A Gene Polymorphism and Hematological Parameters among healthy people in the Northern Districts of the West Bank-Palestine, as well as to determine any demographic and clinical characteristics that might be linked to this genetic variance.

Methods: 100 people were recruited in this cross-sectional study. CBC, followed by PCR-RFLP technology, was used to genotype the HIF-1A polymorphism. Sanger sequencing was conducted to confirm the PCR-RFLP results. SPSS and Python were used to perform statistical analysis. To evaluate relationships between HIF-1A polymorphism and hematological parameters, chi-square tests, and one-way ANOVA. A 0.05 level of significance was used.

Results: The mean age of participants was 31.4 years, and the mean BMI was 24.9 ± 3.46 kg/m2. Most of the participants did not exercise (81.0%), and about 18.0% were current tobacco smokers. Frequency of 582S (T) mutant allele was 47.0% while the frequency of the 582P (C) wild-type allele was 53.0%. No association was found between P582S HIF-1A and hemoglobin level (p=0.56), red blood cell count (p=0.62), hematocrit (p=0.16), mean cell volume (p=0.32), mean corpuscular volume (p=0.68), mean corpuscular hemoglobin concentration (p=0.62), and red blood cell distribution width (p=0.89).Furthermore, there were no apparent differences in the

distribution of P582S genotypes by participant age, body mass index, smoking status, or exercise routine ($p>0.05$).

Conclusions: In conclusion, hematological indices in healthy individuals may not be correlated with the P582S HIF-1A polymorphism. To validate this conclusion, more research in different populations is required.

Keywords: (HIF-1A), P582S, rs11549465, polymorphism, hemoglobin.

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List of Definition of Abbreviations

Title	Abbreviations
Arab American University - Palestine	AAUP
Basic helix-loop-helix	bHLH
Body mass index	BMI
Chronic obstructive pulmonary disease	COPD
Complete blood cell count	CBC
Erythropoietin	EPO
Ethylenediaminetetraacetic acid	EDTA
Hematocrit	Hct
Hemoglobin	Hb
Hypoxia response elements	HREs

Title	Abbreviations
Hypoxia-inducible factor 1-alpha	HIF-1α
Mean corpuscular hemoglobin	MCH
Mean corpuscular volume	MCV
Oxygen species	ROS
Oxygen-dependent degradation	ODD
Per-ARNT-Sim	PAS
Polycythemia Vera	PV
Polymerase Chain Reaction	PCR
Proline	Pro
Prolyl hydroxylases	PHDs
Red blood cell	RBC

Title	Abbreviations
Serine	Ser
Single-nucleotide polymorphisms	SNPs
Vascular endothelial growth factor	VEGF
Von Hippel-Lindau	VHL
World Health Organization Eastern Mediterranean Regional Office	WHO EMRO

Chapter One: Introduction

Background

Hypoxia-inducible factor 1-alpha (HIF-1α) is one of the most important transcriptional factors in eukaryotic cells, regulating gene expression in response to varying oxygen levels and concentrations. (Carmeliet et al., 1998;Sharma et al., 2023). It plays an important role in various physiological processes, including angiogenesis, erythropoiesis, and glucose metabolism(Carmeliet et al., 1998).

HIF-1α emerges as a central orchestrator of cellular adaptation to oxygen deprivation, combining genetic and environmental conditions to maintain homeostasis under hypoxic stress. This master transcription factor, whose discovery was honored with a Nobel Prize, operates as a heterodimer with HIF-1β, employing a developed oxygen-sensing mechanism mediated by prolyl hydroxylases (PHDs) and the von Hippel-Lindau (VHL) tumor suppressor protein to regulate its stability and activity (Ziello et al., 2007).

HIF-1α regulates vital physiological responses by transcriptionally activating more than 60 target genes. This involves vascular endothelial growth factor (VEGF)-induced angiogenesis, metabolic reprogramming toward glycolysis, erythropoiesis-induced erythropoietin (EPO), and iron homeostasis-induced hepcidin suppression. (Bailey et al., 2013a)

HIF-1α genetic variants, especially the P582S polymorphism (rs11549465), have important therapeutic implications because they affect transcriptional activity, protein stability, and patterns of disease susceptibility. The unique genetic history of the Palestinian people, which is characterized by frequent hematological disorders and stressful environmental conditions, provides a crucial background for examining the intended effects of these polymorphisms on hemoglobin ranges, erythrocyte indices, and pathologies linked to hypoxia(Bailey et al., 2013a).

The intricate relationship between genetic diversity, systemic adaptation, and the molecular control and regulation of HIF-1α at the nexus of precision medication treatments for oxygen-sensitive diseases and basic cellular physiology (Ziello et al., 2007).

This study aims to examine whether variations in red blood cell indices and other hematological parameters are associated with the genetic diversity of the HIF-1A P582S polymorphism.

Molecular Structure and Regulatory Mechanisms of HIF-1:

Transcriptional Regulation of Target Genes

The hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor composed of two subunits, each containing basic helix-loop-helix (bHLH) and Per-ARNT-Sim (PAS) domains. Specifically, HIF-1α and HIF-1β (also known as ARNT, aryl hydrocarbon receptor nuclear translocator) (Semenza et al., 1996). Although HIF-1β is constitutively produced, the complex's main oxygen-sensing component is the HIF-1α subunit, which is strictly controlled by cellular oxygen levels. (Semenza et al., 1996; Semenza, 2008) .

The Nobel Prize in Physiology or Medicine was awarded in 2019 for the revolutionary discovery of HIF and its oxygen-sensing mechanism, highlighting its essential role in cellular physiology (C. C. Lee et al., 2020). The structural features of the HIF-1α polypeptide include a nuclear localization signal motif, a PAC (PAS-related C-terminal) domain, a primary helix-loop-helix domain close to the C-terminal, and transactivation domain names (CTAD and NTAD) that are separated by an inhibitory domain that may suppress their transcriptional activities(Wang et al., 1995)—Figure 1.1 Schematic structures of HIF1α and HIF1β domains.

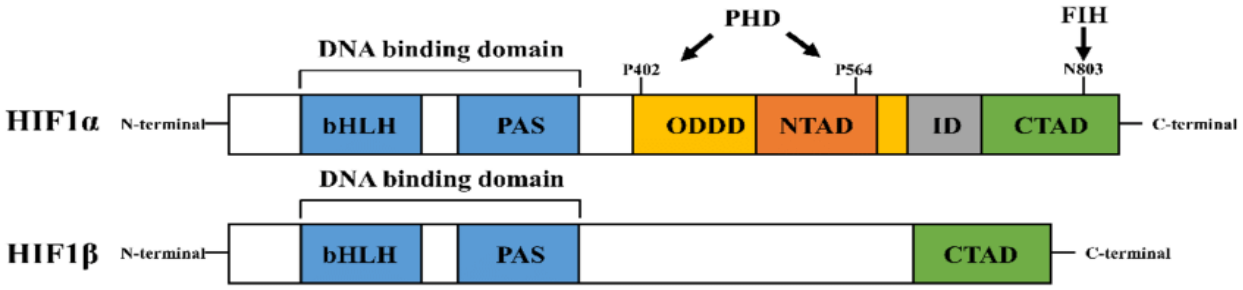


Figure1.1 Schematic structures of HIF1α and HIF1β domains.(H. J. Lee et al., 2019).

Oxygen-Dependent Regulation

HIF-1α is mostly regulated at the post-translational stage by a sophisticated oxygen-sensing mechanism. Under normoxic circumstances, oxygen-based prolyl hydroxylases (PHDs)

rapidly hydroxylate HIF-1α, allowing the von Hippel-Lindau (VHL) tumor suppressor protein to recognize it (Oellinger, 1997).

Due to this interaction, HIF-1α is ubiquitinated and then degraded by proteases, which keeps the protein's constant expression low. In contrast, the oxygen-dependent hydroxylases are blocked below hypoxic conditions, which stops HIF-1α from degrading and permits its buildup inside the cytoplasm(Oellinger, 1997; Semenza, 2008). Once in the nucleus, the accumulated HIF-1α dimerizes with HIF-1β to form the vibrant HIF-1 complex.

When HIF-1α dimerizes with ARNT, it undergoes a significant structural shift in addition to protein stabilization. Studies have confirmed that this dimerization results in an allosteric modification that increases HIF-1α's DNA binding ability and gives it further protection against proteolytic digestion in vitro. Crucially, despite their capacity to dimerize with HIF-1α, shortened versions of ARNT do not accomplish this conformational alternation, suggesting a particular structural need for full activation (Oellinger, 1997). Figure 2.1 shows that.

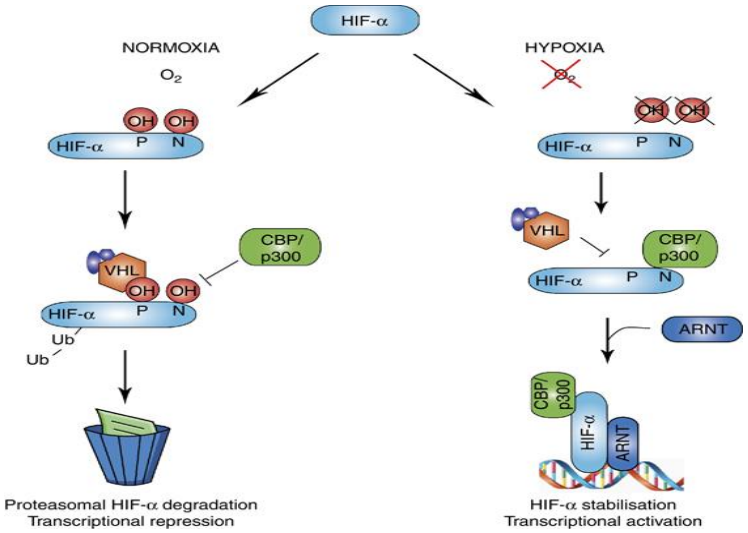


Figure1.2 The oxygen-dependent regulation of the HIF complex.(Martin et al., 2011)

Furthermore, it appears that molecular chaperones such as heat shock protein 90 (hsp90) have a significant role in folding HIF-1α into a shape that can offer an allosteric alteration.

Physiological Roles of HIF-1 in Cellular Adaptation:

Transcriptional Regulation of Target Genes

The active HIF-1 complex responds to hypoxic conditions by binding to certain DNA sequences in the promoter regions of many target genes known as hypoxia response elements (HREs)(Chen & Sang, 2016; Semenza, 2008). According to Peyssonnaux et al. (2007), these HREs often have the consensus sequence 5'-[A/G] CGTG-3'. Using this interaction, HIF-1 coordinates the transcription of over 60 genes, which collectively control the transport of oxygen to hypoxic tissues and promote metabolic adaptation to reduced oxygen availability(Semenza, 2008).

Metabolic Reprogramming and Energy Homeostasis

One of HIF-1's most important roles is mediating the metabolic switch from oxidative phosphorylation to glycolysis in hypoxic conditions (Basheeruddin & Qausain, 2024). This metabolic reprogramming allows cells to generate ATP through less oxygen-dependent pathways, keeping power homeostasis with the reduced amount of accessible oxygen (Semenza, 2008).

According to recent studies, this change is an active cell strategy for steady ATP synthesis while reducing reactive oxygen species (ROS) rather than only a passive reaction to oxygen deprivation (Semenza, 2008).To maximize the stability between glycolytic and oxidative metabolism over the whole range of physiological oxygen tensions, HIF-1 controls important glycolytic enzymes, glucose transporters, and components that prevent mitochondrial respiration (Semenza, 2008; Basheeruddin & Qausain, 2024).

Regulation of Iron Homeostasis

Along with the regulatory function of hepcidin, the master regulator of systemic iron balance, HIF-1 plays a crucial role in maintaining iron homeostasis through a number of pathways (Peyssonnaux et al., 2007;Hintze & Mcclung, 2011). Hepcidin prevents iron absorption from the gut and iron release from macrophages by binding to ferroportin 1 (FPN1), the only mobile iron exporter, and causing its internalization and destruction (Hintze & Mcclung, 2011).

By instantly attaching to consensus HREs in the hepcidin promoter, HIF-1 inhibits the production of hepcidin in hypoxic conditions(Peyssonnaux et al., 2007). To support erythropoiesis and other

oxygen-dependent processes, this inhibition increases the availability of iron. At the same time, HIF-1 increases the expression of genes related to iron acquisition, such as transferrin, transferrin receptors, and other iron delivery machinery (Peyssonnaux et al., 2007; Hintze & McClung, 2011).

Genetic Polymorphisms in HIF-1α and Their Clinical Significance:

Genetic polymorphism within the HIF1A gene

Located on chromosome 14q21-24, the HIF-1α gene has a large number of functionally significant single-nucleotide polymorphisms (SNPs). The protein's oxygen-structured degradation domain has two well-researched polymorphisms: G-to-A substitution at role -1790 (-1790G>A, A588T, or rs11549467) and C-to-T alteration at function-1772 (-1772C>T, P582S, or rs11549465). Whereas the latter results in an alanine to threonine alteration at codon 588, the former generates a proline to serine substitution at codon 582. Due to their closeness to the N-terminal transactivation domain, these changes may have an impact on the protein's stability, transactivation capacity, and functional interest (Bahadori, Uitz, Mayer, ̈rgenHarauer, et al., 2010).

Clinical and Population Associations of Genetic Variation

Meta-analyses have found sizeable associations among the rs11549465 (1772C/T) polymorphism and elevated disorder, mainly among Asian populations(Y. Li et al., 2015). Individual studies have connected these polymorphisms to susceptibility to diverse conditions, together with exceptional most cancers, cardiovascular diseases, renal problems, and hematological abnormalities.

According to research, the P582S polymorphism may also shield regular male blood donors from iron deficiency by affecting red blood cell mobility indices, which is especially pertinent to erythropoiesis (Torti et al., n.d.).This discovery emphasizes how important HIF-1 is for maintaining hematological parameters and coordinating erythropoietin-mediated bone marrow responses to anemia.

Genetic Heritage and Hematological Health in the Palestinian Context:

Genetic Diversity and Research Gaps

The Palestinian populace represents a unique genetic tapestry shaped through centuries of migration, settlement, and admixture throughout the Eastern Mediterranean region. This wealthy

genetic history gives valuable opportunities for information on human genetic variety, evolution mechanisms, and disorder susceptibility pattern(Haber et al., 2013). Despite this ability, complete genetic research focusing on functional polymorphisms like those in the HIF-1α gene remains scarce in this population(Peyssonnaux et al., 2007). This research gap is especially high-quality given the unique genetic architecture that can have developed in response to regional environmental pressures and ancient population actions.

Hematological Challenges and Environmental Factors

Hematological disorders, in particular various types of anemia, represent massive public health challenges in Palestine. These conditions are motivated using multiple factors, which include dietary deficiencies, socioeconomic constraints, and potentially underlying genetic predispositions(Peyssonnaux et al., 2007; Hintze & McCgggg, 2011).

Iron deficiency anemia is specifically normal, women of reproductive age and children, highlighting the complex interplay between environmental conditions and physiological needs. Understanding the superiority and purposeful effect of HIF-1α polymorphisms in this population may want to offer valuable insights into genetic factors modulating iron homeostasis, erythropoiesis, and adaptive responses to hypoxic pressure(Semenza, 2008**;** Peyssonnaux et al., 2007)**.**

To completely understand the impact of the genetic variants altering the form of HIF-1α, population-specific studies are necessary since they show strong correlations with a variety of physiological and pathological situations. A useful setting is provided by the understudied Palestinian population, with its unique genetic background and typical hematological difficulties. In addition to filling up large knowledge gaps, expanding research in this area should likely guide focused therapies that address population-specific health issues about erythropoiesis, iron metabolism, and oxygen sensing.

The purpose of this study is to fill the knowledge gap in the Palestinian community by examining the relationship between hematological parameters and the P582S polymorphism in the HIF-1α gene. It sheds light on how this genetic diversity may alter blood-related characteristics in Palestinians, adding important information to our knowledge of the genetic elements influencing this population's hematological health.

Statement of the Problem

Genetics is one of the numerous elements that affect blood health. One gene that has garnered interest is HIF-1α, which controls iron metabolism and blood cell synthesis and is crucial in how our bodies react to low oxygen levels. Red blood cell counts and hemoglobin levels may be impacted by the P582S polymorphism, a specific mutation in this gene.

Significant obstacles face the Palestinian healthcare system, such as a shortage of finance, unstable political conditions, and restricted access to state-of-the-art diagnostic equipment (Rahim et al., 2009). These restrictions make it difficult to study genetic polymorphisms like HIF-1α P582S and how they relate to hematological markers. Even while research on genetic factors influencing blood health is growing, there is still a lack of studies specifically focusing on how these genetic differences impact Palestinians.

It is increasingly recognized that genetic variations may have different effects depending on gender. However, most studies have not thoroughly investigated these sex-specific differences. Do not fully understand if the P582S mutation in the HIF-1A gene has distinct effects on males and females in the Palestinian population.

Investigating this is essential for advancing individualized treatment and expanding treatment options specific to each gender. In order to get a better understanding of the genetic factors that influence blood fitness in this system, this study looks at how this gene variation affects blood parameters in both men and women in the West Bank.

A shortage of information specific to the Palestinian population about this polymorphism creates a significant knowledge gap that might impede the progressive identification of hereditary risks for hematological illnesses such as polycythemia and anemia. Closing this gap is essential for strengthening diagnostic and therapeutic strategies, especially in an area influenced by unique genetic advancements and environmental factors.

The study's objective

This study's primary objective is to determine the prevalence of the HIF-1α P582S gene polymorphism in Palestinians and whether it is linked to blood-related parameters such as hematocrit, hemoglobin levels, and red blood cell counts. Compare blood parameters with different genotype variations. And to investigate any demographic and clinical characteristics that might be

linked to this genetic variance. And investigate if there are any differences between the two genders.

Research Question

I. What is the prevalence of the HIF-1α P582S gene variant among the Palestinian population?
II. Does the HIF-1α P582S polymorphism influence hematological parameters such as hemoglobin levels, red blood cell count, and hematocrit in the Palestinian population?
III. Are there gender-based differences in the frequency of the HIF-1α P582S genetic variant among Palestinians?
IV. Is the HIF-1α P582S gene variant associated with health conditions such as anemia or other disorders related to reduced blood oxygen levels?

Hypothesis of the Study

The HIF-1α P582S polymorphism is prevalent among individuals of Palestinian descent. There is a significant association between the HIF-1α P582S polymorphism and variations in hematological parameters. There are notable differences in the incidence of the HIF-1α P582S polymorphism between males and females. The HIF-1α P582S polymorphism is linked with health conditions such as anemia or hypoxia-related disorders in the Palestinian population.

Study Limitations

- Regional Generalizability: the effects are particular to the West Bank's northern districts and won't apply to other areas.
- Sample Size and Recruitment: challenges, Political instability, and in some areas may limit participant recruitment. A small or non-representative sample could reduce the statistical power and generalizability of the findings.
- Cross-Sectional Design: the study's cross-sectional design makes it unlikely to demonstrate causal links or the polymorphism's long-term consequences.

- Funding and limited funding might restrict the scope of the study, including sample size, lab testing, and statistical analysis.

Definitions of Concepts and Procedures

- HIF-1α P582S Polymorphism: A single-nucleotide polymorphism (rs11549465, C1772T) in the HIF-1α gene ensuing in a proline-to-serine substitution at codon 582 (Tanimoto et al., 2003).
- Hematological Parameters: Blood measurements, which include hemoglobin (Hb), red blood cell (RBC) count, and hematocrit (Hct), reflecting oxygen-carrying capacity and blood fitness (F. S. Lee & Percy, 2011).
- Polymerase Chain Reaction (PCR): a molecular method used to increase and detect the HIF-1α P582S polymorphism in DNA samples(Akkoub & Khabour, 2023).
- Prevalence: the percentage of observed individuals with the HIF-1α P582S polymorphism at the time of the information series(Beall, 2000).

Study's Structure

The study is organized as follows:

Literature Review: A comprehensive overview of existing research on HIF-1α polymorphisms, hematological parameters, and their relevance to the Palestinian population.

Methods: A detailed description of the study design, participant demographics, data collection methods, and statistical analyses.

Results: Presentation of key findings, including the prevalence of the HIF-1α P582S polymorphism and its association with hematological parameters.

Discussion: Interpretation of findings in the context of existing literature, limitations, and implications for healthcare in Palestine.

Chapter Two: Literature Review

Overview of HIF-A genes

Hypoxia-inducible factor-1 (HIF-1) is a heterodimer consisting of an alpha and a beta subunit. By triggering the transcription of numerous genes, including those related to energy metabolism, angiogenesis, apoptosis, and other genes whose protein products enhance oxygen delivery or facilitate metabolic adaptation to hypoxia, HIF-1 acts as a master regulator of the cellular and systemic homeostatic response to hypoxia. Thus, HIF-1 is crucial for the pathophysiology of ischemic illness, tumor angiogenesis, and embryonic vascularization. For this gene, alternatively spliced transcript variants that produce distinct isoforms have been identified (Semenza, 2008).

Genetic variation in the HIF pathway, specifically within the HIF1A gene, can impact the susceptibility of males and females to various diseases and may affect hematological parameters. This review examines the molecular mechanisms of the hypoxia-inducible factor (HIF) pathway, with a particular emphasis on the functional significance of HIF1A gene polymorphisms, specifically the Pro582Ser (P582S, rs11549465) variant, and their associations with hematological parameters, with a specific focus on their relevance to the Palestinian population.

The Palestinian population is characterized by a unique genetic history formed by historical, geographical, and sociopolitical elements(Wahidmurni, 2017). According to studies, iron deficiency and anemia are fairly prevalent, specifically among women and children, in Palestine(Sirdah et al., 2014). However, there is a lack of records regarding the genetic determinants of hematological parameters in this population.

The HIF pathway: molecular regulation and physiological roles.

HIF Structure and oxygen-dependent regulation.

HIFs are transcription factors made of two main domains: an oxygen-sensitive α-subunit (HIF-1α, HIF-2α, and HIF-3α) and a β-subunit (HIF-1β) that is always present in the cell. Under normal oxygen levels (normoxia), enzymes called prolyl hydroxylase domain proteins (PHDs) add hydroxyl groups to specific proline amino acids on the HIF-α subunit: iron, oxygen, and 2-oxoglutarate act as cofactors for this hydroxylation. The von Hippel-Lindau (VHL) E3 ubiquitin ligase complex detects hydroxylated HIF-α and binds it with ubiquitin molecules. This targeting keeps HIF-α levels extremely low by flagging it for destruction by the proteasome, a protein-degrading complex (Luo et al., 2022; Lim et al., 2013).

When oxygen levels drop (hypoxia), PHD enzymes are inhibited because they need oxygen to function. As a result, HIF-α is no longer hydroxylated and escapes degradation. Stabilized HIF-α accumulates in the cell, moves into the nucleus, and pairs with the always-present HIF-1β subunit. This HIF-α/β complex then binds to specific DNA sequences called hypoxia-response elements (HREs) in the promoters of target genes, activating their expression to help the cell adapt to low oxygen conditions (Luo et al., 2022; J. Li et al., 2020), as shown in Figure 2.1.

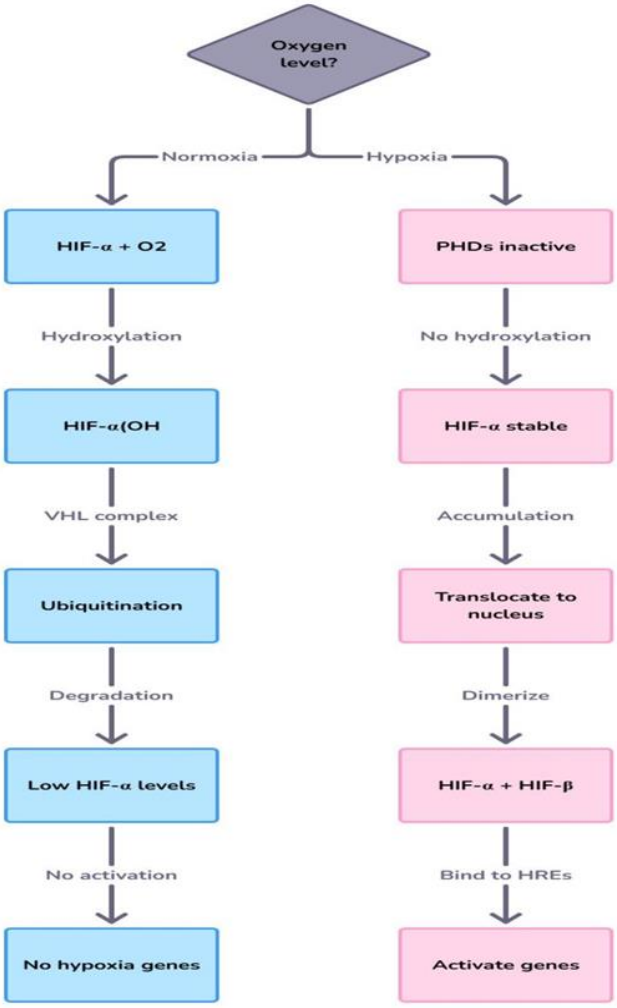


Figure 2.1 illustrates the regulation mechanism of Hypoxia-Inducible Factor alpha (HIF-α) in response to cellular oxygen levels.

Isoform-Specific Functions of the HIF-A gene

HIF-1α: controls acute hypoxic responses, which involve glycolysis (GLUT1, PDK1) and angiogenesis (VEGF). Likewise, by suppressing pyruvate dehydrogenase activity, which alters mitochondrial metabolism, it reduces oxidative phosphorylation (Weidemann & Johnson, 2008b).

HIF-2α:Controls long-term adaptations, including erythropoietin (EPO) synthesis and iron metabolism (Weidemann & Johnson, 2008; Luo et al., 2022). HIF-2α-deficient animals develop

severe anemia, and genetic research highlights its non-redundant role in erythropoiesis (Weidemann & Johnson, 2008b).

As a negative regulator, HIF-3α inhibits transcription and angiogenesis mediated by HIF-1α/2α (Luo et al., 2022).Table 2.1**.** Shown is a summary of publications in presentations to illustrate the molecular regulation and distinct physiological roles of HIF isoforms in oxygen sensing and hypoxia response.

Table 2.1 summarizes publications to illustrate the molecular regulation and distinct physiological roles of HIF isoforms in oxygen sensing and hypoxia response.

Aspect	**Details**	**Key Targets / Notes**
HIF Complex Composition	Heterodimer of oxygen-labile α-subunit (HIF-1α, HIF-2α, HIF-3α) and constitutive β-subunit (HIF-1β)	α-subunit stability is regulated by oxygen-dependent hydroxylation and ubiquitination via PHDs and VHL
Oxygen-Dependent Regulation	Under normoxia, PHD enzymes hydroxylate HIF-α → VHL-mediated ubiquitination → proteasomal degradation	Requires oxygen, iron, and 2-oxoglutarate as cofactors; hypoxia inhibits PHDs → HIF-α stabilization
HIF-1α Functions	Acute hypoxia responses: metabolic reprogramming and angiogenesis	Upregulates VEGF (angiogenesis), GLUT1, PDK1 (glycolysis), and inhibits pyruvate dehydrogenase to reduce ROS
HIF-2α Functions	Long-term hypoxia responses: erythropoiesis and iron metabolism	Drives EPO transcription in renal cells; essential for red blood cell production; non-redundant in anemia

HIF-3α Functions	Negative regulator of HIF-1α and HIF-2α transcription and angiogenesis	Inhibits transcriptional activity and angiogenesis mediated by HIF-1α/2$^{\alpha}$
Physiological Roles	Maintains oxygen homeostasis and metabolic adaptation	Switches metabolism from oxidative phosphorylation to glycolysis; reduces ROS via PDK-mediated mitochondrial inhibition
Erythropoiesis & Angiogenesis	HIF-2α induces EPO → stimulates erythropoiesis; HIF-1α induces VEGF → promotes angiogenesis	In myocardial ischemia, HIF-1α upregulates protective genes (HO-1, iNOS) reducing infarct size

HIF in erythropoiesis, angiogenesis, and metabolic regulation.

HIF-1α and HIF-2α coordinate the body's response to hypoxia by regulating the expression of genes that promote adaptation.

Erythropoiesis: HIF-2α at once binds and stimulates EPO gene expression in the kidneys, leading to increased red blood cell production (Weidemann & Johnson, 200; Lim & Sandison, n.d.).

Angiogenesis: HIF-1α stimulates VEGF and different angiogenic factors, which induce the formation of new blood vessels to get better tissue oxygenation (J. Li et al., 2020).

Metabolic Reprogramming: HIF-1α adjust cell metabolism from oxidative phosphorylation to glycolysis, meanwhile regulating glucose transporters and glycolytic enzymes, and with the aid of inhibiting mitochondrial respiration by pyruvate dehydrogenase kinase (PDK).In pathological conditions, like myocardial ischemia, HIF-1α activation upregulates protective genes (e.g., HO-1, iNOS), decreasing tissue harm (J. Li et al., 2020).

HIF gene polymorphism

Overview of HIF1A Gene Variability

The HIF-1α subunit is encoded by the HIF1A gene, which is situated on chromosome 14q23.2(Semenza, 2001). There are several single-nucleotide polymorphisms (SNPs) in HIF-1α

that have been demonstrated to influence its regulation or function. These SNPs have been associated to an increased risk of various kinds of diseases, including cancer, as well as cardiovascular, respiratory, and hematological problems. (Faculty, 2018).

The Pro582Ser (P582S, rs11549465) Polymorphism

The most widely studied HIF-1α genetic polymorphism is rs11549465. This results in a C-to-T transition in the 12th exon at nucleotide 1772, which causes the amino acid at position 582 of the codon (Pro582Ser) to change from proline (Pro) to serine (Ser) This polymorphism is found in an oxygen-dependent HIF-1α degradation domain, which is crucial for the control of HIF-1α balance(Uznetsova et al., 2013).

The T allele (Ser variation) enhances HIF-1α transcriptional potential by improving protein balance under hypoxic conditions, without significantly altering degradation in normoxic conditions. This benefit-of-characteristic impact may influence the physiological response to hypoxia and the likelihood of illness. (Ali et al., 2022; Zhang et al., 2021).

Other Notable HIF1A Polymorphisms

Rs11549467 (G1790A, Ala588Thr): Another missense version inside the ODD domain, frequently studied along P582S, Linked to increased cancer risk and HIF-1α activity (Cancer, n.d.; Zhang et al., 2021)

Rs2057482 (C/T in 3'-UTR): May affect HIF-1α expression through microRNA binding, although meta-analyses display no regular relationship to disease susceptibility (Cancer, n.d.)

Other SNPs (e.g., rs10873142, rs11549456) have been investigated in ischemic stroke and various other conditions, with certain haplotypes exhibiting protective or risk-enhancing effects. (Jamali et al., 2020). Table 2.2 summarizes the main HIF1A polymorphisms and the diseases they are associated with, primarily based on comprehensive evaluations and meta-analyses of the literature.

Table 2.2 summarizes the main HIF1A polymorphisms and the diseases they promote, primarily based on a comprehensive assessment and meta-analyses of the literature.

SNP (rsID)	Polymorphism	Genomic Location	Disease Associations	Notes	References
rs11549465	1772 C/T (Pro582Ser)	ODD domain (exon region)	Cancer (variable association, e.g., prostate cancer), COPD, skin diseases, diabetic complications, CVD	Conflicting results in some cancers; significant in Caucasians for overall disease risk	(Faculty, 2018;Ali et al., 2022)
rs11549467	1790 G/A (Ala588Thr)	ODD domain (exon region)	Cardiovascular disease, diabetic complications, and other diseases	An allele associated with increased disease risk under the recessive model, especially in Asians	(Faculty, 2018; Ali et al., 2022)
Other SNPs (total 34 studied)	Various (5'-flanking, introns, 3'-UTR)	Various genomic regions	Cancer, cardiovascular, bone, renal, hematological, immunological, respiratory, gastrointestinal diseases	Some SNPs show protective effects; others show no association	(Faculty, 2018; Strauss et al., 2023)

Prevalence of HIF1A P582S Polymorphism in different populations

Genotype frequencies differ considerably among ethnic groups, being more common in Caucasians than Asians. Generally, around 15-20% mutant allele frequency in Caucasians and a decrease of 4% in Asians(Akkoub & Khabour, 2023; D. Li et al., 2013; Bahadori et al., 2010).

In a Jordanian take a look at related to 310 ladies, the frequency of the 582S (T) mutant allele was 17.5%, while the wild-type 582P (C) allele was 82.5%(Akkoub & Khabour, 2023). A meta-analysis comparing Caucasian and Asian populations located Considerable variation in allele frequencies:

Caucasians: C allele 80.74%, T allele 19.26%, **Asians**: C allele 95.9 %, T allele 4.10%.The genotype frequencies for Caucasians were CC 69.17%, CT 23.15%, TT 7.69%, and for Asians CC 91.90%, CT 7.99%, TT 0.11%(D. Li et al., 2013;Akkoub & Khabour, 2023).

These differences can also reflect evolutionary pressures, genetic drift, or founder effects. Data on the prevalence of P582S within the Palestinian population are missing, underscoring the need for research on this location. Table 2.3: Showing the prevalence of the HIF1A P582S polymorphism (allele frequencies and genotype distributions) in different populations worldwide, based on the available data from multiple studies and meta-analyses.

Several studies have reported the frequency of the P582S variation in Caucasian, Asian, and Jordanian populations; there are currently no published records on its incidence amongst Palestinians. This is the first investigation to examine the genotype and allele frequencies of the P582S polymorphism in a consultant sample from the West Bank, Palestine.

Table 2.3. Summarize the prevalence of the HIF1A P582S polymorphism (allele frequencies and genotype distributions) in different populations worldwide, based on the available data from multiple studies and meta-analyses.

Population / Country	Allele Frequency T (582S) %	Genotype Frequencies (%) CC	CT	TT	Reference
Caucasian (overall)	19.26	69.17	23.15	7.69	(D. Li et al., 2013)
Asian (overall)	4.10	91.90	7.99	0.11	(D. Li et al., 2013)
Jordan	17.5	-	-	-	(Akkoub & Khabour, 2023)

Italy	~15.4	-	-	-	(Akkoub & Khabour, 2023)
Iran	~10.6	-	-	-	(Akkoub & Khabour, 2023)
USA	~12.9	-	-	-	(Akkoub & Khabour, 2023)
Korea	~10.5	-	-	-	(Akkoub & Khabour, 2023)
Turkey	~14.7	-	-	-	(Akkoub & Khabour, 2023)
China	~11.0	-	-	-	(Akkoub & Khabour, 2023)
Russia	~7.5	-	-	-	(Akkoub & Khabour, 2023)
Mexico	~10.9	-	-	-	(Akkoub & Khabour, 2023)
Japan	~6.7	-	-	-	(Akkoub & Khabour, 2023)
Poland	~7.0	-	-	-	(Akkoub & Khabour, 2023)
South Asians (subgroup of Asia)	~11.9	-	-	-	(Islam, 2024)
East Asians (subgroup of Asia)	~4.6	-	-	-	(Islam, 2024)

Disease Associations of HIF-1α Polymorphisms

Cancer

The P582S polymorphism has been associated with an increased risk of several cancers, especially in Asian populations, including breast, gastric, and colorectal cancers. The variation

may modulate HIF-1α expression and feature in malignant tissues, influencing tumor growth and prognosis(Xu et al., 2014; Faculty, 2018). Based on previously published research, the Ser allele is linked to less favorable results in tumors where the growth of the tumor is promoted by an increased HIF-1α activity. (Polymorphism on Diabetes Nephropathy, 2013).

Diabetes and Its Complications

Meta-analyses have observed a protective association between the Pro582Ser polymorphism and diabetes risk under the heterozygous genetic version (Ren et al., 2020). According to studies, the Ser variation provides a relative level of protection against hypoxic damage in diabetic kidneys, potentially delaying the progression of diabetic nephropathy by maintaining HIF-1α activity in spite of hyperglycemia-induced suppression(Gu et al., 2013; Ren et al., 2020; Bi et al., 2015).

Cardiovascular and Respiratory Diseases

A greater risk of cardiovascular disorders, vascular and skin disorders, and chronic obstructive pulmonary disease (COPD) has been related to the P582S polymorphism (Ali et al., 2022). The overall extent of these relationships' effects and their placement, however, differ among studies and populations.

According to a thorough meta-analysis and subgroup analysis, the HIF1A 1772 C/T polymorphism is significantly linked to COPD, skin and vascular diseases, and diabetic complications, albeit the risk guidelines vary depending on the condition. For example, the C allele became a protective factor for COPD (ORs < 1) but a high-threat problem for skin and diabetes headaches (ORs > 1)(Ali et al., 2022).

Hematological Parameters

Research on the affiliation between Pro582Ser and hematological indices (e.g., hemoglobin, red blood cell count, hematocrit, ferritin, and erythropoietin) is confined and once in a while contradictory:

Women: Most studies, including those in Middle Eastern populations, document no significant association between P582S and red blood cell indices in females, suggesting a limited functional role for this variant in baseline hematological trends in females (Akkoub & Khabour, 2023).

Men: In evaluations, a few studies have discovered that male carriers of the Ser allele exhibit substantially better hemoglobin, hematocrit, mean corpuscular hemoglobin, and ferritin levels, indicating a gender-specific effect (Torti et al., n.d.).

Notably, previous investigations by Torti et al. (n.d.) have demonstrated that the P582S HIF-1α polymorphism has gender-specific effects. When comparing male blood donors to wild-type people, carriers of the polymorphism showed significantly greater hemoglobin concentrations, hematocrit, mean corpuscular hemoglobin, and serum ferritin levels. However, even in the tiny sample under study, those protective results have not yet been established in female blood donors. Summary of Pro582Ser Associations with diseases is concluded in Table 2.4.

Previous research by Tepebaş et al. (2016) explores the contributions of HIF-1α gene polymorphisms, especially C1772T (P582S) and G1790A (A588T), in the control of hemoglobin levels and the involvement of erythrocytosis, including polycythemia vera (PV). Findings appear that the C1772T T allele is less frequent amongst PV patients, while the G1790A allele is significantly related to elevated hemoglobin levels. These associations indicate that these genetic variants may serve as risk factors for erythrocytosis and PV by modulating the hypoxia response pathway, probably through disabling interaction with the von Hippel-Lindau (VHL) protein and subsequent stabilization of HIF-1α.

Table2.4. Pro582Ser polymorphism of HIF-1A and its associations with diseases.

Disease/Condition	Association Type	Effect Direction	Population/Notes
Diabetes	Protective (heterozygous model)	Decreased risk	Meta-analysis, multiple ethnicities(Ren et al., 2020)
Diabetic complications	Protective	Decreased risk	Includes nephropathy(Ren et al., 2020);*Polymorphism on Diabetes Nephropathy*, 2013)

Digestive tract cancers	Risk factor	Increased risk	Especially gastric and colorectal cancers in Asians (Xu et al., 2014)
Breast cancer	Risk factor	Increased risk	Asian populations (Faculty, 2018)
COPD, CVD, skin diseases	Associated	Varied	Broad hypoxia-related disease (Ali et al., 2022)
Hematological parameters	No significant association	None	Women studied; gender-specific effects(Akkoub & Khabour, 2023)

Methodological Considerations in Genetic Association Studies

Genetic association studies are necessary to confirm the relationship between complex patterns, such as hematological parameters, and certain gene variations, such as the P582S HIF-1α polymorphism. Nonetheless, certain essential methodological elements are required for the reliability and perceptive interpretation of these studies.

Study Design

In this study, a cross-sectional design was employed to evaluate the association between the P582S HIF-1α polymorphism and hematological parameters in a sample of 100 individuals from the northern West Bank, Palestine. This region was selected to provide a representative of the Palestinian populace, considering its particular genetic and environmental traits.

Cross-sectional research, which includes that used in current studies on HIF-1 α P582S, investigates variables at a single time point; however can't set up causality (Akkoub & Khabour, 2023).

Sample Size and Power

Adequate sample size is important to uncover modest genetic effects and avoid false-positive consequences. Underpowered research may additionally fail to discover proper associations, whilst small samples can yield spurious findings due to random variation. In the

present study, a sample of a hundred individuals from the northern West Bank was selected to provide a consultative evaluation of the population.

Population Stratification

Population stratification-systematic differences in allele frequencies among subgroups-can confound genetic affiliation studies if not well addressed. Matching cases and controls by ethnicity or the use of statistical modifications for population structure is mainly crucial in diverse populations, along with the ones inside the Middle East.

Genotyping Methods

Accurate genotyping is important for reliable effects. Common methods include PCR-RFLP, TaqMan assays, and subsequent-technology sequencing. Quality control measures, consisting of replica samples and Hardy-Weinberg equilibrium checking, help in ensuring fact integrity. In this study, the PCR-RFLP method was used for its accessibility and reliability.

Environmental, Sociodemographic, and Gender-Specific Modifiers

Environmental and Lifestyle Factors

External environments can affect the phenotypic regulation of the HIF-1α Pro582S polymorphism significantly. Those living at high altitude experience chronic hypoxia regardless of genotypes, which triggers the HIF route and can increase hemoglobin and hematocrit, particularly in men. (Villafuerte et al., 2022; Akkoub & Khabour, 2023).

Lifestyle and Occupational Exposures Smoking: according the World Health Organization Eastern Mediterranean Regional Office (WHO EMRO) also reported high tobacco use among young adult males in Palestine, with smoking rates reaching nearly 48% in some studies might also result in secondary polycythemia(Seir et al., 2020), doubtlessly amplifying the P582S variant's effect on hemoglobin levels. Physical Labor: Manual labor in agriculture or construction, familiar in rural areas, increases oxygen consumption, which may also promote HIF1α-lead to erythropoiesis in genetically predisposed individuals.

Gender is an important modifier of the relationship between P582 and hematological parameters. Women-specific factors, including menstruation, pregnancy, and menopause, can impact iron

status and erythropoiesis, possibly affecting genetic results. Including both sexes in research is important for complete knowledge of the genotype-phenotype picture.

Research Gap and Rationale for the Present Study

Despite extensive studies on the P582S HIF-1α polymorphism in diverse populations, there is a lack of data regarding its occurrence and relationship with hematological parameters among Palestinians. The special genetic history and environmental risk to this population may have an impact on the frequency of the P582S version and phenotypical results. Exploring this research of association will provide useful knowledge for the health and individual care in this area.

Understanding the interplay among the HIF-1α Pro582Ser polymorphism and environmental, sociodemographic, and lifestyle elements has vital scientific implications for health care consequences. It may help improve iron supplement programs, erythropoiesis-stimulating treatments, and manage anemia by utilizing lifestyle, sociodemographic, and genetic information.

Genetic and Hematological Health in the Palestinian Population

One of the most significant public health issues in Palestine is genetic abnormalities and blood-related illnesses. According to recent studies, a large number of Palestinians continue to have blood disorders linked to malnutrition, which are impacted by both environmental and hereditary factors(Alshawish et al., 2024; Abu-Libdeh et al., 2012).

Genetic Disorders and Awareness

Familial hereditary and genetic problems continue to be prevalent in Palestine, with consanguineous marriage—a commonplace cultural exercise—contributing to the elevated prevalence of autosomal recessive situations (Ghanim et al., 2025). A recent cross-sectional have a look at found that over 1/2 of the surveyed Palestinians reported having their family records of genetic or hereditary problems (Alshawish et al., 2024).

Public knowledge and the sensibility of genetic testing are unexpectedly elevated, with the preponderance of respondents recognizing its importance for disease risk assessment and prevention (Alshawish et al., 2024). However, there are nonetheless gaps in translating this understanding into recurrent fitness behaviors, and in addition, efforts are needed to show the importance of using genetic testing and consultations (Ghanim et al., 2025).

Hematological Disorders: Anemia and Thalassemia

Anemia, particularly iron deficiency anemia (IDA), is a main health problem in Palestine, especially among children, adolescents, and females of bearing age (Mikki et al., 2011; El Bilbeisi, 2025; Sirdah et al., 2014). Studies have suggested that the prevalence of anemia among children in Gaza is as high as 35.6%, with maximum cases categorized as moderate to slight(El Bilbeisi, 2025). Among kindergarten youngsters in marginalized areas of Gaza, the prevalence of iron deficiency anemia was observed to be 33.5%, with enormous variation among the governorates, with rural areas with low parental schooling and socioeconomic status (Sirdah et al., 2014).

Among young people, anemia costs additionally vary by way of region and gender, with higher surveyed observed in certain governorates and amongst girls (Mikki et al., 2011). These findings mirror broader regional tendencies within the Eastern Mediterranean, where anemia remains a persistent public health problem.

In addition to nutritional anemia, inherited hemoglobinopathies, along with β-thalassemia are present within the Palestinian populace. In the Gaza Strip, approximately 0.02% of the population has β-thalassemia predominant, an extreme hereditary anemia requiring lifelong transfusions (Faraon et al., 2019). Genetic studies have identified a spectrum of β-thalassemia mutations in Palestinian patients, with numerous common and a few novel mutations reported (Faraon et al., 2019). The persistent prevalence of these mutations has demonstrated the significance of genetic screening, counseling, and community-based public health interventions.

Another cause related to daily life in this region, chronic stress from political instability and confined healthcare get entry to in Palestine may exacerbate anemia through mechanisms including inflammation-driven hepcidin upregulation, which inhibits iron absorption(Bailey et al., 2013; D'Angelo, 2013). These elements could override the protective effects of HIF-1α polymorphisms, especially in women who face extra iron loss from menstruation and pregnancy.

Implications for Research and Public Health

The high spread of hereditary and nutritional hematological problems in Palestine highlights the need for integrated processes combining genetic, nutritional, and sociodemographic statistics. However, awareness of genetic testing is growing; there continue to be limitations to its

great adoption and effective use in sickness prevention and control (Ghanim et al., 2025). National intervention applications concentrated on enhancing nutrition, expanding get right of entry to genetic counseling, and promoting early detection of hereditary blood problems are urgently needed (Sirdah et al., 2014).

Furthermore, a study on gene polymorphisms, which includes HIF-1α P582S, and their affiliation with hematological tendencies in Palestinians, is limited. Addressing this gap shall provide beneficial insights for personalized medication and public health planning tailored to the unique genetic and environmental conditions of the Palestinian population.

Summery

The HIF-1 α gene, in particular the P582S polymorphism, plays a master role in the hypoxia model and hematological regulation. Its effects can be modulated with the aid of environmental, sociodemographic, and gender-unique elements. However, data on its occurrence and impact on the Palestinian populace are lacking, justifying the need for the present take a look at.

Chapter Three: Methodology and Materials

Study Design

This study employed an observational cross-sectional design to investigate the association between the P582S HIF-1α gene polymorphism and hematological parameters within the Palestinian population in the West Bank, aged between18-50 year's old, healthy individuals from both sexes, especially in the northern district. The cross-sectional design was chosen because it saves time and money. It allows researchers to collect genetic and blood data all at once, without needing to track participants over a long period.

This study design allows the evaluation of the prevalence of specific genetic polymorphisms and hematological parameters in the population, along with the association between them. These designs-cross sectional, also enable direct comparison between the two sexes at the same time point. Furthermore, it grants Pivotal Initial data for the region and builds the foundation for future research and healthcare.

The study was conducted during the period from March to June 2025, utilizing time to enroll participants and conduct comprehensive laboratory tests, such as complete blood cell count (CBC) and polymerase chain reaction (PCR). All of the procedures and protocols of the method were approved by the Ethical Review Committee of the Arab American University - Palestine (AAUP), which were conducted under the principles of the Ethical Statement of the Declaration of Helsinki. This ensured that the research complied with policies targeted to protect and respect participants.

Study Population and Recruitment

The study included healthy people aged 18 to 50 years who visited Primary Health Care Clinics of the Palestine Ministry of Health in Tubas, Nablus, Jenin, and surrounding areas. This approach to recruitment was designed to verify a representative sample from both urban and rural populations, as these clinics operate across diverse demographic and geographic communities. Individuals were selected for participation during routine medical visits, providing the opportunity for the inclusion of individuals from different backgrounds and improving the generalizability of the outcomes related to the prevalence of the P582S polymorphism in the HIF-1A gene.

The Planned sample size was 310 participants. This number was calculated using the G*Power software guided by the following factors: an effect size of 0.15 (a common effect size in genetic association studies), a significance level (alpha) of 0.05, a statistical power of 0.80, and a 95% confidence interval. These determinations were carried out to allow for reaching a sufficient level of strength to recognize important connections between the gene polymorphism and hematological parameters.

However, due to unexpected challenges, which include restrictions associated with the ongoing warfare for the duration of the look at period and budgetary constraints, it is now not feasible to attain the deliberate sample size. Consequently, the very last variety of enrolled participants changed into a hundred. While this reduced pattern length can also have an effect on the statistical strength of the have a look at, it nonetheless affords treasured preliminary facts on the genetic polymorphism and hematological profiles in the Palestinian population. Figure 3.1

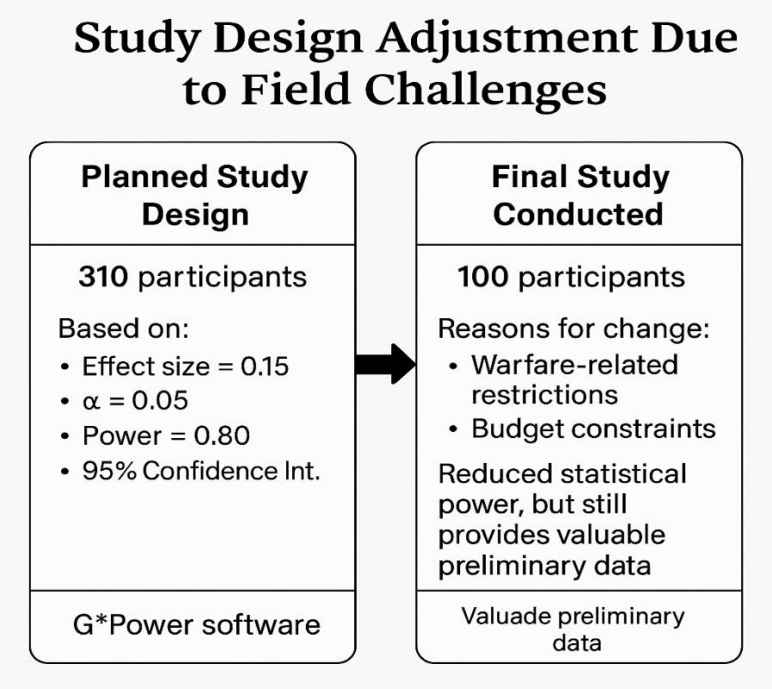


Figure3.1.Comparison of the sample size planned and the actual study design. Designed by the Infogram internet tool.

Inclusion Criteria

Participants were eligible for the study if they met the following criteria:

Age: Adults aged 18 to 50 years were included to represent the general adult population, while reducing the influence of age-related changes in blood parameters.

Health Status: Participants had to be healthy with no known blood disorder, like anemia, thalassemia, or sickle cell disease. Additionally, individuals with recent acute ailments (e.g., febrile situations within the past weeks) were excluded to avoid temporary changes in hematological parameters.

Availability for Data Collection: Eligible members had to be willing and complete study questionnaires and provide a blood sample within the timeframe to ensure accurate and timely data collection.

Ability to Provide Blood Samples: Participants needed to be physically capable of undergoing venous blood collection without any contraindications or discomfort that might affect sample quality or their well-being.

Informed Consent: All participants provided written informed consent, demonstrating that they understood the study procedures and agreed to participate under ethical guidelines.

Exclusion Criteria

The following exclusion criteria were implemented to verify the validity and consistency of the research outcomes

Blood Disorders: people with chronic or acute hematological disorders, such as anemia, thalassemia, sickle cell disease, or other blood-related health problems that could influence hematological parameters.

Pregnancy and Lactation: Women who were pregnant or lactating were excluded because of physiological changes that affect hematological parameters and cope with moral considerations.

Consent and Compliance: Those not able or unwilling to signal a written informed consent form, or observe, had been excluded to ensure moral requirements and file integrity**.**

Iron Supplementation: Individuals who had used iron dietary supplements within the past four months were excluded, as current supplementation may affect hematological parameters and confound the study's effects.

Sample Quality: To maintain laboratory integrity, blood samples that have been hemolyzed or improperly stored were excluded from analysis.

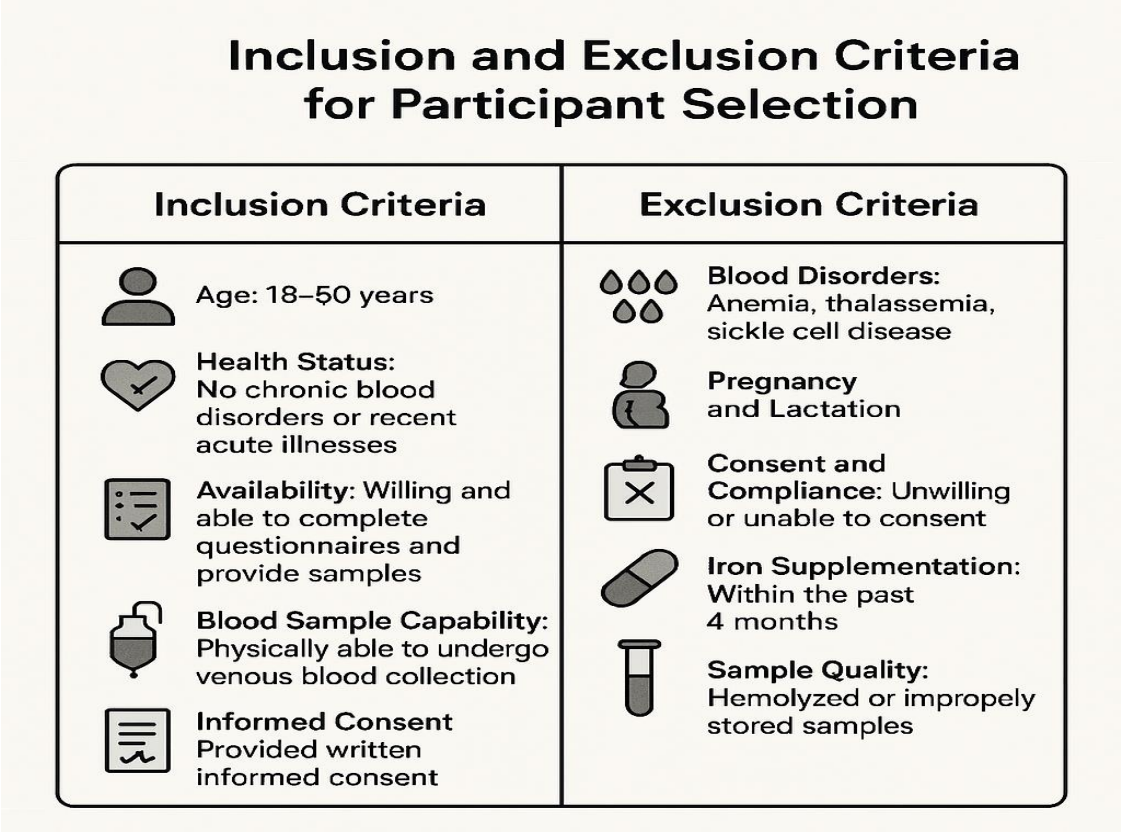


Figure 3.2 Summary of the inclusion and exclusion criteria used for participant selection. Designed by the Infogram internet tool.

Sampling Method

This study utilized a non-probability convenience sampling method. Participants were recruited from primary healthcare facilities in both rural and urban regions throughout the northern

West Bank. The sample was taken from an individual who was easily accessible and willing to participate, which facilitated a faster recruitment process.

Participants had been screened according to the predefined inclusion and exclusion criteria to ensure they met the study requirements. This sampling method simplified the collection of data, given the constraints of time and resources.

Markedly, owing to the fact that participants aren't picked arbitrarily, the sample won't be a representative sample of the wider populace, which restricts the generalizability of the findings. Additionally, this technique may introduce selection bias because it is based on accessibility and willingness to take part in a place rather than random selection.

Regardless of these limitations, convenience sampling was determined to be the most fitting method for this exploratory study, given the logistical constraints and the objective of obtaining initial impressions of hematological parameters in the target population.

The size of the sample

Several key factors were considered in determining the sample size for this study, including effect size, significance level, statistical power, and confidence interval.

Effect Size: Based on conventions in genetic association studies, effect sizes are generally categorized as small (0.2), medium (0.5), or large (0.8). For this study, an effect size of 0.15 was used, reflecting a small to moderate expected effect.

Significance Level (α): The significance level was set at 0.05, indicating a 5% probability of committing a Type I error (rejecting the null hypothesis when it is true).

Power (1 - β): A statistical power of 0.80 was targeted, meaning there is an 80% probability of correctly detecting a true effect.

Confidence Interval: A 95% confidence level was applied to ensure precision in the estimates

The Planned sample size was 310 participants. This number was calculated using the G*Power software guided by the following factors: an effect size of 0.15 (a common effect size in genetic association studies), a significance level (alpha) of 0.05, a statistical power of 0.80, and a 95% confidence interval. These determinations were carried out to allow reaching a sufficient level of

strength to recognize important connections between the gene polymorphism and hematological parameters. However, because of sensible constraints, such as ongoing local conflict and limited funding resources, and time, it is no longer feasible to recruit this number. Consequently, the very last pattern size was reduced to a hundred individuals.

Despite this reduction, the pattern size was enough to fulfill the number one goal of the look at and yielded precious insights into the hematological parameters of the goal population. It is mentioned that a smaller pattern size might also restrict the statistical power and generalizability of the findings, but the layout and analysis accounted for these obstacles.

Ethical Considerations

The Ethical Review Committee at Arab American University-Palestine (AAUP) approved this study with code number "R-2025/A/4/N", confirming that all procedures adhered to the relevant ethical guidelines.

The Declaration of Helsinki's guidelines were strictly adhered to, ensuring the safety and rights of all participants throughout the study. Each participant received a thorough description of the study's goals, procedures, potential risks, and advantages before enrollment. To assist them in fully comprehending, this information was provided via a Participant Information Sheet.

Before participating, each individual provided written consent. For individuals who struggled with reading, the study staff provided verbal explanations to ensure clarity. The entire consent procedure was conducted honestly, respecting people's decision to participate or not.

To keep things confidential, each participant received a unique ID code. All personal information, including age, health status, and test results, was anonymized and maintained securely. Only approved team members had access to data, including names. Computer data was password-protected, while paper documents were kept secure in the main office.

By adhering to these guidelines, the study was able to respect everyone's privacy and dignity while also producing reliable research results.

Data collection

Sample Collection and Handling

Biological samples were collected at the Central Health Clinic, which operates under the Palestinian Ministry of Health. Venous blood samples were drawn using standard venipuncture techniques by skilled medical professionals, ensuring strict adherence to sterile strategies to preserve sample integrity.

Blood was collected into EDTA (ethylenediaminetetraacetic acid) tubes, which acted as anticoagulants through chelating calcium ions- thereby preventing clotting and preserving cell components and DNA integrity. Due to their superior preservation of cell morphology and stability of blood parameters, EDTA tubes are the preferred choice for hematological analyses, including whole blood count (CBC) and genomic DNA extraction (*No Title*, n.d.; Banfi et al., 2007).

Immediately after the collection, the blood in EDTA tubes becomes lightly blended using inversion to ensure the right anticoagulant distribution and keep away from clot formation, as full of life shaking can cause hemolysis or cell damage(*Samples for Hematology*, n.d.).

The samples were centrifuged at 2,500 RPM for 15 minutes to separate the buffy coat from plasma and different blood components. Proper labeling was carried out to ensure accurate sample identification.

DNA extraction was performed within 48 hours of the series. Extracted DNA samples were stored at -20 °C to preserve their integrity for subsequent analysis (Tendulkar et al., 2015). This protocol ensured great samples appropriate for dependable hematological and molecular assays, minimizing pre-analytical variability that could have an effect on the examination outcomes.

Demographic and Clinical Data Collection

Demographic and clinical statistics were collected through the use of established questionnaires administered to every participant. The questionnaire's included variables consisted of age, gender, body mass index (BMI), smoking status, physical activity habits, and the presence of persistent ailments. Participants were additionally asked approximately more natural use, with specific emphasis on iron supplement consumption in the past 4 months, to ensure compliance with the observer’s inclusion standards.

To ensure accuracy and consistency, all accumulated information was de-identified and stored securely to guard participants' privacy and confidentiality. This approach combined a systematic sampling with validated data collection methods to acquire comprehensive and dependable records, supporting the study's research objectives.

Materials and Equipment

The laboratory analyses in this study were conducted using advanced and reliable equipment to ensure the accuracy and reproducibility of results.

Sysmex XP-300 Automated Hematology Analyzer

Complete blood count (CBC) analyses were performed using the Sysmex XP-300, an automated 3-part differential hematology analyzer located at a facility under the Ministry of Health of Palestine. This instrument EDTA-anticoagulated whole blood samples and affords hematological parameters, inclusive of red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and white blood cell (WBC).

The XP-300 supported a high throughput of up to 60 samples per hour, requiring approximately 50 µL of whole blood per test. It provided an operator-friendly control panel touchscreen interface with barcode scanning for efficient sample and reagent management. The analyzer employed direct modern detection methods for RBC and platelet counts and a non-cyanide method for hemoglobin size, ensuring robust and accurate results(*Sysmex-Xp-300*, n.d.).

Every testing session included a three-level quality control (low, normal, and high) to guarantee the reliability and consistency of CBC values (Biotechne, R&D).

NanoDrop Spectrophotometer (IMPLEN, Germany)

The NanoDrop Spectrophotometer was used to evaluate the concentration and purity of DNA isolated from blood samples. Before further molecular testing, this device ensured quality control by enabling rapid, microvolume measurement of nucleic acids.

PCR Thermocycler(biometra - analytik jena thermocycler PCR)

Polymerase chain reaction (PCR) amplification of the P582S HIF-1A gene fragment was performed using a thermo-cycler. The instrument was programmed with specific denaturation,

annealing, and extension temperature cycles tailored to the target sequence to ensure efficient and specific amplification

Agarose Gel Electrophoresis System (Cleaver-Scientific)

The analysis of PCR results was performed using agarose gel electrophoresis. The amplified DNA fragments were separated using a 3% and 2% agarose gel, stained with ethidium bromide, and visualized under UV light. To optimize resolution, electrophoresis was carried out for one hour at 90 V.

Laboratory Procedures

Blood collection and DNA extraction

Approximately 3 mL of venous blood was drawn from each participant using a sterile disposable needle and vacuum collection system, taking care to avoid hemolysis. Blood was directly transferred into EDTA tubes and gently mixed to ensure proper anticoagulation.

Genomic DNA was extracted from the buffy coat layer using the QIAamp Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Briefly, 200 µL of buffy coat was transferred into a sterile 1.5 mL microcentrifuge tube and combined with 20 µL of proteinase K. Then, 200 µL of AL lysis buffer was added, and the mixture was incubated at 56°C for 10 minutes to lyse cells and release DNA.

Following lysis, 200 µL of absolute ethanol was added and vortexed thoroughly. The lysate was incubated at room temperature (15–25°C) for 5 minutes before being transferred to a QIAamp MinElute column. The column was centrifuged at 8,000 rpm for 1 minute to bind DNA to the membrane.

The column changed into washed twice to remove impurities: first with 500 µL of AW1 buffer (centrifuged at 8,000 rpm for 1 minute), then with 500 µL of AW2 buffer (centrifuged at 14,000 rpm for 3 minutes). Finally, DNA was eluted with 100 µL of Buffer AE into a sterile microtube. Extracted DNA samples had been stored at -20°C until further analysis.

DNA Quantification

DNA concentration and purity were assessed using a NanoDrop Spectrophotometer (IMPLEN, Germany). One microliter of Buffer AE was used to blank the device and followed by the addition

of 1 µL of the DNA pattern. The absorbance ratio at 260 nm and 280 nm (OD260/OD280) was calculated to assess DNA purity, with a ratio greater than 1.8 taken into consideration as indicative of excellent DNA suitable for downstream applications.

Genotyping of P582S HIF-1A Polymorphism

Genotype analyses of P582S HIF-1A Polymorphism were performed by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), The PCR amplification was performed in a 50 µL reaction mixture containing 5µL of genomic DNA, 1µL of forward primer (5'-GACTTTGAGTTTCACCTGTTT-3'), 1µL of reverse primer (5'-ACTTGC GCTTTCAGG GCTTGC GGAAC TGCT T-3'), with 25µL of master mix that contains, 1 µL dNTPs, 5µL of 1X PCR buffer, 1.5µL $MgCl_2$, and 1.25 µL of Taq DNA polymerase , Water + Stabilizers 16.25µL(Promega, USA) and ultra-pure water added to reach 50µL total volume .as shown in table 3.1.

Table3.1. PCR Reaction Mixture Components for P582S HIF-1A Genotyping.

Component	Volume (µL) per reaction	Function
Genomic DNA	5µL	Template DNA containing the target sequence
Forward Primer	1µL	Binds to one strand, initiates DNA synthesis
Reverse Primer	1µL	Binds to the complementary strand, initiates DNA synthesis
dNTPs (Deoxynucleotide triphosphates)	1µL	Building blocks for new DNA strand synthesis
10X PCR Buffer	5µL of 1X PCR Buffer	Provides optimal pH and ionic environment for enzyme

$MgCl_2$	1.5µL	Cofactor required for Taq DNA polymerase activity
Taq DNA Polymerase	1.25µL	Thermostable enzyme that synthesizes new DNA strands
Nuclease-Free Water	To a 50µL total volume	Adjusts the final reaction volume

The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 34 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes.

Table 3.2 Shown PCR Amplification Conditions for P582S HIF-1A Polymorphism.

Step	Temperature (°C)	Duration	Number of Cycles	Description
Initial Denaturation	94	5 minutes	1	Denature template DNA
Denaturation	94	60 seconds	34	Separate DNA strands
Annealing	55	60 seconds	34	Primer binding to the target sequence
Extension	72	60 seconds	34	DNA strand elongation by Taq polymerase
Final Extension	72	10 minutes	1	Complete synthesis of all DNA strands

The PCR products (approximately 197 bp) were examined for size via 2% agarose gel electrophoresis at 90 V for 45 minutes, visualized using ethidium bromide staining and UV transillumination.

For genotyping, the amplified fragments were digested with the endonuclease restriction enzyme Tsp451 (R0583S, New England Biolabs) according to the manufacturer's instructions. The digestion products were separated by electrophoresis on a 3% agarose gel at 90 V for an hour and visualized under UV light to determine the genotype based on the pattern of DNA fragments.

Restriction Fragment Length Polymorphism (RFLP) The PCR products were digested with the Tsp451 restriction enzyme (catalog number R0583S from NEB), which recognizes and cleaves specific sites altered by the polymorphism (Gerger et al., 2011). Tsp45I cuts between guanine and thymine in the recognition sequence, as illustrated in Figure 3.3 below.

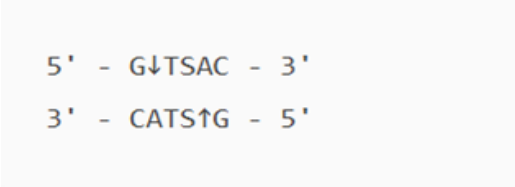


Figure 3.3.Tsp45I cuts between the guanine and thymine in the recognition sequence(*No Title*, n.d.-b).

The digestion mixture for one reaction contained 15 µL of PCR product, 2 µL of diluted Tsp451 enzyme, 3 µL of 10X restriction buffer, and nuclease-free water to a final volume of 5 µL. Table 3.3 shows the RFLP reaction mixture. The digestion was performed at 65°C for 1 hour. The resulting fragments were separated by electrophoresis using a 3% agarose gel at 90 V for 1 hour. DNA fragments were stained with ethidium bromide and visualized under UV light. To make more validation of the genotype, Sanger sequencing was performed on some of the samples.

Table 3.3: RFLP reaction mixture volume.

Component	**Volume**
10X restriction buffer	3µL
Tsp451 restriction enzyme (R0583S from NEB)	2µL after being diluted from 10000U

Nuclease-free water.	5µL
PCR product (amplicon).	15µL

The restricted fragment sample was implemented to determine the genotypes:

- The C allele (Proline) remains undigested, resulting in an unpaired 197 bp fragment.
- The T allele (Serine) introduces a limit website online, resulting in digestion into two fragments of about 150 bp and 47 bp.

Samples showing the presence of only the 197 bp fragment were classified as CC (homozygous wild-type). Those with both 197 bp and smaller fragments were classified as heterozygous (CT), and those with only the smaller fragments (150 bp and 47 bp) were classified as TT (homozygous mutant). As shown in Table 3.4.

Table 3.4. Restriction fragments and gel bands for each genotype.

Genotype	**Restriction Pattern**	**Fragment Sizes (bp)**	**Gel Bands**
CC (Homozygous Wild-Type)	No restriction site; PCR product remains uncut	197	Single band at 197 bp
CT (Heterozygous)	One allele uncut, one allele cut	197, 150, 47	Bands at 197 bp, 150 bp, and 47 bp
TT (Homozygous Mutant)	Both alleles cut	150,47	Bands at 150 bp and 47 bp

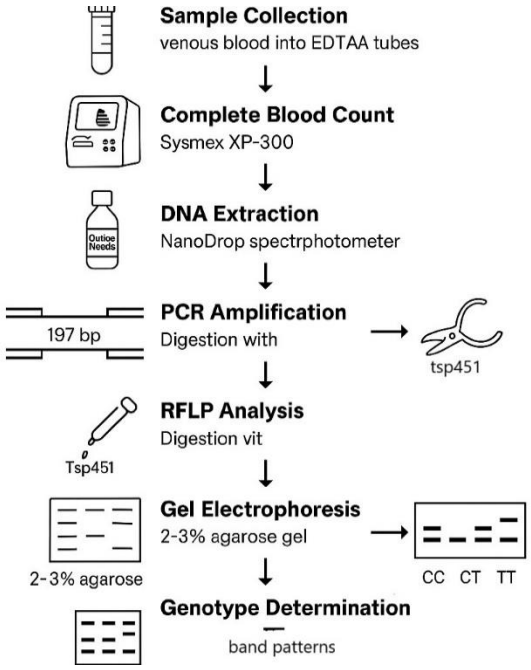


Fig.3.4 Flowchart showing the Step-by-Step Laboratory Process for HIF-A1 P582S polymorphism, starting with collecting the sample, followed by CBC, PCR, RFLP, and the bands appear. Designed by the Infogram internet tool.

Ethical Considerations

Ethical approval for this study was obtained from the Ethical Review Committee at the Arab American University–Palestine (AAUP). Written informed consent was obtained from all participants before their inclusion in the study. Participant confidentiality was strictly maintained through the anonymization of all collected data.

Statistical Analysis Methods

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 and Python. Statistical significance was set at a p-value < 0.05. Descriptive statistics have been used to summarize demographic and clinical data, including continuous variables with their means and standard deviations. Comparative analyses were completed to evaluate variations between groups (e.g., genotype, demographic subgroups). ANOVA and chi-square was used. The

distribution of the different P582S HIF-1A genotypes was examined for their concordance with the Hardy-Weinberg equilibrium.

Correlation evaluation (e.g., Pearson or Spearman correlation) was conducted to evaluate relationships between quantitative variables. All statistical assessments had been two-tailed, and confidence intervals had been calculated at the 95% interval.

Chapter Four: Results

Sociodemographic Characteristics of the Study Population

Overall, the demographic characteristics of the study100 study participants are shown in Table 4.1.

Briefly, the study sample consists of -number comprised (58% females and males 42%), with a mean age of 31.4 ± 11.3 years. , as shown in Figure 4.1.

Table 4.1. Sociodemographic Characteristics of the Study Population (N=100).

Variable	Statistic	Value
Age (years)	Mean ± SD	31.4 ± 11.3
	Range (Min-Max)	18 - 56
Gender	Male (n, %)	42 (42.0%)
	Female (n, %)	58 (58.0%)
Body Mass Index (BMI)	Mean ± SD (kg/m²)	24.9 ± 3.46
BMI Category	Underweight (<18.5 kg/m²)	3 (3.0%)
	Normal (18.5–24.9 kg/m²)	43 (43.0%)
	Overweight (25–29.9 kg/m²)	47(47.0%)
	Obese (≥30 kg/m²)	7 (7.0%)
Smoking Status	Yes (n, %)	18 (18.0%)
	No (n, %)	82 (82.0%)
Exercise Status	Yes (n, %)	19 (19.0%)
	No (n, %)	81 (81.0%)

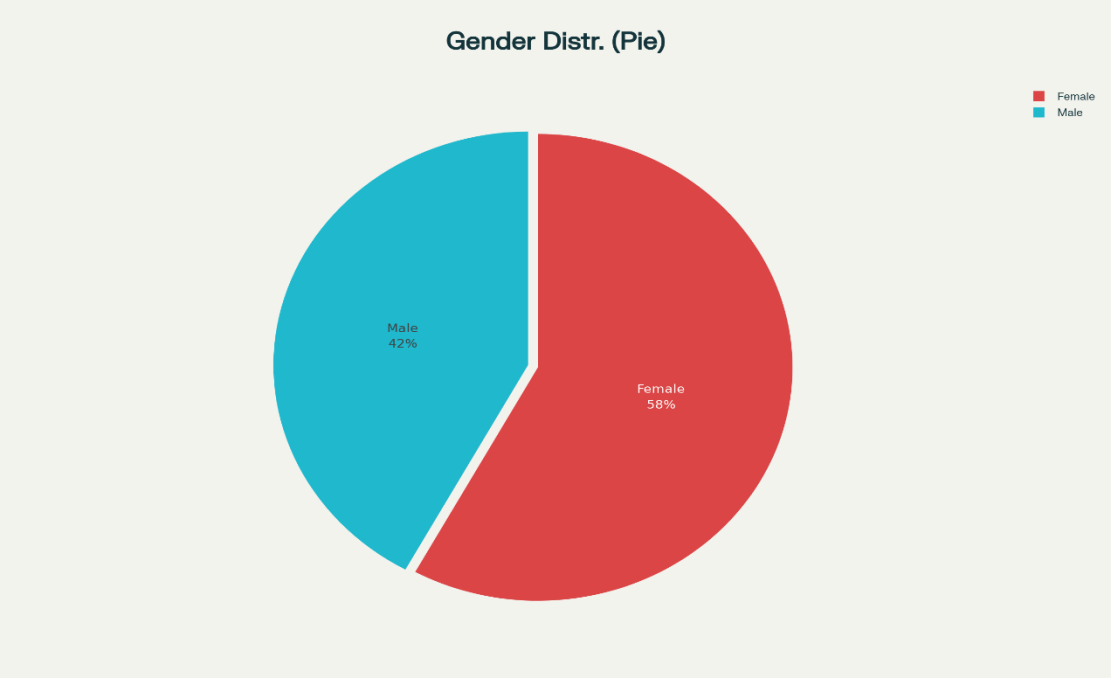


Figure 4.1: Gender distribution of study participants, 3D-style pie chart showing gender distribution with Male 42% and Female 58%.

The age range was from 18 to 56 years, with a predominant representation of younger adults aged 18 to 34. The mean BMI was 24.4 ± 3.46 kg/m², indicating an average in the normal-weight category. A further breakdown revealed that nearly half of the participants (47%) were overweight, while 43% were classified as having a normal weight. A smaller proportion was obese (7%), and three percent of the participants were classified as underweight. A Bar Chart with labeled percentages illustrating the percentage of participants classified in Figure 4.2.

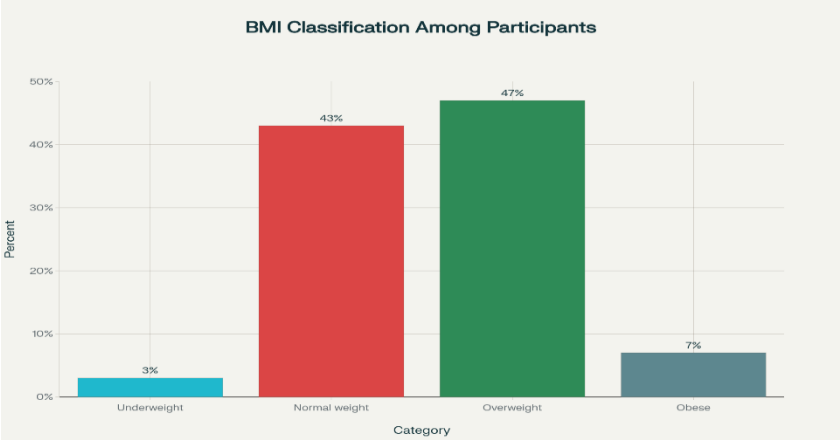


Figure 4.2 is a bar chart illustrating the percentage of participants classified as underweight (3%), normal weight (43%), overweight (47%), and obese (7%) based on their Body Mass Index (BMI).

The majority (82%) of participants were reported to be nonsmoking. While, 79.1% of females were non-smokers 20.9% of them were smokers. Whereas 66.7% of male participants were non-smokers, 33.3% were smokers.

(81%) of the study participants reported no regular exercising, (83.3%) of female participants did not exercise regularly, while 16.7% exercised regularly. For males participants 76.2% did not exercise regularly, and 23.8% exercised regularly. Males are more likely than females to smoke and also slightly more likely to exercise. A bar chart showing smoking and exercise status percentages by gender, Figure 4.3.

The majority of both genders do not exercise regularly, and most are non-smokers, although the proportion of male smokers is notably higher.

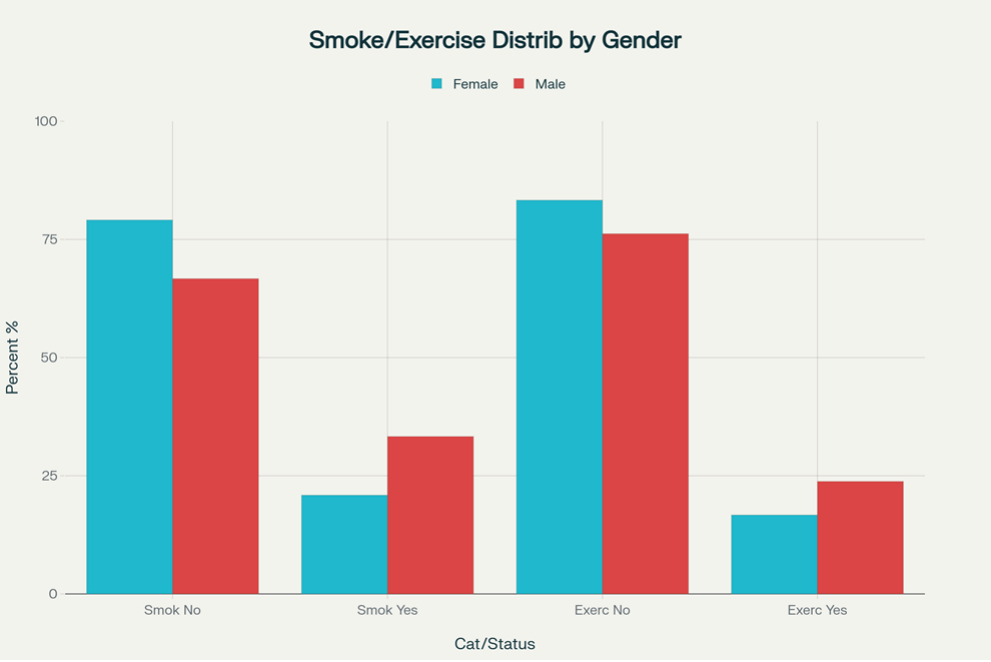


Figure 4.3 grouped bar chart showing smoking and exercise status percentages by gender.

Geographic Distribution

The geographic distribution of the study participants across different areas of the northern West Bank, more than half of the study participants (54%) were from Tubas, making it the most represented area. Jenin accounted for 25% of the participants, followed by Nablus (11%) and Tulkarm (9%). One participant is from another area.

Table 4.2: Geographic Distribution of Study Participants by Address. Figure 4.3 .A bar chart displaying the absolute counts of participants.

Table 4.2: Geographic Distribution of Study Participants by Address (N=100).

Address	**Count (n)**	**Percentage (%)**
Tubas	54	54.0%
Jenin	25	25.0%
Nablus	11	11.0%
Tulkarm	9	9.0%
Others	1	1.0%
Total	**100**	**100.0%**

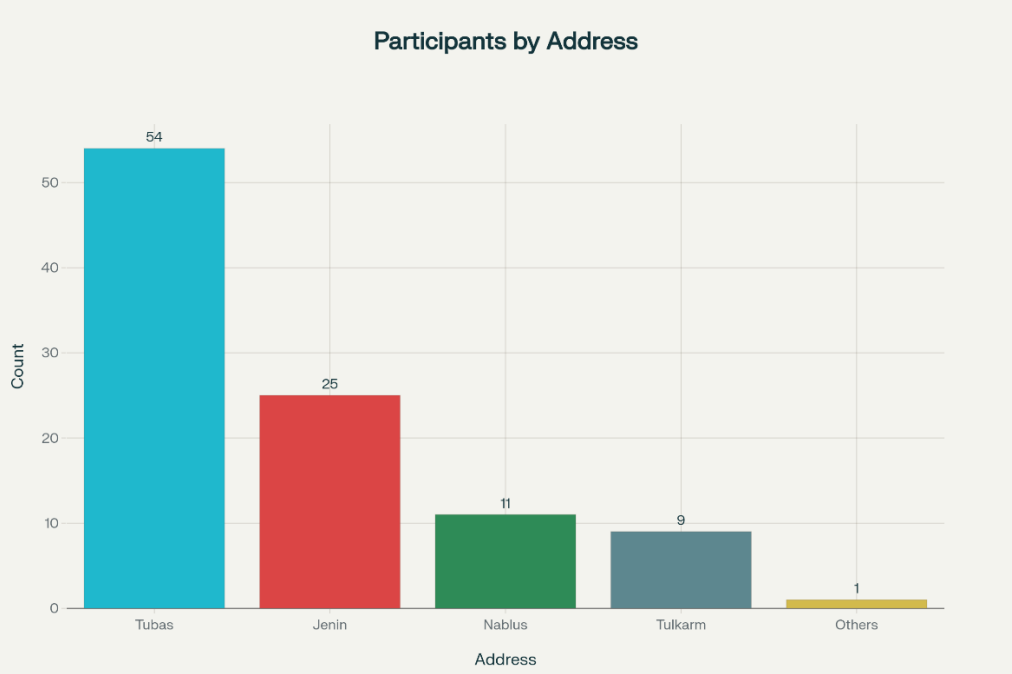


Figure 4.4.A bar chart displaying the absolute counts of participants residing in Tubas, Jenin, Nablus, and Tulkarm, visually emphasizing the higher concentration of participants from Tubas.

Hematological Parameters

The CBC parameters of the study sample are shown in **Table 4.3**. The mean hemoglobin concentration was 12.7 g/dL (SD = 1.17), with most results within adult reference ranges. Red blood cell (RBC) count averaged $4.44 \times 10^6/\mu L$, while hematocrit averaged 37.28%. Other hematological parameters, including WBC, platelet count, and red cell indices, also showed values within normal reference intervals Table 4.3.

Table 4.3: descriptive statistics for key hematological parameters measured in the study (N=100).

Parameter	Mean	SD	25th %ile	Median	75th %ile	Min	Max
Hemoglobin (g/dL)	12.70	1.17	12.00	13.00	14.00	9.00	17.00

RBC (10^6/µL)	4.44	0.63	4.00	4.00	5.00	3.00	6.00
Hematocrit (%)	37.28	4.71	33.00	37.00	41.00	25	48.0
WBC (1000/µL)	7.34	2.13	6.00	7.00	8.00	3.00	14.00
MCV (fL)	81.43	6.61	78.00	81.50	87.00	62.0	96.0
MCH (pg)	27.21	3.15	26.00	27.00	29.00	18.0	35.0
MCHC (g/dL)	33.16	1.73	32.00	33.00	34.00	26	36.0
RDW (%)	12.89	1.56	12.00	13.00	13.00	10.00	20.00
Platelets (1000/µL)	260.00	66.98	217.00	261.10	298.75	121.00	491.00
Lymphocytes (%)	31.29	9.65	25.25	30.50	36.00	4.00	54.00
Monocytes (%)	5.29	2.86	3.00	4.50	7.00	2.00	14.00
Neutrophils (%)	62.63	10.49	54.75	63.50	68.75	41.00	93.00

Presents histograms illustrating the distribution of nine hematological parameters among study participants Figure 4.5.

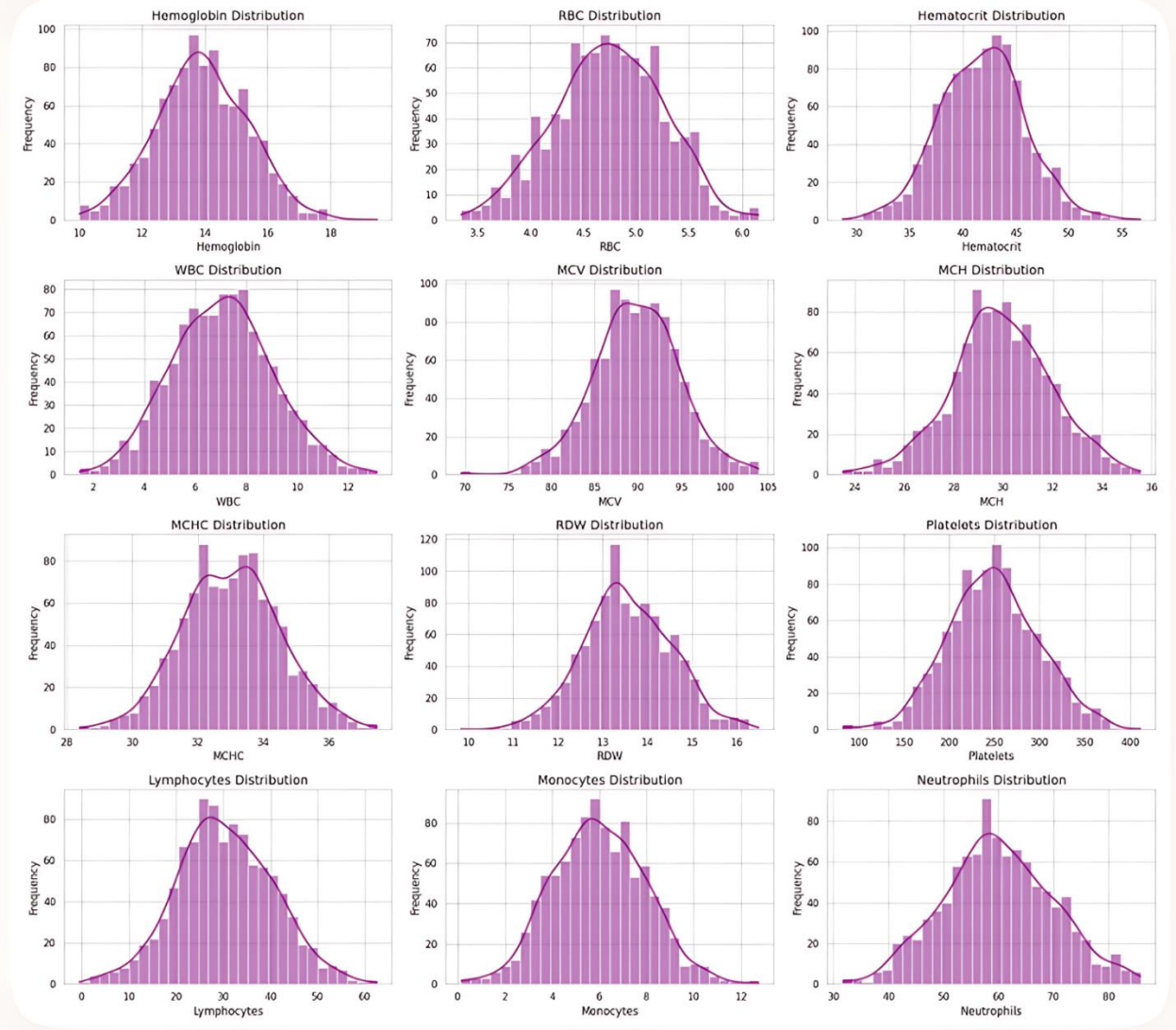


Figure 4.5 presents histograms illustrating the distribution of nine hematological parameters among study participants. Each plot displays the frequency of values for a given marker, accompanied by a smooth density curve to highlight the underlying pattern of distribution. Parameters include Hemoglobin (g/dL), RBC count (10^6/µL), Hematocrit (%), WBCs (10^3/µL), MCV (fL), MCH (pg), MCHC (g/dL), RDW (%), and Platelets (10^3/µL). The x-axes represent the respective parameter ranges, while the y-axes denote count frequency.

Genotype Frequency and Hardy-Weinberg Equilibrium

The distribution of HIF-1α P582S genotypes in the sample was as follows: CT genotype was the most prevalent, observed in 84% of individuals, followed by CC at 11% and TT at 5%. Corresponding allele frequencies were 53% for the C allele and 47% for the T allele. Notably, the

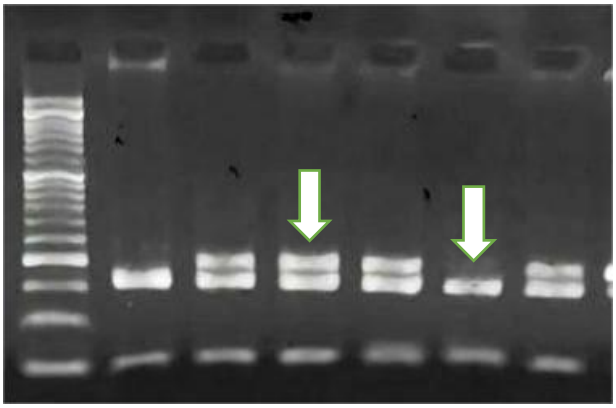
observed genotype frequencies showed a significant deviation from Hardy–Weinberg equilibrium ($\chi^2 = 41.28$, $p < 0.05$), indicating potential population-specific factors or selective pressures influencing this polymorphism, Table 4.4.

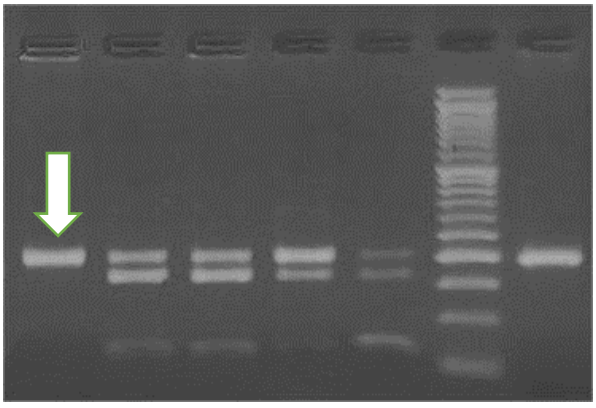
Table 4.4 Frequency Analysis of CC, CT, and TT Genotypes in Sample Population. Hardy-Weinberg equilibrium: Chi-squared value=41.28 and p-value <0.05.

Genotype	Frequency	Percent%
CC	11	11.0%
CT	84	84.0%
TT	5	5.0%
Total	100	100.0%
Alleles	C	106(53%)
	T	94(47%)

Molecular Analysis and Genotyping Validation

Restriction digestion using Tsp451 and gel electrophoresis distinguished genotype-specific fragment patterns (CC = 150bp/47bp; TT = 197bp; CT = 197bp/150bp/47bp, Figures 4.5 and 4.6). Sanger sequencing in a subset confirmed the genotype assignments Figure 4.7.





Figures 4.6 and 4.7 lanes showing a single band indicated the homozygous mutant genotype TT, one fragment was 197bp, two bands represented the homozygous wild-type variant CC, two

fragments were 150bp and 47 bp, and three bands corresponded to the heterozygous genotype. Three fragments were 197bp, 150bp, and 47bp. In both figures, the ladder was 50bp.

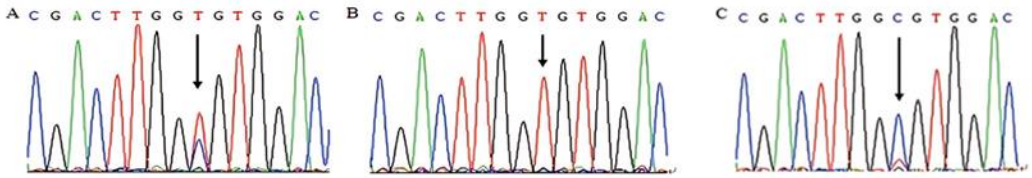


Figure 4.8. Sanger sequencing was performed on a subset of samples to confirm the RFLP genotyping results, with chromatograms illustrating the nucleotide substitution at the polymorphic site and validating the PCR-RFLP data for (A) heterozygotes CT,(B) homozygous mutants TT, and (C) the wild-type CC.

Association of Genotype with Demographic and Lifestyle Factors

The CT genotype was predominant across all age, BMI, smoking, exercise, and gender groups. No statistically significant associations were found between genotype and demographic or lifestyle characteristics (Table 4.5).

Table 4.5. Shows the distribution of P582S (HIF-1α) Genotypes According to Demographic and Lifestyle Variables in the Study Population.

Variable	Category	CC n (%)	CT n (%)	TT n (%)	p-value
Age group	18-30	8 (14.8)	44 (81.5)	2 (3.7)	
	>30-40	1 (4.5)	19 (86.4)	2 (9.1)	
	>40	2 (8.3)	21 (87.5)	1 (4.2)	0.241
Body mass index	<18.5	0 (0.0)	3 (100.0)	0 (0.0)	
	18.5-24.9	3 (6.4)	42 (89.4)	2 (4.3)	

	25-29.9	7 (15.9)	36 (81.8)	1 (2.3)	
	>30	1 (16.7)	3 (50.0)	2 (33.3)	0.115
Smoking	Yes	2 (11.1)	15 (83.3)	1 (5.6)	
	No	9 (11.0)	69 (84.1)	4 (4.9)	0.993
Exercise	Yes	3 (15.8)	15 (78.9)	1 (5.3)	
	No	8 (9.9)	69 (85.2)	4 (4.9)	0.754
Gender	Female	6 (11.3)	45 (84.9)	2 (3.8)	
	Male	5 (12.5)	39 (97.5)	1 (2.5)	0.968

Age group: Among participants aged 18-30 years, the CT genotype was the most prevalent (81.5%), followed by the CC genotype (14.8%) and the TT genotype (3.7%). Similar trends were observed in the >30-40 and >40 age groups, with CT genotype frequencies exceeding 80% in both. No statistically significant difference in genotype distribution was found across age groups ($p = 0.241$).

Body Mass Index (BMI): The genotype distribution across BMI categories showed the CT genotype to be consistently the most frequent. However, the TT genotype proportion was relatively higher in the BMI >30 category (33.3%) compared to other BMI groups, which contributed to a statistically insignificant association between genotype and BMI ($p = 0.115$).

Smoking status: Among smokers, the CT genotype accounted for 83.3% of participants, with CC and TT genotypes comprising 11.1% and 5.6%, respectively. The distribution among non-smokers was comparable, with no significant association between genotype frequencies and smoking status ($p = 0.993$).

Exercise status: Participants who reported exercising had a CT genotype prevalence of 78.9%, slightly lower than non-exercisers (85.2%), but genotype distributions were not statistically different by exercise status ($p = 0.754$).

Gender: The CT genotype predominated in both females (84.9%) and males (97.5%). The CC genotype was observed in 11.3% of females and 12.5% of males, while the TT genotype was rare in both groups. There was no statistically significant difference in genotype distribution between males and females ($p = 0.968$).

Genotype Associations with Hematological Parameters

None of the parameters show statistically significant differences among the genotype groups, as all p-values are well above the conventional significance level of 0.05.

Such as p-value for MCV ($p = 0.324$), MCH ($p = 0.683$), and lymphocyte percentage ($p = 0.557$). (Table 4.6).

Table 4.6.Correlation of P582S with hematological parameters. Significant difference at $p < 0.05$, tested by ANOVA. There is no evidence that the blood parameters measured differ by genotype (C/C, C/T, and T/T).

Parameter	C/C (Mean ± SD)	C/T (Mean ± SD)	T/T (Mean ± SD)	p-value (ANOVA)
WBCs ($\times 10^3/\mu L$)	7.45 ± 2.763	7.27 ± 2.1	8.2 ± 1.09	0.635
RBCs ($\times 10^6/\mu L$)	4.36 ± 0.80	4.46 ± 0.61	4.2 ± 0.44	0.626
Hemoglobin(g/dL)	12.45± 4.50	12.80±1.72	11.80±1.92	0.564
Hematocrit (%)	38.81 ± 5.96	37.27 ± 4.53	34.00 ± 3.74	0.167
MCV (fL)	83.82 ± 6.22	81.27 ± 6.47	78.80 ± 4.02	0.324
MCH (pg)	24.72 ± 3.79	27.27 ± 3.09	26.00 ± 3.08	0.683
MCHC (g/dL)	33.64 ± 1.12	33.10 ± 1.78	33.2 ± 2.28	0.628
RDW (%)	12.482 ± 1.25	12.92 ± 1.64	12.60 ± 0.54	0.898

Parameter	C/C (Mean ± SD)	C/T (Mean ± SD)	T/T (Mean ± SD)	p-value (ANOVA)
Platelets (×10³/µL)	275.09 ± 45.79	255.42 ± 66.10	314.40 ± 101.80	0.119
Lymphocytes (%)	32.45 ± 10.91	30.90 ± 9.49	35.20± 10.59	0.577
Monocytes (%)	4.55 ± 1.91	5.44 ± 3.01	4.40 ± 1.51	0.486
Neutrophils (%)	61.36 ± 11.50	62.9 ± 10.44	60.80 ± 10.91	0.834

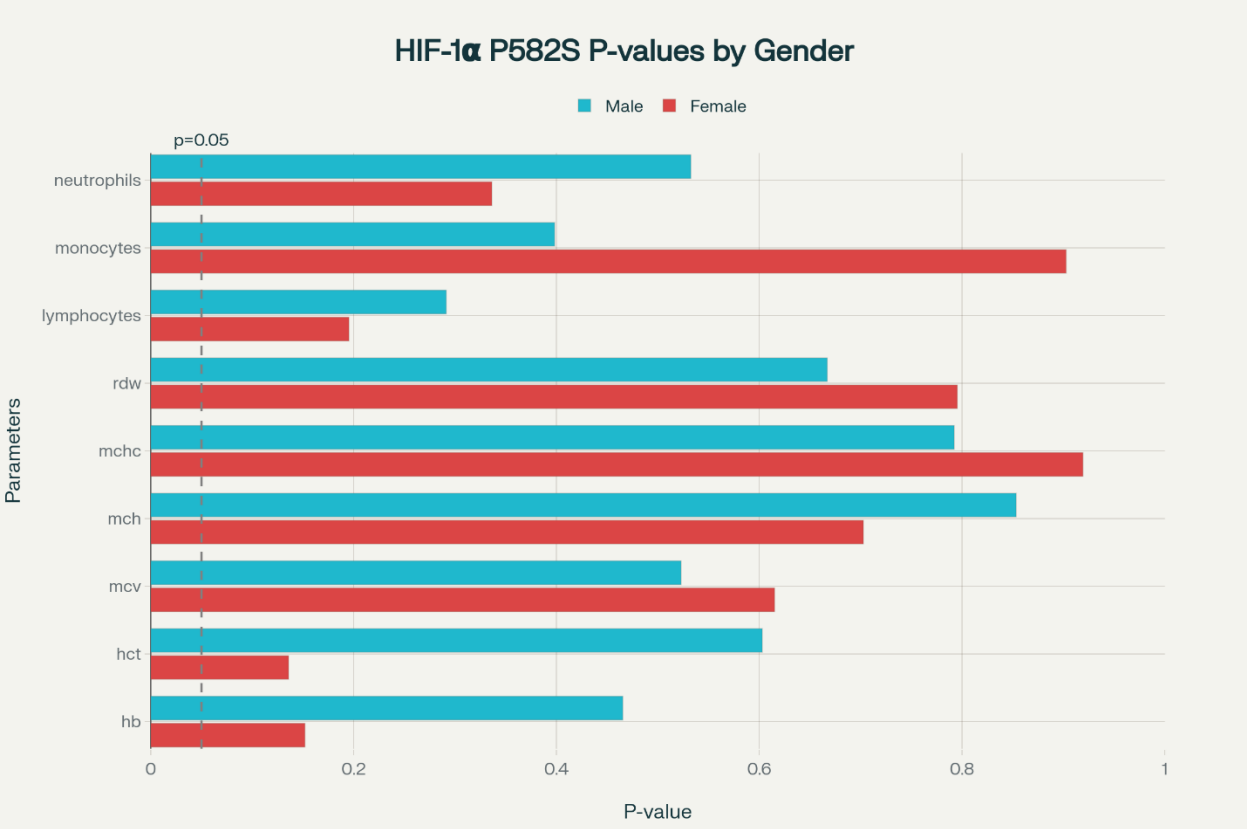


Figure 4.9. HIF-1α P582S Polymorphism vs Hematological Parameters by gender.

No statistically significant associations were observed between HIF-1α P582S polymorphism genotypes and any of the tested hematological parameters in either gender group. All p-values ranged from 0.136 to 0.919, well above the significance threshold. F-statistics ranged from 0.084

to 2.070, with females showing numerically higher F-values for red blood cell indices (Hb F=1.950, p=0.152; Hct F=2.070, p=0.136) compared to males, though these differences remained non-significant.

Genotype Distribution by Hemoglobin Status

The sample was split into hemoglobin levels (Hb≥12 g/dL) called controls and hemoglobin levels of hemoglobin (Hb<12 g/dL) called cases. To investigate the relationship between the P582S polymorphism and hemoglobin among individuals.

The distribution of P582S HIF-1A genotypes and allele frequencies was compared between individuals with normal hemoglobin levels (Controls: Hb > 12.0 g/dL) and those with low hemoglobin levels (Cases: Hb < 12.0 g/dL). The chi-square test did not indicate a significant association between the P582S genotype and hemoglobin status. (Table 4.7).

Among the Controls (n = 66), the most common genotype was C/T (84.8%), followed by C/C (12.1%) and T/T (3.0%). In the Cases group (n = 34), C/T remained the dominant genotype (82.4%), with C/C and T/T genotypes comprising 8.8% each. The genotype distribution did not differ significantly between the two groups (p = 0.42).

Similarly, allele frequency analysis revealed comparable proportions of the C allele in Controls (54.5%) and Cases (50.0%), with T allele frequencies of 45.5% and 50.0%, respectively. There was no statistically significant difference in allele frequencies between groups (p = 0.55).

These findings indicate that the P582S polymorphism of the HIF-1A gene is not significantly associated with differences in hemoglobin levels in this study population.

Table 4.7 Genotype and Allele Frequencies of P582S HIF-1A with Hemoglobin Levels.

Genotype	Controls: n (%) (Hb>12.0 g/dL)	Cases: n (%) (Hb<12.0 g/dL)	p-value
C/C	8 (12.1%)	3 (8.8%)	0.42
C/T	56 (84.8%)	28 (82.4%)	0.42
T/T	2 (3.0%)	3 (8.8%)	0.42

Allele C	72 (54.5%)	34 (50.0%)	0.55
Allele T	60 (45.5%)	34 (50.0%)	0.55

Chapter Five: Discussion

The main goal of this study was to examine the relationship between the Pro582Ser (P582S, rs11549465) polymorphism in the hypoxia-inducible factor-1 alpha (HIF-1α) gene and various hematological parameters in a group of Palestinian healthy adults from the northern West Bank. The study found no significant association between the P582S HIF-1α polymorphism and specific hematological parameters. Besides that, Socio-demographic variables, including age, gender, smoking, BMI, and Physical activity, did not significantly affect genotype distribution. The study also found that the P582S polymorphism is common in this population sample, with genotype frequencies of 11% for CC, 84% for CT, and 5% for TT, along with a high T allele frequency of 47%.

Molecular Mechanisms and Physiological Context

Understanding these findings requires considering the molecular and physiological functions of the HIF-1α P582S polymorphism. HIF-1α encodes a vital component of the hypoxia-inducible factor complex, which acts as a master regulator of cellular responses to oxygen deprivation. This transcription factor plays a key role in linking oxygen sensing with hematopoietic regulation through complex interactions with the erythropoietin (EPO), transferrin, and hepcidin pathways (Cebral, Jua; Mut & Weir, Jane; Putman, 2011; Souvenir et al., 2014).

In the oxygen-dependent degradation (ODD) domain of HIF-1α, the P582S polymorphism is a proline-to-serine alteration from a molecular standpoint. Under hypoxic circumstances, this alteration may impact transcriptional activity and protein stability. Increased transcriptional activity of hypoxia-responsive elements has been linked to the variation, which may exert regulatory effects on key downstream targets, notably VEGF, EPO, and genes implicated in iron homeostasis(Bi et al., 2015; Huang et al., 2022).

Given this, it was suggested that the P582S alterations contributed to hematological parameter variability. In this investigation, there was no significant correlation between the P582S HIF-1A polymorphism with particular hematological parameters.

Gender-Specific of Associations:

Separate tests were performed for males and females to assess whether the polymorphism's effect differs by gender.For males (n=42), none of the hematological parameters (e.g., hemoglobin,

hematocrit, MCV, MCH, MCHC, RDW, lymphocytes, monocytes, neutrophils) showed significant differences across the three genotypes (CC, CT, TT). All p-values were greater than 0.05, indicating no significant effect of genotype on these parameters.

For females (n = 58), no significant differences in hematological parameters were identified across genotypes.

Though the F-statistics for some red blood cell parameters (hemoglobin and hematocrit) were numerically higher than those in males, the p-values did not reach significance ($p > 0.05$).

Comparison with Previous Studies

To properly comprehend our findings, we need to compare them to existing studies on the HIF-1α P582S polymorphism in various groups and symptoms. The results align closely with those of Akkoub and Khabour (2023), who investigated 310 women and found no association between the P582S HIF-1α mutation and hematological parameters, including hemoglobin ($p = 0.37$), RBC count ($p = 0.33$), and hematocrit ($p = 0.96$). The current study found no significant differences when examining cases with low hemoglobin (<12.0 g/dL) versus controls ($p = 0.42$) (Akkoub & Khabour, 2023)

Both males and females were included in the current investigation. Interpretation of gender-related effects remains restricted, as no sex-specific subgroup analysis was conducted. Contrary to prior research, our data reveal differing patterns. From the conclusions of the landmark study. The P582S polymorphism protects healthy blood donors, particularly males, against iron deficiency, according to Torti et al. (n.d.)

Their cross-sectional study reported that individuals carrying the Pro582Ser variant exhibited elevated ferritin and hemoglobin levels and had a reduced likelihood of developing anemia in response to recurrent blood loss. More transcriptional activity is probably the cause of the polymorphism's increased erythropoietic response. Crucially, the protective effect was more noticeable in men, suggesting possible sex-specific modulation (Torti et al., n.d.).

Other studies took these polymorphisms into their studies. Consistent with these findings, Döring et al. (2010)reported a relationship between the Pro582Ser variant and improved staying power overall performance in top athletes, correlating the polymorphism to better maximum oxygen consumption and the variant to staying power training. Prior et al. (2004)also identified HIF1A sequence variants related to maximum oxygen intake, bolstering the functional significance of this genetic variant in oxygen use efficiency.

Building on this insight, McPhee et al. (2011)studied younger women and found that the P582S polymorphism influenced responses to staying power schooling, suggesting that the polymorphism should affect physiological adaptation to exercise in females, although the results might differ from males. Liu & Hu (2006) found that the P582S polymorphism plays a role in hypoxia sensitivity by affecting individual adaptive responses related to HIF-1α gene variants. Given that our study population consisted of healthy participants naïve to both endurance training and acute hypoxic conditions, the anticipated functional effects may not have been reflected in standard blood parameters.

Beyond hematological and athletic ramifications, P582S has been linked to cancer and cardiovascular disease. (Lessi et al., 2014)identified gene variants in HIF-1α and P582S as predictive variables in renal mobile carcinoma. (Meka et al., 2015)linked the C1772T (P582S) polymorphism to an increased risk of breast cancer, whereas Fu et al. (2005)linked it to prostate cancer development via inhibiting HIF-1α breakdown under normal conditions.

Furthermore, Xu et al. (2013)conducted a meta-analysis that demonstrated a link between the P582S variant and gastrointestinal malignancies. (Resar et al., 2005) emphasized the polymorphism's influence on coronary collateral development in ischemic coronary heart disease, demonstrating its cardiovascular importance.

In addition, under conditions of metabolic illness, the P582S polymorphism has been reported to confer protective effects against diabetes-related complications. (Ekberg et al., 2019)discovered that the variant protects against severe diabetic retinopathy, while Pichu et al. (2015) found that the polymorphism influences diabetic foot ulcer outcomes. (Zhao et al., 2022) found genetic polymorphisms, including P582S, to influence diabetic foot hazard in a systematic analysis.

Our investigation highlighted no significant correlation between the P582S HIF-1α polymorphism with hematological parameters in the Palestinian community. However, abundant evidence links this variation to cancer development and cardiovascular remodeling. These implications across multiple disease states and functional outcomes are significant beyond baseline blood indices, implying that their harmful consequences may be more obvious in illness circumstances than in healthy individuals.

Genotype Distribution and Hardy-Weinberg Equilibrium

Among Palestinians from the northern West Bank, the study found a T (582S) allele frequency of 47%, which is significantly higher than the frequency observed in populations

worldwide. A Jordanian survey of 310 women revealed a T allele frequency of 17.5%, which is consistent with local trends. The Palestinian frequency, however, is far above the typical levels found in other groups, with Asians exhibiting the maximum effective T allele frequency of 4.10 % and Caucasians exhibiting around 19.26%. Compared to the stated frequencies in nearby Arab communities, which include Iran (~10.6%) and Turkey (~14.7%), the Palestinian prevalence is significantly higher(Akkoub & Khabour, 2023; D. Li et al., 2013).

This greater proportion in Palestinians could reflect inherited genetic variations of the Levantine Arab population. Previous genetic research has demonstrated that Levantine Arabs (Palestinians, Jordanians, Lebanese, and Syrians) correspond to genetic similarities and exhibit unique patterns from other Arab groups. Genetic variation among Arab populations is well-established in the literature, with notable differences observed across locations in allele frequencies for a few genetic markers (Hajjej et al., 2018).

The considerable deviation from Hardy–Weinberg equilibrium ($\chi^2 = 41.28$, $p < 0.05$) indicates that the HIF-1A P582S locus is not in genetic equilibrium within the population being studied. This imbalance may be caused by migration, non-random mating, population stratification, or natural selection acting on this particular genetic variety. These influences may have affected allele frequencies, leading to the observed divergence in genotype distribution (Hardy (Castle) Weinberg Equilibrium : Deviations from Hardy-Weinberg Equilibrium, n.d.; Saadat, 2024).

In genetic affiliation research, it is essential to detect deviations from HWE as they may indicate actual biological occurrences, population stratification, or technological errors. Strong factors are at work in this genetic variance within the Palestinian population, as evidenced by the large size of this deviation (chi-squared = 41.28) (Saadat, 2024). Additionally, the relatively small sample size (n=100) may amplify the effects of genetic drift, leading to random fluctuations in allele frequencies.

Methodological Comparisons

Our cross-sectional, convenience-sampled study has benefits and challenges common in genetic epidemiology research. While molecular techniques (PCR-RFLP) provide accurate genotyping, the pattern length (n=100) restricts the potential to detect tiny genetic effects and interactions. This aligns with methodological considerations, indicating that several association

studies may yield inconsistent or limited conclusions due to underpowered designs or populations. (Akkoub & Khabour, 2023).

More multi-center research is necessary to properly comprehend the multifactorial impacts on HIF-1α functioning and associated phenotypes, as demonstrated by comparisons to larger meta-analyses. Our findings give essential population-specific information for future studies and individualized treatment strategies in the Middle Eastern environment, complementing worldwide genetic research on HIF-1α polymorphisms.

Study Limitations, Future Directions, and Clinical Implications

- Sample Size and Statistical Power. The research's small sample size of 100 participants may restrict its ability to identify subtle genetic changes in hematological markers, maybe lead to false negative or positive results.
- Cross-sectional design limits the ability to determine causal links or temporal changes in blood parameters related to the P582S polymorphism.
- Deviation from Hardy-Weinberg Equilibrium: The detected significant deviation may imply population stratification, non-random mating patterns, or genotyping mistakes, thus biasing data and affecting interpretability. A small sample and the population from the same region can make hidden true deviations detectable.
- Gender Distribution and Lack of Stratified Analysis: With 58% female contributions and no separate statistical examination applying sex, the sex-specific effects of the P582S polymorphism, as shown in previous work, may go undiscovered. Also, to make a fair judgment should be an equal number of both sexes.
- Limited Representation of Lifestyle Data: The majority of subjects were physically inactive and did not smoke, which precluded investigation of gene-environment interactions that could modify the effects of the polymorphism. Different groups that should be included.
- Focus on Healthy Individuals: Researching issues that appear to be healthy may fail to keep an eye on institutions that become evident beneath disorders, hypoxia, or stresses related to blood loss. Many other factors should be considered, even metal or psychological conditions within the population sample

Future Directions:

- Expanding the sample size and studying male and female individuals independently improves statistical power and clarifies gender-specific effects.
- Longitudinal studies can explore how a polymorphism affects hematological changes during strain events such as blood donation, anemia improvement, or hypoxia exposure.
-
- Gene-Environment Interaction Analysis, including lifestyle factors including smoking, physical activity, iron consumption, and altitude exposure, and more factors susceptible to effects.
- Clinical Correlation in Disease States, investigating the P582S polymorphism in patients with anemia, cancer, cardiovascular diseases, or diabetes, might reveal clinically meaningful relationships beyond baseline blood indices and not just for healthy individuals.
- Population Genetics Research, to fully understand the Hardy-Weinberg deviation and allele distribution seen, more genetic research is needed to examine population structure, linkage disequilibrium, and allele frequency changes in Palestinians and traditions in this region, and ensure a sample to examine. Which that enough to make a correct interpretation of any deviation and avoid falsely positive results.

Conclusion

This study provides the first insight into the genotype distribution of the HIF-1α P582S polymorphism and its potential hematological implications in the Palestinian population. While no significant associations were found between genotype and blood parameters, the marked deviation from Hardy–Weinberg equilibrium and the high prevalence of the T allele suggest underlying genetic dynamics that merit further exploration.

More studies are needed to confirm this finding; future research should incorporate more gender-balanced populations. To evaluate gene–environment interactions, take lifestyle and environmental data into account. Examine more SNPs in the HIF-1α pathway and how they affect hematological health throughout time.

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Appendices

1. Appendix A: Institutional Review Board (IRB) Approval

R-2025 A 4 N (1).pdf

2. Appendix B: Task Facilitation Document (تسهيل المهمة)

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133903.pdf

3. Appendix C: The QIAamp DNA Mini Kit

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8_Pcard_QIAamp_DNA

4. Appendix D: Participant Questionnaire

questionnaire-sana
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الارتباط بين تعدد الأشكال الجينية برولين582سيرين في جين العامل المحفز لنقص الاوكسجين ومعايير القيم الدموية لدى الافراد الاصحاء في المناطق الشمالية من الضفة الغربية – فلسطين.

سناء حسان صالح خطيب

دكتور فكري سماره

دكتور مهند خضر

دكتور كمال ضميدي

ملخص

الخلفية :يُعد عامل الاستجابة لنقص الأكسجين HIF−1α منظِّمًا رئيسيًا لنسخ أكثر من 60 جينًا، ويلعب دورًا حيويًا في العديد من العمليات الخلوية، بما في ذلك تكوّن الأوعية الدموية، وتكوين خلايا الدم الحمراء، واستقلاب الحديد، وتنظيم توازن الأكسجين. وقد ارتبطت التغيرات الجينية في جينHIF−1α ، وخاصة تعدد الأشكال P582S (rs11549465)، بتغيرات في التعبير الجيني، واستقرار البروتين، وزيادة القابلية للإصابة بعدة اضطرابات مرضية، بما في ذلك أنواع مختلفة من السرطانات والأمراض الدموية.

الأهداف :هدفت هذه الدراسة إلى التحقيق فيما إذا كان هناك ارتباط بين تعدد الأشكال الجيني P582S في جين HIF−1α والمعايير القيم الدموية لدى الأفراد الأصحاء في المناطق الشمالية من الضفة الغربية – فلسطين، بالإضافة إلى تحديد أي خصائص ديموغرافية وسريرية قد تكون مرتبطة بهذا التباين الجين.

المنهجية:تم اختيار 100 مشارك في هذه الدراسة المقطعية. وقد تم إجراء تحليل العد الدموي الكامل (CBC)، تلاه استخدام تقنية PCR–RFLP لتحديد النمط الجيني لتعدد الأشكال في جين HIF–1α. وتم تأكيد نتائج تقنية PCR–RFLP باستخدام تسلسل سانجر. استخدمت برامج SPSS وPython لإجراء التحليل الإحصائي. ولتقييم العلاقة بين تعدد الأشكال في جين HIF–1α والمعايير الدموية، تم استخدام اختبار كاي–تربيع (Chi–square) وتحليل التباين الأحادي (One–way ANOVA). وتم اعتماد مستوى دلالة إحصائية قدره 0.05..

النتائج: كان متوسط عمر المشاركين 31.4 سنة، ومتوسط مؤشر كتلة الجسم 24.9 ±3.46 (BMI) كغم/م². معظم المشاركين لا يمارسون الرياضة (81.0%)، وحوالي 18.0% منهم من المدخنين الحاليين. بلغت نسبة تكرار الأليل الطافر 582(T) S حوالي 47.0%، في حين بلغت نسبة تكرار الأليل السائد 582(C) P حوالي 53.0%. لم يُلاحظ وجود ارتباط بين تعدد الأشكال P582S في جين HIF–1α ومستوى الهيموغلوبين (p=0.56)، وعدد خلايا الدم الحمراء (p=0.62)، والهيماتوكريت (p=0.16)، ومتوسط حجم الخلية (MCV) (p=0.32)، ومتوسط حجم الكريات الحمراء (p=0.68)، وتركيز الهيموغلوبين الكروي المتوسط (MCHC) (p=0.62)، وعرض توزيع خلايا الدم الحمراء (p=0.89) (RDW). علاوة على ذلك، لم تُلاحظ فروق واضحة في توزيع الأنماط الجينية لتعدد الأشكال P582S حسب عمر المشاركين، أو مؤشر كتلة الجسم، أو حالة التدخين، أو نمط ممارسة الرياضة (p>0.05).

الاستنتاجات: في الختام، قد لا تكون المؤشرات الدموية لدى الأفراد الأصحاء مرتبطة بتعدد الأشكال الجيني P582Sفي جين .HIF–1α ولتأكيد هذا الاستنتاج، هناك حاجة إلى إجراء المزيد من الدراسات على مجموعات سكانية مختلفة.

الكلمات المفتاحية :((HIF–1A، P582S، rs11549465، الدم ، الهيموغلوبين.