Arab American University
Faculty of Graduate Studies
Department of Health sciences
Master Program in Immunohematology

Correlation of Breakpoint Cluster Region - Abelson Murine Leukemia (BCR-ABL) transcript variants with hematological parameters and demographic characteristics in Palestinian patients with chronic myeloid leukemia

Mohammed Fathi Qalalwah

202112984

Supervision Committee:
Dr. Mohannad Khader
Dr. Fekri Samarah
Dr. Adham Abu Taha

This Thesis Was Submitted in Partial Fulfillment of the Requirements for the Master Degree in Immunohematology

Palestine, 7 / 2025

© Arab American University. All rights reserved.

Arab American University
Faculty of Graduate Studies
Department of Health sciences
Master Program in Immunohematology

Thesis Approval

Correlation of Breakpoint Cluster Region - Abelson Murine Leukemia (BCR- ABL) transcript variants with hematological parameters and demographic characteristics in Palestinian patients with chronic myeloid leukemia

Mohammed Fathi Qalalwah

202112984

This thesis was defended successfully on 10 / 7 / 2025 and approved by:

Thesis Committee Members:

Name	Title	Signature
1. Dr. Mohannad Khader	Main Supervisor	
2. Dr. Fekri Samarah	Members of Supervision Committee	
3. Dr. Adham Abu Taha	Members of Supervision Committee	

Palestine, 7 / 2025

Declaration

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

Student Name: Mohammed Fathi Qalalwah

Student ID: 201812865

Signature: Mohammed Qalalwah

Date of Submitting the Final Version of the Thesis: 28/8/2025

Dedication

"All praise is due to Allah most exalted, outwardly and inwardly, for his endless grace and guidance. Without His help and assistance not, a single word would have been put to paper, nor would a single step have been taken. I thank Allah most exalted for the strength, forbearance, and clarity of thinking that He gave me throughout this process. I pray Allah most exalted accepts this work solely for His sake, and that He makes this work beneficial to knowledge and all those who seek." I am incredibly grateful to my beloved father, Fathi Qalalwah, and my loving mother Asmahan Qalalwah. Your love, encouragement, and prayers have been my source of strength. Thank you for believing in me, supporting me every step of the way, and teaching me patience, hard work, and perseverance. This accomplishment is as much yours as it is mine. I would like to express my appreciation for my dear wife, Haneen Qalalwah. Thank you for your love, patience, and support. You have grounded me through every difficulty, and you continue to support me. You have faith in me, which has empowered me. I would like to express my appreciation for my children, Nour, Ammar, Fathi, and Yaffa. You are the light of my life, and you are why I do what I do. Every time I saw your smile, heard your laughter, or felt your love; I was motivated to continue moving forward through the hardest of days. This accomplishment is for you and because of you. To my beloved brother and soul mate, Mustafa — your friendship, inspiration, and unwavering presence in my life have been absolutely priceless. You haven't just been a brother — you've been an ever-present buddy in all the steps of this journey. To my loving sisters — Rania, Suheir, Rasha, Rola, Sawsan, and Hasnia — thank you for your love, prayers, and encouragement. You have all touched my heart in a special way, and your faith in me has meant more to me than I can say. I am truly thankful for having been blessed with such loving and supportive family members.

Mohammed Fathi Qalalwah

Acknowledgments

I would like to thank my supervisor, Dr. Mohanned Khader, for his precious advice, support, and constructive comments throughout the process of this research. His directions, forbearance, and support have been invaluable in the development of this project and in building my research and academic abilities. It was an honor to work under his guidance, and I am really indebted for being given a chance to learn from him. I would like to extend my heartfelt thanks to Dr. Fekri Samarah, whose exceptional support and dedication were second only to that of my supervisor. His generous assistance, valuable insights, and unwavering willingness to help at every stage of this research have left a lasting impact on both the quality of this work and my personal academic growth. I am deeply grateful for his guidance, kindness, and the genuine care he showed throughout this journey. I would also like to use this chance to thank Dr. Adham Abu Taha, my supervisor at the An-Najah National University Hospital, for his unlimited support and encouragement. His dedication to science, educational advancement, and professional practice has been very inspiring. I am particularly thankful for the opportunity he afforded me and for believing in the importance of clinical and academic practice development. His motivation played an important role in helping me balance my responsibilities and carry out this research successfully. I am also truly thankful to all the esteemed professors and lecturers in the university who have opened my eyes and guided my studies. All of you have contributed to me in your own manner, and I am very grateful to your toil and devotion. I hope that nobody has been neglected, and I would like to thank to the core of my heart to all those who assisted me in completing this work directly or indirectly.

Correlation of Breakpoint Cluster Region - Abelson Murine Leukemia (BCR-ABL) transcript variants with hematological parameters and demographic characteristics in Palestinian patients with chronic myeloid leukemia

Mohammed Fathi Qalalwah
Dr. Mohannad Khader
Dr. Fekri Samarah
Dr. Adham Abu Taha

Abstract

Background: Chronic myeloid leukemia (CML) is classified as a myeloproliferative neoplasm connected to the Philadelphia chromosome. This connection arises from the BCR-ABL fusion gene, resulting from a chromosome translocation between chromosomes 9 and 22. The gene BCR-ABL produces a continuous form of tyrosine kinase, which contributes to the progression of leukemia. There are various transcript variants, with E13a2 (B2a2) and E14a2 (B3a2) being the most prevalent. These variants are linked to specific diseases, their treatment outcomes, and general prognosis.

Objectives: Our goal was to evaluate the connection between BCR-ABL transcript variants and various blood parameters (such as WBC, Hb, platelet count) in Palestinian patients diagnosed with CML, alongside their clinic pathological characteristics.

Methodology: A cross-sectional sample of fifty patients with CML was selected from An-Najah National University Hospital in Palestine for this study. RNA was extracted from peripheral venous blood collected in EDTA tubes, and the variants of BCR-ABL transcripts were verified using RT-PCR. Additionally, data from the patients' medical records were gathered. Statistical analysis was used to examine the connection between the different transcript variants and various clinical and demographic factors.

Results: The ratio of males to females was 1.27:1. Among the patients, twenty-nine had the b3a2 transcript, while twenty-one tested positives for the b2a2 variant. The total leukocyte count was higher in the b3a2 group compared to the b2a2 group, but the b2a2 variant had notably higher platelet counts than those with the b3a2 transcript.

Conclusion: Our study showed a significant difference in blood-related measurements between b3a2 and b2a2 in CML, particularly regarding white blood cell and platelet counts. The variation in transcript expression linked to age might also suggest biological differences. These findings support the importance of transcript typing for diagnosing and predicting outcomes in CML, providing a useful reference for future studies within the Palestinian community.

Keywords: Chronic Myeloid Leukemia (CML), BCR-ABL Transcript Variants, Philadelphia Chromosome, Hematological Parameters, Palestinian Patients

Table of Contents

Declaration I

Dedication II

Acknowledgments III

Abstract IV

List of Tables VIII

List of Figures IX

List of Appendices X

List of Definitions of Abbreviations XI

Chapter One: Introduction 1

1.1 Introduction 1

1.2 Study Problem 2

1.3 Justification 2

1.4 Specific Objective 3

1.5 Research Questions and Hypotheses 3

Chapter Two: Literature Review 4

2.1 Myeloproliferative neoplasms (MPN) and Classification 4

2.2 Chronic myeloid leukemia (CML) 5

2.2.1 Definition and epidemiology 5

2.2.2 Historical perspective 6

2.3 Gene Expression Analysis 6

2.3.1 Techniques used for gene expression profiling in CML 6

2.3.2 Identification of gene signatures associated with disease progression and treatment response 8

2.4 Philadelphia Chromosome (BCR-ABL Gene) 9
2.4.1 Structure and Function of BCR-ABL Fusion Protein 9
2.4.2 Role of BCR-ABL in dysregulation of signaling pathways 10
2.4.3 Implications for targeted therapy development 12
2.5 BCR-ABL Transcript Variants 13
2.5.1 Description of common BCR-ABL transcript variants (e.g., e13a2, e14a2) 13
2.5.2 Clinical significance and prognostic implications of different transcript variants 14
2.6 Laboratory Evaluation 16
2.6.1 Blood counts and peripheral smear examination 16
2.6.2 Bone marrow aspiration and biopsy findings 17
2.6.3 Molecular tests for BCR-ABL transcript detection 18
2.7 Previous studies related to the study 19
Chapter Three: Methodology 21
3.1 Study deign: 21
3.2 Study area: 21
3.3 Study Participants and setting 21
3.4 Sample Size Justification: 21
3.4.1. Sample Size Justification and Limitations 22
3.4.2. Timing of Sample Collection: 22
3.5 Inclusion and exclusion criteria: 23
3.6 RNA extraction and blood collection: 23
3.6.1 RNA extraction: 23
3.6.2 RNA Quantification: 24
3.6.3 RNA Quantification: 24
3.7 Complementary DNA synthesis: 24

3.8 Gene amplification and detection of fusion type: ... 25
3.9 Statistical analysis: ... 26
Chapter Four: Results ... 27
4.1 Demographic characteristics of the study population ... 27
4.2 Hematological characteristics of the study population ... 29
Hemoglobin and Packed Cell Volume ... 29
Red Blood Cell (RBC) Indices ... 29
White Blood Cell Count and Differential ... 29
Platelet Count ... 30
4.3. The frequency of different BCR-ABL fusion variants: ... 31
4.4 Demographic data based on BCR-ABL fusion transcripts: ... 32
4.5. Hematological data based on BCR ABL fusion transcript variants: ... 35
4.6. RBC indices based on BCR-ABL fusion transcript variants: ... 37
Chapter Five: Discussion ... 38
5.1 Conclusions ... 43
5.2 Limitations of the Study ... 43
5.3 Recommendations ... 44
5.4 Clinical Follow-Up and Patient Outcomes: ... 44
References ... 47
Appendices ... 52
ملخص ... 57

List of Tables

Table 4.1: Baseline demographic characteristics of the study group 28
Table 4.2: Hematological data of CML patients 30
Table 4.3: Patient's demographic data based on BCR-ABL fusion transcript variants 33
Table 4.4: Clinical Data Summary 35
Table 4.5: Patient's hematological data based on BCR-ABL fusion transcript variants 36
Table 4.6: Patient's RBC indices data based on BCR-ABL fusion transcript variants 37
Table 5.1: Incidence of b2a2 and b3a2 transcripts in Eastern, African, European, and Western countries 40
Table 5.2: Main Published Studies about The Association Between P210bcr-ABL1 Transcript variants And Laboratory Data In Patients With CML 42

List of Figures

Figure 2.1: BCR-ABL oncogene induces pathological conditions through multiple mechanisms, all of which may be attributed to the initial upregulation of tyrosine kinase (El-Tanani et al., 2024). 10

Figure 2.2: Formation of Philadelphia (Ph) chromosome. Translocation of chromosome 9 and 22 forms Ph chromosome with BCR-ABL1 oncogene with either of three breakpoint regions in BCR resulting into p190, p210 and p230 transcripts (Poudel, 2023). 11

Figure 2.3: In this set of pictures, myeloid precursors are evident in the peripheral blood smear of a patient diagnosed with chronic myeloid leukemia (CML). 17

Figure 4.1: Multiplex RT-PCR products were analyzed using gel electrophoresis, and then visualized by UV light following staining with ethidium bromide. The first lane, labeled M, contains a DNA ladder ranging from 100 to 1500 bp. Lanes 2, 7, 9, 10, and 12 show the b3a2 band leinght 385 and lane 3,4,5,6,8 and 11 show the b2a2 band leinght 310. 31

Figure 4.2: The Frequency of Different BCR-ABL Fusion Variants: 32

Figure 4.3: Incidence of b3a2 and b2a2 BCR-ABL Transcripts Across Countries. 41

List of Appendices

Appendix A: IRB Approval 52
Appendix B: تسهيل مهمه بحثية 53
Appendix C: Consent form 54
Appendix D: Material 55
Appendix E: Media Approval -CRC 56

List of Definitions of Abbreviations

Abbreviation	Title
ABL1	Abelson
BCR	Breakpoint cluster region
cDNA	Complementary DNA
CML	Chronic myelogenous leukemia
CNL	Chronic neutrophilic leukemia
ET	Essential thrombocythemia
EUTOS	European treatment and outcome study
FISH	Fluorescence in situ hybridization
JAK	Janus kinase
MBCR	Major breakpoint cluster region
mBCR	Minor breakpoint cluster region
MPNs	Myeloproliferative neoplasms
NGS	Next-generation sequencing
NNUH	An- Najah National university hospitals
PCR	Polymerase chain reaction
Ph	Philadelphia
PI3K	Phosphoinositide 3-kinase
PIP3	(3,4,5)-trisphosphate
PMF	Primary myelofibrosis
PV	Polycythemia Vera
qRT-PCR	Quantitative real-time polymerase chain reaction
RNA-seq	RNA sequencing
RT-PCR	Real-time PCR apparatus
scRNA-seq	Single-cell RNA sequencing
TKIs	Tyrosine kinase inhibitors
WHO	World Health Organization

Chapter One: Introduction

1.1 Introduction

Chronic myelogenous leukemia (CML) is a type of blood cancer that occurs when myeloid cells grow uncontrollably (O'Brien et al., 2004). CML is classified as a long-term disease because it can arise from multipotent stem cells that have the ability to develop into various important cell types, such as granulocytic, erythroid, megakaryocytic, and monocytic lineages. A key feature of this disease is the existence of the BCR-ABL fusion gene (Gen Bank accession NC 000022.11) (Pasternak et al., 1998). In chronic myeloid leukemia (CML), the main chromosomal change is t(9;22)(q34;q11), which leads to the creation of two types of BCR/ABL fusion transcripts, known as b2a2 and b3a2, or sometimes both (Zenebe et al., 2023).

The b3a2 transcript can be alternatively spliced, which enables the presence of different transcript variants. Research shows mixed findings regarding how these transcripts affect blood test results at the time of diagnosis and the progression of the disease later in life. The break in the ABL1 gene (GenBank accession NC 000009.12) is found in the second exon (a2), while the break in the BCR gene can happen in any part of the three areas called the major breakpoint cluster region (MBCR), minor breakpoint cluster region (mBCR), and microBCR (Hovorkova et al., 2024).

Chronic myeloid leukemia affects 1-2 adults per 100,000 and the underlying cause of 15% of newly diagnosed adult leukemia cases (Karunarathna et al., 2024). Where, the current medical techniques mostly focus on "treatment after disease start," whereas molecular medicine offers "prevention before illness begins" (Marques et al., 2024). It is essential to explore the relationship between BCR-ABL transcript variations and chronic myeloid leukemia. This exploration can help identify the genetic factors that might make Palestinians more likely to develop long-lasting leukemia. Additionally, understanding these variations could lead to better insights into how the disease develops and foster the creation of effective preventive treatments for at-risk groups. This research enhances our knowledge of how blood parameters and demographic traits are related to chronic myeloid leukemia, specifically in connection with the b3a2 and b2a2 transcripts. This is achieved by using reverse transcriptase polymerase chain

reaction (RT-PCR) to analyze the occurrence of different transcripts in CML patients, alongside their blood parameters when they first seek treatment.

1.2 Study Problem

Chronic Myeloid Leukemia (CML) is a clinically significant hematological malignancy that demands focused attention in both research and healthcare planning. Although it constitutes a smaller proportion of overall leukemia cases, its impact is substantial due to its chronic nature, the need for lifelong treatment, and the economic burden of targeted therapies. In Palestine, data from a retrospective study conducted in the Gaza Strip between 2017 and 2019 revealed that myeloid leukemias—including CML—accounted for approximately 12% of all hematologic malignancies, with an estimated leukemia incidence of 15 per 100,000 population annually (Abu-El-Noor et al., 2021). While specific national CML statistics remain limited, the findings underscore the presence and ongoing burden of this disease in the Palestinian population. Globally, CML has an estimated incidence of 0.6–2.0 per 100,000 and a growing prevalence due to improved survival outcomes with tyrosine kinase inhibitors (TKIs) (Bower et al., 2016; Jemal et al., 2009). These figures emphasize the necessity for localized data, early diagnosis, and sustained patient monitoring, particularly in under-resourced health systems like that of Palestine (Ali et al., 2021). Nevertheless, no study has yet explored the link between blood-related factors and demographic details in those suffering from chronic myeloid leukemia and variations in BCR/ABL transcripts within the Palestinian context. Currently, there is a lack of recorded information regarding the connection between the b3a2 and b2a2 variations and chronic myeloid

This research aims to investigate possible relationships among hematological factors, demographic information, and BCR/ABL gene variants in patients with CML in Palestine.

1.3 Justification

Investigating the BCR-ABL transcript variant in patients with chronic leukemia in Palestine will enhance our understanding of how chronic myeloid leukemia relates to genotype frequencies (b3a2, b2a2, or both), as well as their hematological features and demographic data. This research could support the creation of new treatment methods and improve control programs for chronic myeloid leukemia. The value of this study lies in its simple method, utilizing reverse transcriptase polymerase chain reaction (RT-PCR) to assess the frequency of

different transcripts and their related blood characteristics at the time of diagnosis for CML patients.

While molecular medicine aims for “prevention before illness occurs,” existing medical approaches predominantly focus on “treatment after the disease has begun.” This research will clarify the connection between chronic myeloid leukemia and its BCR-ABL transcript variants. By pinpointing individuals at risk based on these variants, we can gain deeper insights into the disease's development and design effective preventive strategies.

1.4 Specific Objective

The study's purpose is to implement RT-PCR to evaluate the occurrence of various transcripts in a sample of CML patients and examine their blood parameters upon initial evaluation.

1.5 Research Questions and Hypotheses

1. How common is the BCR-ABL fusion gene genotype in CML patients?
2. Do these genotypes alter hematological characteristics of patients with CML, such as leukocyte and platelet counts, in addition to hemoglobin concentration?

Hypotheses:

- Palestinian CML patients will exhibit varying frequencies of b2a2 and b3a2 transcript variants
- Transcript variants will correlate with specific hematological profiles at diagnosis

Chapter Two: Literature Review

2.1 Myeloproliferative neoplasms (MPN) and Classification

Myeloproliferative neoplasms (MPNs), hematological disorders, are characterized with atypical growth of myeloid cells in the bone marrow due to genetic flaws that matures red blood cells too quickly; increasing and accumulating their quantity in the bone marrow and peripheral circulation (Jain & Sharma, 2024). These acquired genetic abnormalities interferes with signaling pathways, the development and proliferation of hematopoietic stem cell, and are considered MPNs' primary cause (Luque Paz et al., 2023). In the past, MPNs were mainly categorized based on their morphologic and clinical features. However, advancements in molecular genetics have enhanced our understanding of these disorders. This progress has led to better classification by identifying key mutations like MPL, CALR, and JAK2 V617F, significantly influencing how MPNs are diagnosed and classified (Gerke et al., 2023).

The World Health Organization (WHO) classifies MPNs into two main categories based on the **presence or absence of the Philadelphia (Ph) chromosome** (i.e., BCR-ABL1 fusion gene):

1. **Philadelphia chromosome–positive (Ph-positive) MPNs**
   - **Chronic Myeloid Leukemia (CML)**
     Characterized by the presence of the BCR-ABL1 fusion gene resulting from the t(9;22)(q34;q11) translocation. This leads to constitutive tyrosine kinase activity, which drives uncontrolled proliferation of myeloid cells.
2. **Philadelphia chromosome–negative (Ph-negative) MPNs**
   These disorders lack the BCR-ABL1 fusion gene and include:
   - **Polycythemia Vera (PV)**
   - **Essential Thrombocythemia (ET)**
   - **Primary Myelofibrosis (PMF)**
   - **Chronic Neutrophilic Leukemia (CNL)**
   - **Chronic Eosinophilic Leukemia, NOS (CEL-NOS)**

Each subtype has distinct clinical features, treatment options, and prognostic implications (Thiele et al., 2023).

Since these conditions can progress to acute leukemia and increase therapeutic challenges, researchers have developed specialized drugs targeting aberrant signaling pathways. This expanded molecular knowledge provides patients with more therapeutic options, though these complex conditions remain difficult to identify and manage precisely (Gregory et al., 2006).Each of these subtypes has distinct clinical features, treatment options, and prognostic implications (Thiele et al., 2023). Since these symptoms have the potential to develop into acute leukemia and increasing the obstacles of therapeutic management, better or specialized drugs that target aberrant signaling pathways have been developed due to the expanded knowledge of molecular processes behind MPNs, through giving the patient more therapeutic choices. However, these complicated conditions are still difficult to be identified and managed precisely(Gregory et al., 2006).

2.2 Chronic myeloid leukemia (CML)

2.2.1 Definition and epidemiology

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm featuring excessive myeloid cells, particularly granulocytes, in the bone marrow (Elhadary et al., 2023). The Philadelphia (Ph) chromosome marks this condition, resulting from translocation between chromosomes 9 and 22 (t(9;22)(q34;q11)) (L'Abbate et al., 2023). This creates the BCR-ABL fusion gene when the breakpoint cluster region (BCR) gene on chromosome 22 connects with the ABL (Abelson) gene on chromosome 9, producing a constitutively active tyrosine kinase.

CML represents 15-25% of adult leukemias. While CML can affect any age group, it primarily affects individuals over 60 years, with males predominating in most statistics. The sixth and seventh decades represent peak incidence years. Estimates show 1-2 CML cases per 100,000 individuals annually (Jabbour & Kantarjian, 2020).

Although the exact cause remains unknown, genetic predisposition and ionizing radiation exposure are potential risk factors. Tyrosine kinase inhibitors (TKIs) like imatinib, nilotinib, and dasatinib have significantly advanced CML diagnosis and treatment, allowing many patients long-term survival (Kantarjian et al., 2021).

2.2.2 Historical perspective

The 19th century brought developing knowledge of CML causes, symptoms, and treatment management. In 1845, Scottish physician John Hughes Bennett discovered the illness as a separate clinical entity, observing splenomegaly and abnormal blood appearance classified as splenic anemia (Aljamali et al., 2021). Throughout the 19th and 20th centuries, doctors used advanced microscopes to identify unusual cells in blood and bone marrow of CML patients (Bain & Leach, 2024).

German physician Rudolf Virchow coined the term "leukemia" in 1847 to describe the high white blood cell count associated with CML (Lloyd, 2023). In 1960, Peter Nowell and David Hungerford discovered the Philadelphia chromosome (Ph chromosome), though understanding of CML's genetic basis was limited compared to current knowledge (Balk et al., 2021). This discovery enabled further investigation into the disease's molecular origins.

The 1970s introduced bone marrow transplantation as a potential therapeutic option for certain CML patients, despite significant risks and side effects (Granot & Storb, 2020). The late 1990s brought a significant transformation in CML treatment through tyrosine kinase inhibitors (TKIs). These drugs, like imatinib, specifically target the BCR-ABL fusion protein and initiate molecular and cytogenetic responses (Osman & Deininger, 2021).

The 21st century saw development of several second- and third-generation tyrosine kinase inhibitors following imatinib approval. These include nilotinib, ponatinib, bosutinib, and dasatinib, providing alternative treatment options for patients who cannot tolerate imatinib. TKIs transformed deadly CML into a manageable chronic condition with impressive long-term survival rates. Patients may discontinue medication after achieving sustained molecular responses. Researchers continue developing new treatments including immunotherapies, combination therapies, and targeted medications to improve outcomes and reduce treatment side effects (Cohen et al., 2021).

2.3 Gene Expression Analysis

2.3.1 Techniques used for gene expression profiling in CML

Studying gene expression patterns in leukemia cells allows researchers and doctors to gain insights into molecular mechanisms. This analysis helps identify potential biomarkers and

tailor treatment plans to each patient's genetic profile. Various sophisticated methods profile gene expression, each offering distinct benefits. This approach is crucial for CML diagnosis and management (Haferlach et al., 2005).

Microarray Analysis Microarray analysis represents the principal method for gene expression profiling. It applies hundreds of DNA probe-containing patches on a solid surface (Drăghici, 2019). Using complementary DNA (cDNA) made from RNA, researchers can measure thousands of gene levels simultaneously when placed on microarray probes. This technology enables identification of gene expression patterns related to specific CML phases and treatment outcomes.

Quantitative Real-Time PCR (qRT-PCR) qRT-PCR measures gene expression levels with high sensitivity and accuracy. This technique uses specific primers to amplify cDNA created from reverse transcription in real-time PCR machines. qRT-PCR effectively monitors specific gene expression, such as BCR-ABL, and confirms microarray study findings (Liu et al., 2020).

Next-Generation Sequencing (NGS) NGS provides comprehensive, high-resolution transcriptome interpretation. RNA sequencing (RNA-seq), an NGS type, can sequence every RNA transcript in samples, including those from unknown or poorly understood genes. It produces complete information on alternative splicing events, transcript identification, and gene expression levels. This method has significantly improved CML understanding and facilitated innovative therapeutic target discovery (Kumar et al., 2024).

Single-Cell RNA Sequencing (scRNA-seq) scRNA-seq examines leukemic cell variety in CML at individual cell levels. This technique identifies subgroups with unique gene expression patterns important for understanding disease progression, treatment failure, or recurrence. This analysis provides crucial cellular behavior information and supports personalized treatment strategy creation (Petti et al., 2019).

Fluorescence In Situ Hybridization (FISH) FISH uses fluorescent probes that attach to specific RNA sequences within whole cells. Traditionally identifying genetic abnormalities, it is also flexible for gene expression profiling. This allows researchers to understand gene interactions with each other and cellular structure.

2.3.2 Identification of gene signatures associated with disease progression and treatment response

Identifying gene signatures and connecting them to disease progression and treatment success is vital for understanding CML. Extensive studies enabled researchers to discover specific genetic markers tracking disease development and patient treatment responses by analyzing leukemic cell gene expression patterns. These findings make personalized treatment plans, enhanced prognostic assessments, and improved patient outcomes possible (Ginsburg & Willard, 2009).

Gene signatures represent expression patterns of gene groups related to specific clinical outcomes, disease progression, or patient treatment response (Buch & Liston, 2021). The combination of differential gene expression changes and CML evolution from chronic phase to aggressive accelerated and blast stages gives physicians advantages to forecast disease progression, intervene earlier, and use more potent medicines (Jabbour & Kantarjian, 2022).

High-Throughput Approaches Microarray analysis and RNA-seq are high-throughput approaches for finding CML gene signatures through characterizing thousands of gene expressions simultaneously. Researchers can identify specific genes and pathways linked to disease development and therapy resistance by comparing gene expression patterns between different CML stages or between treatment responders and non-responders (Kok et al., 2019).

BCR-ABL and Additional Markers The BCR-ABL fusion gene represents a well-recognized CML gene marker. Its detection and expression levels serve as important diagnostic and treatment effectiveness indicators. Beyond BCR-ABL signatures, additional genetic markers provide insights into tyrosine kinase inhibitor (TKI) resistance. These markers connect to increased gene expression related to cell cycle regulation, programmed cell death, and DNA repair processes (Amarante-Mendes et al., 2022).

Single-Cell Analysis Single-cell RNA sequencing (scRNA-seq) enhanced gene signature knowledge by visualizing heterogeneity within leukemic cell populations, identifying cell subpopulations with unique gene expression patterns involved in treatment resistance development. This technique can trace rare cell populations with mutations causing TKI resistance and create combination treatments targeting these mutations (Lei et al., 2021).

Risk Stratification Gene signature analysis allows patient categorization according to illness response. Patients with low-risk gene expression patterns typically achieve prolonged remission with standard treatment programs, while high-risk profile patients require intensive surveillance and customized therapy approaches (Bomprezzi et al., 2003).

Treatment Optimization CML therapy optimization occurs through linking gene signatures with treatment response. Identifying specific gene expression patterns sensitive or resistant to TKIs helps select first-line therapy or switch treatments during resistance. Customized therapy regimens based on these profiles can maximize benefits and reduce side effects, improving overall outcomes (Adnan Awad et al., 2020).

2.4 Philadelphia Chromosome (BCR-ABL Gene)

2.4.1 Structure and Function of BCR-ABL Fusion Protein

The BCR-ABL fusion protein structure derives from major regions in BCR and ABL genes. The BCR component domains allow ABL kinase domain dimerization and oligomerization, activating it to create a coiled-coil domain that improves protein function and stability (Zhu & Gao, 2019). The ABL portion contains a tyrosine kinase domain along with SH2, SH3, and DNA-binding domains. These components are essential for kinase function and interaction with various biological substrates (Bracco et al., 2021). ABL kinase loses regulatory constraints during BCR fusion, causing the enzyme to become continuously activated (Loscocco et al., 2019).

The BCR-ABL tyrosine kinase triggers several cancer-related processes by phosphorylating key signaling pathway molecules that regulate cell adhesion, growth, and survival. It stimulates several downstream pathways, including PI3K/AKT, JAK/STAT, and RAS/RAF/MEK/ERK, leading to increased cell growth and higher resistance to cell death. BCR-ABL also alters cell adhesion and internal structure, further boosting leukemia cell aggressiveness (Poudel et al., 2022).

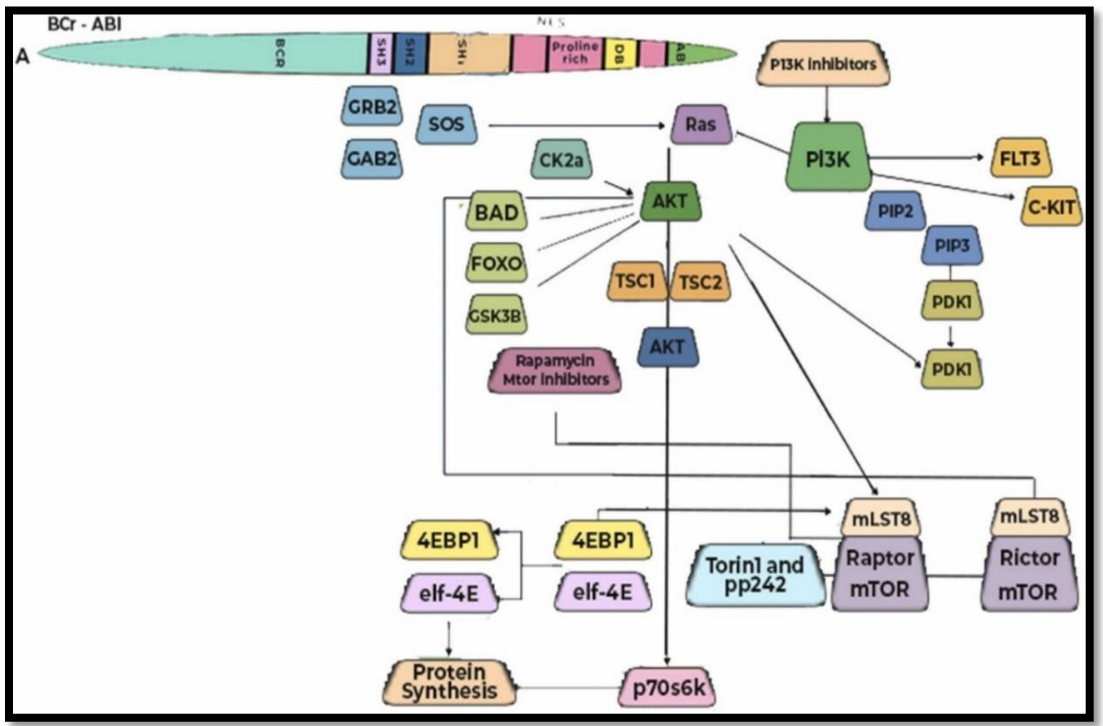


Figure 2.1: BCR-ABL oncogene induces pathological conditions through multiple mechanisms, all of which may be attributed to the initial upregulation of tyrosine kinase (El-Tanani et al., 2024).

Understanding BCR-ABL structure and function greatly benefited targeted CML therapeutic development. Tyrosine kinase inhibitors (imatinib, nilotinib, and dasatinib) selectively block BCR-ABL kinase activity, preventing leukemic cell spread and transforming CML from a deadly illness to a manageable chronic condition (Zhang et al., 2020).

2.4.2 Role of BCR-ABL in dysregulation of signaling pathways

The BCR-ABL fusion protein represents an important oncogenic driver for CML. The Philadelphia chromosome or reciprocal translocation of chromosomes 9 and 22 creates a fusion gene that constitutively modifies cellular signaling and promotes malignant transformation through tyrosine kinase activation (Adnan-Awad et al., 2021). BCR-ABL dysregulation encourages unchecked cell growth, survival, and apoptosis resistance, representing a key factor in CML pathophysiology.

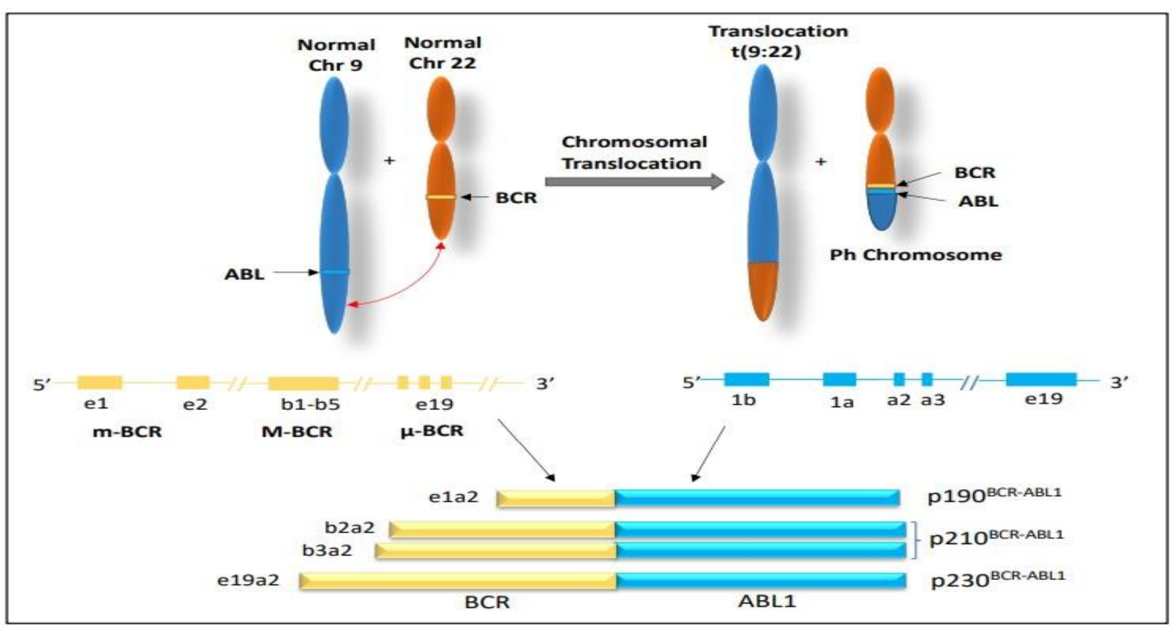


Figure 2.2: Formation of Philadelphia (Ph) chromosome. Translocation of chromosome 9 and 22 forms Ph chromosome with BCR-ABL1 oncogene with either of three breakpoint regions in BCR resulting into p190, p210 and p230 transcripts (Poudel, 2023).

Constitutive Tyrosine Kinase Activity The normal, highly controlled ABL protein kinase domain remains present in the BCR-ABL fusion protein but loses regulatory activity after fusion. This creates ongoing, unchecked kinase activity that phosphorylates many substrates for different important signaling pathways (K. Bhanumathy et al., 2021).

RAS/RAF/MEK/ERK Pathway This pathway plays a vital role in cell growth and division and is primarily influenced by BCR-ABL activation. When BCR-ABL phosphorylates substrates, RAS activates, which subsequently activates RAF, MEK, and ERK pathways. This promotes unregulated cell division and contributes to leukemia cell proliferation (Amarante-Mendes et al., 2022).

PI3K/AKT Pathway BCR-ABL influences the phosphoinositide 3-kinase (PI3K)/AKT pathway. BCR-ABL activation of PI3K leads to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) creation, which activates AKT. This pathway inhibits cell death-promoting factors while activating cell survival-supporting proteins, helping leukemia cells escape programmed cell death and enhancing their growth and longevity (Sanchez et al., 2019).

JAK/STAT Pathway BCR-ABL activates and phosphorylates Janus kinase (JAK) proteins, which then phosphorylate signal transducer and activator of transcription (STAT) proteins. This causes activation of genes encouraging cell proliferation and preventing apoptosis (Hu et al., 2021).

Cytoskeleton and Cell Adhesion Effects BCR-ABL affects cytoskeleton and cell adhesion by altering integrin behavior and actin filament dynamics. This weakens leukemic cell adherence to bone marrow stroma, allowing free movement (Windisch et al., 2019).

Therapeutic Insights Understanding how BCR-ABL influences various signaling pathways has led to specific treatment development. Targeted therapies like imatinib, dasatinib, and nilotinib selectively inhibit these faulty signaling routes. This targeted approach has greatly transformed patient outcomes, reshaping CML treatment (Nemkov et al., 2019).

2.4.3 Implications for targeted therapy development

TKI development following BCR-ABL fusion protein identification revolutionized CML treatment, establishing the disease as a model for effective targeted therapy development. Understanding molecular processes behind BCR-ABL oncogenic characteristics clarified CML molecular pathophysiology and opened doors for highly targeted treatment drug creation (Rahm, 2024).

Targeting Constitutive Tyrosine Kinase Function Targeting constitutive tyrosine kinase activity represents the therapeutic key for BCR-ABL's oncogenic role in CML. Researchers created small molecule inhibitors to precisely target the ATP-binding region of the ABL kinase domain (Alves et al., 2021). These TKIs efficiently block BCR-ABL activation-mediated signaling pathways, suppressing cell growth and triggering apoptosis (Singh et al., 2021). Focusing on BCR-ABL kinase activity for CML treatment produces long-lasting responses and enhances patient outcomes.

Overcoming Resistance Mechanisms Resistance mechanisms limit TKI efficiency, including point mutations in the BCR-ABL kinase domain that alter signaling pathway activation (Alves et al., 2021). Expanded knowledge of resistance mechanisms created second- and third-generation TKIs. Drugs like nilotinib, ponatinib, and dasatinib increased treatment options for

CML patients by proving effective against several BCR-ABL mutations resistant to first-generation TKIs (Jabbour & Kantarjian, 2020).

Researchers investigated combination medicine concepts to improve therapeutic efficacy according to BCR-ABL-mediated signaling complexity and the possibility of targeting multiple resistance pathways simultaneously. Combining TKIs with conventional chemotherapeutic drugs or additional targeted medicines reduces resistance establishment, provides profound responses, and enhances long-term CML patient prognosis (Huang et al., 2020).

Personalized Medicine Approaches Molecular diagnostic development (gene expression profiling and mutation testing) now enables personalized CML treatment plans. Clinicians can customize treatment according to each patient's unique BCR-ABL mutation profile, illness stage, and medication response to increase therapeutic benefits and reduce side effects. Molecular monitoring tools facilitate early resistance diagnosis and treatment response assessment, stimulating medication modification (Saeed et al., 2023).

2.5 BCR-ABL Transcript Variants

2.5.1 Description of common BCR-ABL transcript variants (e.g., e13a2, e14a2)

Two typical BCR-ABL transcript variants linked to CML are b2a2 (also called e13a2) and b3a2 (also called e14a2). These variations result from different BCR and ABL gene fusion sites:

1. **b2a2 (e13a2)** This variant forms through combining ABL exon 2 (a2) and BCR exon 13 (b2). The resulting BCR-ABL fusion protein has 210 kilodaltons (kDa) molecular weight and primarily links to CML diagnosis. The fusion involves adding 3' sequence to the ABL transcript, resulting from inserting 927 nucleotides from BCR into the ABL gene.
2. **b3a2 (e14a2)** This variant forms through combining ABL exon 2 (a2) and BCR exon 14 (b3). The resulting BCR-ABL fusion protein has reduced 190 kDa molecular weight. CML patients commonly have the b3a2 variant, making up a sizable case fraction (Shareefa, 2023).

Clinical Consequences Although b2a2 and b3a2 variants link to CML, they may associate with clinical characteristics and prognostic consequences. TKI treatment may produce different responses in certain studies with unpredictable results about linking

variants to outcomes. In some cases, the b3a2 variant commonly links to other chromosomal abnormalities, affecting patient response and illness progression (Shareefa, 2023).

Detection Methods RT-PCR and qRT-PCR are commonly used for molecular identification of BCR-ABL transcript variations, enabling BCR-ABL transcript measurement and description. These methods provide important data for CML patient diagnosis, risk assessment, and therapy response tracking (Nachi et al., 2020).

2.5.2 Clinical significance and prognostic implications of different transcript variants

Treatment Response and Outcomes Different BCR-ABL transcript variants' impact on treatment response and CML outcomes remains an active investigation area. While both b2a2 and b3a2 variants respond to TKI therapy, accumulating evidence suggests transcript type may influence molecular response depth and speed. Several studies reported that patients harboring the b3a2 transcript tend to achieve faster and deeper molecular responses compared to those with the b2a2 variant, despite receiving the same TKI therapy (Hochhaus et al., 2020).However, study findings are not always consistent, and these transcript-related differences' clinical significance remains under debate. Some reports failed to demonstrate statistically significant differences in long-term outcomes, such as progression-free survival or overall survival, between transcript groups. These discrepancies highlight CML biology complexity and other factor influences, such as age, disease burden, comorbidities, and treatment adherence. Therefore, further prospective and large-scale studies are essential to clarify transcript variants' prognostic value and their role in guiding personalized treatment strategies (Hanfstein et al., 2014; Hochhaus et al., 2020; Jain et al., 2016; Shanmuganathan et al., 2018).

Prognostic Implications Prognostic evaluations indicate that the b3a2 transcript links to better prognosis compared to the b2a2 transcript (Molica et al., 2020). Additional variables affect CML prognostic evaluations, including the Sokal score, European Treatment and Outcome Study (EUTOS) score, and molecular treatment response to improve risk assessment and patient therapeutic choices (Hochhaus et al., 2020).

Detection Methods RT-PCR and qRT-PCR identify and accurately quantify BCR-ABL transcript variants to determine transcript type, track transcript level changes over time, and enhance doctors' abilities and patient outcomes (Jovanovski et al., 2020).

Determining the transcript type (13a2 (b2a2) or e14a2 (b3a2)) present in BCR-ABL protein is a critical point for molecular characterization of CML (Musteață, 2022). However, the transcript variants rise from certain fusion divisions on ABL and BCR genes; it has been connected to distinct clinical attributes and consequences for prognosis. It's important to follow the transcript kind for CML patients to Comprehend their risk evaluation, therapy selections, and prognosis evaluation (Genthon et al., 2020).

Although e13a2 and e14a2 are the most common BCR-ABL1 transcript variants in chronic myeloid leukemia (CML), several studies have shown that these variants may be associated with distinct clinical presentations. Patients expressing the e13a2 transcript (also known as b2a2) are often found more frequently than those with e14a2 (b3a2) in certain populations. Clinically, e13a2-positive patients tend to present with higher white blood cell (WBC) counts and larger spleen size (splenomegaly) at diagnosis, which may reflect a more aggressive disease phenotype.

In contrast, the e14a2 transcript has been associated in some studies with a younger age at diagnosis and a higher frequency of additional chromosomal abnormalities, although these findings remain variable across populations. Differences in response to tyrosine kinase inhibitors (TKIs), such as imatinib, have also been observed between transcript types, with some reports suggesting a better molecular response in patients with the e14a2 variant. These transcript-related distinctions underscore the potential value of molecular subtyping in the clinical assessment and therapeutic management of CML(Deininger et al., 2020).

The impact of different BCR-ABL1 transcript variants on treatment response and outcomes in chronic myeloid leukemia (CML) remains an active area of investigation. While both e13a2 (b2a2) and e14a2 (b3a2) variants respond to tyrosine kinase inhibitor (TKI) therapy, accumulating evidence suggests that transcript type may influence the depth and speed of molecular response. Several studies have reported that patients harboring the e14a2 transcript tend to achieve faster and deeper molecular responses compared to those with the e13a2 variant, despite receiving the same TKI therapy (Hochhaus et al., 2020).

Prognostic Implications: prognostic evaluations indicated that e14a2 transcript linked to a better prognosis compared to e13a2 transcript (Molica et al., 2020). CML's Prognostic evaluations are affected additional variables including the Sokal score, the European Treatment and Outcome

Study (EUTOS) score, and the molecular response to treatment in order to improve risk assessment and therapeutic choices for patients (Hochhaus et al., 2020).

2.6 Laboratory Evaluation

2.6.1 Blood counts and peripheral smear examination

- CML, a myeloproliferative disease, features abnormally proliferated myeloid cells (granulocytes) in bone marrow (Jabbour & Kantarjian, 2020). The disease connects to the Philadelphia chromosome, formed by chromosome 9 and 22 translocation, producing the BCR-ABL fusion gene that generates an active tyrosine kinase protein contributing significantly to CML pathophysiology (Sampaio et al., 2021).
- Peripheral smear examinations and blood counts represent conventional CML diagnostic methods and treatment follow-up, providing information about illness, therapeutic response, and duration (Elhadary et al., 2023). Blood counts provide patient hematological health information including red blood cells (RBC), white blood cells (WBC), and platelets (Ware, 2020). High WBC counts indicated by immature and mature granulocytes (leukocytosis) characterize CML (Kantarjian et al., 2024).
- Peripheral smear examination of blood cells differentiates between diverse leukemia configurations and identifies aberrant cell types (Rastogi et al., 2022). CML diagnostic examination often demonstrates myeloid cells with leftward lineage shift and increased granulocytes including bands, myelocytes, and metamyelocytes. When peripheral smear examination shows basophilia and eosinophilia, it suggests dysregulated hematopoiesis in CML (Gorczyca, 2021).

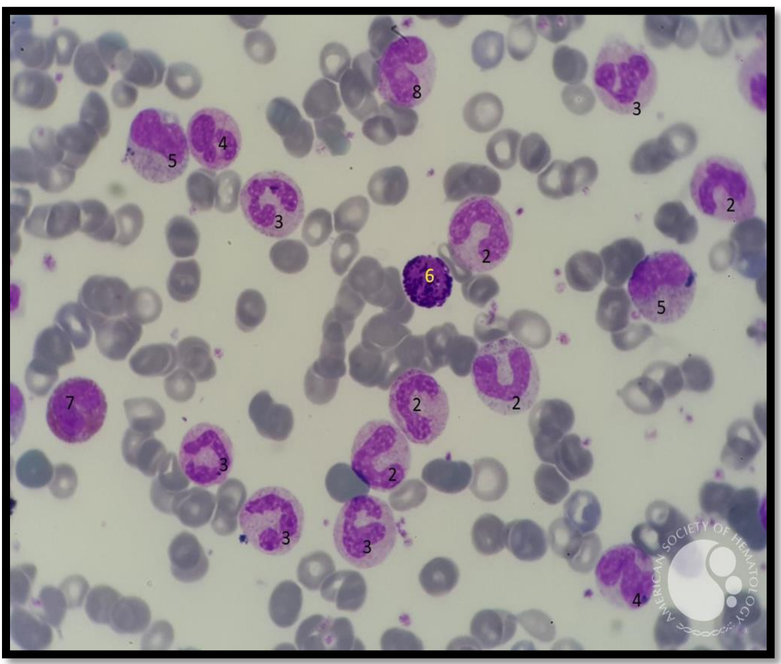


Figure 2.3: In this set of pictures, myeloid precursors are evident in the peripheral blood smear of a patient diagnosed with chronic myeloid leukemia (CML).

https://imagebank.hematology.org/image/61888/chronic-myeloid-leukemia-presentation-in-peripheral-blood-1).

Each cell has been described and labeled using the corresponding numbers:

1) Blast cell (probably basophilic normoblast), 2) Band cell, 3) Band cells going to be segmented, 4) Dysplastic cell, 5) Metamyelocyte, 6) Basophil, 7) Eosinophilic myelocyte and 8) Late metamyelocyte.

Blood level monitoring and peripheral smear examinations are vital for therapeutic planning, assessing medication response, and identifying CML advancement in patients. Treatment effectiveness or disease progression is determined by leukocyte count variations, blast cell presence, and differential counts. Observing these markers is crucial for improving patient outcomes and deciding treatment routines (Osman & Deininger, 2021).

2.6.2 Bone marrow aspiration and biopsy findings

Bone marrow aspiration and biopsy represent necessary tools for diagnosing and treating hematological malignancies including CML (Dogan & Demircioglu, 2022). CML is differentiated from others by abnormal myeloid cell presence, especially granulocytes (Patnaik & Tefferi, 2023). Bone marrow examination provides greater information than peripheral blood

tests about cellular composition, maturation, and myeloid leukemia genetic abnormalities (Pimenta et al., 2021).

Bone marrow aspirate and biopsy specimen analysis provides prognostic information and diagnosis for CML patients. Bone marrow aspiration indicates leftward alteration in mature myeloid cells, hypercellularity, and granulocytic proliferation. CML diagnostic results using this method can be confirmed by heightened basophilia presence (Orazi, 2007). Both bone marrow specimens and morphological assessment are important for cytogenetic and molecular testing to follow CML disease development.

The Philadelphia chromosome genetic basis is detectable using fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) methods (Nath & Johnson, 2000). Quantitative BCR-ABL transcript level analysis (using RT-PCR) and bone marrow testing provide insights about medication response (Foroni et al., 2009). Decreased BCR-ABL transcript levels, normalized mature myeloid cells, and reduced bone marrow cellularity all indicate positive treatment response. Conversely, unusual genetic mutation occurrence and cytogenetic abnormality preservation indicate drug resistance, demanding different treatment approaches (Wang et al., 2019).

2.6.3 Molecular tests for BCR-ABL transcript detection

Philadelphia chromosome is an indication for hematologic malignancy, which it's the consequence pathways, produces active tyrosine kinase then allowing unchecked growth of myeloid cells in bone marrow and chronic myeloid leukemia. The molecular testing is also another vital approach for precisely identifying BCR-ABL transcript levels in peripheral blood or bone marrow samples, progressing and treating of CML (Kang et al., 2016).

The used molecular assessment techniques for CML include polymerase chain reaction (PCR)-based assays, which sensitively reveals the presence of BCR-ABL transcripts, and real-time quantitative PCR (qPCR) approach that tracks BCR-ABL transcript levels for the assessment of therapeutic response and disease progression. Besides, using qPCR for quantitative assessment the level of BCR-ABL transcripts, allows establishing disease baseline for tracking patient's responses to treatment throughout time (Hughes & Branford, 2003). As, results of elevated transcript levels indicates treatment resistance, whereas a positive response is

indicated by the drop-in ratio values of BCR-ABL transcript levels to a reference gene (such as ABL1) (Rossari et al., 2018).

Moreover, molecular approaches, such as direct sequencing or mutation-specific PCR tests, can recognize BCR-ABL kinase domain mutations that resist TKIs, changing treatment choices for CML patients, or combining different approaches to overcome resistance and improve patient outcomes. Likewise, having integrated molecular testing within stander clinical practice allows modifying the treatment plans, matching the medication with patient's response, determining risk profiles, early detecting of treatment resistance, and enhancing quality of life for CML patients (Jabbour et al., 2013).

2.7 Previous studies related to the study

Currently, limited research exists on BCR-ABL transcript variant significance. Early studies showed that identifying transcript type might have therapeutic implications or assist in understanding t(9;22) positive leukemic cell pathobiology.

Syrian Study In Syria, 45 patients tested positive for BCR-ABL fusion gene rearrangement. Among the 45 CML patients studied, 23 (51.1%) had b2a2 transcript, 21 (46.7%) had b3a2 fusion transcript, and 1 (1.2%) had a rare variant (Al-Achkar et al., 2016). No patient exhibited b3a2/b2a2 co-expression. Syria's BCR-ABL transcript type distribution was comparable to populations in India, the Middle East, Africa, and Europe (Khazaal et al., 2019). The M-BCR rearrangement types did not affect patients' WBC, platelet count, hemoglobin level, or gender. Patients with b3a2 rearrangements were younger than patients with b2a2 transcripts, implying that younger patients' prognosis may be less favorable (Khazaal et al., 2019).

Sudanese Study In Sudan, three of 46 Sudanese patients tested negative for BCR-ABL fusion transcript. Among 43 positive patients, the majority had b2a2 fusion transcript (53.5%), followed by b3a2 transcript (41.9%) and co-expression of b3a2/b2a2/e19a (4.6%) (Osman et al., 2010). Neither p210/p190 nor e1a2 were co-expressed alone. Male patients tended to express b2a2 ($p = 0.017$), while female patients tended to express b3a2. One in four patients had a single nucleotide mutation in BCR exon 13, and this patient expressed only b2a2 (Osman et al., 2010). Researchers concluded a significant relationship exists between sex and BCR-ABL transcript type, indicating additional study is needed (Osman et al., 2010).

Iranian Study In Iran, researchers found BCR-ABL rearrangement in all examined patients. Those with b3a2 gene fusion numbered 53 (62.35%), b2a2 numbered 25 (29.41%), one (1.17%) had other variants, and six (7.05%) had b3a2 and e1a2 co-expression (YAGHMAEI et al., 2008). No significant differences existed between sex and b2a2 (P=0.61), b3a2 (P=0.79), or e1a2 (P=0.20) (Ayatollahi, Keramati, et al., 2018). Substantial differences existed in mean age between patients testing positive for b3a2 (44.07 years) and those testing negative (50.35 years). Northeast Iran CML patients showed higher b3a2 transcript frequencies than b2a2 and e1a2 transcripts, with no association between e1a2 transcript frequencies and peripheral blood monocytosis (Ayatollahi, Keramati, et al., 2018).

Pakistani Study The b3a2 transcript was most common among Islamabad CML patients (Amin & Ahmed, 2021). Patients in the b2a2 subgroup had significantly higher mean white blood cell counts at presentation (Amin & Ahmed, 2021). The b3a2 transcript expressed in a substantially higher percentage of male patients than the b2a2 transcript (Pfirrmann et al., 2017).

Iraqi Study Iraqi evidence revealed M-BCR-ABL with b3a2 subtype preference over b2a2 subtype (Khazaal et al., 2019). BCR-ABL transcript variant gender distribution was skewed, with b3a2 transcript being more prevalent in males. At diagnosis, different leukocyte and platelet counts reveal BCR-ABL transcript type, which may imply distinct phenotype and disease biology (Khazaal et al., 2019).

Chapter Three: Methodology

3.1 Study deign:

A cross-sectional study.

3.2 Study area:

This study was conducted at the Oncology Department of An-Najah National University Hospital (NNUH) in Nablus, Palestine. Palestinian patients diagnosed with Chronic Myeloid Leukemia (CML) were recruited between July 2024 and December 2024. NNUH is considered one of the leading tertiary care centers in the country, particularly recognized for its comprehensive services in diagnosing and managing hematological malignancies, including CML. The hospital provides centralized molecular diagnostic facilities and long-term treatment follow-up for leukemia patients from across the West Bank and Gaza.

3.3 Study Participants and setting

From the Arab American University (Reference: R-2024/A/42/N) on March 3, 2024, in accordance with the Declaration of Helsinki. All participants signed written informed consent prior to data collection. A structured questionnaire was used to collect demographic and clinical information, including age, gender, place of residence, smoking status, and family history of hematological malignancies. For seven participants residing in Gaza, data were obtained through structured interviews and phone communication due to travel and accessibility limitations.

3.4 Sample Size Justification:

Although the study included a relatively small sample size of 50 patients, this number reflects real-world limitations associated with conducting molecular research in low-resource settings such as Palestine. Factors such as limited funding, restricted access to advanced laboratory reagents, and the high cost of molecular testing (including RT-PCR kits, RNA extraction materials, and gel electrophoresis supplies) significantly constrained the ability to enroll a larger cohort. Additionally, the study was conducted in a single center with limited molecular diagnostic infrastructure and staff. Despite these constraints, the sample size was sufficient to yield statistically meaningful results and offers valuable preliminary data for future larger-scale studies.

Control Sample Specifications:

- **Positive Control:** Confirmed CML patient sample with known BCR-ABL fusion
- **Negative Control:** Healthy donor sample or nuclease-free water
- **Internal Control:** β-actin amplification for RNA integrity verification

3.4.1. Sample Size Justification and Limitations

Financial Constraints Acknowledgment: The sample size of 50 patients was primarily determined by budgetary limitations imposed by university funding allocation, which restricted the study to this number of samples. While this represents a limitation for statistical power, it provides a foundation for preliminary analysis and hypothesis generation in the Palestinian population.

Regional Prevalence Considerations: The relatively small sample size also reflects the limited number of newly diagnosed CML cases in the regional population, making this study size appropriate for the demographic context while acknowledging limitations for broader generalization.

3.4.2. Timing of Sample Collection:

Based on the study implementation, clearly distinguish between diagnostic and follow-up sampling protocols. In this study, while initial BCR-ABL positivity was confirmed at diagnosis through the hospital's internal system for all fifty patients, the actual research samples were collected during treatment follow-up visits after obtaining written informed consent from all patients in the oncology department. This approach, while practical given clinical constraints, may introduce treatment-related variables that should be acknowledged in future research designs.

Enhanced Storage Protocol: a standardized cold-chain protocol for sample preservation:

- Immediate refrigeration at 2-8°C upon collection
- Same-day RNA extraction to minimize degradation
- Post-extraction storage at -30°C until analysis
- Pre-PCR nanodrop analysis for all samples to ensure RNA quality

3.5 Inclusion and exclusion criteria:

The diagnosis and classification of Chronic Myeloid Leukemia (CML) in all patients were based on the World Health Organization (WHO) criteria, which include a complete blood count (CBC), bone marrow examination, and confirmation of the BCR-ABL fusion gene. Only patients who met these diagnostic standards were included in the study. Medical histories and CBC data were retrieved from patient records maintained by the Oncology Department at An-Najah National University Hospital. Patients, who had received prior treatment with tyrosine kinase inhibitors (TKIs), were in advanced disease phases, or had other hematologic malignancies were excluded from the study.

3.6 RNA extraction and blood collection:

About three milliliters of EDTA-anticoagulated peripheral blood were collected from each patient, and total RNA was extracted using the NucleoSpin® RNA Blood Kit (Macherey-Nagel GmbH & Co. KG, Germany), following the manufacturer’s instructions.

3.6.1 RNA extraction:

In a sterile microtube of 1.5ml capacity, a sample of 200µL of blood buffy coat was combined. To this, 200µL of AL lysis buffer and 5µL of liquid proteinase K were added. The resulting mixture was allowed to incubate at room temperature (15–25°C) for 10 minutes, followed by a brief centrifugation at 2,000 X g for one second to clear the lid. Next, 200µL of 70% ethanol was incorporated into the mixture, which was then vortexed thoroughly. Another short spin at 2,000 X g for one second was performed to clean the lid again. After that, 610µL of the lysate was carefully pipetted into a Nucleo^{R}Spin RNA Blood Column and spun at 11,000 X g for 30 seconds. Next, 350µL of Membrane Desalting Buffer (MDB) was added to the column, and it underwent centrifugation for 30 seconds at 11,000 X g. Following this, 95µL of rDNase was applied to the column and incubated at room temperature for 15 minutes. Then, 200µL of Buffer RB2 was introduced to the NucleoRSpin RNA column and centrifuged for 30 seconds at 11,000 X g. The flow-through and collection tube were discarded, and the column was positioned in a fresh collection tube. Subsequently, 600µL of Buffer RB3 was added to the NucleoRSpin RNA column, followed by another 30 seconds of centrifugation at 11,000 X g. Again, the flow-through and collection tube were removed, and the column was placed in a new collection tube. Lastly, 250µL of Buffer RB3 was added to the NucleoRSpin RNA blood column, and it was

spun for 2 minutes at 11,000 X g. Finally, 60µL of RNase-free H2O was added to the column, followed by a 30-second centrifugation at 11,000 X g. The isolated RNA was stored at -20°C until it was needed.

3.6.2 RNA Quantification:

A Nano Drop analyzer (IMPLEN, Germany) was used to determine the concentrations and quality (OD260/OD280). One µL of Buffer AVE was used as a blank, followed by 1µL of RNA. The ratio OD260/OD280 was computed to represent the quantity of RNA to protein, which should be > 1.8 to be considered appropriate for analysis.

3.6.3 RNA Quantification:

A Nano Drop analyzer (IMPLEN, Germany) was used to determine the concentrations and quality (OD260/OD280). One µL of Buffer AVE was used as a blank, followed by 1µL of RNA. The ratio OD260/OD280 was computed to represent the quantity of RNA to protein, which should be > 1.8 to be considered appropriate for analysis.

3.7 Complementary DNA synthesis:

RNA was reverse-transcribed into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific™, Thermo Fisher Scientific Inc., USA), which served as the template for the subsequent PCR reaction. The reverse transcription was performed following the manufacturer's protocol. This kit is specifically designed for RT-qPCR applications and is provided as a ready-to-use lyophilized master mix containing all the necessary components for efficient first-strand cDNA synthesis from an RNA template.

Technical Methodology Clarifications

Weak DNA Band Analysis: Several samples demonstrated weak or faint bands during electrophoresis analysis. This phenomenon could be attributed to:

- RNA degradation despite careful handling protocols
- Technical variations in laboratory processing
- Inherent differences in BCR-ABL expression levels among patients

To address this issue, we must:

- Implementation of RNA integrity assessment using additional markers
- Standardization of sample processing timeframes
- Regular equipment calibration and maintenance protocols

3.8 Gene amplification and detection of fusion type:

The cDNA was subjected to multiplex conventional PCR. Four primers were used for qualitative determination of BCR-ABL fusion in the following sequences:

BCR-e1: 5′ ACCGCATGTTCCGGGACAAAAG-3’.

BCR-b2: 5′ ACAGAATTCCGCTGACCATCAATAAG-3′.

BCR-rev: 5′-ATAGGATCCTTTGCAACCGGGYCYGAA-3′.

ABL a2: 5′-TGTTGACTGGCGTGATGTAGTTGCTTGG-3′ (Khazaal et al., 2019).

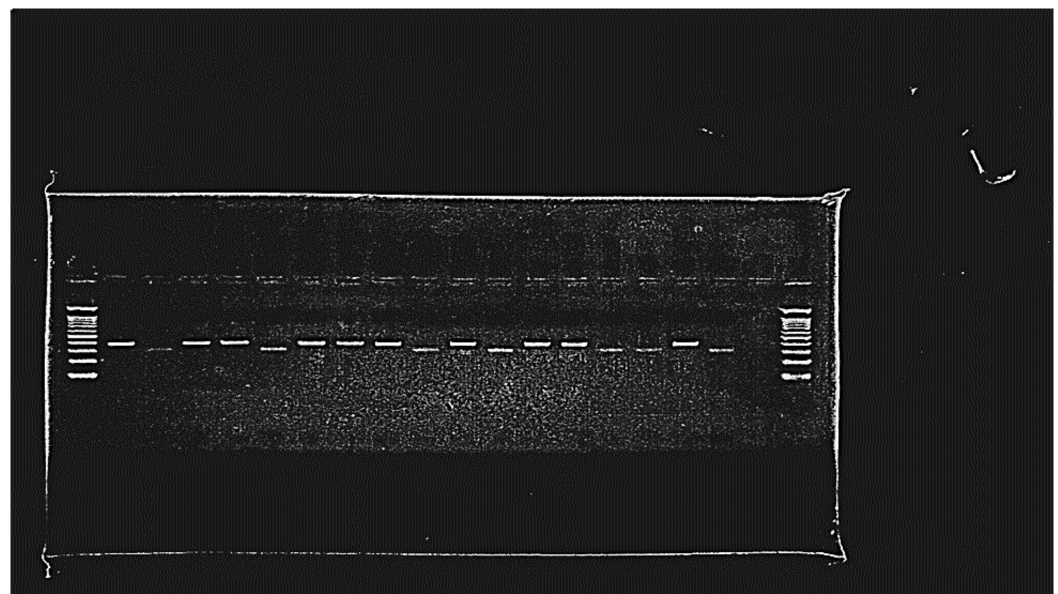
A ready green master mix of 12.5 µl was utilized for creating the reaction mixture. To the master mix tube, 2 µl of template cDNA and 0.5 µl of each primer were introduced. The total volume was then brought up to 25 µl using nuclease-free distilled water. Afterward, the mixture was vortexed for 10 seconds and placed in a thermo cycler (Biometra TAdvanced/Germany), which had been set with the following parameters: an initial denaturation of 3 minutes at 95°C, succeeded by 40 cycles consisting of 30 seconds of denaturation at 95°C, 30 seconds of annealing at 63°C, and 1 minute of extension at 72°C. Finally, a 10-minute elongation step was performed at 72°C. The anticipated fragment sizes are: 481 bp for ela2, 385 bp for b3a2, 310 bp for b2a2, 209 bp for b3a3, and 103 bp for b2a3. The PCR products underwent 2% agarose gel electrophoresis, were stained with ethidium bromide, and then visualized using a UV transilluminator.

Laboratory Quality Control Enhancements

Gel Electrophoresis Visualization Improvements: Future studies should implement enhanced gel documentation protocols:

- Use 2% agars gel with ethidium bromide (2 drops per gel)

- Load 6 μL of each PCR product
- Include DNA ladder (50 bp) at both beginning and end of gel
- Ensure proper band identification: B3a2 (385 bp - darker bands) and B2a2 (310 bp - lighter bands)
- Include both positive and negative controls in every gel run



Ladder(black) 385B3a2 / / 310 B2a2 (light)

Weak DNA Band Analysis: Several samples demonstrated weak or faint bands during electrophoresis analysis. This phenomenon could be attributed to:

- **RNA degradation despite careful handling protocols**
- **Technical variations in laboratory processing**
- **Inherent differences in BCR-ABL expression levels among patients**

3.9 Statistical analysis:

For statistical analysis, we utilized the Statistical Package for the Social Sciences (SPSS, version 21.0). Continuous variables were presented as mean ± standard deviation (SD) and assessed using Student's t-test. Dichotomous variables were reported as frequency and percentage, analyzed through the Chi-squared test. All p-values were considered two-sided, with significance set at $p < 0.05$.

Chapter Four: Results

4.1 Demographic characteristics of the study population

Fifty patients diagnosed as CML were enrolled in the study. Demographic data of the study group is shown in Table 1. For the 50 enrolled patients, the substantial number of those aged 45 years or older was 34 (68%). On the other hand, 16 patients (32%) were younger than 45 years with a mean age of 47.6818 ± 15.24328 years (Table 1) (21-85 years) with male mean age: 51.1786 ± 15.42017 years, and female mean age: 47.68 ±15.14183 years. This suggests that most patients were elderly, who might have various clinical manifestations or responses to treatment in the context of CML. The study sample had a slightly higher number of male patients (28 patients, 56%) compared to female patients (22 patients, 44%) (M:F ratio of 1.27:1) suggesting a relatively balanced gender distribution in this cohort. The Body Mass Index (BMI) of the patients was categorized into three groups:

- 17 patients (34%) had a BMI of less than 25, indicating they were within the normal weight range.
- 16 patients (32%) had a BMI between 25 - 30, categorizing them as overweight.
- 17 patients (34%) had a BMI greater than 30, placing them in the obese category. These results suggest that there is a fairly even distribution across the three BMI categories in the study group, with no clear dominance of one category over the others.

Patients in the study were from various regions, with the largest group residing in Nablus (15 patients, 30%). Other notable regions include the Gaza Strip (6 patients, 12%), Jenin (9 patients, 18%), and Tulkarm (9 patients, 18%). Smaller numbers of patients were from Ramallah (2 patients, 4%), Qalqilya (2 patients, 4%), Tubas (2 patients, 4%), Jericho (1 patient, 2%), Salfit (1 patient, 2%), and Hebron (3 patients, 6%). This geographic diversity provides insights into the distribution of CML cases across different areas. In terms of smoking habits, 23 patients (46%) reported a history of smoking, while 27 patients (54%) were non-smokers. This finding suggests a relatively balanced smoking history in the cohort, which may be useful when evaluating potential lifestyle-related risk factors in the study. Regarding family history, 24 patients (48%) had a family history of CML or another hematologic malignancy, while 26 patients (52%) did not. This result indicates a significant proportion of patients with a potential genetic

predisposition to CML, though no direct correlation with transcript variants or other clinical factors has been made in this preliminary analysis.

Table 4.1: Baseline demographic characteristics of the study group

Variable		No (%)
Age (years)	< 45	16 (32%)
	≥ 45	34 (68%)
Gender	Male	28 (56%)
	Female	22 (44%)
Body mass index (kg/m2)	<25	17 (34%)
	25-30	16 (32%)
	>30	17 (34%)
Residence	Ramallah	2
	Gaza Strip	6
	Jenin	9
	Nablus	15
	Tulkarm	9
	Qalqilya	2
	Tubas	2
	Jericho	1
	Salfit	1
	Hebron	3
Smoking	No	27 (54%)
	Yes	23 (46%)
Family history	No	26 (52%)
	Yes	24 (48%)

4.2 Hematological characteristics of the study population

Hemoglobin and Packed Cell Volume

- The mean hemoglobin level was 11.14 ± 2.59 g/dL, which is slightly below the normal range, indicating mild anemia in this cohort of patients.
- The mean packed cell volume (PCV) was 33.44 ± 7.68%, further supporting the presence of mild anemia in these patients, which is consistent with CML-related hematologic abnormalities.

Red Blood Cell (RBC) Indices

- The mean RBC count was 3.86 ± 0.92 × 10^{12}/L, which is within the normal range for adults.
- The mean MCV (Mean Corpuscular Volume) was 86.85 ± 5.35 fL, indicating normocytic red blood cells, which is typical in CML patients.
- The mean MCH (Mean Corpuscular Hemoglobin) was 29.07 ± 2.26 pg, suggesting normochromic red blood cells.
- The mean MCHC (Mean Corpuscular Hemoglobin Concentration) was 33.40 ± 0.93 g/dL, which is also within the normal range.
- The mean RDW-CV (Red Cell Distribution Width) was 17.10 ± 2.29%, which is mildly elevated, potentially reflecting the heterogeneity of red blood cell sizes, commonly seen in anemia of chronic disease such as CML.

White Blood Cell Count and Differential

- The mean total white blood cell count (WBC) was 57.87 ± 17.58 × 10^9/L, indicating leukocytosis, a hallmark of CML in the chronic phase.
- The mean neutrophil percentage was 63.28 ± 20.78%, which is elevated, as neutrophils typically predominate in CML.
- The mean lymphocyte percentage was 14.56 ± 15.96%, which is relatively low, consistent with the neutrophilic predominance in CML.

- The mean monocyte percentage was 7.07 ± 8.55%, slightly elevated, but within the normal range.
- The mean eosinophil percentage was 1.67 ± 1.55%, and the mean basophil percentage was 2.32 ± 1.98%, both of which are elevated relative to normal values, and may reflect CML-associated abnormalities in hematologic lineages, particularly eosinophilia and basophilia that are often seen in chronic phase CML.

Platelet Count

- The mean platelet count was 283.96 ± 153.22 × 10^9/L, which is within the normal range, though the platelet count can often be elevated in CML, especially in the chronic phase, where it can fluctuate based on disease dynamics and treatment response.
- The laboratory data of the 50 CML patients in this study is presented in Table 2.

Table 4.2: Hematological data of CML patients

Variable		Mean ± SD
Hemoglobin (g/dL)		11.14 ± 2.58637
Packed cell volume %		33.438 ± 7.67798
Red blood cell total count & indices	Total × 10^{12}/L	3.8626 ± 0.92015
	MCV (fL)	86.85 ± 5.34554
	MCH (pg)	29.0720 ± 2.25543
	MCHC (g/dL)	33.4020 ± 0.93427
	RDW-CV %	17.1040 ± 2.28571
White blood cells	Total × 10^9/L	57.8730 ± 17.58368
	Neutrophil %	63.2810 ± 20.77706
	Lymphocyte %	14.5586 ± 15.95778
	Monocyte %	7.0698 ± 8.54957
	Eosinophil %	1.6680 ± 1.54686
	Basophil %	2.3166 ± 1.97768
Platelets total count	Total × 10^9/L	283.9600 ± 153.22319

4.3. The frequency of different BCR-ABL fusion variants:

Figure1. The gel electrophoresis displayed results from multiplex RT-PCR products created with four different primers. These combinations of primers made it possible to reliably identify common p210 transcripts like b2a2 or b3a2, as well as unusual types, including transcripts missing ABL exon a2 (b2a3), all in a single reaction.

Figure 4.1: Multiplex RT-PCR products were analyzed using gel electrophoresis, and then visualized by UV light following staining with ethidium bromide. The first lane, labeled M, contains a DNA ladder ranging from 100 to 1500 bp. Lanes 2, 7, 9, 10, and 12 show the b3a2 band leinght 385 and lane 3,4,5,6,8 and 11 show the b2a2 band leinght 310.

The b3a2 transcript variant was identified as a product measuring 385 bp in length, while the b2a2 variant was noted to be 310 bp long. In total, all 50 patients, representing 100%, showed expression of one of the P210 BCR-ABL rearrangements, which are b3a2 or b2a2. Every patient in the study tested positive for M-BCR, and none were positive for m-BCR or µ-BCR. Out of the patients, 29, or 58%, exhibited the b3a2 transcript, while the b2a2 variant was present in 21 cases, accounting for 42%.

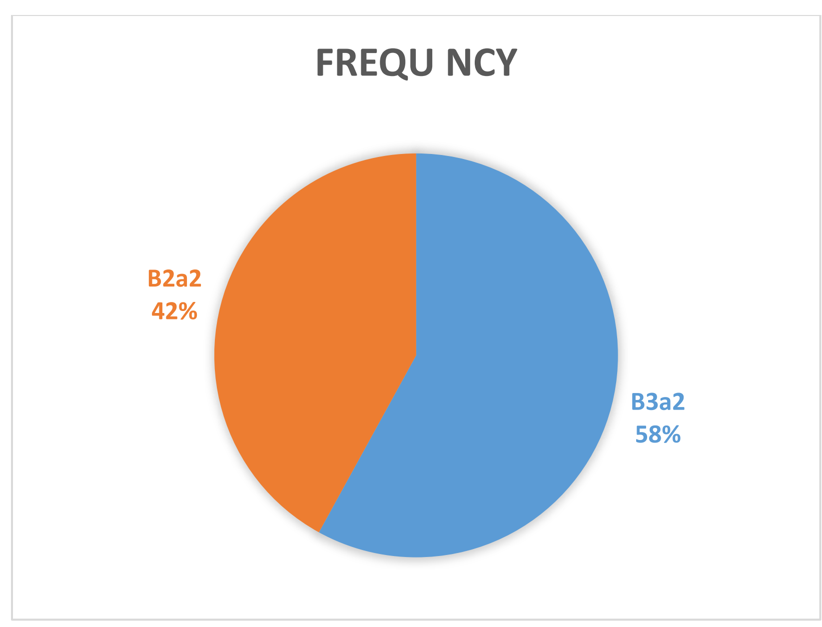


Figure 4.2: The Frequency of Different BCR-ABL Fusion Variants:
The distribution of BCR-ABL transcript variants among the study population is illustrated in Figure 4.2, highlighting the predominance of the e13a2 transcript, followed by e14a2 and a small number of co-expression cases.

4.4 Demographic data based on BCR-ABL fusion transcripts:

Patient's demographic data based on BCR-ABL fusion transcript variants are presented in Table 4.3. The age distribution between the two BCR-ABL fusion transcript groups showed a significant difference. Among patients with the b3a2 fusion transcript, 23 patients (79.3%) were aged ≥ 45 years, while only 11 patients (52.4%) with the b2a2 fusion transcript were in the same age group. This difference was statistically significant with a p value of 0.044 depend on the Chi-square test, suggesting that older patients tend to present more frequently with the b3a2 transcript. There was no significant difference in the gender distribution between the two groups. Among b3a2 patients, 18 (62.1%) were male, and 11 (37.9%) were female. Similarly, among b2a2 patients, 10 (47.6%) were male, and 11 (52.4%) were female. The p value of 0.310 depend on the Chi-square test indicates that gender is not significantly associated with the BCR-ABL transcript type. The BMI distribution did not show any significant differences between the two BCR-ABL transcript variants. In the b3a2 group, 12 patients (41.4%) had a BMI greater than 30, while 5 patients (23.8%) in the b2a2 group had a BMI greater than 30. The p value of 0.375

depend on the Chi-square test suggests no significant association between BMI and transcript type. There was no significant difference in residence between the two groups. Both b3a2 and b2a2 patients came from diverse regions, including Nablus, Gaza Strip, Jenin, and others. The p value of 0.421 depend on the Chi-square test indicates that residence is not significantly associated with the BCR-ABL transcript type. The smoking status also did not show a significant association with the BCR-ABL fusion transcript variants. 15 patients (51.7%) in the b3a2 group were non-smokers, and 12 patients (57.1%) in the b2a2 group were non-smokers. The p value of 0.704 depend on the Chi-square test suggests no significant relationship between smoking and transcript type. There was no significant difference in family history of CML between the two groups. In the b3a2 group, 14 patients (48.3%) had a family history of CML or other hematologic malignancies, while in the b2a2 group, 10 patients (47.6%) had a family history. The p value of 0.963 depend on the Chi-square test indicates no significant association between family history and the BCR-ABL transcript type.

Table 4.3: Patient's demographic data based on BCR-ABL fusion transcript variants

Variable No (%)		BCR-ABL b3a2 N = 29	fusion variants b2a2 N = 21	p value
Age (years)	< 45	6	10	0.044
	≥ 45	23	11	
Gender	Male	18	10	0.310
	Female	11	11	
BMI (kg/m2)	< 25	8	9	0.375
	25-30	9	7	
	> 30	12	5	
Residence	Ramallah	1	1	0.421
	Gaza Strip	4	2	
	Jenin	7	2	
	Nablus	5	10	
	Tulkarm	5	4	
	Qalqilya	1	1	

	Tubas Jericho Salfit Hebron	1 1 1 3	1 0 0 0	
Smoking	No Yes	15 14	12 9	0.704
Family history	No Yes	15 14	11 10	0.963

The dataset represents 49 patients (Table:4.4) with what appears to be chronic myeloid leukemia (CML) based on the phase classifications and imatinib treatment. The vast majority of patients (87.8%) were in the chronic phase, which is typically associated with better prognosis, while smaller proportions were in accelerated (8.2%) and blast crisis phases (4.1%). Philadelphia chromosome (Ph) status was negative in 61.2% of cases and positive in 38.8%. Nearly all patients (98%) received imatinib therapy, the standard tyrosine kinase inhibitor for CML treatment. The overall mortality rate was low at 6.1% (3 deaths out of 49 patients), with all three deaths occurring in chronic phase patients who were Ph-positive and receiving imatinib treatment. Notably, no deaths were observed in the accelerated or blast crisis phases within this cohort, though the small sample sizes in these advanced phases limit definitive conclusions. The low mortality rate suggests effective disease management, likely reflecting imatinib's established efficacy in CML treatment, particularly in chronic phase disease.

Table 4.4: Clinical Data Summary

Data Overview	**Count**	**Percentage**	**Phase Distribution**	**Count**	**Percentage**
Total Patients	49	100%	Chronic	43	87.8%
Deaths	3	6.1%	Accelerated	4	8.2%
Survivors	46	93.9%	Blast	2	4.1%
Ph Status Distribution	Count	Percentage	Treatment Distribution	Count	Percentage
Negative	30	61.2%	Imatinib	48	98.0%
Positive	19	38.8%	Cytoreduction with hydroxyurea	1	2.0%

4.5. Hematological data based on BCR ABL fusion transcript variants:

The comparison of hematological parameters between patients expressing the b3a2 (n = 29) and b2a2 (n = 21) BCR-ABL fusion transcripts is shown in Table 4. Most parameters were comparable between the two groups, with the exception of white blood cell count and platelet count, which showed statistically significant differences.

Interpretation of Key Findings

- **White Blood Cell (WBC) Count**:

  Patients with the b3a2 transcript had a significantly higher WBC count ($64.34 \times 10^9/L$) compared to those with b2a2 ($48.94 \times 10^9/L$, $p = 0.003$ Student's t test), indicating a possible association between b3a2 and more marked leukocytosis.

- **Platelet Count**:

  In contrast, platelet counts were significantly higher in b2a2 patients (368.29 × 10^9/L) than in b3a2 patients (222.90 × 10^9/L, p = 0.001 Student's t test), suggesting a potential link between the b2a2 variant and thrombocytosis.

- **Hemoglobin, PCV, RBC Count, and Differential WBC Counts**:

  No statistically significant differences were observed between the two transcript variants for these parameters. Both groups showed mild anemia with relatively normal red blood cell indices.

- **Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Basophil Percentages**: These values varied slightly but did not differ significantly between transcript variants.

Table 4.5: Patient's hematological data based on BCR-ABL fusion transcript variants

Variable	BCR-ABL b3a2 Mean ± SD N = 29	fusion variants b2a2 Mean ± SD N = 21	p-Value
Packed cell volume %	33.5345 ± 7.74879	33.3048 ± 7.76772	0.918
Hemoglobin (g/ dL)	11.2245 ± 2.60211	11.0233 ± 2.62388	0.789
Red blood cells × 10^{12}/L	3.8314 ± 0.85934	3.9057 ± 1.01839	0.781
White blood cells × 10^9/L	64.3434 ± 13.44163	48.9376 ± 18.97413	0.003
Neutrophil %	60.6141 ± 23.42831	66.9638 ± 16.26769	0.291
Lymphocyte %	16.0314 ± 19.38762	12.5248 ± 9.49602	0.449
Monocyte %	6.0814 ± 4.53564	8.4348 ± 12.12062	0.342
Eosinophil %	1.6497 ± 1.43278	1.6933 ± 1.72833	0.932
Basophil %	2.5683 ± 2.24481	1.9690 ± 1.51955	0.266
Platelets × 10^9/L	222.8966 ± 114.05116	368.2857 ± 162.59063	0.001

4.6. RBC indices based on BCR-ABL fusion transcript variants:

The red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW-CV), were compared between patients expressing the b3a2 and b2a2 BCR-ABL fusion transcripts. As shown in the table 4.5 below, no statistically significant differences were observed in any of the RBC indices between the two BCR/ABL transcript variants.

Interpretation

- Although slight differences were observed in mean values of MCV, MCH, and MCHC, these differences were not statistically significant.
- The RDW-CV values were nearly identical between the two groups, indicating similar levels of red cell size variability in both transcript variants.
- The lack of significant variation suggests that BCR-ABL transcript type may not have a substantial impact on red blood cell morphology or production parameters in CML patients.

Table 4.6: Patient's RBC indices data based on BCR-ABL fusion transcript variants

Red blood cell Indices Variable	BCR-ABL b3a2 Mean ± SD N = 29	fusion variants b2a2 Mean ± SD N = 21	p-Value
MCV (fL)	87.5000± 3.70125	85.9524 ± 7.02642	0.365
MCH (pg)	29.4241 ± 1.54565	28.5857 ± 2.94793	0.244
MCHC (g/dL)	33.4690 ± 0.83756	33.3095 ± 1.06813	0.557
RDW-CV %	17.0241 ± 2.07083	17.2143 ± 2.60275	0.775

Chapter Five: Discussion

In our study, 58% of patients expressed the b3a2 transcript and 42% expressed b2a2, which is consistent with many Middle Eastern and Asian studies. For instance, similar distributions were observed in Iraq (59% b3a2) India (63.5%), Iran (62.35%) , and Saudi Arabia (63.33%), suggesting a regional pattern where b3a2 is slightly more prevalent.(Alanazi et al., 2023; Ayatollahi, Keramati, et al., 2018; Kagita et al., 2018; Khazaal et al., 2019) In contrast, certain populations show a different trend. For example, in Pakistan, b2a2 (65%) was more common than b3a2 (26%), and in Ecuador, b2a2 was overwhelmingly dominant (94.6%).(Irshad et al., 2012; Paz-y-Miño et al., 2002) This highlights how ethnic, genetic, and perhaps environmental factors may influence transcript variant expression. Western and European countries generally show more balanced or variable distributions. The USA had a relatively equal distribution (41% b3a2, 42% b2a2), while Germany and the UK reported lower b3a2 frequencies (44.89% and 39%, respectively). (Hanfstein et al., 2014; Jain et al., 2016; Lucas et al., 2009) Interestingly, Korea showed high prevalence of b3a2 (67.9% and 67.66%), aligning more closely with Asian populations. (Goh et al., 2006) African countries such as Tunisia (64% b3a2) and Sudan (41.9% b3a2) also reflect regional differences. (Bennour et al., 2013; Osman et al., 2010) Sudan, notably, had a higher b2a2 rate (53.5%) than b3a2, again reinforcing the variability of transcript distribution across continents.(Osman et al., 2010) These comparisons suggest that while the b3a2 transcript is generally more prevalent worldwide, its dominance varies significantly by geographic location and ethnicity. Our study falls within the typical Middle Eastern pattern, reinforcing the regional consistency of molecular subtypes in CML (Table 6). Our findings on the association between BCR-ABL transcript variants and hematological parameters are consistent with several international reports, reflecting a global trend in chronic myeloid leukemia (CML) profiles.

In our study, the b3a2 transcript was significantly associated with higher WBC counts ($p = 0.003$), while the b2a2 transcript was associated with significantly higher platelet counts ($p = 0.001$). These hematological distinctions align with previously published studies. A similar association of elevated WBC in b3a2-positive patients was noted in: Iraq, India and Germany. (Hanfstein et al., 2014; Kagita et al., 2018; Khazaal et al., 2019) On the other hand, higher platelet counts in b2a2-positive cases were reported in: Germany and Brazil. (de Oliveira et al., 2022; Hanfstein et al., 2014) Some studies, such as those from Tunisia and Bulgaria, observed

increased platelet counts in b3a2-positive patients, suggesting possible regional or population-based variability.(Balatzenko et al., 2011a; Bennour et al., 2013) Notably, the Indian study found both WBC and PLT counts elevated in b3a2 cases, supporting a trend toward more proliferative disease in b3a2 carriers.(Kagita et al., 2018) These findings may point to intrinsic differences in the biological behavior of the two transcript variants. The b3a2 transcript has been associated with greater leukocytosis and may reflect more active tyrosine kinase signaling, while b2a2 may influence megakaryopoiesis more strongly, reflected in elevated platelet levels. Overall, these hematological patterns across various populations underscore the clinical utility of identifying BCR-ABL transcript variants, not only for diagnosis but also for better prognostic stratification and potentially tailored management strategies.

Most often in the great majority of the above studies from Far-East to Far-West, b3a2 transcript was more expressed than b2a2 transcript among CML patients of the many series, the present work is one of the few examining a relatively large series of CML patients. The reason for the difference of the data from this series could be due to four reasons. First, the ethnicity of population in whom the study was done; different population have diverse composition of certain transcript due different genetic back ground. Also, likely the geographical distribution Secondly, sample size; larger the sample size, the more representative and reproducible we believe the results are. Surprisingly, the sampling time: getting blood samples from newly diagnosed patients is more accurate than during do imatinib treatment (even by presence of imatinib resistance as it was shown to alter rare transcripts such as e1a2 (Verma et al., 2009), though co-expression of b3a2 and b2a2 might be seen in beginning but as disease progresses only one of them will be continued. (Khazaal et al., 2019) Table 3, Only age was the only single demographic factor that exhibited a significant association with the BCR-ABL fusion type available. We obtained mixed results from global data on this correlation. Many wonder if that the ethnicity of the study subject and sample size, technique of detection would explain possible variations in it. In the same manner that so many researchers around the world have dealt with the association of BCR-ABL fusion variants. In many studies an unambiguous rise of WBC count was observed among CML patients presenting b3a2 transcript and particularly those with b2a2 variant but high platelet count. Other studies state the exact opposite, namely that leukocyte and platelet counts are low in b2a2, b3a2 transcript, respectively. Additional studies showed no differences in this sense. Table 7 lists main issued articles at laboratory characteristics and BCR-

ABL transcript. The authors of several papers could not explain the observed variability in correlation between particular fusion type and WBC or platelet count. Some groups interpreted this as difference in conformation between the two transcripts with b3a2 being longer than b2a2 by full 25 amino acids in BCR. This region lacks tyrosine kinase activity but the extra 25 residues may alter on overall conformation of BCR-ABL in a manner which decreases the kinase activity of it (Lin et al., 2016). However, this explanation is inadequate in the present study since the b3a2 transcript was associated with high total WBC and also regardless of demographic patient subgroup; b2a2 transcript was associated with raised platelets count in any explanation. The second group connects this variability to b3a2 transcript effect on thrombopoietic activity, which not justified the results obtained in the current analysis. (Inokuchi et al., 1991). Another possibility is that numerous leukopoietic and thrombopoietic processes, alongside tyrosine kinase should be included in this multiple pathway (of signaling ect.) any defect on single/ more pathways therefore have relation with the imbalance of entire hematopoietic process. Another support for this hypothesis in the study carried out by Balatzenko et al. (2011) who analysed how combined variants of BCR-ABL fusion gene in co-morbid multi drug resistance (MDR1) gene influence the hematological parameters of 89 CML patients from India.(Balatzenko et al., 2011b). The human MDR1 gene encodes an integral membrane protein (P glycoprotein (Pgp)) which is an energy-dependent exporter of substances from inside of cells and membranes to outside of them [1]. Physiological function of the gene : protection cells against toxic substances, or metabolites(Brinkmann, 2002).

Table 5.1: Incidence of b2a2 and b3a2 transcripts in Eastern, African, European, and Western countries

Country	No. of patients	b3a2%	b2a2%
Present study	50 patients	58 %	42 %
Iraq (Khazaal et al., 2019)	100 patients	59 %	39 %
India(Kagita et al., 2018)	170 patients	63.53 %	36.36 %
Iran(Ayatollahi, Tavassoli, et al., 2018)	85 patients	62.35 %	29.41 %
Syria(Al-Achkar et al., 2016)	45 patients	51.1 %	46.7 %
Pakistan(Irshad et al., 2012)	23 patients	26 %	65 %
Saudi Arabia(Iqbal, 2014)	30 patients	63.33 %	36.66 %

Korea(Goh et al., 2006)	548 patients	67.66 %	32.34 %
Germany(Hanfstein et al., 2014)	1,105 patients	44.89 %	40.81 %
Bulgaria(Balatzenko et al., 2011a)	98 patients	54 %	45 %
UK(Lucas et al., 2009)	71 patients	39 %	31 %
Brazil(Vasconcelos et al., 2017)	203 patients	64 %	34 %
USA(Jain et al., 2016)	481 patients	41 %	42 %
Argentine(Sastre et al., 2007)	24 patients	37.5 %	41.7 %
Ecuador(Paz-y-Miño et al., 2002)	40 patients	5.4 %	94.6 %
Mexico(Arana-Trejo et al., 2002)	250 patients	35 %	48 %
Tunisia(Bennour et al., 2013)	45 patients	64 %	36 %
Sudan(Osman et al., 2010)	46 patients	41.9 %	53.5 %

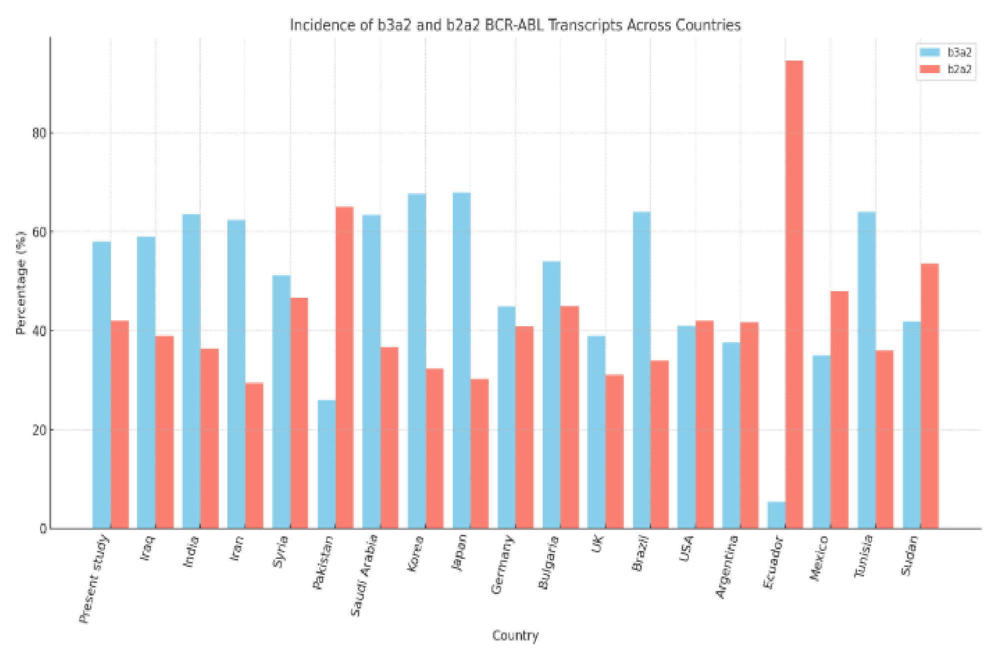


Figure 5.1: Incidence of b3a2 and b2a2 BCR-ABL Transcripts Across Countries.

Table 5.2: Main Published Studies about The Association Between P210bcr-ABL1 Transcript variants And Laboratory Data In Patients With CML

Study	BCR-ABL fusion gene transcripts			
	b3a2		b2a2	
	WBC's	PLT's	WBC's	PLT's
Present study	Increased			Increased
Iraq (Khazaal et al., 2019)	Increased			Increased
India (Kagita et al., 2018)	Increased	Increased		
Bulgaria (Balatzenko et al., 2011a)		Increased		
Japan (Al-Achkar et al., 2016)		Increased	Increased	
Germany (Hanfstein et al., 2014)	Increased			Increased
Brazil (Vasconcelos et al., 2017)		Increased	Increased	
Tunisia (Bennour et al., 2013)		Increased		

5.1 Conclusions

This study contributes significant information regarding the prevalence and clinical implications of BCR-ABL transcript variants in Palestinian CML patients. b3a2 variant was detected in 58% of the 50 patients studied, as opposed to b2a2 variant which made up 42%; this is in line with similar findings in other regional and global studies. This transcript type was associated with age ($P<0.05$), with b3a2 type being found significantly more frequently in patients aged ≥45 years. Hematological analysis indicated that b3a2 was significantly linked with increased white blood cell counts and that b2a2 was significantly linked with increased platelet counts ($p = 0.003$ and $p = 0.001$, respectively). None were observed between transcript type and other hematological variables or demographic characteristics like gender, BMI, or smoking status. These data indicate that transcript variants are not merely significant molecular markers but also clinically and prognostically significant in hematologic presentation. Differences in transcript distribution and their attendant blood parameters further support routine molecular typing as a part of CML diagnosis and management. Larger cohort studies with follow-up for a longer period would be ideal to investigate the influence of transcript variants on response to treatment and survival in the Palestinian population.

5.2 Limitations of the Study

This study has several constraints. Firstly, the participant count was relatively limited (n=50), which might influence the extent to which the findings are applicable to the broader Palestinian demographic or various ethnicities. Additionally, the research was conducted at a single site, An-Najah National University Hospital, potentially introducing selection bias due to the specific circumstances surrounding patient selection in that location. Furthermore, the investigation did not consider the treatment history of the patients or the progression of their disease at the time of sample collection, factors that could have affected their blood parameters. Lastly, while RT-PCR was employed to detect transcript variants, other molecular assessments such as mutation profiling or monitoring treatment responses were not performed.

5.3 Recommendations

According to the results of this research, a series of recommendations are put forward:

1. It is advised that routine molecular characterization of BCR-ABL transcript variants (b3a2 and b2a2) be incorporated into the diagnostic framework for individuals diagnosed with chronic myeloid leukemia (CML), especially during the initial diagnosis phase.
2. Additional multicenter research with an expanded sample size is suggested to validate the identified correlations and enhance the applicability of findings for the Palestinian demographic.
3. Longitudinal studies are advocated to evaluate the prognostic importance of transcript variants regarding treatment efficacy, disease advancement, and overall survival.
4. The inclusion of factors related to treatment (such as the kind and length of TKI therapy) in upcoming research may aid in understanding their influence on hematological variations between transcript categories.
5. Efforts should be made to increase awareness among medical professionals about the significance of identifying transcript variants for effective CML management and risk evaluation.
6. The creation of a national registry for CML patients in Palestine could facilitate future research efforts and inform public health initiatives aimed at managing leukemia.

5.4 Clinical Follow-Up and Patient Outcomes:

The clinical follow-up data of 50 CML patients were analyzed to assess their current disease status, treatment outcomes, and molecular response. At the time of sample collection, all patients were Philadelphia chromosome-positive. However, follow-up results revealed that 24 patients (48%) became Philadelphia-negative, suggesting a favorable molecular response to tyrosine kinase inhibitor (TKI) therapy.

Regarding disease stage, the majority of patients (82%) were in the chronic phase, while 12% were in the accelerated phase and 6% in blast crisis. Treatment with imatinib was the standard approach for all patients.

Transcript variant analysis showed that 70% of patients remained positive for BCR-ABL transcripts, while 30% became variant-negative over time. This shift may indicate the success of therapy in suppressing the fusion gene expression.

In terms of survival, 6 patients (12%) passed away during the follow-up period, whereas 44 patients (88%) were alive and under ongoing monitoring. These outcomes highlight the clinical benefit of early diagnosis, standardized treatment, and continuous molecular monitoring in the management of CML.

Category	**Patient Count**	**Percentage**
Chronic Phase	41	82%
Accelerated Phase	6	12%
Blast Phase	3	6%
Philadelphia Positive	26	52%
Philadelphia Negative	24	48%
Transcript Variant Present	35	70%
Transcript Variant Absent	15	30%
Alive	44	88%
Deceased	6	12%

Clinical Follow-Up and Patient Outcomes:

The clinical follow-up data in this study provide important insights into disease progression, treatment response, and overall patient outcomes in CML. The transition of nearly half the patients from Philadelphia chromosome-positive (Ph+) to Philadelphia-negative (Ph–) status is particularly noteworthy. This shift suggests that tyrosine kinase inhibitor (TKI) therapy, primarily imatinib, was effective in achieving cytogenetic or even molecular remission in a significant proportion of patients. These findings align with existing literature indicating that TKIs can induce long-term remission and improve survival in CML patients.

The distribution of disease phases—with most patients in the chronic phase—reflects typical patterns in clinical practice, where early diagnosis has become more feasible due to increased awareness and improved diagnostic tools. The small number of patients in the accelerated and blast phases highlights the importance of timely detection, as these advanced stages are associated with poorer prognoses and lower treatment response rates.

Interestingly, 30% of patients became transcript variant-negative during follow-up. This observation may serve as a molecular marker of treatment efficacy and reinforces the importance of continued transcript monitoring in routine care. The decline in detectable BCR-ABL transcripts can be interpreted as a surrogate for molecular remission and is consistent with previous studies that linked transcript disappearance with better prognosis.

Regarding survival outcomes, the mortality rate (12%) among this cohort is within the expected range for CML populations in low-to-middle income countries. It underscores the need for improved patient access to advanced therapies, consistent follow-up, and supportive care. On the other hand, the 88% survival rate reflects the significant strides made in CML management, particularly in countries that have begun integrating molecular diagnostics into public health systems.

These follow-up findings contribute to a more comprehensive understanding of CML in the Palestinian population and demonstrate the dynamic impact of targeted therapies on disease biology. They further highlight the necessity of longitudinal studies, especially in underrepresented populations, to evaluate real-world treatment effectiveness and long-term patient survival.

In conclusion, the follow-up data of CML patients in this study provide compelling evidence of the clinical value of molecular monitoring in routine patient care. The observed transitions from Philadelphia-positive to negative status, as well as the disappearance of BCR-ABL transcript variants in a subset of patients, demonstrate the therapeutic impact of TKIs and underscore the potential for long-term disease control. These outcomes highlight the importance of early diagnosis, consistent follow-up, and access to targeted therapies in improving patient prognosis. Continued research and longitudinal surveillance are essential to further optimize treatment strategies and better understand the disease dynamics within the Palestinian population.

References

Adnan Awad, S., Kankainen, M., Ojala, T., Koskenvesa, P., Eldfors, S., Ghimire, B., Kumar, A., Kytölä, S., Kamel, M. M., & Heckman, C. A. (2020). Mutation accumulation in cancer genes relates to nonoptimal outcome in chronic myeloid leukemia. *Blood Advances*, *4*(3), 546-559. https://doi.org/10.1182/bloodadvances.2019000943

Adnan-Awad, S., Kankainen, M., & Mustjoki, S. (2021). Mutational landscape of chronic myeloid leukemia: More than a single oncogene leukemia. *Leukemia & Lymphoma*, *62*(9), 2064-2078. https://doi.org/10.1080/10428194.2021.1913135

Al-Achkar, W., Moassass, F., Youssef, N., & Wafa, A. (2016). Correlation of p210 BCR-ABL transcript variants with clinical parameters and disease outcome in 45 chronic myeloid leukemia patients. *Journal of B.U.ON.*, *21*(2), 444-449.

Alanazi, N., Iqbal, Z., Akhtar, T., Khalid, A. M., Aleem, A., Shahzadi, S., Akram, A. M., Rasool, M., Shah, I. H., & Khalid, M. (2023). Association of BCR-ABL alternative splice variants with disease progression, treatment response and survival in chronic myeloid leukemia patients treated with first-line imatinib monotherapy. *Advancements in Life Sciences*, *9*(4), 612-617.

Alves, R., Gonçalves, A. C., Rutella, S., Almeida, A. M., De Las Rivas, J., Trougakos, I. P., & Sarmento Ribeiro, A. B. (2021). Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia—From molecular mechanisms to clinical relevance. *Cancers*, *13*(19), 4820. https://doi.org/10.3390/cancers13194820

Amarante-Mendes, G. P., Rana, A., Datoguia, T. S., Hamerschlak, N., & Brumatti, G. (2022). BCR-ABL1 tyrosine kinase complex signaling transduction: Challenges to overcome resistance in chronic myeloid leukemia. *Pharmaceutics*, *14*(1), 215. https://doi.org/10.3390/pharmaceutics14010215

Ayatollahi, H., Keramati, M. R., Kooshyar, M. M., Raiszadeh, M., Shakeri, S., & Sadeghian, M. H. (2018). BCR-ABL fusion genes and laboratory findings in patients with chronic myeloid leukemia in northeast Iran. *Caspian Journal of Internal Medicine*, *9*(1), 65-70. https://doi.org/10.22088/cjim.9.1.65

Balatzenko, G., Vundinti, B. R., & Margarita, G. (2011). Correlation between the type of BCR-ABL transcripts and blood cell counts in chronic myeloid leukemia—A possible influence of mdr1 gene expression. *Hematology Reports*, *3*(1), e3. https://doi.org/10.4081/hr.2011.e3

Bennour, A., Ouahchi, I., Achour, B., Zaier, M., Youssef, Y. B., Khelif, A., Saad, A., & Sennana, H. (2013). Analysis of the clinico-hematological relevance of the breakpoint location within M-BCR in chronic myeloid leukemia. *Medical Oncology*, *30*(1), 478. https://doi.org/10.1007/s12032-012-0478-z

de Oliveira, M. B., de Alcântara Maneschy, C., de Castro, J. A. A., dos Santos Barile, K. A., Palmeira, M. K., & de Melo Amaral, C. E. (2022). Association between the BCR-ABL gene transcripts and the laboratory hematological profile. *Health Sciences Journal*, *12*(3), 61-66. https://doi.org/10.21767/1791-809X.1000912

Deininger, M. W., Shah, N. P., Altman, J. K., Berman, E., Bhatia, R., Bhatnagar, B., DeAngelo, D. J., Gotlib, J., Hobbs, G., & Maness, L. (2020). Chronic myeloid leukemia, version 2.2021, NCCN clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network*, *18*(10), 1385-1415. https://doi.org/10.6004/jnccn.2020.0047

Foroni, L., Gerrard, G., Nna, E., Khorashad, J. S., Stevens, D., Swale, B., Milojkovic, D., Reid, A., Goldman, J., & Marin, D. (2009). Technical aspects and clinical applications of measuring BCR-ABL1 transcripts number in chronic myeloid leukemia. *American Journal of Hematology*, *84*(8), 517-522. https://doi.org/10.1002/ajh.21445

Goh, H.-G., Hwang, J.-Y., Kim, S.-H., Lee, Y.-H., Kim, Y.-L., & Kim, D.-W. (2006). Comprehensive analysis of BCR-ABL transcript types in Korean CML patients using a newly developed multiplex RT-PCR. *Translational Research*, *148*(5), 249-256. https://doi.org/10.1016/j.trsl.2006.05.004

Hanfstein, B., Lauseker, M., Hehlmann, R., Saussele, S., Erben, P., Dietz, C., Fabarius, A., Proetel, U., Schnittger, S., & Haferlach, C. (2014). Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. *Haematologica*, *99*(9), 1441-1447. https://doi.org/10.3324/haematol.2014.107383

Hochhaus, A., Baccarani, M., Silver, R. T., Schiffer, C., Apperley, J. F., Cervantes, F., Clark, R. E., Cortes, J. E., Deininger, M., & Guilhot, F. (2020). European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*, *34*(4), 966-984. https://doi.org/10.1038/s41375-020-0776-2

Hovorkova, L., Winkowska, L., Skorepova, J., Krumbholz, M., Benesova, A., Polivkova, V., Alten, J., Bardini, M., Meyer, C., & Kim, R. (2024). Distinct pattern of genomic breakpoints in CML and BCR::ABL1-positive ALL: Analysis of 971 patients. *Molecular Cancer*, *23*(1), 138. https://doi.org/10.1186/s12943-024-02045-0

Inokuchi, K., Inoue, T., Tojo, A., Futaki, M., Miyake, K., Yamada, T., Tanabe, Y., Ohki, I., Dan, K., & Ozawa, K. (1991). A possible correlation between the type of BCR-ABL hybrid messenger RNA and platelet count in Philadelphia-positive chronic myelogenous leukemia. *Blood*, *78*(12), 3125-3127. https://doi.org/10.1182/blood.V78.12.3125.3125

Irshad, S., Butt, M. A., & Joyia, A. (2012). Frequency of different BCR-ABL fusion transcripts in chronic myelogenous leukemia patients in Pakistan. *International Journal of Animal and Veterinary Medical Sciences*, *6*(6), 418-423.

Jabbour, E., & Kantarjian, H. (2022). Chronic myeloid leukemia: 2022 update on diagnosis, therapy, and monitoring. *American Journal of Hematology*, *97*(9), 1236-1256. https://doi.org/10.1002/ajh.26642

Jain, P., Kantarjian, H., Patel, K. P., Gonzalez, G. N., Luthra, R., Shamanna, R. K., Sasaki, K., Jabbour, E., Romo, C. G., & Kadia, T. M. (2016). Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. *Blood*, *127*(10), 1269-1275. https://doi.org/10.1182/blood-2015-10-674242

Jovanovski, A., Petiti, J., Giugliano, E., Gottardi, E. M., Saglio, G., Cilloni, D., & Fava, C. (2020). Standardization of BCR-ABL1 p210 monitoring: From nested to digital PCR. *Cancers*, *12*(11), 3287. https://doi.org/10.3390/cancers12113287

Kagita, S., Mamidi, T. K., Digumarti, L., Gundeti, S., & Digumarti, R. (2018). Assessment of BCR-ABL1 fusion transcripts and their association with response to imatinib treatment in chronic myeloid leukemia patients. *Indian Journal of Medical and Paediatric Oncology*, *39*(2), 165-171. https://doi.org/10.4103/ijmpo.ijmpo_148_17

Kantarjian, H. M., Hughes, T. P., Larson, R. A., Kim, D.-W., Issaragrisil, S., le Coutre, P., Etienne, G., Boquimpani, C., Pasquini, R., & Clark, R. E. (2021). Long-term outcomes with frontline nilotinib versus imatinib in newly diagnosed chronic myeloid leukemia in chronic phase: ENESTnd 10-year analysis. *Leukemia*, *35*(2), 440-453. https://doi.org/10.1038/s41375-020-01050-5

Khazaal, M. S., Hamdan, F. B., & Al-Mayah, Q. S. (2019). Association of BCR/ABL transcript variants with different blood parameters and demographic features in Iraqi chronic myeloid leukemia patients. *Molecular Genetics & Genomic Medicine*, *7*(8), e809. https://doi.org/10.1002/mgg3.809

Kok, C. H., Yeung, D. T., Lu, L., Watkins, D. B., Leclercq, T. M., Dang, P., Saunders, V. A., Reynolds, J., White, D. L., & Hughes, T. P. (2019). Gene expression signature that predicts early molecular response failure in chronic-phase CML patients on frontline imatinib. *Blood Advances*, *3*(10), 1610-1621. https://doi.org/10.1182/bloodadvances.2018028027

L'Abbate, A., Moretti, V., Pungolino, E., Micheloni, G., Valli, R., Frattini, A., Barcella, M., Acquati, F., Reinbold, R. A., & Costantino, L. (2023). Occurrence of L1M elements in chromosomal rearrangements associated to chronic myeloid leukemia (CML): Insights from patient-specific breakpoints characterization. *Genes*, *14*(7), 1351. https://doi.org/10.3390/genes14071351

Lin, H. X., Sjaarda, J., Dyck, J., Stringer, R., Hillis, C., Harvey, M., Carter, R., Ainsworth, P., Leber, B., & Pare, G. (2016). Gender and BCR-ABL transcript type are correlated with molecular response to imatinib treatment in patients with chronic myeloid leukemia. *European Journal of Haematology*, *96*(4), 360-366. https://doi.org/10.1111/ejh.12608

Loscocco, F., Visani, G., Galimberti, S., Curti, A., & Isidori, A. (2019). BCR-ABL independent mechanisms of resistance in chronic myeloid leukemia. *Frontiers in Oncology*, *9*, 939. https://doi.org/10.3389/fonc.2019.00939

Lucas, C. M., Harris, R. J., Giannoudis, A., Davies, A., Knight, K., Watmough, S. J., Wang, L., & Clark, R. E. (2009). Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. *Haematologica*, *94*(10), 1362-1367. https://doi.org/10.3324/haematol.2009.009134

Nachi, M., Kihel, I., Entasoltane, B., Brahimi, M., Yafour, N., Guella, D., Abed, A., & Bekadja, M. A. (2020). Impact of the major BCR-ABL1 transcript type on clinical and biological parameters and molecular response in patients with chronic myeloid leukemia. *Hematology/Oncology and Stem Cell Therapy*, *13*(4), 207-213. https://doi.org/10.1016/j.hemonc.2020.01.004

Osman, A. E., & Deininger, M. W. (2021). Chronic myeloid leukemia: Modern therapies, current challenges and future directions. *Blood Reviews*, *49*, 100825. https://doi.org/10.1016/j.blre.2021.100825

Osman, E.-A. I., Hamad, K., Elmula, I. M. F., & Ibrahim, M. E. (2010). Frequencies of BCR-ABL1 fusion transcripts among Sudanese chronic myeloid leukaemia patients. *Genetics and Molecular Biology*, *33*(2), 229-231. https://doi.org/10.1590/S1415-47572010005000027

Paz-y-Miño, C., Burgos, R., Morillo, S. A., Santos, J. C., Fiallo, B. F., & Leone, P. E. (2002). BCR-ABL rearrangement frequencies in chronic myeloid leukemia and acute lymphoblastic leukemia in Ecuador, South America. *Cancer Genetics and Cytogenetics*, *132*(1), 65-67. https://doi.org/10.1016/S0165-4608(01)00531-2

Pfirrmann, M., Evtimova, D., Saussele, S., Castagnetti, F., Cervantes, F., Janssen, J., Hoffmann, V. S., Gugliotta, G., Hehlmann, R., & Hochhaus, A. (2017). No influence of BCR-ABL1 transcript types e13a2 and e14a2 on long-term survival: Results in 1494 patients with chronic myeloid leukemia treated with imatinib. *Journal of Cancer Research and Clinical Oncology*, *143*(5), 843-850. https://doi.org/10.1007/s00432-017-2364-2

Poudel, G., Tolland, M. G., Hughes, T. P., & Pagani, I. S. (2022). Mechanisms of resistance and implications for treatment strategies in chronic myeloid leukaemia. *Cancers*, *14*(14), 3300. https://doi.org/10.3390/cancers14143300

Sastre, D. A., Argaraña, C. E., Heller, V. B., Gallo, M., Fernández, E. N., & Rodríguez, C. M. (2007). An analysis of multiplex-PCR in the detection of BCR-ABL transcripts in hematological disorders. *Genetics and Molecular Biology*, *30*(3), 520-523. https://doi.org/10.1590/S1415-47572007000400001

Shanmuganathan, N., Branford, S., Yong, A. S., Hiwase, D. K., Yeung, D. T., Ross, D. M., & Hughes, T. P. (2018). The e13a2 BCR-ABL1 transcript is associated with higher rates of molecular recurrence after treatment-free remission attempts: Retrospective analysis of

the Adelaide cohort. *Blood*, *132*(Supplement 1), 1731. https://doi.org/10.1182/blood-2018-99-113908

Thiele, J., Kvasnicka, H. M., Orazi, A., Gianelli, U., Gangat, N., Vannucchi, A. M., Barbui, T., Arber, D. A., & Tefferi, A. (2023). The international consensus classification of myeloid neoplasms and acute leukemias: Myeloproliferative neoplasms. *American Journal of Hematology*, *98*(1), 166-179. https://doi.org/10.1002/ajh.26751

Vasconcelos, A., Azevedo, I., Melo, F., Neves, W., Azevedo, A., & Melo, R. (2017). BCR-ABL1 transcript types showed distinct laboratory characteristics in patients with chronic myeloid leukemia. *Genetics and Molecular Research*, *16*(2), gmr16029541. https://doi.org/10.4238/gmr16029541

YAGHMAEI, M., Ghafari, S., Ghavamzadeh, A., Alimoghaddam, K., Jahani, M., Mousavi, S., Iravani, M., Bahar, B., & Bibordi, I. (2008). Frequency of BCR-ABL fusion transcripts in Iranian patients with chronic myeloid leukemia. *Archives of Iranian Medicine*, *11*(3), 247-251.

.

Appendices

Appendix A: IRB Approval

Institutional Review Board - Ramallah

الجامعـــة العربيـــة الأمريكيـــة

مجلس اخلاقيات البحث العلمي — رام الله

IRB Approval Letter

Study Title: "Correlation of Breakpoint Cluster Region - Abelson Murine Leukemia (BCR-ABL) Transcript Variants with Haematological Parameters and Demographic Characteristics in Palestinian Patients with Chronic Myeloid Leukemia".

Submitted by: Mohammed Fathi Qalalwah

Date received: 3rd February 2024

Date reviewed: 11th February 2024

Date approved: 3rd March 2024

Your Study titled **"Correlation of Breakpoint Cluster Region - Abelson Murine Leukemia (BCR-ABL) Transcript Variants with Haematological Parameters and Demographic Characteristics in Palestinian Patients with Chronic Myeloid Leukemia"** with the code number **"R-2024/A/42/N"** was reviewed by the Arab American University Institutional Review Board - Ramallah and it was approved on the 3rd of March 2024.

Sajed Ghawadra, PhD
IRB-R Chairman
Arab American University of Palestine

الجامعة العربية الأمريكية . فلسطين
مجلس اخلاقيات البحث العلمي - رام الله
IRB-R
ARAB AMERICAN UNIVERSITY-PALESTINE
INSTITUTIONAL REVIEW BOARD - RAMALLAH

General Conditions:

1. Valid for 1 year from the date of approval.
2. It is important to inform the IRB-R with any modification of the approved study protocol.
3. The Bord appreciates a copy of the research when accomplished.

رام الله — فلسطين

Tel: 02-294-1999 **E-Email:** IRB-R@aaup.edu **Website:** www.aaup.edu

Appendix B: تسهيل مهمه بحثية

Arab American University

Faculty of Graduate Studies

الجامعة العربية الأمريكية

كلية الدراسات العليا

2024/3/6

السادة مستشفى النجاح الوطني الجامعي المحترمين

<u>تسهيل مهمة بحثية</u>

تحية طيبة وبعد،

تُهديكم كلية الدراسات العُليا في الجامعة العربية الأمريكية أطيب التحيات، وبالإشارة الى الموضوع أعلاه، تشهد كلية الدراسات العُليا في الجامعة أن الطالب **محمد فتحي قلالوة** والذي يحمل الرقم الجامعي 202112984 هو طالب ماجستير في برنامج علم الدم والمناعة ويعمل على رسالة الماجستير الخاصة به بعنوان:

"Correlation of Breakpoint Cluster Region - Abelson murine Leukemia (BCR-ABL) transcript variants with hematological parameters and demographic characteristics in Palestinian patients with chronic myeloid leukemia"

تحت إشراف الدكتور مهند خضر.نأمل من حضرتكم الإيعاز لمن يلزم لمساعدته للحصول على المعلومات اللازمة للدراسة، علماً أن المعلومات ستستخدم لغاية البحث فقط وسيتم التعامل معها بغاية السرية، وقد أعطي هذه الرسالة بناءً على طلبه.

وتفضلوا بقبول فائق الاحترام

عميد كلية الدراسات العليا

د. نوار قطب

Page 1 of 1

Jenin Tel: +970-4-2418888 Ext.:1471,1472 Fax: +970-4-2510810 P.O. Box:240
Ramallah Tel: +970-2-2941999 Fax: +970-2-2941979 Abu Qash - Near Alrehan
E-mail: FGS@aaup.edu ; PGS@aaup.edu Website: www.aaup.edu

Appendix C: Consent form

Arab American University
Scientific Research Deanship
Ethical Review Committee

الجامعـــة العربيـــة الأمريكيـــة
عمادة البحث العلمي
لجنة اخلاقيات البحث العلمي

موافقة مسبقة

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

أنا .... (اسم المشارك / اختياري) أوافق بموجبه على المشاركة في البحث السريري (دراسة سريرية المحددة أدناه:

عنوان الدراسة: اختلافات نسخ BCR / ABL ومعلمات الدم المختلفة والخصائص الديموغرافية لدى المرضى الفلسطينيين الذين يعانون من سرطان الدم النخاعي المزمن.

الطبيعة والغرض الذي تم شرحه لي من قبل محمد فتحي قلالوة ,وفسره الى أقصى حد ممكن في اللغة العربية. ,حاصل على شهادة البكالوريوس في العلوم الطبية المخبرية في الجامعة العربية الأمريكية.

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

التاريخ : .... التوقيع...
(المشارك)

الشاهد :

الاسم: ....

تعيين:....................................... التوقيع:..............................

(شاهد على توقيع المشارك)

أؤكد انني اوضحت للمريض طبيعة وهدف البحث المذكور أعلاه.

التاريخ:.. التوقيع:..
(محقق حضور)

جنين - ص.ب: 240 هاتف: 970-4-2418888 فاكس: 970-4-2510813
Jenin - P.O. Box: 240 Tel: 970-4-2418888 Fax: 970-4-2510813 E-mail: src@aaup.edu Website: www.aaup.edu

Appendix D: Material

Table 1: Chemicals and Reagents used in the study

Reagents	Provider
Nucleo^{R}Spin RNA Blood Kit	MACHEREY-NAGEL
Ethanol absolute anhydrous	Carlo Erba Reagent
Green Master Mix 2X	Thermo Scientific
Nuclease-free water	Thermo Scientific
Agarose powder	Invitrogen
Ethidium bromide	Hylabs
Ladder 100bp	Promega

Table 2: Materials and Consumables

Materials	Provider
Sterile EDTA vacutainer tubes	Vacuumed
Needles	BD Microlance
Syringes	Consultant medical company
Centrifuge	Hettich
Microcentrifuge	HERMLE
Mini microcentrifuge	Qik Spin
Micropipettes	Human
Sterile aerosol pipet tips	Labcon
Vortex	Stuart
Heating block	Labmet
Microwave	Mega
Flask	SCHOTT
Electrophoresis	BIORAD
Gel tray	BIORAD
Thermo cycler	Biometratadvanced
NanoDrop Analyzer	Implen
Analytical balance	Adam Equipment

Appendix E: Media Approval -CRC

مركز البحث العلمي السريري
Clinical Research Centre

Approval date: 2024-03-11
Ref: CRC_2024_0257

Subject: Approval to conduct a research project at An-Najah National University Hospital

Dear Mr. Mohammed Qalalwah,

I am writing this letter to grant you permission to conduct your research project titled "Correlation of Breakpoint Cluster Region - Abelson murine Leukemia (BCR-ABL) transcript variants with hematological parameters and demographic characteristics in Palestinian patients with chronic myeloid leukemia.". I hope your study will provide new insights and contribute the advancement of knowledge and evidence. Furthermore, I would like to emphasize the importance of adhering to the ethical guidelines set forth by the hospital throughout the research process.

On behalf of An-Najah National University Hospital, I extend my best wishes and support for your research endeavors.

Sincerely,

Sa'ed H. Zyoud, Ph.D.

Clinical Toxicology

Director of Clinical Research Center

CC:

Chief Medical Officer

Chief Nursing Officer

Note: this approval letter is not valid unless signed and stamped by the

اختلافات النسخ بي سي ار اي بي ال ومعلمات الدم المختلفة والخصائص الديموغرافية لدى المرضي الفلسطينيين الذين يعانون من سرطان الدم النخاعي المزمن.

محمد فتحي قلالوة

د. مهند خضر

د. فكري سمارة

د. أدهم ابو خضر

ملخص

الخلفية:

يُصنف سرطان الدم النخاعي المزمن (CML) كنوع من الأورام النقوية المتزايدة، ويرتبط بكروموسوم فيلادلفيا. ينشأ هذا الارتباط من جين الاندماج BCR–ABL، الذي يتكون نتيجة انتقال الكروموسومات بين الكروموسومين 9 و22. ينتج جين BCR–ABL نوعًا مستمرًا من كيناز التيروزين، مما يسهم في تقدم مرض السرطان. توجد عدة متغيرات للنسخ، حيث يُعتبر (b2a2) e13a2 و(b3a2) e14a2 الأكثر شيوعًا. ترتبط هذه المتغيرات بأمراض معينة، ونتائج علاجها، والتشخيص العام.

الأهداف:

كان هدفنا تقييم العلاقة بين متغيرات نسخ BCR–ABL ومختلف معايير الدم (مثل عدد كريات الدم البيضاء، مستوى الهيموغلوبين، عدد الصفائح الدموية، وغيرها) لدى المرضى الفلسطينيين الذين تم تشخيصهم بسرطان الدم النقوي المزمن، بالإضافة إلى خصائصهم السريرية والمرضية.

المنهجية:

تم اختيار عينة عرضية تضم خمسين مريضًا مصابًا بسرطان الدم النقوي المزمن (CML) من مستشفى النجاح الوطني في فلسطين لإجراء هذه الدراسة. تم استخراج RNA من عينات الدم المأخوذة من هؤلاء المرضى، وتم التحقق من أنواع نسخ BCR–ABL باستخدام تقنية .RT–PCR بالإضافة إلى ذلك، تم جمع

بيانات من السجلات الطبية للمرضى. تم استخدام التحليل الإحصائي لدراسة العلاقة بين المتغيرات المختلفة للنسخ والعوامل السريرية والديموغرافية المتنوعة.

النتائج:

كانت نسبة الذكور إلى الإناث 1.27:1. من بين المرضى، كان هناك تسعة وعشرون شخصًا يحملون النسخة b3a2، بينما أظهر واحد وعشرون شخصًا نتائج إيجابية للنسخة b2a2. كان إجمالي عدد كريات الدم البيضاء أعلى في مجموعة b3a2 مقارنة بمجموعة b2a2، لكن النسخة b2a2 سجلت عدد صفائح دموية أعلى بشكل ملحوظ من تلك التي تحمل النسخة b3a2.

الخلاصة:

أظهرت دراستنا وجود فرق ملحوظ في القياسات المتعلقة بالدم بين النمطين b3a2 و b2a2 في حالات اللوكيميا النقوية المزمنة (CML)، خاصة فيما يتعلق بعدد كريات الدم البيضاء والصفائح الدموية. قد تشير التغيرات في تعبير النسخ المرتبطة بالعمر إلى وجود اختلافات بيولوجية. تدعم هذه النتائج أهمية تصنيف النسخ في تشخيص المرض وتوقع النتائج في حالات CML، مما يوفر مرجعًا مفيدًا للدراسات المستقبلية ضمن المجتمع الفلسطيني.

الكلمات المفتاحية: ابيضاض الدم النقوي المزمن(**CML**) ، متغيرات نسخ **BCR–ABL**، كروموسوم فيلادلفيا، المؤشرات الدموية، المرضى الفلسطينيون.