Arab American University – Palestine

Faculty of Graduate Studies

Correlation of Genetic Polymorphisms of Glutathione S-Transferase and Acute Leukemia Development in Palestine.

By

Ayat Bassam Hussein Theeb

Supervisor

Dr. Fekri Samarah

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

Molecular Genetics and Genetic Toxicology

March/ 2022

Thesis Approval

Correlation of Genetic Polymorphisms of Glutathione S-Transferase and Acute Leukemia Development in Palestine.

By

Ayat Bassam Hussein Theeb

This thesis was defended successfully on 2/3/2022 and approved by:

Committee members	Signature
1. Dr. Fekri Samarah Supervisor:	
2. Dr. Zaidoun Salah Internal Examiner: …….	 .....
3. Dr. Mahmoud Srour External Examiner: …	 ..

Declaration

I declared that my dissertation “Correlation of Genetic Polymorphisms of Glutathione S-Transferase and Acute Leukemia Development in Palestine.” submitted by me, under the supervision of Dr. Fekri Samarah for the partial fulfillment for the award of Master degree in Molecular Genetics and Genetic Toxicology, is original and was written independently with no other sources than quoted.

Ayat Bassam Theeb

Signature: 

Date: 25/7/2022

Dedication

This thesis is dedicated to my ***parents***, my wonderful husband ***Mohammad Turkman,*** and my two children, ***"Eyas"*** and ***"Yahia"***, who have been a constant source of inspiration for me. This endeavour would not have been feasible without their love and support.

Acknowledgment

I wish to express my sincere gratitude to Dr. Fekri Samarah for giving me the opportunity and teaching me to do this distinguished work, and for his enthusiastic supervision and support during my work in the laboratory, and for all the good he did for me. I am also indebted to Dr. Riad Amer from An-Najah National University hospital and Dr. Zaidoun Salah from Augusta Victoria Hospital for their collaboration and patience.

I am grateful to my professors and instructors, Dr. Kamal Dumaidi and Dr. Mohannad Khader from the faculty of Allied Medical Sciences, Arab American University-Jenin for their kind concerns and continuous support.

I also thank the colleagues in hospitals, Sajida Ahmad, Mohammad Qalalwa, Rand Zaidan, Tammam Al-Sadi, Kawther Nassar, and Ahlam Mojahid for their concerns and for being the surrogate family during the data collection.

I am also indebted to Miss Fatima Irshaid for her collaboration in the statistical analysis of the study results.

My thanks are also due to my parents, husband, and family for their love enthusiasm and financial support, and the continuous care of my little children.

I acknowledge all those who helped us for the successful completion of our study.

Abstract

Background:

Glutathione S-Transferase (GST), is a primary superfamily of phase II drug-metabolizing enzymes that catalyze the conjugation of large number of xenobiotic compounds, which include environmental carcinogens. Additionally, these enzymes play an essential role in the metabolism of drugs including chemotherapeutic drugs and their metabolites. The presence of polymorphism within these transferases will reduce the body's ability to detoxify carcinogenic agents, thus increasing susceptibility to leukemia.

Objectives:

The aim of this study was to determine the association between genetic polymorphisms of Glutathione S-Transferase and acute myeloid leukemia (AML) development among Palestinian patients.

Methodology:

This study was carried out on thirty-six AML patients from different medical facilities in the West Bank [An- Najah National University Hospital (Nablus), Istishari Arab Hospital (Ramallah), and Augusta Victoria Hospital (Jerusalem)], and 72 healthy volunteers. Blood samples were collected in EDTA tubes for all patients and healthy volunteers. DNA was extracted from all EDTA blood samples. The two polymorphisms GSTM1 (NC_000001.10: g. [0])**,** and GSTT1 (NC_000022.10: g. [0]) were genotyped by using the conventional allele-specific polymerase chain reaction (PCR), while the third polymorphism GSTP1 (NM_000852.4: c.313A>G) was genotyped by restriction fragment length polymorphism (RFLP). Sanger sequencing was conducted to confirm the PCR-RFLP results.

Results:

The null genotypes of GSTM1 showed no association with AML [OR 1.50 (0.62–3.59); P = 0.362]. The presence of GSTP1 (NM_000852.4: c.313A>G) polymorphism showed no association with AML [OR= 0.59 (CI: 0.26–1.36); P = 0.218]. In contrast, the null genotypes of GSTT1 showed a significant association with AML [OR = 2.40 (CI: 1.03–5.59); P = 0.04].

Further, a haplotype analysis between AML cases and controls showed a positive association (P < 0.05). It was found that the subjects carrying (GSTT1 present/GSTP1 heterozygous Ile/Val) genotype [OR= 0.35 (CI: 0.13–0.96); P = 0.037], or carrying (GSTT1 present/GSTM1 present/GSTP1 heterozygous Ile/Val) genotype [OR= 2.67 (CI: 0.08–0.84); P = 0.018] had a protective effect from developing AML.

Conclusion:

In conclusion, there was a statistically significant association between the deletion of the GSTT1 gene with increased risk of AML. On the other hand, the haplotype analysis showed that the combination of (GSTT1present/GSTP1 heterozygous Ile/Val) genotypes played a protective role in developing AML. This protection increases in the presence of (GSTT1 present/GSTM1 present/ GSTP1 heterozygous Ile/Val).

Table of Contents

Contents **Page**

List of Tables

List of Figures

LIST OF ABBREVIATIONS

A	Adenine
Ala	Alanine
ALL	Acute Lymphocytic Leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
BC	Breast cancer
BCL-2	B-cell lymphoma 2
BM	Bone marrow
Bp	base pair
C	Cytosine
CBC	Complete blood count
CI	Confidence interval
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
CNS	Central nervous system
CRC	Colorectal cancer
DIC	Disseminated intravascular coagulation
DNA	deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELN	European Leukemia Net
FAB	French-American-British

FC	Flow cytometry
FISH	Fluorescence in situ hybridization
G	Guanine
GSTM 1	Glutathione S-Transferase Mu 1
GSTP1	Glutathione S-Transferase Pi 1
GSTs	Glutathione S-Transferase
GSTT1	Glutathione S-Transferase Theta 1
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency viruses
HSCs	Hematopoietic stem cells
HSCT	Hematopoietic Stem Cell Transplantation
HTLV-1	Human T cell Leukemia Virus type 1
IHC	Immunohistochemistry
Ile	Isoleucine
Kb	Kilo base
MAPEG	membrane-associated proteins in eicosanoid and glutathione
MDS	Myelodysplastic syndromes
Min	Minute
Ml	Millilitre

MOH	Ministry of Health
MPO	Myeloperoxidase
MRD	Minimal residual disease
NGS	Next-generation sequencing
Nmol	Nano mole
NNUH	An-Najah National University Hospital
OR	Odds ratio
OS	Overall survival
PCR	Polymerase chain reaction
PHRC	Palestinian Health Research Council
Pmol	Picomolehttps://myhealth.alberta.ca/Health/Pages/conditions.aspx?hwid=stp1694 &lang=en-ca
P-value	Probability value
RBC	Red blood cell
RFLP	Restriction fragment length polymorphism
RFS	Recurrence-Free Survival
Rpm	Rotations per minute
SD	standard deviation
Sec	Second
SNPs	Single nucleotide polymorphisms
SSC	Side scatter
T	Thymine
TBE	Tris borate - EDTA

TRM	Transplant-related mortality
Val	Valine
WBC	White blood cell
WHO	World Health Organizationhttps://dndi.org/viewpoints/2021/who-2030-ntds-roadmap-how-science-and-partnerships-can-support-fight-end-neglected-diseases/
μL	Microliter

Chapter 1

Introduction and Literature Review

1.1 Introduction

Leukemia, one of the hematological malignancies, occurring as a consequence of the neoplastic proliferation of myeloid or lymphoid cells. The combing form ‘leuk/o’ literally meaning "White”, is used to indicate neoplastic proliferation white blood cells. In leukemia, the leukocytosis is chronic and progressively elevated without obvious cause (Bain, 2010).

It was found that the genetic and environmental factors play role in the progression of leukemia such as chromosomal changes, chemical agents like benzene, chemotherapeutic agents, radiation, smoking, sex (male), and age (elderly). These risk factors can lead to malignant transformation that can occur in any precursors during the hematopoiesis process: such as a lymphoid precursor, a myeloid precursor, or a pluripotent hemopoietic stem cell. In myeloid leukemia, it can arise in a lineage-restricted cell, in a multipotent stem cell capable of differentiating into cells of erythroid, granulocytic, monocytic, and megakaryocytic lineages (Bain, 2010)(Short et al., 2018).

The human body has developed a series of mechanisms to prevent the DNA damage of the hematopoietic stem cells (HSCs) (Nasr et al., 2015). Glutathione S-transferases (GSTs) enzymes are one of the detoxifying enzymes, which play a significant role in the conservation of cell integrity, the maintenance of oxidative stress, and the protection against DNA damage. These enzymes are involved in catalyzing the conjugation of glutathione to a large set of electrophilic substrates. Though enzymes of the glutathione synthesis and clearance pathways have been well identified in the past, there is a lack of

comprehensive knowledge of their separated and cooperative regulatory mechanisms in carcinogenesis (Chatterjee & Gupta, 2018).

1.1.1 Study Problem

The association of genetic polymorphisms in xenobiotic-metabolizing genes with acute myeloid leukemia (AML) has not been investigated in Palestine. Therefore, the GSTM1, GSTT1and GSTP1loci were analyzed for polymorphism among the Palestinian population regarding their association with acute myeloid leukemia (AML).

1.1.2 Justification

Acute leukemia is the fourth common malignancy in Palestine; with idiopathic causes. However, there are factors that increase the individual chances to develop leukemia such as chemicals, physical agents radioactive, and others.

Nowadays, medical practices focus mostly on the “treatment after the disease onset” while molecular medicine promises “the prevention before the disease onset”. The study of GST gene polymorphisms in acute myeloid leukemia (AML) patients in Palestine will help to understand the correlation between GST polymorphisms and the development of AML, which in turn will help in identifying the alleles that may be associated with the risk of developing leukemia. This might help in understanding the mechanism of disease and identifying the susceptible populations, and subsequently help in the development of an effective preventative interventions.

1.1.3 Specific Objective

The aim of this study was to determine the correlation between genetic polymorphisms of Glutathione S-Transferase and acute leukemia development among the Palestinian patients.

1.1.4 Study hypothesis

We hypothesized that the identification of genetic polymorphisms in xenobiotic-metabolizing genes (GSTP1, GSTM1, and GSTT1) is risk factors for AML would allow individual characterization which would help to design the preventive strategies that are of greatest benefit.

1.2 Literature Review

1.2.1 Leukemia

Leukemia is driven by the mutation of pluripotent or primitive stem cells inside the bone marrow. These mutations lead to abnormal production of leukemic cells and reduce the manufacture of normal red blood cells, leukocytes, platelets. The presence of these mutant cells and the impaired hematopoiesis mechanism could push the leukemic cells to spill into the peripheral blood and overspread in specific tissue such as the spleen, kidneys, lymph nodes, and possibly the central nervous system. Leukemic cells have exceptional morphologic, cytogenetic, and immunophenotypic features that help in the category of the particular type of leukemia (Bain, 2010).

Incidence:

Leukemia is ranked as the 15th most common malignancy in the world with 437,033 new cases of leukemia (2.4% of all new cancers) diagnosed annually (Tebbi, 2021). Chronic lymphoid leukemia (CLL) represents the most frequent type of leukemia with 25% of cases, chronic myeloid leukemia (CML) represents 20% of cases, and acute myeloid leukemia (AML) represents 20% of cases that have been estimated in Western countries (Bray et al., 2018). According to the Palestinian health information center in 2018, leukemia is considered the fourth common malignancy in Palestine with a percentage of 6.7% and with an average incidence of 7.9 cases per 100000 inhabitants (MOH, 2019).

Mortality:

The global 5-year survival rate is 20% of all leukemia lumped together. In developed countries, it has been estimated that the 5 or more years survival rate is 31%, compared to 15% in developing countries. This can be attributed to the deficiency of access to

high-tech cures in the developing world. In 2018, the world health organization (WHO) reported 309,006 (3.2%) deaths globally for all types of leukemia worldwide (Bray et al., 2018).

1.2.2Classification of leukemia:

The World Health Organization (WHO) and the European Association of Hematopathology published a new scheme classification for hematopoietic and lymphoid neoplasms. Their classification of hematopoietic and lymphoid neoplasms relies not only on morphologic findings but it includes also genetic, epigenetic, immunophenotypic and clinical presentation to define the disease. For the lymphoid neoplasms, the Revised European-American Lymphoma (REAL) Classification has been recently adopted by the WHO.

The WHO classification of myeloid neoplasms includes many of the criteria of the French-American-British (FAB), Cooperative Group classifications of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) as well as guidelines of the Polycythemia Vera Study Group (PVSG) for the chronic myeloproliferative neoplasms (CMPNs) (Nizar et al., 2019).

1.2.3Acute myeloid leukemia:

AML is a kind of leukemia that affects hemopoietic stem cells and is marked by clonal growth of improperly myeloid cells (Short et al., 2018). One of the implications of this proliferation of improperly myeloid cells is the accumulation of immature progenitors (myeloblasts) in the bone marrow and peripheral circulation. Red blood cells, platelets, and white blood cells are examples of common myeloid progenitors (Khwaja et al., 2016).

Genomic aberrations have been recognized to have a role in the pathophysiology and cytogenetic abnormalities of acute myeloid leukemia (AML) for more than 30 years. Cytogenetic aberrations have become well-established diagnostic and prognostic indicators. In the past 15 years, progress has been made in microarray and next-generation sequencing (NGS) –based "-omics" technologies, increasing the knowledge of the molecular heterogeneity of AML (Bullinger et al., 2017). AML is the most common form of acute leukemia in adults, being 3–4-times more common in adults than ALL (Khwaja et al., 2016).

1.2.4 Classification of AML:

The French-American-British (FAB) classification system, the most generally used classification system, and the World Health Organization (WHO) classification system, the most recently established system, are the two most extensively used classification systems for AML.

1.2.4.1The French-American-British (FAB) classification of AML:

The French-American-British (FAB) categorization system for acute leukemia was created in the seventies. It was based on the morphologic evaluation of lymphoblasts and myeloblasts using cytochemical stains (Arber et al., 2017).

Table 1.1: FAB classification of AML**.**

M0	**Undifferentiated AML**
M1	**AML with minimal maturation**
M2	**AML with maturation**
M3	**Acute promyelocytic leukemia**
M4	**Acute myelomonocytic leukemia**
M4eso	**Acute myelomonocytic leukemia with eosinophilia**

M5	**Acute monocytic leukemia**
M6	**Pure erythroid leukemia**
M7	**Acute megakaryoblastic leukemia**

(American Cancer Society (ACS), 2018)

Table (1.1) shows that the presence of immature leukocytes distinguishes AML subtypes M0 through M5. While M6 and M7 AML subtypes are restricted to immature precursors of red blood cells and platelets, respectively (American Cancer Society (ACS), 2018).

1.2.4.2The World Health Organization (WHO) classification of AML:

Hematologists and pathologists are now using immunophenotyping, chromosomal aberrations, and genetic abnormalities detected in patients to classify leukemia more precisely. These abnormalities could be linked to oncogenes, tumor suppressor genes, and other genes involved in cell proliferation, maturation, apoptosis, and other essential processes (Bories, 1974).

In the fourth revised edition of the WHO classification, published in 2017, AML falls into six categories: AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes (MRC), therapy-related myeloid neoplasms (t-MN), AML, not otherwise specified (NOS), myeloid sarcoma; and myeloid proliferations related to Down syndrome (DS) (Table 1.2)(Hwang, 2020).

(Table 1.2) World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia**.**

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22q22.1); RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11

APL with PML-RARA
AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
AML with t(6;9)(p23;q34.1); DEK-NUP214
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutation of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, not otherwise specified (NOS)
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic and monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations associated with Down syndrome

Transient abnormal myelopoiesis (TAM) associated with Down syndrome

(Hwang, 2020).

1.2.5 Clinical manifestation of AML:

Manifestations of AML are driven mainly by pancytopenia (leukopenia, anemia, thrombocytopenia) and blast cell proliferation (common symptoms of leukemia are summarized in figure 1.2). Common signs of leukemia such as splenomegaly and hepatomegaly are directly connected to the invasion of normal organs by leukemic blasts with organomegaly observed in up to half of AML patients. Fever is prevalent and is the presenting characteristic in 15-20% of AML patients. Weight loss was observed only occasionally. Skin infiltration also occurs with the involvement of extramedullary sites, including gum. Although 20% of AML patients had clinical manifestations of central nervous system (CNS) involvement as demonstrated by leukemic blast cells in the cerebrospinal fluid AML symptoms included continuous headaches, mental and visual changes, sleepiness, palsies, symptoms of CNS bleeding, and spinal column compression. However, others may present with asymptomatic CNS involvement as well (Tamamyan et al., 2017).

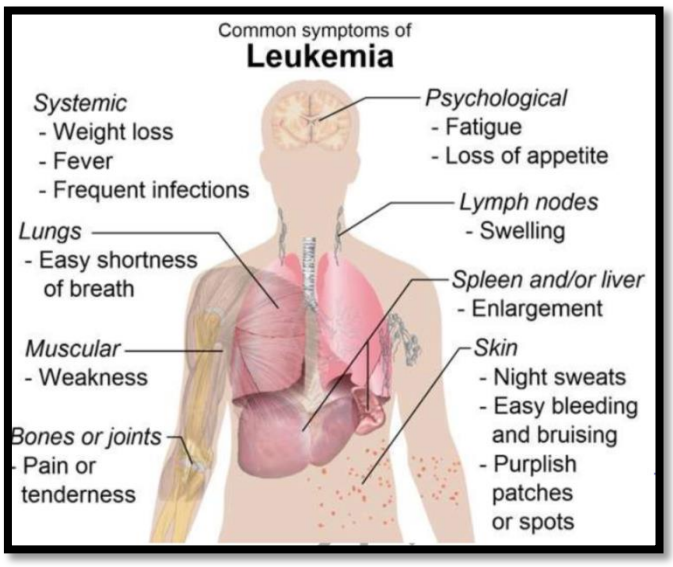


Figure (1.1): Symptoms of leukemia (Bain, 2010).

1.2.6 Laboratory evaluation

1.2.6.1 Blood findings

In AML patients, the complete blood count test (CBC) reveals a quantitative shift in three cell lineages: red blood cells (RBC), white blood cells (WBC), and platelets. Anemia and thrombocytopenia are often observed, while WBC count is variable, high, normal, or low (Khwaja et al., 2016).

Blood smear: Several datasets were used for leukemia diagnosis from microscopic blood images (Nizar et al., 2019). In blood smear, the presence of more than 20% of myeloblasts can be diagnosed as AML, but a lower level does not exclude this diagnosis (Khwaja et al., 2016). Figure (1.2) shows the different blood images for French-American-British (FAB) classification of acute myeloblastic leukemia.

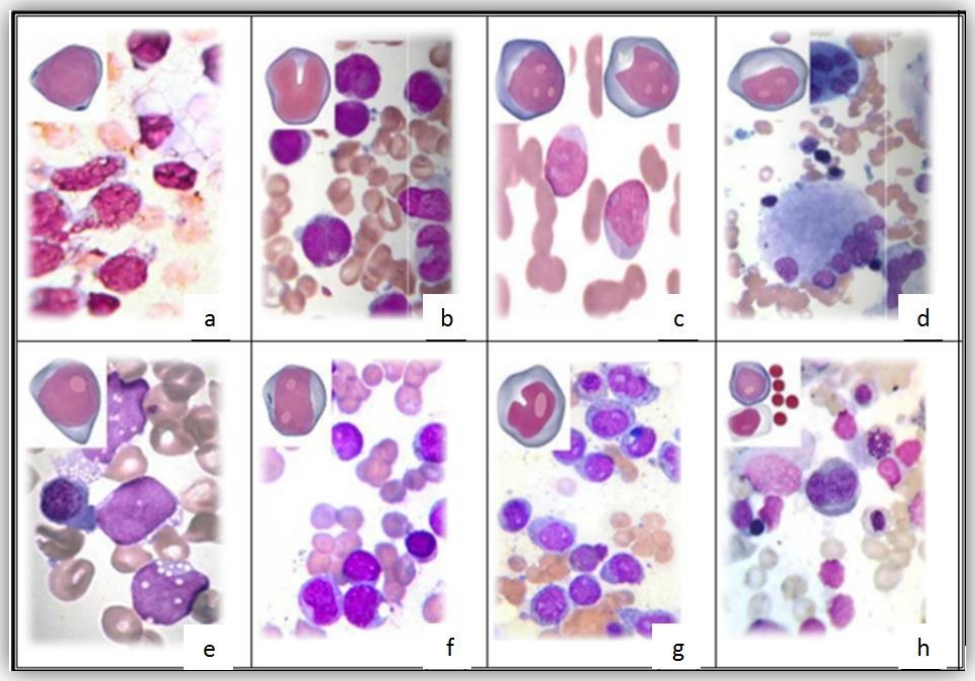


Figure (1.2): The different blood images for French-American-British (FAB) classification of acute myeloblastic leukemia (Ladines-castro, 2016).

1.2.6.2 Bone marrow aspiration

It is important and obligate to do the bone marrow smear to facilities molecular and cytogenetic analysis. At low magnification, the invaded bone marrow containing myeloblasts may be seen in 10 to 20 fold increase. Auer rods, red staining needle-like structures seen in the cytoplasm of these myeloblasts, are also present in myelodysplastic syndromes MDS. There are also additional neoplastic cells that may be differentiated based on their morphologies such as abnormal promyelocytes, neoplastic monoblasts, promonocytes, and ultimately atypical megakaryoblasts.

The expression of myeloperoxidase (MPO) is used to distinguish between normal myeloblast and leukemic ones. The MPO enzyme is usually expressed in leukemic myeloblasts. In addition, the detection of α-naphthylacetate esterase, which is expressed in monocytes, is used to differentiate the monocytic lineage from the lymphoid and other myeloid lineage cell types (Khwaja et al., 2016).

1.2.6.3 Immunophenotyping by Flow Cytometric Analysis

It plays an important role in the diagnosis and classification of AML because it allows blast detection, lineage assignment, and immunophenotypic characterization, which distinguishes aberrant blast populations from normal progenitors.

CD45 against side scatter (SSC) gating is a first-line method for blast detection based on the strength of CD45 and SSC expression on hematopoietic lineages.

This method allows for reliable identification of mature lymphocytes, monocytes, maturing granulocytes, myeloid blasts, and lymphoid blasts (Chen & Cherian, 2017). Figure1.3 summarizes the cell markers involved in each stage of AML.

FC's potential weaknesses in gating analysis, cell lysis, and cell preservation might result in an erroneous blast count. FC may also be ineffective in situations when the aspirate yield is limited (marrow fibrosis), necessitating IHC staining.

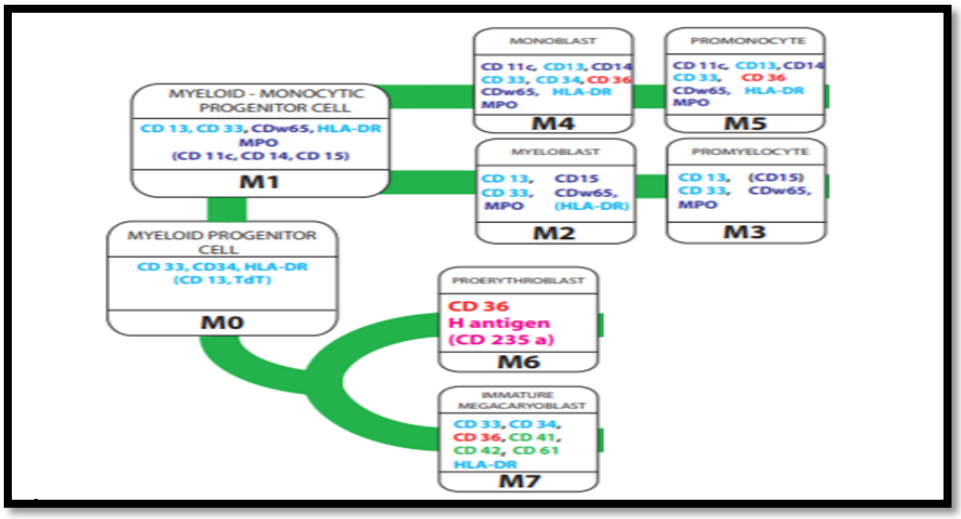


Figure (1.3): Schematic figure of myeloid differentiation including the corresponding acute leukemia and cell markers involved in each stage (Lyon, 2008).

1.2.6.4 Immunohistochemistry (IHC)

IHC investigations, which are often done on bone marrow biopsy specimens, serve as a supplemental or alternative technique of immunophenotyping and evaluating blast cell count, especially when BM aspirates are unavailable or insufficient.

To detect myeloblasts and count blasts, CD34 is the most often utilized IHC stain. Some AML subtypes have blasts that are CD34-negative, and in these circumstances, IHC stains for other markers such as CD117 can be utilized to count blasts. An IHC panel of CD117, CD33, CD71, MPO, CD61, and CD34 can be utilized to detect the relative percentage of cells in the distinct lineages in instances with severe fibrosis. In addition to counting blasts and identifying cell types, IHC tests for specific proteins can be used as an alternative for identifying underlying mutations. For example, IHC studies have shown that leukemic cells with NPM1 mutations exhibit abnormal cytoplasmic NPM1 expression (Narayanan & Weinberg, 2020).

1.2.6.5 Genetic finding

Chromosomal abnormalities

Traditional karyotyping and Fluorescence *in-situ* hybridization (FISH) investigations are said to be essential for determining the subtype of AML, predicting prognosis, and determining therapy response. Translocation of 11q23.3 with 9p21.3 is classified as AML with t(9;11) (p21.3;q23.3), whereas translocation of 11q23.3 with 16p13.3 is classified as AML-MRC.

Despite the fact that karyotyping is the gold standard in cytogenetics, it may miss cryptic deletions and subtle translocations such inv(16) (p13.1q22), PML-RARA, and t (9;22). AML with t(8;21)(q22;q22.1) and AML with inv(16)(p13.1q22) are linked to a good response to high-dose cytarabine therapy(Narayanan & Weinberg, 2020).

Recurrent mutations

Detecting genetic anomalies is important for AML diagnosis, prognosis, and categorization.

AML is typically associated with mutations in genes that control multiple functions, including promoting cell proliferation and survival (*FLT3* and *KIT*), impairing differentiation and apoptosis of hematopoietic cells (*CEBPA*, *RUNX1*, and *NPM1*), and modulating epigenetic mechanisms (*DNMT3A*, *TET2*, *IDH1*, and *IDH2*) (Narayanan & Weinberg, 2020).

Chromatin-modifying genes (ASXL1, EZH2) or KMT2A fusions, splicing process (SRSF2, SF3B1, U2AF1, ZRSR2), Cohesin-complex genes STAG2, RAD21, impaired the function of Tumor-suppressor genes (TP53, WT1, PHF6), as well as a mutation in genes recently implicated in leukemogenesis, including EZH2, U2AF1, SMC1A, and SMC3 (Bullinger et al., 2017).

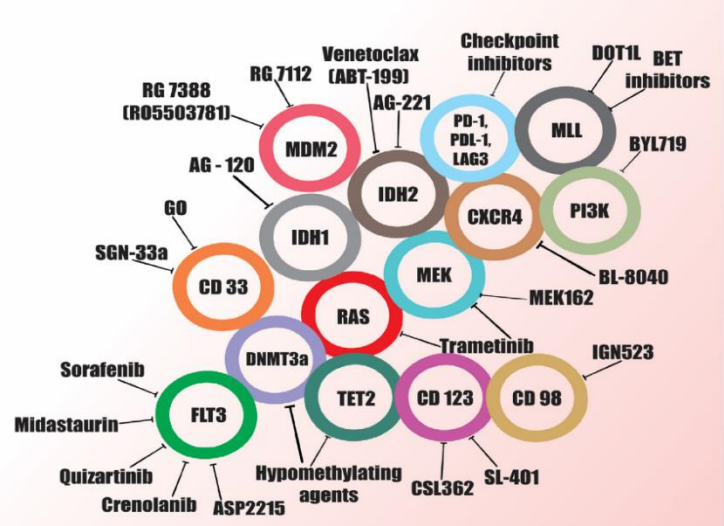
The majority of AML patients contain several mutations. Mutations can also assist in determining if AML is caused by a de novo or secondary pathway. It is also possible to determine the pathogenicity and severity of the genetic defect. For example, translocation or mutation of RUNX1 can cause AML, with mutant RUNX1 having a poorer prognosis than translocated RUNX1, especially in the case of normal karyotype AML. This can aid in the identification of prospective therapeutic targets as well as therapy response prediction. In AML with t(8;21)(q22;q22.1) and AML with inv(16), the KIT D816V mutation causes non-responsiveness to imatinib, a chemotherapeutic drug (p13.1q22) (Narayanan & Weinberg, 2020).

1.2.7 Treatment

The phrase "young age" refers to those who are between the ages of 18 and 60. Patients with AML should begin treatment as soon as the diagnosis is confirmed. However, several recent studies have found that the interval between diagnosis and treatment has no negative consequences. Furthermore, the discovery of unidentified remaining leukemic cells, termed as minimum residual disease (MRD), leads to customized AML patient management. Treatment for AML is determined by the patient's overall condition. While induction treatment induces remission in the majority of patients, the recurrence rate is significant, and it is dependent on the patient's age, the amount of minimum residual disease MRD, and the leukemia's cytogenic and molecular profile. In patients with a high risk of recurrence, hematopoietic stem cell transplantation is regarded the most effective post-remission therapy for preventing relapses. Furthermore, individuals with AML needed therapy for symptoms and consequences of the disease, as well as antifungal and antibiotics to manage infections and platelet transfusion to reduce bleeding risks (Khwaja et al., 2016).

New therapies

Due to the heterogeneity of the AML, new targeted therapies were designed to improve the treatment of adult patients who are suffering from AML. Figure (1.4) summarizes some of these targeted therapies (Khwaja et al., 2016)(Tamamyan et al., 2017).



Figure(1.4): The new targeted therapies used in the treatment of adult AML (Tamamyan et al., 2017)

1.2.8 Glutathione S transferase

Glutathione S-transferases (GSTs) are phase II detoxifying enzymes that are involved in oxidative stress, the cell integrity maintenance, and catalyzing the conjugation of glutathione to a wide variety of electrophilic substrates that protect DNA from damage (Chatterjee & Gupta, 2018)

1.2.8.1 Classification

GSTs are grouped into three different classes: cytosolic, mitochondrial and microsomal membrane-associated proteins in eicosanoid, and glutathione metabolism (MAPEG) (Sha et al., 2018).

In humans, the cytosolic Glutathione S-transferase GST isozymes contain at least 16 genes subdivided into seven major classes : Alpha (α), Sigma (σ), Mu (μ), Omega (ω), Pi (π), Theta (θ), and Zeta (ζ), which are encoded by the GSTA1, GSTA2, GSTA3,

GSTA4, GSTA5, GSTS1, GSTM1, GSTM2, GSTM3, GSTM4, GSTM5, GSTO1, GSTP1, GSTT1, GSTT2, and GSTZ1genes, respectively (Safarinejad et al., 2013)(Chatterjee & Gupta, 2018).

1.2.8.2 Function

Glutathione S-transferases catalyze the conjugation of reactive electrophiles to the –SH group of glutathione leading to neutralizing the electrophilic sites and making the products more soluble (Simic et al., 2009).

As non-enzymatic proteins, GSTs can also modulate signaling pathways that control DNA damage processing, cell proliferation, differentiation, and death (Weich et al., 2015).

These enzymes also play an important role in the metabolism of drugs including chemotherapeutic drugs (Makhtar et al., 2017).

1.2.8.3 GST polymorphism

Some functional polymorphisms have been identified in the GSTM1, GSTT1, and GSTP1 genes coding for GSTs enzymes (Safarinejad et al., 2013):

1.2.8.3.1 GSTM1 (NC_000001.10:g.[0]) polymorphism

The 100-kb gene cluster that codes for the GST (GSTM) subfamily is found on chromosome 1p13.3 and is arranged in the following order: 5-GSTM4-GSTM2-GSTM1-GSTM5-GSTM3-3 as shown in figure (1.5). GSTM1 has eight exons ranging in size from 36 to 112 bp, as well as introns ranging in size from 87 to 2641 bp. GSTM1 is placed between two identical 4.2 kb areas with a high degree of similarity. There is a 4.2 kb repetition in the case of homologous recombination. This deletes the 16 kb area containing the full GSTM1 sequence on both sides of the GSTM1 gene, resulting in a

null allele. If both alleles are deleted, the GSTM1-/- is produced, which may be tested via PCR (null genotype) (Makhtar et al., 2017).

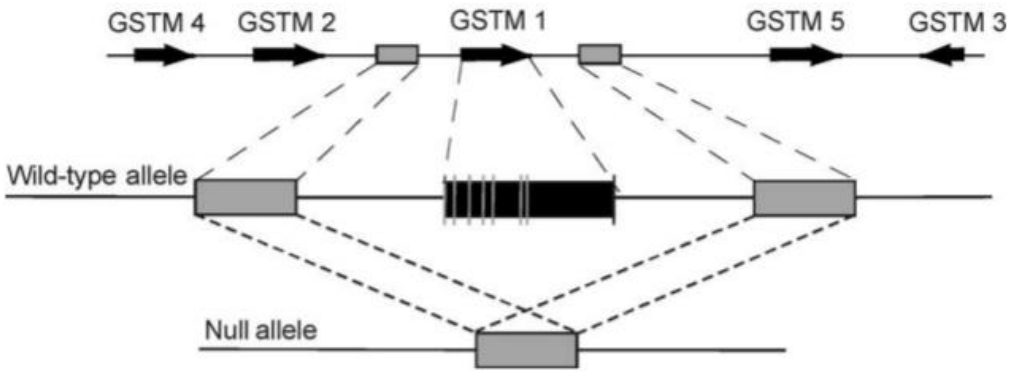


Figure (1.5): Structural localization of gene cluster encoding the GSTμ (GSTM) subfamily (chromosome 1p13.3) (Parl, 2005).

1.2.8.3.2 GSTT1(NC_000022.10:g.[0]) polymorphism

As shown in figure (1.6), theta-class GST gene cluster (GST) on chromosome 22q11.2 is made up of two genes (GSTT1 and GSTT2) that are separated by roughly 50 kb. The GSTT1 gene has five exons with sizes ranging from 88 to 195 bp, and introns with sizes ranging from 205 to 2363 bp. The GSTT1 gene is located in a region between two homology-rich 18 kb regions called HA3 and HA5, which have more than 90% homology. The 403 bp sequences of HA3 and HA5 are identical in all aspects. When the left and right 403 bp repeats of GSTT1 are homologously recombined, it can result in a 54-kb deletion of the whole GSTT1 sequence, resulting in a GSTT1 null allele. GSTT1 is the only gene affected by this null deletion, while GSTT2 is not affected (Makhtar et al., 2017).

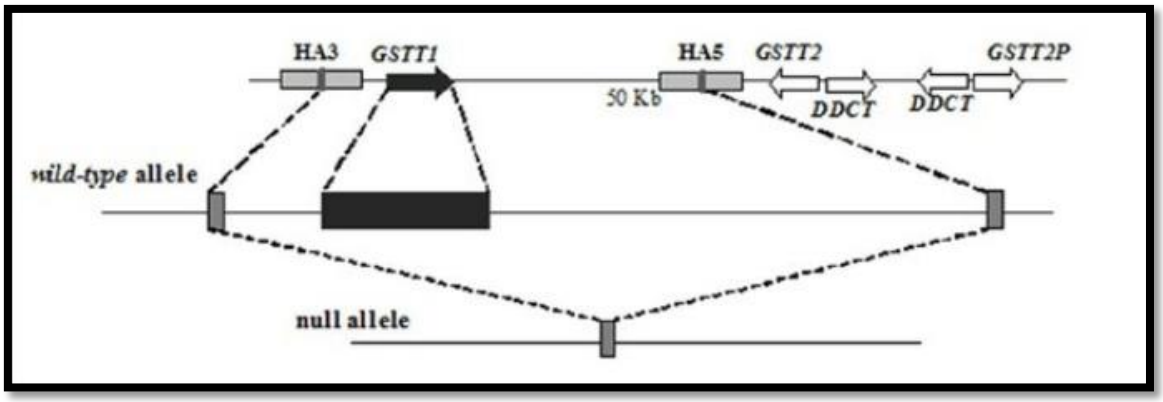


Figure (1.6): Structural localization of gene cluster encoding the GST subfamily theta (chromosome 22q11.2) (Lima & Teixeira, n.d.).

1.2.8.3.3 GSTP1 (NM_000852.4:c.313A>G) polymorphism

In figure (1.7), the GSTP1 which found on chromosome 11q13, is 2.48 kb in size, has seven exons, is one of the pi (π) class gene families. There are two primary polymorphisms in this gene that have been found in all prior research. The first is a polymorphism in nucleotide 313 A>G. This polymorphism causes isoleucine (lle) to be replaced with valine (Val) at codon 105 in exon 5 (lle105Val). The second is the C>T conversion at nucleotide 341, which leads alanine to be replaced by valine at position 114 in exon 6 (Ala114Val).

The enzymatic activity of these polymorphisms varies, and they play a key role in pharmacokinetic variability, treatment results, and resistance or drug-related toxicity (Makhtar et al., 2017).

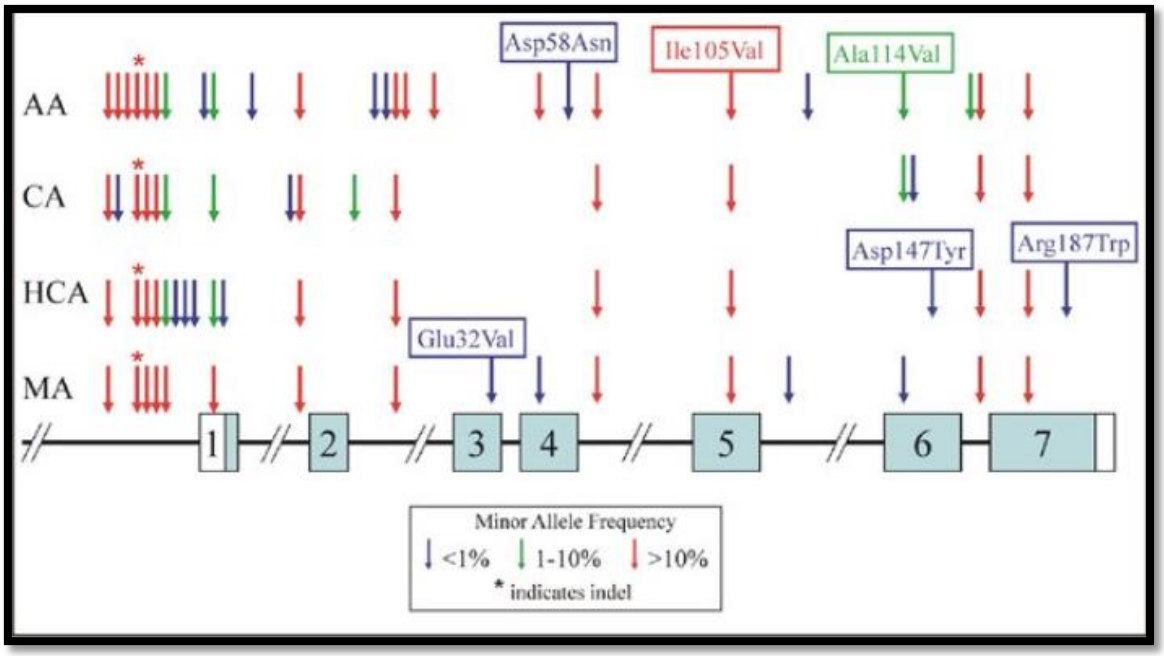


Figure (1.7): Human GSTP1 genetic polymorphisms (Salavaggione et al., 2008).

1.2.8.4 Previous studies

Several previous studies have shown that these genetic polymorphisms are associated with many malignancies, including lung, bladder, kidney and prostate cancer along with leukemia.

1.2.8.4.1 The correlation of GST polymorphisms and different types of cancer

Data from a population-based case-control study in Iran revealed an association between the presence of genotypes (GSTT1 null and GSTM1 null) and the GSTP1 Val allele and the risk of prostate cancer (Shafiei & Safarinejad, 2011).

While the GSTP1 Ala114Val variant was considered as a protective factor against the development of small cell lung cancer (Vural, 2012). However, results are still inconsistent in colorectal cancer (CRC). According to a recent meta-analysis, the GSTM1 null genotype, the GSTT1 null genotype, and their combined impact on CRC risk were investigated. GSTM1 and GSTT1 null genotypes have been linked to an elevated risk of CRC in Asians and Caucasians, but not in Africans and Indians. In

addition, the GSTM1 null genotype was associated with colon cancer risk but not rectal cancer, whereas the GSTT1 null genotype was associated with rectal cancer but not colon cancer (Song & Yang, 2020).

A meta-analysis study, done by Miao et al. (2020) to evaluate the individual and combined effects of GSTM1, GSTT1, and GSTP1 polymorphisms on breast cancer (BC) risk, showed a negative association between these polymorphisms and the development of BC (Miao et al., 2020).

1.2.8.4.2 The correlation of GST polymorphisms with acute leukemia

In diverse populations, the relationship between GST polymorphisms and acute lymphoid leukemia is indeed in conclusive. An Indian study found that the co-existence of GSTT1 and GSTM1 null genotypes are associated with childhood ALL (Moulik et al., 2014). Conversely, a study from Turkey found that the status of heritable GST polymorphisms might not influence the risk of developing childhood ALL (Guven et al., 2015).

Moreover, data from a population-based case-control study in Argentina demonstrated that the GST polymorphisms may reduce or eliminate the enzyme activity, which lead to increase the risk of relapse and shortened the Recurrence-Free Survival (RFS) in pediatric patients with ALL. These polymorphisms are linked to not only the development of ALL, but also to the prognosis and therapy of ALL (Leonardi et al., 2017).

The evidence on the association between GST polymorphisms and AML risk is likewise contradictory. A study from Saudi Arabia revealed a negative association between genetic markers and susceptibility to AML (Farasani, 2019). While, a study from Iran showed that GSTT1 null genotype and its combination with GSTM1 null, and GSTP1

Val hetero/homozygote with increased risk of acute promyelocytic leukemia (APL) (Mandegary et al., 2011).

In an Indian population, Chauhan *et al* reported that the GSTT1 null genotype seems to play a protective role in adult AML (Chauhan et al., 2011), whereas in a Romanian population, Claudia *et al*. found that the presence of GSTP1 (NM_000852.4:c.313A>G) polymorphism may increase the risk of developing AML (Claudia et al., 2016).

The GSTP1 (NM_000852.4:c.313A>G) polymorphism exhibited a quicker neutrophil and platelet recovery in a cohort analysis of German- Austrian Acute Myeloid Leukemia patients. It was thought to be a good prognostic factor because the detoxification of chemotherapeutic drugs was reduced, intensifying the cytotoxic impact of these drugs (Groner & Spa, 2008).

Nasr *et al*. from Cairo University also found that the presence of GSTP1 (NM_000852.4:c.313A>G) polymorphism may be considered as a prognostic factor. Moreover, the GSTM1null and GSTT1 null genotypes were found to be statistically significant among Egyptian patients (Nasr et al., 2015).

It was hypothesized that the increasing activity of the GST enzyme will accelerate the metabolism of the chemotherapy, which reduces the cytotoxic effects of chemotherapeutic agents on cancer cells and consequently leads to poor therapeutic response and poor survival (Shafiei & Safarinejad, 2011). Therefore, recent studies conducted research on the development of GSTP inhibitors that in turn help reverse multidrug resistance in tumors and increase the therapeutic index of anticancer drugs (Sha et al., 2018).

Most of the aforementioned investigations have provided a correlation between the diverse GST polymorphisms and the development, prognosis, and responsiveness to

therapy of acute leukemia among the different ethnicities with varying degrees. This inconclusive association could be attributed to the different genetic susceptibility between populations, sample size, gene-gene interactions, gene-environment interactions and the different treatment protocols (Guven et al., 2015).

The main objective of our research was to see if there was an association between the frequency of (GSTP1, GSTM1, and GSTT1) gene polymorphisms with the development of AML in a cross-sectional study to better understand the current state of AML illness in our region.

Chapter 2
Materials and Methods

2.1. Study design

A hospital-based case-control study

2.2. Study area

This multicenter case- control study conducted among Palestinian AML patients in the period between February 2021 and August 2021. AML patients were recruited from three major hospitals; An- Najah National University Hospital (Nablus), Istishari Arab Hospital (Ramallah), and Augusta Victoria Hospital (Jerusalem).

2.3. Sampling method and sampling size

Using the open Epi software and the equation below, the sample size for the unmatched case-control study was calculated using a ratio of cases-to-controls 1: 2. P-value of < 0.05, a 95% two- sided confidence interval, 80% power of study (β), the hypothetical proportion of controls with GSTP1 (NM_000852.4:c.313A>G) polymorphism 16%, and an odds ratio (OR) of 3.6 as extracted from literature. The sample size calculation was based on Kelsey, Fleiss and Fleiss with a continuity correction equation (Sullivan & Soe, 2007).

A total of 36 patients with Acute Myeloid Leukemia (AML) were included in the study along with 72 healthy individuals as controls.

The study was approved by the Palestinian MOH and the Palestinian Health Research Council-Gaza in its meeting on 01/02/2021 under the number: PHRC/HC/841/21, according to the principles of the Helsinki Declaration. Written informed consent was obtained from patients or their guardians in case of minors. A special questionnaire was used to collect demographic and medical data. The data were collected from the

participants by the investigator during an interview and by phone call from those living in Gaza.

2.4. Inclusion and exclusion criteria

The World Health Organization (WHO) criteria and the French–American–British (FAB) classification based on complete blood count, bone marrow examination, and flow cytometry were used in the diagnosis and typing of leukemia among all cases. The selection criteria for controls were healthy volunteers who had no medical history of any type of malignancy or other diseases and were not relatives to any of the patients. The medical history and clinical biochemistry tests of the AML patients were retrieved from patients' files at the oncology department of the different hospitals during the study period.

2.5. DNA extraction and blood collection

About three milliliters of EDTA blood were collected from each subject, and genomic DNA was extracted using the QIAamp mini kit (QIAGEN, Heldin/Germany) according to manufacturer's guidelines. In a sterile 1.5ml-microtube, 200µL blood buffy coat sample was mixed with 25µL proteinase K. An exact volume of 200µL of AL lysis buffer was added to the microtube. The mixture was incubated at 56°C for 15 minutes. After this, 250µL of absolute ethanol was added to the mixture and vortexed well. The lysate was then incubated at room temperature (15–25°C) for 5 minutes. The lysate was transferred to a QIAamp MinElute column and centrifuged at 8000 rpm for 1 minute. The MinElute column was then washed twice with the washing buffers AW1 and AW2, respectively. Then, 500µL of ethanol (96–100%) was added to MinElute column and centrifuged at 8000 rpm for 1 minute. Finally, using 50µL of Buffer AVE from the kit,

DNA was eluted into a sterile microtube. The extracted DNA was kept at -20°C until use.

DNA Quantification

A NanoDrop analyzer (IMPLEN, Germany) was used to determine the concentrations and quality (OD_{260}/OD_{280}). One µL of Buffer AVE was used as a blank, followed by 1µL of DNA. The ratio OD_{260}/OD_{280} was computed to represent the quantity of DNA to protein, which should be > 1.8 to be considered appropriate for analysis.

2.6. Genotype Analysis

DNA samples of patients and controls were genotyped for GSTP1, GSTM1, and GSTT1 polymorphisms. Genotype analyses of GSTP1 were performed by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), while GSTM1 and GSTT1 were performed by using conventional allele-specific polymerase chain reaction (PCR).

2.6.1 GSTP1 (NM_000852.4:c.313A>G) polymorphism

For the GSTP1 gene, DNA samples were amplified with the following primer pair: forward primer (5' ACC CCA GGG CTC TAT GGG AA 3'); reverse primer (5' TGA GGG CAC AAG AAG CCC CT 3'), using polymerase chain reaction (PCR) (Table 2.1, Appendix 2). PCR was performed in a final volume of 25µL, consisting of 12.5µL Master mix (1X) (Promega Corp., USA), 2µL forward primer (20pmole), 2µL reverse primer (20pmole), 4.5µL nuclease‑ free water and 4µL genomic DNA (68ng/µL). The PCR thermocycling protocol was as follows: An initial denaturation at 95°C for 5 minutes, followed by 35 cycles of amplification at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec. A final extension step of 72°C for 5 min to complete the elongation

processes. Reaction products were analyzed on a 2% agarose gel stained with 1mg/mL ethidium bromide.

Table 2.1: Primer sequences used in PCR

Primer	Sequence	Product size	Method used
GSTP1313AF GSTP1313AR	5' ACCCCAGGG CTC TAT GGG AA 3' 5' TGA GGG CACAAG AAG CCC CT 3'	176bp	RFLP
GSTM1F GSTM1R	5'GAACTCCCTGAAAAGCTAAAGC3' 5'GTTGGGCTCAAATATACGGTGG3'	219bp	conventional PCR
GSTT1F GSTT1R	5'TTCCT TACTGGTCCTCACATCTC3' 5'TCACCGGATCATGGCCAGCA3'	480bp	conventional PCR
BCL-2F BCL-2R	5'GCAATTCCGCATT TAATTCATGG3' 5'GAAACAGGCCACGTAAAGCAAC3'	154bp	conventional PCR

The PCR products were then digested with BsmBI (New England Biolabs, Hertfordshire, United Kingdom) restriction enzyme, according to manufacturer's instructions. The RFLP conditions were performed for 1 hour at 55℃ according to the protocol shown in table 2.2, using a thermo-cycler for incubation:

Table 2.2: RFLP reaction mixture

Component	Volume
NEBuffer 3.1.	1.5µL
Restriction enzyme BsmBI (New England Biolabs, Hertfordshire, United Kingdom).	0.25µL (2 units)
Nuclease‑ free water.	3.2µL

PCR product (amplicon).	10µL

The restriction DNA amplification products were loaded into wells of 4% Metaphore agarose gel (Lorenzo, Italy) stained with 1mg/mL ethidium bromide. A horizontal standard electrophoresis system (BIO-RAD, China) was carried out in 1X TBE buffer, at 100V, for approximately 4 hours.

DNA sequencing analysis

To confirm the RFLP results, two randomly AML PCR products were sent for sequencing. The PCR products were initially cleaned up by adding 5µL of PCR product with 1µL of clean up reagent Eppic FAST (A&A biotechnology) and incubating for 15 minutes at 37°C. The BigDyeTM Direct Cycle Sequencing Kit (Thermo Fisher Scientific, USA) was used to perform Sanger sequencing on the Biosystem 3500 Genetic Analyzer. The Finch TV chromatogram viewer was used to look at nucleotide variations.

2.6.2 GSTM1(NC_000001.10:g.[0]) polymorphism

The GSTM1 deletion was identified by conventional polymerase chain reaction (PCR) technique, including the housekeeping gene BCL-2 as internal control as described (Nasr et al., 2015).

For the GSTM1 gene, DNA samples were amplified with the following primer pair: Forward primer (5'GAACTCCCTGAAAAGCTAAAGC3'); reverse primer (5'GTTGGGCTCAAATATACGGTGG3'), using polymerase chain reaction (PCR). The primers used for the BCL-2 were as follows; forward primer (5'GCAATTCCGCATT TAATTCATGG3'); reverse primer (5'GAAACAGGCCACGTAAAGCAAC3') (Table 2.1).

PCR was performed in a final volume of 25µL, consisting of 12.5µl Master mix (1X) (Promega Corp. USA), 2µL forward primer (20pmole), 2µL reverse primer (20pmole), 4.5µL nuclease- free water and 4µL genomic DNA (68ng/ µL). The PCR protocol was as follows: An initial denaturation at 95°C for 5 minutes, followed by 35 cycles of amplification: 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min. A final extension step of 72°C for 7 min to complete the elongation processes. Reaction products were analyzed by 2% agarose gel stained with 1mg/mL ethidium bromide, to check for the existence of the GSTM1 allele, which was detected by a 219-bp fragment. In 1X TBE buffer, at 100V, for approximately 35 min, a horizontal standard electrophoresis apparatus (BIO-RAD, China) was used. A null genotype is indicated by the lack of amplifiable GSTM1. BCL-2 produced a 154-bp fragment band.

2.6.3 GSTT1 (NC_000022.10:g.[0]) polymorphism

The GSTT1 deletion was identified by conventional polymerase chain reaction (PCR) technique, including the housekeeping gene BCL-2 as internal control as described (Nasr et al., 2015).

For the GSTT1 gene, DNA samples were amplified with the following primer pair: Forward primer (5'GAACTCCCTGAAAAGCTAAAGC3'); reverse primer (5'GTTGGGCTCAAATATACGGTGG3'), using polymerase chain reaction (PCR). The primers used for BCL-2 were as follows; forward primer (5'GCAATTCCGCATT TAATTCATGG3'); reverse primer (5'GAAACAGGCCACGTAAAGCAAC3')**.**

PCR was performed in a final volume of 25µL, consisting of 12.5 µL Master mix (1X) (Promega Corp. USA), 2µL forward primer (20pmole), 2µL reverse primer (20pmole), 4.5µL nuclease- free water and 4µL Genomic extracted DNA (68 ng/µL). The PCR protocol was as follows: An initial denaturation at 95°C for 5 minutes, followed by 35

cycles of amplification: 94°C for 15 sec, 60°C for 30 sec, and 72°C for 45 sec. A final extension step of 72°C for 7 min to complete the elongation processes. Reaction products were analyzed by 2% agarose gel to check for the existence of the GSTT1 allele, which was detected by a 480-bp fragment. In 1X TBE buffer, at 100V, for approximately 45 min, a horizontal standard electrophoresis apparatus (BIO-RAD, China) was used. A null genotype is indicated by the lack of amplifiable GSTT1. BCL-2 produced a 154-bp fragment band.

2.7. Statistical analysis

For data input and analysis, a predesigned Statistical Package for Social Science Version 23 (IBM SPSS Statistics) software was used. Unpaired t-test and odds ratio were used to assess the risk given by a specific allele and genotype, with 95% confidence intervals (95% CI). Chi square ($\chi 2$) test was used to study genotype frequency differences between cases and controls were employed. Statistical significance was defined as a P value of less than 0.05.

Chapter 3
Results

3.1 Demographic and clinical features of the studied subjects

Our study was carried out on 36 patients with AML, in addition to 72 age- and sex-matched apparently healthy volunteers as a control group. The demographic data of patients with AML included in our study are shown in Table (3.1). Patients comprised 36 individuals with AML, of whom 25 males (69.4%) and 11 females (30.6%) as aged 3 to79 years (34.53 ± 18.21years) (mean ± SD), the control group included 72 apparently healthy individuals [50 males (69.4%) and 22 females (30.6%)], age ranged between 3 and 80 years with a mean 34.51±17.89 years. The distribution of demographic variables was compared between cases and controls using two-sided unpaired t-tests. The results showed no significant differences in gender or age, as shown by the high P- values, P=0.924 and P=1.00, respectively.

Table3.1: Age and sex distribution among AML patients and controls**.**

Variable	**AML Patients N=36 (%)**	**Controls N=72 (%)**	**P-value**
Age (year) Mean ± SD	34.53±18.21	34.51±17.89	0.924
Age group n (%)			
≤18	8 (22.2)	14 (19.4)	0.735
>18	28 (77.8)	58 (80.6)	
Sex n (%)			
Male	25 (69.4)	50 (69.4)	1.00
Female	11 (30.6)	22 (30.6)	

Twenty-eight of the AML patients (78%) included in our study were above 18 years old, and 8 patients (22%) were less than or equal to 18, with a ratio of 3.2:1. While 69%

of the AML's patients included in our study were males, and 31% of patients were females.

The geographic distribution of AML patients in our study is summarized in figure 3.1. Approximately 31% of AML cases were from the Gaza Strip, who were admitted to An-Najah National University Hospital (Nablus) for treatment, while 22% of the AML patients were from Jenin, followed by Nablus (17%). The majority of our study cases were inpatients at An-Najah National University Hospital (Nablus) (86%), while the rest were from Istishari Arab Hospital (Ramallah) (6%), and from Augusta Victoria Hospital (Jerusalem) (8%).

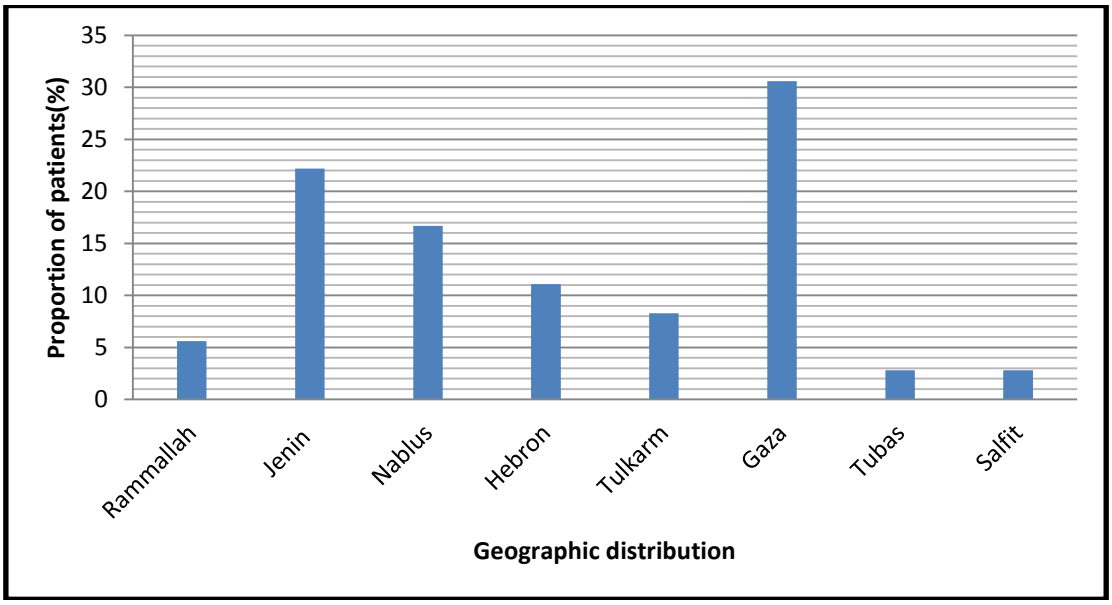


Figure (3.1): Geographic distribution of AML patients by district.

The medical history of AML patients in the present study were retrieved from patients' files at the oncology department of the different hospitals during the study period as shown in table (3.2). Most of the AML patient (58%) were untreated

Table 3.2: Medical history of AML patients

Medical status	AML patients (%)
The patient history of other types of malignancy	2 (5.6%)
Family history of leukemia	3 (8.3%)
Family history of other types of malignancy	8 (22.2%)
Smokers	6 (16.7%)
Phase: In remission	13 (38.1%)
Untreated	21 (58.3%)
Relapsed	2 (5.6%)

The clinical biochemical tests as well as Immunophenotyping and flow cytometry data were also retrieved from the patient`s files. Concerning the kidney function tests, BUN was normal in 32 patients (89%), and the creatinine level was low in 19 patients (53%). Specific myeloid antigens were distributed as follows: CD13 was positive in 31 patients (86%), CD33 was positive in 34 patients (94%) and CD117 was positive in 33 patients (92%). The CD3, CD10, CD20, CD45, 7-AAD and cCD79a were found to be negative in all the 36 AML patients (100%). Cytochemical staining revealed that 26 patients (72%) were positive for MPO. Subtypes according to the French–American–British classification were available for all the patients, 1 patient with M0, 2 with M1, 8 with M2, 18 with M3, 5 with M4, 2 with M5, none for M6 and M7 were reported in this study.

3.2 Genetic analysis of the studied subjects

3.2.1. Molecular analysis

The deletion of GSTT1 and GSTM1 genes (null genotype), was confirmed by the absence of the 480bp and 219bp bands, respectively, resulting in the absence of particular enzymes in both patients and controls. The housekeeping gene BCL2 (154bp) was used as an internal control, as shown in figure (3.2).

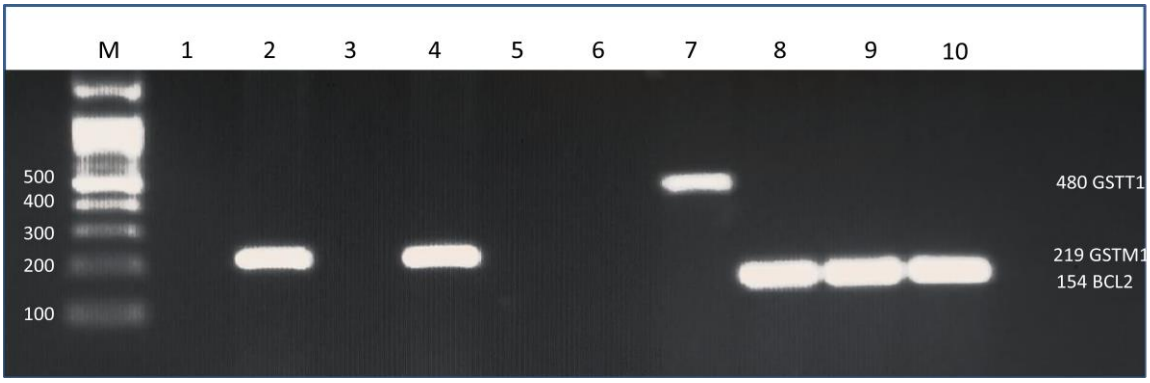


Figure (3.2): Gel electrophoresis illustration of PCR products analyzed on 2% agarose gel. The presence of GSTT1 was confirmed by the presence of a band at (480bp) and the presence of GSTM1 was confirmed by a band at (219bp). A band at (154bp), corresponds to BCL2 housekeeping gene as an internal control. Lane 1 represents a negative control. Lanes 2 and 4 correspond to samples with GSTM1 present (219bp). Lane 3 represents an individual with null alleles for GSTM1 gene. Lanes 5 and 6 represent samples with GSTT1 null. Lane 7 is an individual with GSTT1 present (480bp). Lanes 8, 9, and 10 correspond to BCL2 housekeeping gene as an internal control. M is a DNA molecular marker (100bp).

The GSTP1 (NM_000852.4:c.313A>G) polymorphism was identified with the PCR-restriction fragment length polymorphism (PCR-RFLP).The normal homozygous Ile/Ile was confirmed by the presence of a single DNA fragment (176bp), two DNA fragments for mutant homozygote Val/Val (91 and 85bp), whereas the heterozygous Ile/Val genotype was confirmed by the presence of three fragments (176, 91, and 85bp) as shown in figure (3.3A). Sanger sequencing was performed to confirm the genotypes of GSTP1 (NM_000852.4:c.313A>G) polymorphism for randomly selected samples as demonstrated in figure (3.3B).

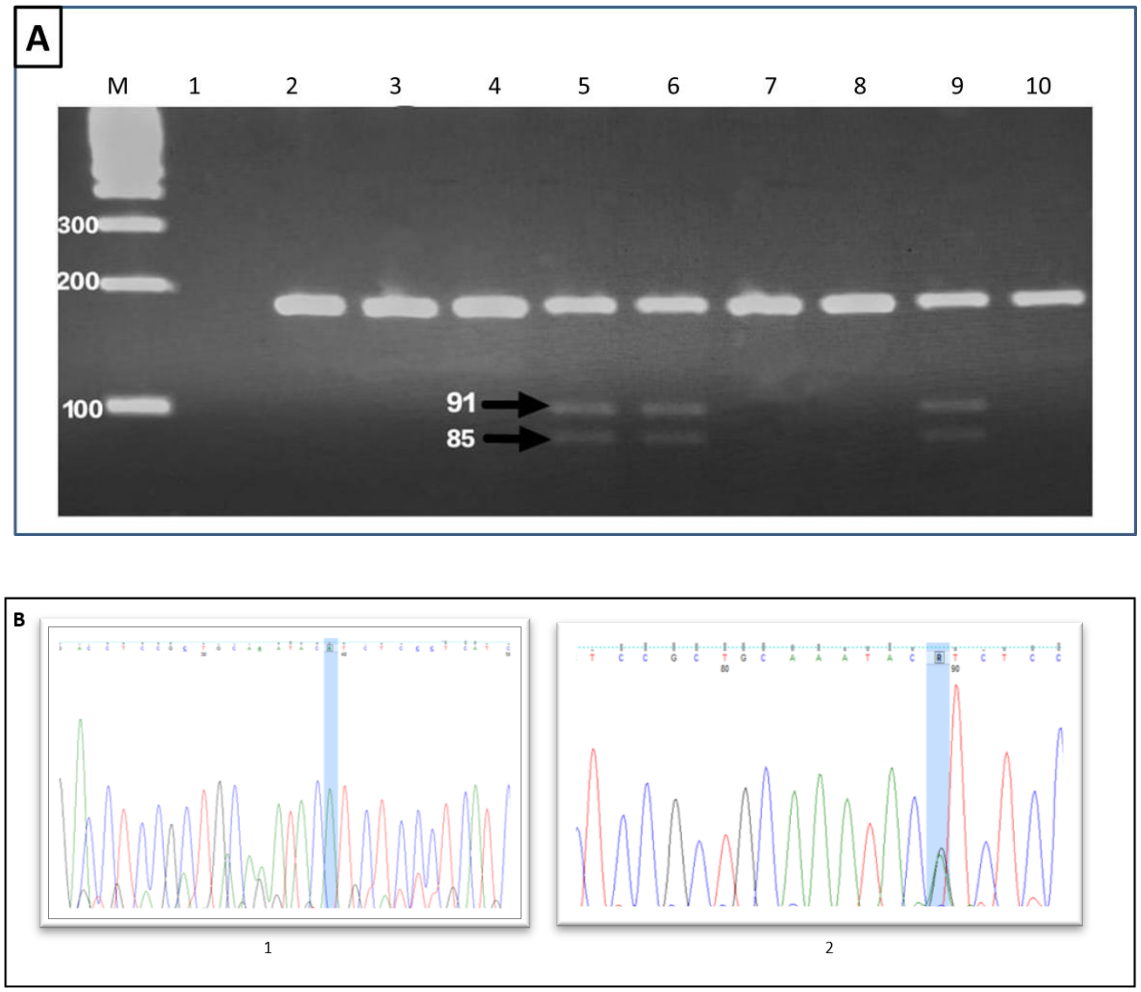


Figure (3.3): Genotyping of the GSTP1 (NM_000852.4:c.313A>G)**. A.** Metaphore agarose gel electrophoresis (4%) pattern of selected RFLP products of the GSTP1 (NM_000852.4:c.313A>G)**.** M: DNA ladder (100bp). Lane 1 is negative control. Lanes 2, 3, and 4 represent PCR amplified DNA fragment (176bp) of the GSTP1 genotype. Lanes 5, 6, and 9 are heterozygous (AG genotype) BsmBI digested products confirmed by the presence of three bands (176, 91, and 85 bp). Lanes7, 8, and 10 correspond to the wildtype homozygous (AA genotype) resulting in one band (176bp). **B.** Sanger sequencing chromatograms showing the sequence for two random samples, sample 1 with AA genotype, and sample 2 with AG genotype.

3.2.2 Genotypic analysis

The Chi-square ($\chi 2$) was used to test the association between the genotypes and alleles in the cases and controls. Regarding the results of PCR-RFLP for GSTP1, 64% of the cases were homozygous for the Ile/Ile genotype, compared to 51% in the controls. The Ile/Val heterozygous genotype was reported in 36% in cases, compared to 49% in the controls. Neither cases nor controls had GSTP1 Val/Val genotype. It was found that

there was no significant association between the frequency of Ile and Val alleles in patients compared to controls (P=0.218).

For the GSTM1 (NC_000001.10:g.[0]) polymorphism, the frequency of GSTM1 null genotype was found to be 33% and 25% in cases and controls, respectively. While in the GSTT1 (NC_000022.10:g.[0]) polymorphism, the frequency of GSTT1 null genotype was 44% and 25% in cases and controls, respectively. These results were statistically significant, with a higher frequency among patients (P=0.04). The results of genotype frequency are summarized in Table 3.3.

Table3.3: Association analysis between individual GST polymorphisms and AML risk.

Genes	**Genotypes/Alleles**	**AML N=36 (%)**	**Controls N=72 (%)**	**OR (95%CI)**	**P-value**
GSTM1	Present	24 (66.7)	54 (75)	1.50 (0.62-3.59)	0.362
	Null	12 (33.3)	18 (25)		
GSTT1	Present	20 (55.6)	54 (75)	2.40 (1.03-5.59)	0.04*
	Null	16 (44.4)	18 (25)		
GSTP1	Ile/Ile	23 (63.9)	37 (51.4)	1.67 (0.73-3.80)	0.218
	Ile/Val	13 (36.1)	35 (48.6)	0.59 (0.26-1.36)	0.218
	Val/Val	0 (0.0)	0 (0.0)	-	-
	Ile	59(82)	109(75.7)	0.69(0.34-1.39)	0.298
	Val	13(18)	35(24.3)		

3.2.3. Haplotype analysis

The combinations of GSTM1 and GSTT1 null genotypes were also evaluated in our study. The frequency of GSTT1 null/GSTM1 null was 16.7%, 5.6% in cases and controls, respectively. These results indicate no association with AML susceptibility as the P-value was 0.060. On the other hand, the frequency of GSTT1 present/ GSTP1 (Ile/Val) was 36.1% and 16.7% in controls and cases, respectively. These results may indicate a slightly significant association, as the P-value was 0.037.

The evaluation of triple combined genotypes showed that the frequency of GSTM1 null/GSTT1 null/ GSTP1 (Ile/ Ile) was 8.3% and 1.4% in cases and controls, respectively. Although the OR was high (6.45), these triple combined genotypes is not associated with AML susceptibility since the P-value was 0.072.

Finally, the frequency of GSTM1 present/GSTT1 present/ GSTP1 (Ile/Val) was 31.9% and 11.1% in controls and cases, respectively (Table 3.4). These results are statistically significant (P-value=0.018).

Table3.4: Association analysis between combined GSTs genotypes and AML risk.

Genotypes	**AML N=36(%)**	**Controls N=72(%)**	**OR (95%CI)**	**P-value**
GSTM1/GSTT1				
M1+/T1+	14(38.9)	40(55.6)	0.51 (0.22-1.15)	0.102
M1+/T1-	10(27.8)	14(19.4)	1.59 (0.62-4.05)	0.326
M1-/T1+	6(16.7)	14(19.4)	0.83 (0.29-2.37)	0.726
M1-/T1-	6(16.7)	4(5.6)	3.40 (0.89-12.93)	0.060
GSTM1/GSTP1				
M1+/P1(Ile/Ile)	16(44.4)	25(34.7)	1.50 (0.66-3.40)	0.326

M1+/P1(Ile/Val)	8(22.2)	29(40.3)	0.42 (0.17-1.05)	0.062
M1-/ P1(Ile/Ile)	7(19.4)	12(16.7)	1.21 (0.43-3.38)	0.721
M1-/ P1(Ile/Val)	8(22.2)	29(40.3)	0.42 (0.17-1.05)	0.062
GSTT1/GSTP1				
GSTT1+/ P1(Ile/Ile)	14(38.9)	28(38.9)	1.00 (0.44-2.27)	1.000
GSTT1+/ P1(Ile/Val)	6(16.7)	26(36.1)	0.35 (0.13-0.96)	**0.037***
GSTT1-/ P1(Ile/Ile)	9(25)	9(12.5)	2.33 (0.83-6.52)	0.100
GSTT1-/ P1(Ile/Val)	7(19.4)	9(12.5)	1.69 (0.57-4.98)	0.338
GSTM1/GSTT1/GSTP1				
M1+/T1+/ P1(Ile/Ile)	10(27.8)	17(23.6)	1.24 (0.50-3.09)	0.637
M1+/T1+/ P1(Ile/Val)	4(11.1)	23(31.9)	2.67 (0.08-0.84)	**0.018***
M1-/T1+/ P1(Ile/Ile)	4(11.1)	11(15.3)	0.69 (0.20-2.35)	0.555
M1-/T1+/ P1(Ile/Val)	2(5.6)	3(4.2)	1.35 (0.22-8.48)	0.746
M1+/T1-/ P1(Ile/Ile)	6(16.7)	8(11.1)	1.6 (0.51-5.02)	0.418
M1+/T1-/P1(Ile/Val)	4(11.1)	6(8.3)	1.38 (0.36-5.22)	0.639
M1-/T1-/ P1(Ile/Ile)	3(8.3)	1(1.4)	6.45 (0.65-64.41)	0.072
M1-/T1-/ P1(Ile/Val)	3(8.3)	3(4.2)	2.09 (0.40-10.92)	0.373

M1+: GSTM1 present; M1 -: GSTM1-null; T1+: GSTM1-present; T1 -: GSTT1

Chapter 4
Discussion

To the best of our knowledge, this is the first genetic study among the Palestinian population to examine the genotypic and allelic distributions of GST gene polymorphisms in AML cases. Accordingly, the aim of this study was to investigate the association of (GSTP1, GSTM1, and GSTT1) gene polymorphisms with AML in Palestinian population. The fact that these polymorphisms may become risk factors for acute myeloid leukemia, according to our hypothesis, would allow individual characterization. Therefore, 36 AML patients and 72 healthy volunteers were studied for the three GST genes (GSTP1/M1/T1). In terms of age and gender distribution, there was no statistical difference between the two groups.

Our findings are in agreement with other previous studies suggesting that AML can occur at any age, but it becomes more common with older age (Khwaja et al., 2016). However, this study disagreed with other studies as it reported that 78% of adult AML patients have a median age of thirty-six years old compared to sixty-seven years old in the United States (Miller et al., 2016). The difference in median age in our study is unsurprising, and can be attributed to varying levels of exposure to mutagens such as ionizing radiation, lifestyle, smoking, nutrition, and occupational chemical exposure, and population structure.

Concerning gender distribution, about 69% of AML patients (25/36) who participated in this study were males. Congruently, it was reported previously that AML is more common in males (Tamamyan et al., 2017). The high prevalence among males in this study might be due to physiologic and genetic differences between men and women, as well as males' increased exposure to mutagenic chemicals. Thus, we can conclude that

advanced age and male gender may be considered as risk factors for AML development among our study samples.

Regarding the geographic distribution of AML patients, our results showed that 31% of the AML patients were from Gaza strip who were admitted to NNUH for treatment. This high prevalence among AML patients from Gaza, could be associated to the population's constant exposure to chemicals and the remnants of wars. Indeed, Jenin and Nablus recorded the highest prevalence, 22%, and 17%, respectively, as the NNUH is the referral center for treating oncology and hematology cases in the northern district of the West Bank of Palestine. The majority of AML cases in this study (80%) were recruited from NNUH.

The etiology of most cases of AML is unknown, but it was found that smoking and family history could be risk factors for developing leukemia (Khwaja et al., 2016). In this study, the results showed that 16.7% of patients were smokers, 5.6% had a history of other types of malignancy, 22.2% had a family history of other types of malignancy, and 8.3% had a family history of leukemia. When we compared the relationship between cases with a family history of leukemia and those without a family history with the null genotype of GSTT1, using the Chi-Square test, no statistical association was found (P= 0.106) (Data not shown). Small sample size among the two groups may explain this lack of association and further studies are needed to investigate the influence of this factor.

The literature showed that 4% of adults with acute myeloid leukemia (AML) carry inherited harmful genetic mutations in the cancer susceptibility genes (Churpek, 2017), which confirms the multifactorial nature of AML.

The present study revealed that the frequency of GSTT1 null genotype in our AML patients was (44.4%), which is similar to that reported in Iran (40%) (Mandegary et al., 2011). Our results were higher than those reported in Caucasian (17%), Saudi (13%), Indian (21.6%), and with those documented for Moroccan (38%) population (Groner & Spa, 2008)(Farasani, 2019)(Chauhan et al., 2011)(Kaltoum et al., 2020). On the other hand, the results of the present study were lower than those reported in Egypt (60%) and Sudan (60%) (Nasr et al., 2015)(Babekir et al., 2019). The GSTM1 null genotype frequency (33.3%) reported in the current study was lower when compared to that observed in Chinese 53.5%, American 53.5%, Iranian 56.2%, and Romanian 57.8% populations (Zi et al., 2014)(Group et al., 2006)(Mandegary et al., 2011)(Claudia et al., 2016), but similar to that observed in the Saudi (34%) and Indian populations (37%) (Farasani, 2019)(Chauhan et al., 2011).

Regarding the GSTP1 (NM_000852.4:c.313A>G) polymorphism in the current study, the frequency of GSTP1 Ile/Val genotype in AML patients was 36.1% which is consistent with other Chinese (34.6%), Iranian (37.3%), Egyptian (36%), and Saudi (37) populations (Zi et al., 2014)(Mandegary et al., 2011)(Nasr et al., 2015)(Farasani, 2019), yet quite different from Moroccan (52.6%), Romanian (56.9%), and Indian (43.3%) populations (Kaltoum et al., 2020)(Claudia et al., 2016)(Chauhan et al., 2011). These differences in the frequency of GSTP1 Ile/Val genotype support the importance of environmental impact and ethnic/genetic variations between these distinct groups.

Several previous studies had shown a significant association between GSTM1 (NC_000001.10:g.[0]), GSTT1(NC_000022.10:g.[0]) and GSTP1 (NM_000852.4:c.313A>G) polymorphisms and the risk of AML. The GSTM1null, GSTT1 null, and GSTP1 Ile/Val genotypes were reported to be associated with the risk

of AML in a recent case-control research in Egypt. Other researchers from China and Sudan found a similar association (Nasr et al., 2015)(Zi et al., 2014)(Babekir et al., 2019).

It was found that inherited homozygous deletions of the GSTT1 and GSTM1 gene may lead to the complete lack of enzyme activity, leading to decreased efficiency at processing carcinogens and radical's oxygen species (Chatterjee & Gupta, 2018). In the current study, the GSTT1 null genotype was significantly ($P < 0.05$) associated with susceptibility to the AML, while the GSTM1 null genotype showed no association ($P=0.362$). Our data was similar to those reported in Iranian and Moroccan studies (Mandegary et al., 2011)(Kaltoum et al., 2020). Moreover, a meta-analysis, which included twenty-nine association studies between AML and GST gene polymorphisms, reported that the GSTT1 null genotype was associated with the risk of AML in Caucasians (He et al., 2014).

According to our study the GSTP1Ile/Val polymorphism had no influence on the development of AML. However, other studies reported a significant association between the presence of GSTP1 Ile/Val genotype and increased risk of developing AML (Claudia et al., 2016). On the other hand, it was found that GSTP1 gene plays a role in drug metabolism by increasing chemotherapy resistance (Phillip et al., 2008). Moreover, it was found that the GSTP1Ile/Val genotype is associated with reduced activity of the enzyme (Groner & Spa, 2008). Therefore, it was demonstrated as a prognostic factor, and it was shown that the patients who have the GSTP1Ile/Val genotype are significantly better with long relapse-free survival (RFS), and over survival (OS) (Nasr et al., 2015).

Other reports had revealed no correlation between the inheritance of GSTT1, GSTM1, and GSTP1 gene polymorphisms with the predisposition of AML. A Saudi case-control study conducted on 100 AML patients confirmed the negative association of the investigated genetic markers with susceptibility to AML (Farasani, 2019). Another case-control study conducted in the United States by Crump *et al* did not support the hypothesis that the deletion of GSTT1 and GSTM1 genes are related to the risk of AML (Crump et al., 2000). Moreover, the GSTT1null and GSTM1 null genotypes in Israeli Jewish and Arab populations did not influence the predisposition of AML (Phillip et al., 2008).

Many studies had focused on the possible association between the combination of GSTT1 and GSTM1 null genotypes and various diseases, but the results were variables (Shafiei & Safarinejad, 2011)(Song & Yang, 2020)(Miao et al., 2020). Other studies had reported a relationship between the combination of the GST genotypes and the risk of AML, with few of which suggesting a possible synergistic effect between GST genotypes (Mandegary et al., 2011)(Zi et al., 2014).

In the present study, we investigated the combined impact of GSTM1(NC_000001.10:g.[0]), GSTT1(NC_000022.10:g.[0]) and GSTP1 (NM_000852.4:c.313A>G) polymorphisms in an attempt to conclude whether carrying the GSTT1 Present and GSTP1 Ile/Val genotypes protected people from developing AML. Subjects carrying the GSTM1 Present, GSTT1 Present, and GSTP1 Ile/Val genotypes had a higher protective effect. However, Farahani *et al* found that carrying the GSTM1 Present, GSTT1 null and GSTP1 Ile/Val genotypes had a protective role among Saudi population (Farasani, 2019). To explain why GSTP1 Ile/Val is considered as a protective factor rather than the GSTP1 Ile/ Ile, the Genome Aggregation Database

(gnomAD) shows the frequency of (G) allele to be 0.3532. This single nucleotide variation leads to the substitution of Ile at codon 105 to Val causing a missense variant; see source, https://gnomad.broadinstitute.org/variant/11-67352689-A-G

In nonpolar amino acids substitution for another is an advantage according to the local substitution matrix. In BLOSUM62 the substitution of Ile for Val has log-odds ratio 3 which means no negative change. Moreover, Ile is likely to be replaced with Val in nature;https://www.ncbi.nlm.nih.gov/Class/FieldGuide/BLOSUM62.txt?fbclid=IwAR0nvTvxrYwzXcAugI_Zncq5PyVqDYNjvnN_CWiUb9Te4JQjSj2QMUZl66I

The two amino acids participate in forming the GSTP1 H site. The presence of isoleucine versus valine in position 105 could change substrate binding and, consequently, enzyme activity due to the differences between these two amino acids in bulkiness and hydrophobicity. The terminal methyl group of Ile has the closest contact, 0.36 nm, with the y-carbonyl group of glutamic acid in glutathione; for Val, the distance rises to 0.47 nm. The bulkier isoleucine residue could result in a small size of H site which is fitter and have high affinity for small substrates. This high affinity leads to a decrease in the Michaelis-Menten constant Km, although both enzymes attained the same maximum velocity (Vmax) and catalyst rate constant (kcat), indicating that the I-V substitution did not significantly alter the catalytic event (Zimniak et al., 1994). The Ile -Val substitution could also create an environment of different hydrophobicity, that could cause significant shifts in the side chains of several active site amino acid residues and resulting in geometry changes of the region of the H site bordered by Tyr108 by providing hydrogen-bond, van der Waals, and hydrophobic contact sites for the electrophile which will affect enzyme activity This single, conservative amino acid substitution has a considerable influence on enzyme specificity and stability by playing

a strong modulatory role on enzyme activity of amino acid residues that are not directly engaged in the catalytic process (Ali-osman et al., 1997).

. Furthermore, it indicates that naturally occurring isoforms of glutathione S-transferase P1-1 that differ by a single amino acid residue are functionally distinct (Zimniak et al., 1994).

Although, Chauhan *et al.,* demonstrated a negative association between the combination of GSTT1 null, GSTM1 null, and GSTP1 Ile/Val genotypes and the risk of AML (Chauhan et al., 2011), different trials suggested a possible association between the combination of GSTT1 null, GSTM1 null, and GSTP1 Ile/Val genotypes and AML (Mandegary et al., 2011)(Zi et al., 2014).

In a case-control study conducted in China, researchers discovered a statistically significant link between the combination of GSTM1 null, GSTT1 null, and the risk of AML, with the risk increasing with the GSTM1 Present, GSTT1 null. This confirms the hypothesis of the absence of an association between the GSTM1 null genotype and AML (Zi et al., 2014). A similar finding was reported by Moroccoan study (Kaltoum et al., 2020).

The insignificant correlation of the double combined genotypes of GSTM1 null/GSTT1 null in our study (P-value= 0.060) may be attributed to the small sample size. Six patients and four controls were reported to have this haplotype combination. Similarly, the triple combined genotypes of GSTM1 null/GSTT1 null/ GSTP1 (Ile/ Ile) were also reported to show insignificant association (P-value= 0.072) with AML development although the OR was high (6.45), as the studied cases and controls were three and one, respectively. Small sample size among the two groups may explain this lack of

association and further studies are needed to investigate the influence of this haplotype combination in AML development in our patients.

Regarding GSTT1 (NC_000022.10:g.[0]) polymorphism, the sample size of the GSTT1null genotype was representative to do the correlations with a frequency of 16 (44%) and 18 (25%) in cases and controls, respectively. The GSTT1null genotype was reported to have a higher frequency among AML patients with a significant statistical association (P- value = 0.04).

The limited sample sizes in other studies may explain the contradictory results in the connection of GST polymorphisms with developing AML. Furthermore, it appears that these genes are population-specific due to the different factors involved in tumor formation and progression, including changes in environmental exposure between populations. This is also due to the fact that AML is a rare malignancy, making it difficult to assess risk directly between societies due to a lack of comparability (Phillip et al., 2008).

There are some significant limitations in the present study including small sample size. The limited number of AML patients poses a challenge to make a reasonable statistical inference and therefore draw solid conclusions. Furthermore, our AML patients had a limited life expectancy, making it difficult to keep track of them. Also, the limited time frame to conduct this research made it unlikely to recruit sufficient sample size. The main objective of our research was to see if there was an association between the frequency of (GSTP1, GSTM1, and GSTT1) gene polymorphisms with the development of AML in a cross-sectional study to better understand the current state of AML illness in our region. A longitudinal cohort research design and patient follow-up will undoubtedly provide us with more information. The poor filing system of AML

patients in the Palestinian MOH centers was a major obstacle in recruiting and following up of these patients, as well as the patients, are referred to the private sector for treatment which in some instances refrained from cooperation.

Chapter 5
Conclusion and Recommendations

In conclusion, our current findings provide conclusive proof that the GSTT1 gene deletion is associated with susceptibility to AML. Carrying a combination of all three of GSTM1 present, GSTT1 present, and heterozygous (105Ile/105Val) GSTP1 genotypes, on the other hand, appears to protect against AML.

This study suggests that having a GSTT1 null genotype is an independent risk factor for developing AML. Increased solubility in water due to reduction of glutathione permits their elimination, and this detoxification activity prevents DNA damage, genomic instability and mutagenesis. GSTs modulate the non-enzymatic proteins and signaling pathways which control cell proliferation, differentiation and apoptosis. Future clinical and genetic investigations pertaining to variations in response or toxicity to xenobiotics or medications known to be glutathione-S-transferase substrates would benefit from this data. To demonstrate a concrete link between these polymorphisms and AML risk, more studies with bigger cohorts and different study designs such as cohort study are needed. A cohort study design will allow the follow up of patients for at least 18 months which will enable researchers to clearly investigate the differences in the outcome of patients. Finally, if we can identify an association between SNPs and therapy response, we may be able to incorporate this test into the standard blood testing protocols before the initiation of treatment.

References:

Ali-osman, F., Akande, O., Antoun, G., Mao, J., & Buolamwini, J. (1997). Molecular Cloning, Characterization, and Expression in Escherichia coli of Full-length cDNAs of Three Human Glutathione S -Transferase Pi Gene Variants. Journal of Biological Chemistry, 272(15), 10004–10012. https://doi.org/10.1074/jbc.272.15.10004

American Cancer Society (ACS). (2018). Acute Myeloid Leukemia - Early Detection , Diagnosis , and Types. American Cancer Society, August 2018, 1–21. www.cancer.org/

Arber, D. A., Borowitz, M. J., Cessna, M., Etzell, J., Foucar, K., Hasserjian, R. P., Rizzo, J. D., Theil, K., Wang, S. A., Smith, A. T., Rumble, R. B., Thomas, N. E., Ascp, C. T., & Vardiman, J. W. (2017). Initial Diagnostic Workup of Acute Leukemia. Arch Pathol Lab Med, 141, 1342–1393. https://doi.org/10.5858/arpa.2016-0504-CP

Babekir, E. A., Abdelateif, N. M., Adelrahim, S. O., Hassan, R., & Ibrahim, I. K. (2019). GSTM1 and GSTT1 Polymorphisms and Susceptibility to Acute Myeloid Leukemia : A Case-control Study of the Sudanese Population. Asian Pacific Journal of Cancer Biology, 4(1), 7–10.https://doi.org/10.31557/APJCB.2019.4.1.7

Bain, B. (2010). leukemia Diagnosis. In leukemia Diagnosis (fourth, p. 404). wiley.

Bories, H. (1974). Acute leukemias. L'Infirmiere Francaise, 156, 19–25.

Bray, F., Ferlay, J., & Soerjomataram, I. (2018). Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. A Cancer Journal for Clinicians, 68, 394–424. https://doi.org/10.3322/caac.21492

Bullinger, L., Döhner, K., & Dohner, H. (2017). Genomics of acute myeloid leukemia diagnosis and pathways. Journal of Clinical Oncology, 35(9), 934–946. https://doi.org/10.1200/JCO.2016.71.2208

Chatterjee, A., & Gupta, S. (2018). The multifaceted role of glutathione S-transferases in cancer. Cancer Letters, 433, 33–42. https://doi.org/10.1016/j.canlet.2018.06.028

Chauhan, P. S., Ihsan, R., Yadav, D. S., Mishra, A. K., Bhushan, B., Soni, A., Kaushal, M., Devi, T. R., & Saluja, S. (2011). Association of Glutathione S-Transferase, EPHX, and p53 codon 72 Gene Polymorphisms with Adult Acute Myeloid Leukemia. DNA AND CELL BIOLOGY, 30(1), 39–46. https://doi.org/10.1089/dna.2010.1092

Chen, X., & Cherian, S. (2017). Acute Myeloid Leukemia Immunophenotyping by Flow Cytometric Analysis. Clinics in Laboratory Medicine, 37(4), 753–769. https://doi.org/10.1016/j.cll.2017.07.003

Churpek, J. E. (2017). Best Practice & Research Clinical Haematology Familial myelodysplastic syndrome / acute myeloid leukemia. Best Practice & Research Clinical Haematology, 3–5. https://doi.org/10.1016/j.beha.2017.10.002

Claudia, B., Iancu, M., Trifa, A. P., Cândea, M., Lazar, E. B., Moldovan, V. G., Duicu, C., Tripon, F., Crauciuc, A., & Dobreanu, M. (2016). From Six Gene Polymorphisms of the Antioxidant System , Only GPX Pro198Leu and GSTP1 Ile105Val Modulate the Risk of Acute Myeloid Leukemia. Oxidative Medicine and Cellular Longevity These, 2016, 1–10. https://doi.org/10.1155/2016/2536705

Crump, C., Chen, C., Appelbaum, F. R., Kopecky, K. J., Schwartz, S. M., Willman, C. L., Slovak, M. L., & Weiss, N. S. (2000). Glutathione S-Transferase Theta 1 Gene Deletion and Risk of Acute Myeloid Leukemia. Cancer Epidemiology, Biomarkers & Prevention, 9(May), 457–460.

Farasani, A. (2019). Saudi Journal of Biological Sciences Genetic variants of glutathione S-transferase and the risk of acute myeloid leukemia in a Saudi population. Saudi Journal of Biological Sciences, 26(7), 1525–1530. https://doi.org/10.1016/j.sjbs.2018.12.011

Groner, S., & Spa, D. (2008). Prognostic role of glutathione S -transferase polymorphisms in acute myeloid leukemia. Leukemia, 22(2008), 1685–1691. https://doi.org/10.1038/leu.2008.169

Group, S. O., Repository, S. M., Weiss, J. R., Dna, G. P., Bio-, B., & Gstm, F. (2006). Glutathione S -transferase (GSTM1 , GSTT1 and GSTA1) polymorphisms and outcomes after treatment for acute myeloid leukemia : pharmacogenetics in Southwest Oncology Group (SWOG) clinical trials. Leukemia, 20, 2169–2171. https://doi.org/10.1038/sj.leu.2404421

Guven, M., Unal, S., Erhan, D., Ozdemir, N., Baris, S., Celkan, T., Bostanc, M., & Batar, B. (2015). Role of glutathione S-transferase M1 , T1 and P1 gene polymorphisms in childhood acute lymphoblastic leukemia susceptibility in a Turkish population. Meta Gene, 5, 115–119. https://doi.org/10.1016/j.mgene.2015.06.002

He, H., You, H., Sun, J., Hu, S., Ma, Y., Dong, Y., & Lu, J. (2014). Glutathione S - transferase Gene Polymorphisms and Susceptibility to Acute Myeloid Leukemia : Meta-analyses. Jpn JClin Oncol 2014;44(11), 44(11), 1070–1081. https://doi.org/10.1093/jjco/hyu121

Hwang, S. M. (2020). Classification of acute myeloid leukemia. Bloodresearch.or.Kr, 55, 1–4.

Kaltoum, O., Boujmia, A., Nadifi, S., Dehbi, H., & Kassogue, Y. (2020). Genetic Polymorphisms of Glutathione S-Transferase and Risk of Acute Myeloid Leukemia : Case-Control Study and Meta-Analysis. Middle East Journal of Cancer, 11(2), 127–139.

Khwaja, A., Bjorkholm, M., Gale, R. E., Levine, R. L., Jordan, C. T., Ehninger, G., Bloomfield, C. D., Estey, E., Burnett, A., Cornelissen, J. J., Scheinberg, D. A., Bouscary, D., & Linch, D. C. (2016). Acute myeloid leukaemia. Nature Reviews Disease Primers, 2(March), 1–22. https://doi.org/10.1038/nrdp.2016.10

Ladines-castro, W. (2016). Morphology of leukaemias. Rev Med Hosp Gen Méx, 79(2), 107–113.

Leonardi, D. B., Abbate, M., Riccheri, M. C., & Nuñez, M. (2017). Improving risk stratification of patients with childhood acute lymphoblastic leukemia : Glutathione-S-Transferases polymorphisms are associated with increased risk of relapse. Oncotarget, 8(1), 110–117.

Lima, R., & Teixeira, D. F. (n.d.). Tuberculosis Pharmacogenetics : State of The Art. May 2014.

Lyon, H. C. De. (2008). Diagnostics of the AML with immunophenotypical data. Math. Model. Nat. Phenom, 2(1), 104–123.

Makhtar, S. M., Husin, A., Baba, A. A., & Ankathil, R. (2017). Association of GSTM1, GSTT1 and GSTP1 Ile105Val polymorphisms with clinical response to imatinib mesylate treatment among Malaysian chronic myeloid leukaemia patients. Journal of Genetics, 96(4), 633–639. https://doi.org/10.1007/s12041-017-0819-2

Mandegary, A., Rostami, S., Alimoghaddam, K., & Ghahremani, M. H. (2011). Gluthatione-S-transferase T1-null Genotype Predisposes Adults to Acute Promyelocytic Leukemia ; a Case-Control Study. Asian Pacific J Cancer Prev, 12, 1279–1282.

Miao, L., Ye, X., & Id, X. H. (2020). Individual and combined effects of GSTM1 , GSTT1 , and GSTP1 polymorphisms on breast cancer risk : A meta-analysis and re-analysis of systematic meta-analyses.

Miller, K. D., Siegel, R. L., Lin, C. C., Mariotto, A. B., Kramer, J. L., Rowland, J. H., Stein, K. D., Alteri, R., & Jemal, A. (2016). Cancer Treatment and Survivorship Statistics , 2016. 66(4), 271–289. https://doi.org/10.3322/caac.21349.

MOH. (2019). Health. www.moh.ps

Moulik, N. R., Parveen, F., Kumar, A., & Agrawal, S. (2014). Glutathione-S-transferase polymorphism and acute lymphoblastic leukemia (ALL) in north Indian children : a case –

control study and meta-analysis. Journal of Human Genetics, April, 1–7. https://doi.org/10.1038/jhg.2014.66

Narayanan, D., & Weinberg, O. K. (2020). How I investigate acute myeloid leukemia. International Journal of Laboratory Hematology, 42(1), 3–15. https://doi.org/10.1111/ijlh.13135

Nasr, A. S., Sami, R. M., Ibrahim, N. Y., & Darwish, D. O. (2015). Glutathione S transferase (GSTP 1 , GSTM 1 , and GSTT 1) gene polymorphisms in Egyptian patients with acute myeloid leukemia. Indian Journal of Cancer, 52(4), 490–495. https://doi.org/10.4103/0019-509X.178408

Nizar, A., Yigit, A., Isik, Z., & Alpkocak, A. (2019). Identification of Leukemia Subtypes from Microscopic Images Using Convolutional Neural Network. Diagnostics, 9(3), 104.

Parl, F. F. (2005). Glutathione S-transferase genotypes and cancer risk. Cancer Letters, 221(2), 123–129. https://doi.org/10.1016/j.canlet.2004.06.016

Phillip, M., Asher, N., Heled, M., Bar, S., Risch, A., & Rund, D. (2008). Polymorphisms in transporter and phase II metabolism genes as potential modifiers of the predisposition to and treatment outcome of de novo acute myeloid leukemia in Israeli ethnic groups. Leukemia Research, 32, 919–929. https://doi.org/10.1016/j.leukres.2007.10.011

Safarinejad, M. R., Safarinejad, S., Shafiei, N., & Safarinejad, S. (2013). Association of genetic polymorphism of glutathione S-transferase (GSTM1, GSTT1, GSTP1) with bladder cancer susceptibility. Urologic Oncology: Seminars and Original Investigations, 31(7), 1193–1203. https://doi.org/10.1016/j.urolonc.2011.11.027

Salavaggione, O., Eckloff, B., Wieben, E., & Weinshilboum, R. (2008). Glutathione S-Transferase P1: Gene Sequence Variation and Functional Genomic Studies. Cancer Res, 68(12), 4791–4801. Glutathione. https://doi.org/10.1158/0008-5472.CAN-07-6724

Sha, H., Hu, T., Wu, J., & Dan, C. (2018). Glutathione S -transferase π : a potential role in antitumor therapy. Drug Design, Development and Therapy, 12, 3535–3547.

Shafiei, N., & Safarinejad, S. H. (2011). Glutathione S-transferase gene polymorphisms (GSTM1 , GSTT1 , GSTP1) and prostate cancer : a case-control study in Tehran , Iran. Prostate Cancer and Prostatic Diseases, 14(2), 105–113. https://doi.org/10.1038/pcan.2010.54

Short, N. J., Rytting, M. E., & Cortes, J. E. (2018). Acute myeloid leukaemia. The Lancet, 392(10147), 593–606. https://doi.org/10.1016/S0140-6736(18)31041-9

Simic, T., Savic-Radojevic, A., Pljesa-Ercegovac, M., Matic, M., & Mimic-Oka, J. (2009). Glutathione S-transferases in kidney and urinary bladder tumors. Nature Reviews Urology, 6(5), 281–289. https://doi.org/10.1038/nrurol.2009.49

Song, L., & Yang, C. (2020). Individual and combined effects of GSTM1 and GSTT1 polymorphisms on colorectal cancer risk : an updated meta-analysis. Portland Press, 40.

Sullivan, K. M., & Soe, M. M. (2007). Sample Size for an Unmatched Case-Control Study Sample Size for Unmatched Case-Control Study.

Tamamyan, G., Kadia, T., Ravandi, F., Borthakur, G., Cortes, J., Jabbour, E., Daver, N., Ohanian,

M., Kantarjian, H., & Konopleva, M. (2017). Frontline treatment of acute myeloid leukemia in adults. Critical Reviews in Oncology/Hematology, 110, 20–34. https://doi.org/10.1016/j.critrevonc.2016.12.004

Tebbi, C. K. (2021). Etiology of Acute Leukemia : A Review. Cancers, 13(2256), 1–19.

Vural, B. (2012). Evaluation of Glutathione S-Transferase P1 Polymorphisms (Ile105Val and Ala114Val) in Patients with Small Cell Lung Cancer. 16(7), 701–706. https://doi.org/10.1089/gtmb.2011.0315

Weich, N., Nuñez, M. C., Galimberti, G., Elena, G., Acevedo, S., Larripa, I., & Fundia, A. F. (2015). Polymorphic variants of gstm1, gstt1, and gstp1 genes in childhood acute leukemias: A preliminary study in Argentina. Hematology, 20(9), 511–516. https://doi.org/10.1179/1607845415Y.0000000007

Zi, Y. M., Wu, S., Ma, D., Yang, C., Yang, M., Huang, Y., & Yang, S. J. (2014). Association of GSTTI and GSTM1 variants with acute myeloid leukemia risk. Genet. Mol. Res, 13(2), 3681–3685.

Zimniak, P., Nanduri, B., Pikula, S., Bandorowicz-pikula, J., Singhal, S. S., Srivastava, S. K., Awasth, S., & Awasthi, Y. C. (1994). Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur. J. Biochem, 224, 893–899.

Appendices

Appendix 1

Acute Myelogenous Leukemia Questionnaire

Name (Last, 1st, M.I.)		**Female□Male □**		**DOB:**	
Phone		**Leukemia type**			
Address		**Institute**			

Health History

1. Name and address of hematologist or other physician seen most recently for your heart condition.

_____ Date of last consultation

_____/_____/_____

2. Age at diagnosis: _____

3. Any history of the following (check all that apply)?

I. Any recurrence after initial treatment? □

Yes □ No

II. History of any other cancer(s)? □

Yes □ No

III. History of HIV virus, HCV or HBV □

Yes □ No

IV. History of Human T-Lymphotropic Virus (HTLV) □

Yes □ No

V. Family history of Leukemia □

Yes □ No

VI. Family history of other types of cancer □

Yes □ No

4. Do you currently smoke cigarettes? □

Yes □ No

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

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

5. For Acute Myelogenous Leukemia (AML), check the appropriate phase:

I. Untreated Phase □
II. Remission Phase □
III. Recurrent Phase □

6. Date & results of most recent CBC (complete blood count):
_____

7. Date & results of last Immunophenotyping (Flowcytometry):
_____

8. Date & results of last Bone marrow biopsy (FAB):
_____

9. Date and results of last Biochemical tests
(KFT):_____

I certify that the statements and responses to the questions are accurate to the best of my knowledge and belief.

Patient signature _____ Date _____/_____/_____

Appendix 2

Table 2.1: Chemicals and Reagents used in the study

Reagents	**Provider**
QIAamp DNA Mini Kit	Qiagen/ Germany
Ethanol absolute anhydrous	Carlo Erba Reagent
Master Mix	Promega
Nuclease-free water	Promega
BsmB1	New England BioLabs
NEBuffer 3.1	BioLabs
Agarose powder	Invitrogen
Ethidium bromide	Hylabs
TBE	Sigma
Ladder 100bp	Promega

Table 2.2: 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

الملخص

الخلفية:

Glutathione S-Transferase (GST)، عائلة كبيرة من إنزيمات استقلاب الدواء في المرحلة الثانية والتي تحفز اقتران مجموعة كبيرة ومتنوعة من المركبات الغريبة الحيوية، بما في ذلك المواد المسببة للسرطان. وتلعب هذه الإنزيمات أيضًا دورًا مهمًا في استقلاب الأدوية بما في ذلك أدوية العلاج الكيميائي. تؤثر الأشكال المتعددة داخل إنزيمات التمثيل الغذائي (GSTT1 وGSTM1 وGSTP1) على قدرة الجسم على إزالة السموم الناتجة من العوامل المسببة للسرطان التي قد تكون موجودة في البيئة. مما يجعل الجسم عرضة للإصابة باللوكيميا.

الأهداف:

الهدف من دراستنا هذه، هو التحقق من العلاقة بين تعدد الأشكال الجيني (GSTP1 وGSTM1 وGSTT1) وحدوث سرطان الدم النخاعي الحاد في فلسطين.

المنهجية:

تم جمع 36 مريض بسرطان الدم النخاعي الحاد من عدة مراكز في الضفة الغربية [مستشفى جامعة النجاح الوطنية (نابلس)، والمستشفى الاستشاري العربي (رام الله)، ومستشفى أوغستا فيكتوريا (القدس)]، و72 شخص سليما. في جين GST، تم التنميط الجيني لمتغيرات GSTM1 وGSTT1 عن طريق تفاعل البلمرة المتسلسل التقليدي للأليل (PCR)، وتم التنميط الجيني لمتغيرات GSTP1 عن طريق تحليل PCR-RFLP. بالإضافة إلى انه تم إجراء تحليل التسلسل لتأكد من النتائج الناتجة عن تحليل (RFLP).

النتائج:

لم تظهر الأنماط الجينية الصفرية لـ GSTM1 أي ارتباط مع سرطان الدم النخاعي الحاد [p = 0.362]. أيضًا، لم يظهر وجود GSTP1 105 Val allele أي ارتباط [p = 0.218]. من ناحية أخرى، أظهرت الأنماط الجينية الفارغة للنمط الجيني GSTT1 ارتباطًا كبيرًا بوجود مرض سرطان الدم النخاعي الحاد [p = 0.04].

علاوة على ذلك، أظهرت التفاعلات الجينية مع توليفات تحليل الأنماط الجينية لـ GSTs بين حالات سرطان الدم النخاعي الحاد والضوابط ارتباطًا إيجابيًا ($p < 0.05$). حيث وجد أن المزيج الثنائي بين GSTT1و GSTP1 105 Val allele [p = 0.037]، والمزيج الثلاثي من (GSTT1 + / GSTM1 + / GSTP1 105 Val allele) [p = 0.018] تلعب دورًا وقائيًا من خطر الإصابة بسرطان الدم النخاعي الحاد بين الفلسطينيين.

الخلاصة:

في الختام، كان هناك ارتباطا إحصائيا بحذف جين GSTT1 مع خطر الإصابة بسرطان الدم النخاعي الحاد. من ناحية أخرى، يبدو أن وجود GSTT1 مع GSTP1 105 Val allele يلعب دورًا وقائيًا من سرطان الدم النخاعي الحاد بين الفلسطينيين.

وتكون الحماية أعلى في وجود التركيبة الثلاثية من GSTM1 وGSTT1 مع GSTP1 105 Val allele.