Chapter one

Introduction and literature review

1.1 Introduction
Leukemia’s are group of disorders characterized by the accumulation of themalignant white cells in the bone marrow and blood. These abnormal cells cause symptoms because of bone marrow failure (i.e. anemia, neutropenia, and thrombocytopenia) and infiltration of organs (e.g. liver, spleen, lymph Nodes, meninges, brain, skin or testes (hoffbrand  et al., 2006).

Glutathione s transfers M1 (GSTM1) is an enzyme encoded by the GSTM1 gene on chromosome band 1p13. GST is able to detoxify various harmful oxidants, oxidative stress product, xenobiotics and carcinogens, all by catalyzing their conjugation to reduce glutathione (8,9) (Yam-Kau Poon et al., 2012).

1.2 Literature review
Leukemia is a disease, usually of leukocytes, in the blood and bone marrow (Turgeon, 2010).

1.2.1 Classification of leukemia
The main classification is into four types: Acute and chronic leukemia, which are further subdivided into: Lymphoid or myeloid, as we see in the table (1.1)

<table>
<thead>
<tr>
<th>Classification of leukemia’s</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute:</td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukemia: M0-M7</td>
<td></td>
</tr>
<tr>
<td>Acute lymphoid leukemia: L1-L3</td>
<td></td>
</tr>
<tr>
<td>Chronic:</td>
<td></td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td></td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td></td>
</tr>
</tbody>
</table>
1.2.1.1 Chronic lymphocytic leukemia

1.2.1.1.1 Introduction

Within the broad category of B-cell lymph proliferative disorders include a number of disease entities arising from mature B lymphocytes and which involve primarily the blood, bone marrow and other lymphoid organs such as the lymph nodes and spleen. All these disorders are classified by the world health organization (WHO), On the basis of their histopathological features. Their clinical course is often chronic and they affect mainly adults. A constant finding in all these entities is the presence in peripheral blood of leukemic cells in various degrees. (Hoffbrand et al, 2011).

The world health organization (WHO) classification of tumors of the Hematopoietic and Lymphoid Tissues has enhanced the classification of lymphoid neoplasm by including immunophenotypic features and genetic abnormalities to define different disorders. Examples of the disorders of the mature B-cell neoplasm classification:-

- CLL/small lymphocytic lymphoma (SLL)
- B-cell prolymphocytic leukemia
- Hairy cell leukemia (vHCL)
- Plasma cell neoplasms (Turgeon, 2010)

The primary B-cell leukemia include chronic lymphocytic leukemia (CLL), which is by far the most common (Hoffbrand et al, 2011).

CLL is a neoplasms composed of small B lymphocytes in the peripheral blood, bone marrow, spleen, and lymph nodes, mixed with prolymphocytes and paraimmunoblasts forming proliferation centers in tissue infiltrates. (A Victor Hoffbrand et al, 2011).
1.2.1.1.2 Epidemiology:
CLL is the most common form of leukemia in adults in Western countries but it is very rare in far Eastern countries. CLL/SLL accounts for almost 7% of non-Hodgkin lymphomas (NHLs) in biopsies. The median age of onset is 65 years. This form of leukemia is rare before age 20 and uncommon before age 50. But it is now diagnosed more often in younger persons, more males than females (1.5 to 2.1:1) are affected by the disorder. CLL has the highest genetic predisposition of all hematologic neoplasms. A family predisposition can be documented in 5% to 10% of patients with CLL. The overall risk is two to seven times greater in first-degree relatives of CLL patients. (Hoffbrand et al., 2011).

The chronic lymphocytic leukemia differ from acute lymphoblastic leukemia (ALL) in that the cells appear mature and have a mature phenotype. Acute lymphoblastic leukemia is a proliferation of immature cells (blasts). The distinction between chronic lymphocytic leukemia and non-Hodgkin’s lymphoma (NHL) primarily depends on the presence or absence of peripheral blood involvement; however, the distinction is sometimes arbitrary and often clinically insignificant. (Kern, 2002).

1.2.1.1.3 Etiology and pathology:
The cause of CLL is unknown. It is not certain at what stage in lymphocyte maturation the CLL cell arises, since roughly equal numbers seem to come from a pre- and post-germinal center B-lymphocyte. Diseases originating at these two stages of maturation have vastly different prognoses. However, gene expression profiling suggests that both subtypes are
similar, differing from each other by relatively few expressed genes (Saba, Mufti, 2011).

The immunologic deficiency in CLL is complex, Hypogammaglobulinemia is common; therefore, patients are predisposed to infections with encapsulated organisms such as *Streptococcus pneumoniae*. There are also abnormalities in T-cell numbers and function, including a decreased ratio of T helper to T suppressor (CD4/CD8) cells and impaired Cell-mediated immunity. Therapy for CLL may increase the immune suppression (Kern, 2002).

There is much confusion about aggressive transformation of CLL. Transformation to acutelymphoblastic leukemia, a myth. Richter’s syndrome is now recognized as a rare but regular culmination of CLL. In the largest series of cases, the incidence was 2.8%. It has been accepted that any type of aggressively lymphoma occurring in a patient with CLL maybe called Richter’s syndrome (Hussain I. Saba, Ghulam J. Mufti, 2011).

1.2.1.1.4 Clinical Features

Many patients with CLL are asymptomatic at diagnosis; lymphocytosis is detected as an incidental finding on a routine CBC. Symptoms, when present, are usually due to anemia and include fatigue, dizziness, or dyspnea on exertion. Patients may have nonspecific systemic symptoms such as fever, night sweats, and weight loss. The physical examination in CLL may be completely normal, the most common abnormality is lymphadenopathy, which may be localized or generalized. The lymph nodes are usually slightly to moderately enlarged, firm or rubbery, nontender, and freely movable. Mild or moderate hepatosplenomegalymay be present. (Kern, 2002)

1.2.1.1.5 The Laboratory Features of Chronic Lymphocytic Leukemia:
The features vary quite a lot with various stages of the disease and with the presence of complications. The early phase: The patient may present only with a mild chronic lymphocytosis. Atypical lymphocytes may be present but not primitive ones. In this stage, the patient may not have any lymphocytosis or splenomegaly – in fact there may well be no positive features. The established phase: This is the typical appearance, with high absolute lymphocyte count, sometimes showing atypical features. However, the more progressive and aggressive variants may show cells of increasing primitiveness, anemia, and thrombocytopenia, These features are often accompanied by clinical features of weight loss, malaise, and weakness. These findings have significant implications for prognosis and therapy. (Beck, 2009).

1.2.1.1.6 Complications:
The most important of these is the Richter syndrome, which is a form of acute transformation. The leukemic phase of a low-grade lymphoma is much less common, but otherwise has much the same features. However, there are rare other lymphoproliferative disorders causing a lymphocytosis and their distinction can be very difficult – and the distinction is important, since the malignant status of some of these is open to question. In all these cases, bone marrow aspiration, biopsy, cytogenetic, and immunophenotyping are essential investigations (Beck, 2009).

1.2.1.1.7 Factors Associated with a Poor Prognosis
Clinical:
- Lymphadenopathy
- Splenomegaly
- Hepatomegaly
- "Bulky" disease
• Poor performance status
• Hematologic
• Anemia
• Thrombocytopenia
• Large and atypical lymphocytes in blood
• Diffuse bone marrow lymphocytic infiltration

Laboratory abnormalities:
- Increased serum lactate dehydrogenase level
- Hypoalbuminemia
- Increased serum calcium level
- Cytogenetic abnormalities
- Complex and multiple cytogenetic abnormalities and 17p, 11q

Immunologic:
- Hypogammaglobulinemia
- Immunophenotype (different abnormalities related to poor prognosis (e.g. CD5, CD23)
- Increased serum-soluble CD25 receptors
- Increased serum-soluble CD23 receptors
- Kinetic parameters
- Rapid doubling time

Others:
- Poor response to therapy. (Hoffmanet al, 2000).

Peripheral Blood:
Although a persistent lymphocyte count of greater than 10×10⁹/L was originally required for the diagnosis of CLL (153), the diagnosis is now made with a count of greater than 5×10⁹/L and the presence of typical
immunophenotypic markers (1). The median lymphocyte count at diagnosis is 30×10⁹/L, and in most patients, there is a continuous increase in the lymphocyte count over time. In half the patients, it takes more than 12 months for the lymphocyte count to double (John P. Greer et al., 2003).

A leukemic cell seen in the figure (1.1) from CLL patient.

**Figure 1.1 CLL in blood smear** (Tkachuk, 2007)

Prognostic markers:

Cytogenetic:

The four most common chromosome abnormalities are deletion of 13q14, trisomy 12, deletions at 11q23 and structural abnormalities of 17p involving the p53 gene. These abnormalities carry prognostic significance. The 13q14 deletion prevents expression of microRNAs which control expression of proteins relevant to the CLL lifespan (p. 137). Expression of a novel gene CLLU.1 is substantially greater in unmutated than mutated cases (Hoffbrand et al., 2006).

1.2.1.1.8 Staging
Two staging systems are used for CLL: the Rai or modified Rai (Table 2) and the Binet system (Table 3). The modified Rai system is commonly known as Table 1.2 the Rai and modified Rai staging (Kern, 2002).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Rai Staging</th>
<th>Modified Rai Staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:</td>
<td>lymphocytosis only (blood Low risk Rai stage 0 and marrow)</td>
<td></td>
</tr>
<tr>
<td>I:</td>
<td>Lymphocytosis plus enlarged Nodes</td>
<td></td>
</tr>
<tr>
<td>II:</td>
<td>Lymphocytosis plus spleen and/or liver, ± nodes enlarged</td>
<td></td>
</tr>
<tr>
<td>III:</td>
<td>Lymphocytosis plus anemia (Hgb &lt;11 g/dL), ± above</td>
<td></td>
</tr>
<tr>
<td>IV:</td>
<td>Lymphocytosis plus thrombocytopenia (&lt;100 _ 10^9/L) ± above</td>
<td></td>
</tr>
</tbody>
</table>

Rai Staging: Modified Rai Staging

Low risk Rai stage 0
Intermediate risk Stages I and II
High risk Stages III and IV
The management of these cancers is adjusted to their natural course. Cure can rarely be achieved, and the median overall survival in most series is 8-10 years. Prognosis relates to age (poorer when older) and particularly to the extent of disease judged in terms of bulk and effect of tumor. The outlook for chronic leukemia worsens with increasing extent of disease at presentation and cytopenias (Binet stage B and C). (Provan, 2003).

1.2.1.1.9 Treatment:
Cures are rare in CLL and so the approach to therapy is conservative, aiming for symptom control rather than a normal blood. Indeed, chemotherapy given too early in the disease can shorten rather than prolong life expectancy. Many

Table 1.3 Binet Staging System for CLL (Kern, 2002).

<table>
<thead>
<tr>
<th>Stage</th>
<th>≥3 Lymphoid Sites</th>
<th>Hemoglobin &lt;10 g/dL</th>
<th>Platelets &lt;100,000/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>C</td>
<td>_/+</td>
<td>+</td>
<td>_</td>
</tr>
</tbody>
</table>

A = lowest stage; B = intermediate; C = highest.
_ = present; _ = absent;_/ _ = present or absent.
Patients never receive treatment; Treatment is given for troublesome organomegaly, hemolytic episodes and bone marrow suppression. The lymphocyte count alone is not a good guide to treatment. Usually, patients in Binet stage C will need treatment as will some in stage B (Hoffbrand et al., 2006).

1.2.1.10 Supportive care:

The main objective of treatment for CLL is to prolong survival with a good quality of life; therefore care should be taken to prevent and/or treat promptly any complications arising from the immunodeficiency associated with the disease or its treatment. Thus, prompt antibiotic treatment for seemingly benign upper respiratory tract infections and antiviral measures to prevent the spread of herpetic infections are as important as specific treatment for the disease. Patients with repeated infections and very low serum immunoglobulin levels may benefit from courses of intravenous gamma - globulin injections.

Erythropoietin is now an accepted treatment for anemia associated with cancer therapy (Hoffbrand et al., 2011).

1.2.2 Glutathione S Transferase Mu

Glutathione sulfur transferases (GSTs) are a group of enzymes involved in the detoxification process of carcinogens and other substances (Tsabouri et al., 2004).

Glutathione S-transferase M1 (GST M1) is an enzyme encoded by the GST M1 gene on chromosome band 1p13. GST M1 is able to detoxify various harmful oxidants, oxidative stress products, xenobiotics and carcinogens, all by catalyzing their conjugation to reduced glutathione (8, 9) (Yam Kau Poon et al., 2012).
Eight classes of GSTs have been identified, including alpha (GSTA), mu (GSTM), theta (GSTT), Pi (GSTP), zeta (GSTZ), sigma (GSTS), Kappa (GSTK) and omega (GSTO). *(Kassogue et al, 2015).*

In addition, these enzymes are also believed to play a crucial role in the protection of DNA from oxidative damage. The genes encoding the mu class of enzymes are organized in a gene cluster on chromosome 1p13.3 and are known to be highly polymorphic. These genetic variations can change an individual’s susceptibility to carcinogens and toxins as well as affect the toxicity and efficacy of certain drugs. GSTM1 products catalyze the conjugation of glutathione to oxide derivatives of polycyclic aromatic hydrocarbons. Polymorphisms in the GSTT1 and GSTM1 genes are caused by a deletion, which consequently results in virtual absence of enzyme activity, especially in individuals with deletion in both genes (null genotype). Many studies have demonstrated great concordance (> 95%) between the genotype and phenotype *(Syed et al, 2010).*
1.3 Previous studies:

A previous study done by (Tsabouri et al., 2004) to examine whether the GSTM1 and GSTT1 homozygous null genotypes altered the risk of CLL and they found a significantly increased incidence of the GSTM1 null genotype was found in the group of patients compared to the controls (74.07 versus 34.69%, P=0.0002). (Tsabouri et al., 2004).

Another study was done to examine whether polymorphic variation in GSTs confers susceptibility to chronic lymphocytic leukemia (CLL), GSTM1, GSTT1, and GSTP1 genotypes. They found that the frequency of both GSTM1 and GSTT1 null genotypes and the GSTP1-Ile allele was higher in cases than in controls and there was evidence of a trend in increasing risk with the number of putative “high-risk” alleles of the GST family carried (P=0.04). The risk of CLL associated with possession of all 3 “high-risk” genotypes was increased 2.8-fold (OR = 2.8, 95% confidence interval: 1.1-6.9), the findings suggest that heritable GST status may influence the risk of developing CLL (Yuille et al., 2002).

Other studies done to examine whether null genotypes of GSTM1 and GSTT1 confer susceptibility to chronic myeloid leukemia (CML), they found that heritable GST status may influence the risk of developing CML (Bajpai et al., 2007).

The study of (Syed et al., 2010) was designed to assess the GSTM1 gene variant in patients with coronary artery disease (CAD), they found that there was no significant association of GSTM1 gene (null) polymorphism (χ² =
3.72, P = 0.053, OR = 1.17, 95% CI = 0.95 – 3.07) with CAD, (Syed et al., 2010).

Chapter two
Rationale and objectives

2.1 Rationale:
Chronic lymphocytic leukemia has increased prevalence in Sudan according to radiation and isotype center records, and many studies showed association between GSTs gene polymorphisms specifically (GSTT1 and GSTM1) and many types of cancer. The frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1 have been reported in various cancers and there are several studies have been published on the relationship between GSTM1 null polymorphism and various types of cancers. To the best of our knowledge, there are no published reports about the association between GSTM1 null polymorphism and CLL patients in Khartoum State, so this study may fill the gap regarding this polymorphism and its association with Sudanese CLL patients attended to radiation and isotype center Khartoum.
2.2 objectives

2.2.1 General objectives:
To study the association between GSTM1 null gene polymorphism and newly diagnosed patients of chronic lymphocytic leukemia in Khartoum state, Sudan.

2.2.2 Specific objectives:
- To determine the frequency of GSTM1 null genotype among firstly diagnosed Sudanese patients of chronic lymphocytic leukemia.
- To correlate the presence of this polymorphism with patient's hematological parameter.
- To examine the GSTM1 null polymorphism as risk factor for chronic lymphocytic leukemia.
Chapter Three
Materials and Methods

3.1 Study Design
It was prospective case control study conducted in central research laboratory, Khartoum State in the period from February 2016 to January 2017.

3.2 Study Population
Forty chronic lymphoblastic leukemia patients referred to RISK and thirty apparently healthy volunteers were enrolled as a control group.

3.3 Inclusion Criteria
Newly diagnosed Patients with chronic lymphoblastic leukemia.

3.4 Exclusion Criteria
Patients of chronic lymphoblastic leukemia which have take therapy for this disease.

3.5 Ethical Consideration
Approval was taken from selected patients after told about details of the study.

3.6 Data Collection
A questionnaire was filled for each of the patients (see appendix)

3.7 Data Presentation
The data were presented in tables and figures.

3.8 Blood collection
Three milliliter of venous blood sample was collected in plastic container containing EDTA anticoagulant from each participant using a sterile disposable syringe and applying a standard septic non-traumatic vein puncture
technique for CBC and preserved at -20°C for DNA extraction.

3.9 Methodology

3.9.1 Hematological parameters:
Complete blood count was done using automated hematology analyzer sysmex KX-21N from whole blood sample.

3.9.2 DNA Extraction by salting out method:
Procedure:
It was extracted by Saturated sodium chloride method, 300 µl of EDTA-blood sample was placed in 1.5 Eppendorf's tube, 1000 µl red cell lysis buffer was added, mixed well and was centrifuged at 2500 r.p.m for 5 minutes, Supernatant was discarded and the pellet (WBC) washed again with 1000 µl of RCLB (repeated 3 times), 300 µl of white cell lysis buffer, 10 µl of 10% SDS, and 10 µl proteinase K was added to the clear white pellets for break the nuclear membrane and denaturation of proteins, the mixture was incubated for 1 hour at 65°C, 100 µl of 6M NaCl was added to precipitate proteins and mixed well by vortexing. (NaCl allows DNA molecules aggregate instead of repelling each other, making it easier for DNA to precipitate out of solution when alcohol is added), 200 µl of ice cold chloroform was added to the tube (to precipitate the protein down and the DNA remain in the aqueous layer) then centrifuged at full speed (11000rpm) for 6 minutes, the aqueous phase was transferred carefully to clean Ependorf tube, and to which double volume of cold absolute ethanol was added to precipitate the DNA, the tube was centrifuged at 11000rpm for 5 minutes, the supernatant was poured off without disturbing the precipitate, and then washed with 600 µl 70% ethanol (Rehydration), the tube content was centrifuged at 6000rpm for 5 minutes, the ethanol was discarded and the tube is left to air dry, the pellets was
resuspended in 100 µl TE (buffer EDTA chelates any Mg2+ helping to inactivate DNases) and leaved to dissolve overnight.

3.9.3 Determination of DNA quality and purity
Part of the DNA solution was mixed with loading dye 1 in 5 and DNA quality and purity was determined using gel electrophoresis.

3.9.4 DNA Storage
DNA was preserved at -20ºC until PCR was performed.

3.9.5 Molecular Analysis:
3.9.5.1 Detection of GSTMI null genotype
All patients with CLL were screened for the presence of GSTMI null genotype using Allele specific PCR, the primers sequence used were as follow:

**Table 3.1 The primers sequence**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>Product Size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GSTM1</td>
</tr>
<tr>
<td>GSTM 1 Forward</td>
<td>5'-GACTCCCTGAAAAGCTAAAGC-3'</td>
<td></td>
</tr>
<tr>
<td>GSTM 1 Reverse</td>
<td>5'-GTTGG-GCTAAATATACGGTG-3'</td>
<td>215</td>
</tr>
</tbody>
</table>

PCR mixture of 20µ was prepared using premix master mix tube (maxime PCR premix kit {i-Tag™}) for each sample, in sterile Ependof tube as follow:

**Table 3.2 PCR mixture content:-**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>13 µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1µl</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1µl</td>
</tr>
</tbody>
</table>
DNA  
5 µl  

Total volume  
20 µl  

The PCR protocol as follow :-

### Table 3.3 The PCR protocol

<table>
<thead>
<tr>
<th>Profile</th>
<th>Temperature</th>
<th>Time duration</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94º C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95º C</td>
<td>1 minutes</td>
<td>35</td>
</tr>
<tr>
<td>Annealing</td>
<td>62 ºC</td>
<td>1 minutes</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72º C</td>
<td>1 minutes</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72º C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
</tbody>
</table>

#### 3.9.5.2 Detection of PCR product:-

Detection of the product done by gel electrophoresis by using 2% agarose gel which stained by ethedium bromide and 1X TBE buffer used as running buffer, 5 µl of the product was applied into the gel, the voltage was 100 volt with time of 30 minute and DNA ladder (50_100 bp) was used as molecular weight marker

#### 3.10 Interpretation of result:

The GSTM1 normal genotype will give band (219 bp) while GSTM1 null give no band.

#### 3.11 Data Analysis

Data was analyzed by using spssprogram.
4.1 Demographic data:
Study included 70 samples 40 of them were cases and 30 of them were control. The results showed that $28/40$ (70%) of the samples were males and $12/40$ (30%) were females.

Figure (4.1) Gender distribution among the samples
The results showed that 6/40 (15%) of the cases were GSTM1 null genotype and 34/40 (85%) of them were normal GSTM1 genotype.
The result showed that 17/30 (56.6%) of the control were GSTM1 null and 13/30 (43.4%) are normal GSTM1 genotype.

**Table 4.1 GSTM1 genotype among case and control subjects**

<table>
<thead>
<tr>
<th>Case and Control</th>
<th>Case</th>
<th>Count</th>
<th>GSTM1 Normal genotype</th>
<th>GSTM1 Null genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>34</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>85%</td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>Control</td>
<td>Count</td>
<td>13</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>43.4%</td>
<td></td>
<td>56.6</td>
</tr>
</tbody>
</table>
Figure 4.2 GSTM1 genotype among case and control subjects

4.2 Comparisons of hematological findings in GSTM1 null group and normal GSTM1 group

Mean of TWBCs counts was not statistically significantly ($p$-value=0.767) lower in patients with normal genotype (mean ±SD: 71.338 ± 57.91) compared to those with null genotype (mean ±SD: 63.85 ± 50.73).
Mean of Hb level was not statistically significantly \( (p.value=0.989) \) higher in patients with normal genotype (mean±SD: 10.265 ± 2.53) compared to those with null genotype (mean ±SD: 10.25 ± 1.86).

Mean of PCV was not statistically significantly \( (p.value=0.451) \) higher in patients with normal genotype (mean ±SD: 34.80 ±9.804) compared to those with null genotype (mean ±SD: 31.67 ± 4.676).

Mean of MCV was not statistically significantly \( (p.value=0.224) \) higher in patients with normal genotype (mean±SD: 90.24 ±9.046) compared to those with null genotype (mean ±SD: 85.17 ± 10.629).

Mean of MCH was not statistically significantly \( (p.value=0.111) \) higher in patients with normal genotype (mean ±SD: 28.37 ±3.344) compared to those with null genotype (mean ±SD: 26.00±2.828).

Mean of MCHC was not statistically significantly \( (p.value=0.790) \) higher in patients with normal genotype (mean±SD: 30.89±1.998) compared to those with null genotype (mean ±SD: 30.67 ±1.211).

Mean of TRBCs count was not statistically significantly \( (p.value=0.839) \) lower in patients with normal genotype (mean ± SD: 3.6941±1.00301) compared to those with null genotype (mean ± SD: 3.7833 ±0.85186).

Mean of blast percentage was not statistically significantly \( (p.value=0.680) \) higher in patients with normal genotype (mean ± SD: 0.12 ± 0.686) compared to those with null genotype (mean ± SD: 0.00 ±0.0).

Mean of platelets counts was not statistically significant \( (p.value=0.369) \) lower in patients with normal genotype (mean ±SD: 199.44 ± 64.193) compared to those with null genotype (mean ± SD: 202.67± 157.073).
Mean of lymphocyte percentage was not statistically significantly ($p$.value$=0.198$) higher in patients with normal genotype (mean ± SD: 77.88 ± 9.716) compared to those with null genotype (mean ± SD: 72.50 ±5.648).
Mean of neutrophil percentage was not statistically significantly ($p$.value$=0.066$) lower in patients with normal genotype (mean ± SD: 17.12 ± 7.244) compared to those with null genotype (mean ± SD: 23.00±5.404).
Mean of monocyte percentage was not statistically significantly ($p$.value$=0.587$) higher in patients with normal genotype (mean ± SD: 4.06 ±3.892 ) compared to those with null genotype (mean ± SD: 3.17 ±1.722).
Mean of eosinophil percentage was not statistically significantly ($p$.value$=0.764$) lower in patients with normal genotype (mean ± SD: 1.15 ±1.417) compared to those with null genotype (mean ± SD: 1.33 ±1.211).
**Table 4.3 Comparisons of hematological findings in GSTM1 null group and normal GSTM1 group**

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>GSTM1 genotype</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBCs count</td>
<td>Normal</td>
<td>71.388</td>
<td>±7.9125</td>
<td>0.767</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>63.850</td>
<td>±50.7287</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>Normal</td>
<td>90.24</td>
<td>±9.046</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>85.17</td>
<td>±10.629</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>Normal</td>
<td>30.89</td>
<td>±1.998</td>
<td>0.790</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>30.67</td>
<td>±1.211</td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>Normal</td>
<td>10.265</td>
<td>±2.5311</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>10.250</td>
<td>±1.8641</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>Normal</td>
<td>28.37</td>
<td>±3.344</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>26.00</td>
<td>±2.828</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte%</td>
<td>Normal</td>
<td>77.88</td>
<td>±9.716</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>72.50</td>
<td>±5.648</td>
<td></td>
</tr>
<tr>
<td>PCV</td>
<td>Normal</td>
<td>34.80</td>
<td>±9.804</td>
<td>0.451</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>31.67</td>
<td>±4.676</td>
<td></td>
</tr>
<tr>
<td>Neutrophil%</td>
<td>Normal</td>
<td>17.12</td>
<td>±7.244</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>23.00</td>
<td>±5.404</td>
<td></td>
</tr>
<tr>
<td>TRBCs count</td>
<td>Normal</td>
<td>3.6941</td>
<td>±1.00301</td>
<td>0.839</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>3.7833</td>
<td>±0.85186</td>
<td></td>
</tr>
<tr>
<td>Blast%</td>
<td>Normal</td>
<td>.12</td>
<td>±.686</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>.00</td>
<td>±.000</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Normal</td>
<td>169.44</td>
<td>±64.193</td>
<td>0.369</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>202.67</td>
<td>±157.073</td>
<td></td>
</tr>
<tr>
<td>Eosinophil%</td>
<td>Normal</td>
<td>1.15</td>
<td>±1.417</td>
<td>0.764</td>
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<tr>
<td></td>
<td>Null genotype</td>
<td>1.33</td>
<td>±1.211</td>
<td></td>
</tr>
<tr>
<td>Monocyte%</td>
<td>Normal</td>
<td>4.06</td>
<td>±3.892</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>Null genotype</td>
<td>3.17²⁴</td>
<td>±1.722</td>
<td></td>
</tr>
</tbody>
</table>
4.3 Correlation between CLL and GSTM1 null polymorphism

The result showed that GSTM1 null genotype was not a risk factor for CLL and there is a statistically significant correlation between GSTM1 null polymorphism and CLL (OR: 0.3137, P=0.0293)

4.4 Correlation between gender and GSTM1 polymorphism

The results showed that no statistically significant correlation between gender and GSTM1 polymorphism (p.value =0.440).

Table (4.4) Correlation between gender and GSTM1 polymorphism

<table>
<thead>
<tr>
<th>Gender</th>
<th>GSTM1</th>
<th>Total</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Null genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (12.5%)</td>
<td>23 (57.5%)</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (2.5%)</td>
<td>11 (27.5%)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (15%)</td>
<td>34 (85%)</td>
<td>40 (100%)</td>
</tr>
</tbody>
</table>
Chapter Five
Discussion, Conclusion and Recommendations

5.1 Discussion
This case control study was conducted to determine the frequency of GSTM1 null genotype among Sudanese firstly diagnosed patients with CLL and to examine the association between GSTM1 polymorphism and risk of CLL.

In this study the result showed that the GSTM1 null genotype was not risk factors for CLL (OR =0.3137, P=0.0293) and this findings disagreed with study of Tsabouri et al (2004) to examine whether the GSTM1 and GSTT1 homozygous null genotypes altered the risk of CLL and they found a significantly increased incidence of the GSTM1 null genotype was found in the group of patients compared to the controls (74.07 versus 34.69%, P=0.0002).

Also our findings disagreed with study which was done by Martin Yuille et al to examine whether polymorphic variation in GSTs Confers susceptibility to chronic lymphocytic leukemia (CLL) and they found that the risk of CLL associated with possession of all 3 “high-risk” genotypes was increased 2.8-fold (OR = 2.8, 95% confidence interval: 1.1-6.9).

The mean of blasts percentage in GSTM1 null cases was insignificantly higher than in normal GSTM1 (P.value =0.680). The mean of TWBCs count was lower among patients with GSTM1 null genotype than those with normal GSTM1 genotype and the difference was statistically insignificant (P.value =0.767). The mean of Hb was higher among patients with GSTM1 null genotype than those with GSTM1 normal genotype and the difference was not statistically significant (p.value =0.989). The mean of PCV in GSTM1 null cases was insignificantly higher than in normal GSTM1 (P.value = 0.451).
The mean of MCV was higher among patients with GSTM1 null genotype than those with normal GSTM1 genotype and the difference was statistically insignificant \((P . value = 0.224)\). The mean of MCH was higher among patients with GSTM1 null genotype than those with GSTM1 normal genotype and the difference was not statistically significant \((p . value = 0.111)\). The mean of MCHC in GSTM1 null cases was insignificantly higher than in normal GSTM1 \((P . value = 0.790)\). The mean of lymphocyte and monocyte percentage was higher among patients with GSTM1 null genotype than those with normal GSTM1 genotype and the difference was not statistically insignificant. The mean of neutrophil and eosinophil percentage was lower among patients with GSTM1 null genotype than those with GSTM1 normal genotype and the difference was not statistically significant. The mean of platelets in GSTM1 null cases was insignificantly lower than in normal GSTM1 \((P . value = 0.369)\). There is insignificant association between GSTT1 polymorphism and gender \((P . value = 0.440)\) and this findings is in agreement with previous study done by Yaya Kassoue et al who reported that there were no significant association between GSTM1 and chronic myeloid leukemia in Morocco \((p . value = 0.13)\).
5.2 Conclusions

- GSTM1 null genotype is not a risk factor for CLL and there is statistically insignificant association between GSTM1 null polymorphism and CLL.
- GSTM1 null genotype is associated with statistically insignificant higher blast percentage, Hb, PCV, MCV, MCH, MCHC, lymphocyte percentage and monocyte percentage. And statistically insignificant lower TWBC count, platelets count, neutrophil percentage and eosinophil percentage.
- GSTM1 null genotype is not correlated with patient’s gender.
5.3 Recommendations

- Another study should be conducted to evaluate the impact of Null genotype on patient prognosis.
- Another study must be conducted with more information about subtype and variants of CLL in each patient.
References


**Yam Kau Poon Peter, Cheuk-Chun Szeto, Bonnie Ching-Ha Kwan, Kai-Ming Chow, Philip Kam-Tao Li, (2012).** Relationship between glutathione S-transferase M1 polymorphism and clinical outcomes in Chinese peritoneal dialysis patients. *JNEPHROL*; 25(03) 310-316.
Appendixes

Appendix 1:

Questionnaire

ID: ............................................................

Gender: ...........................................................

Do you have take therapy: Yes( ) No( )

Hematological parameters:

HB:................................. PCV:..............................

TRBCs:......................... MCV:..............................

MCH:......................... MCHC:..............................

TWBCs:......................... Lymphocyte %:..................

Neutrophil %:.................. Monocyte %:..................

Eosinophil %:.................. Blast %:......................

Platelets:......................

GSTM1 genotype: Normal () Null( )
Appendix 2:

Reagent

Red Cell Lysis Buffer (RCLB):
Add 8.3 gm of NH4Cl, 1gm KHCO3, 1.8 ml 5% EDTA and 1 liter of distilled water.

White Cell Lysis Buffer (WCLB):
1.576 gm Tris-HCL, 1.088 gm EDTA, 0.0292 gm NaCl, 0.2% SDS, and 100 ml distilled water.

TE buffer:
2.42 Tris base, 0.57 ml acetic acid, 50 μl EDTA (0.01 M), and 100 ml distilled water.

6 M NaCl:
35 gm of NaCl added to 1 Liter of distilled water
Appendix 3: Images

PCR machine = ESCO
Gel electrophoresis documentation system: Biometra, Germany
Gel electrophoresis: analytik jena, Germany.
PCR result detected by gel electrophoresis