Chapter one

Introduction and literature review

1.1introduction

Leukemia's are group of disorders characterizedby the accumulation of themalignant white cells inthe bone marrow and blood. These abnormal cellscause 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 *etal*, 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 *etal*,2012).

1.2 Literaturereview

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 andchronic leukemia, which are further subdividedinto:Lymphoid or myeloid, as we see in the table (1.1)

Table 1.1 Classification of leukemia(hoffbrand *etal*,2006)

Classification of leukemia's
Acute:
Acute myeloidleukemia :Mo-M7
Acute lymphoid leukemia: L1 -L3
Chronic:
Chronic myeloid leukemia
Chronic lymphoid leukemia

1.2.1.1Chronic 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 matureB lymphocytes and which involve primarily the blood, bonemarrow and other lymphoid organs such as the lymph nodesand spleen.All these disorders are classified by the world health organization (WHO) , On the basis oftheir histopathological features.

Their clinical course is oftenchronic and they affect mainly adults, A constant Finding in allthese entities is the presence in peripheral blood of leukemiccells in various degrees. (Hoffbrand*etal*, 2011).

The world health organization (WHO) classification oftumors of the Hematopoietic and Lymphoid Tissues has enhanced the classification of lymphoidneoplasm by including immunophenotypic features andgenetic 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 *etal*,2011).

CLL is a neoplasms composed of smallB lymphocytes in the peripheral blood, bone marrow,spleen, and lymph nodes, mixed with prolymphocytes andparaimmunoblasts forming proliferation centers in tissueinfiltrates.(A Victor Hoffbrand *etal*, 2011).

1.2.1.1.2Epidemiology:

CLL isthe most common form of leukemia in adults in Westerncountries 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 leukemiais rare before age 20 and uncommon before age 50. But it isnow diagnosed more often in younger persons,more malesthan females (1.5 to 2.1:1) are affected by the disorder.CLL has the highest genetic predisposition of all hematologicneoplasms, A family predisposition can be documentedin 5% to 10% of patients with CLL. The overall risk is two to seven times greater in first-degree relatives of CLL patients.(Hoffbrand *etal*, 2011).

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

1.2.1.1.3Etiologyand pathology:

The cause of CLL is unknown.It is not certainat what stage in lymphocyte maturation the CLL cell arises, since roughly equal numbers seem to comefrom a pre- and post-germinal center B-lymphocyte.Diseases originating at these two stagesof maturation have vastly different prognoses.However,gene expression profiling suggests that bothsubtypes are

similar, differing from each other byrelatively few expressed genes(Saba, Mufti, 2011).

The immunologic deficiency in CLL is complex, Hypogammaglobulinemia Is common; therefore, patients are predisposed to infections withencapsulated organisms such as *Streptococcus pneumoniae*.There are alsoabnormalities in T-cell numbers and function, including a Decreased ratioof T helper to T suppressor (CD4/CD8) cells and impaired Cell-mediatedimmunity. 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 nowrecognized 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 aggressivelymphoma occurring in a patient with CLL maybe called Richter's syndrome (Hussain I. Saba, Ghulam J. Mufti, 2011).

1.2.1.1.4Clinical 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 mostcommon abnormality is lymphadenopathy, which may be localized or generalized.The lymph nodes are usually slightly to moderately enlarged, firmor rubbery, nontender, and freely movable. Mild or moderate hepatosplenomegalymay be present. (Kern,2002)

1.2.1.1.5The Laboratory Features of Chronic LymphocyticLeukemia:

The features vary quite a lot with various stages of thedisease and with the presence of complications.The early phase:- The patient may presentonly with a mild chronic lymphocytosis. Atypical lymphocytesmay be present but not primitive ones. Inthis stage, the patient may not have any lymphocytosisor splenomegaly – in fact there may well be nopositive features.The established phase:- This is the typicalappearance, with high absolute lymphocyte count,Sometimes showing atypical features. However, themore progressive and aggressive variants may showcells of increasing primitiveness, anemia, and thrombocytopenia,These features are often accompanied byclinical features of weight loss, malaise, and weakness.These findings have significant implications for prognosisand therapy.(Beck,2009).

1.2.1.1.6 Complications:

The most important of these isthe Richter syndrome, which is a form of acute Transformation. The leukemic phase of a low-grade lymphoma is much less common, but otherwisehas much the same features. However, there are rareother lymphoproliferative disorders causing a lymphocytosisand their distinction can be very difficult – and thedistinction is important, since the malignant status ofsome of these is open to question,In all these cases, bone marrow aspiration, biopsy,cytogenetic, and immunophenotyping are essentialinvestigations(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. (Hoffman*et al*, 2000).

Peripheral Blood:

Although a persistent lymphocyte count of greater than 10×10^9/L was originallyrequired for the diagnosis of CLL (153), the diagnosis is now made with a count of greaterthan 5×10^9/L and the presence of typical

immunophenotypic markers (1). The medianlymphocyte count at diagnosis is 30×10^9/L, and in most patients, there is a continuousincrease in the lymphocyte count over time In half the patients, it takes morethan 12 months for the lymphocyte count to the double (John P. Greer et al, 2003).

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

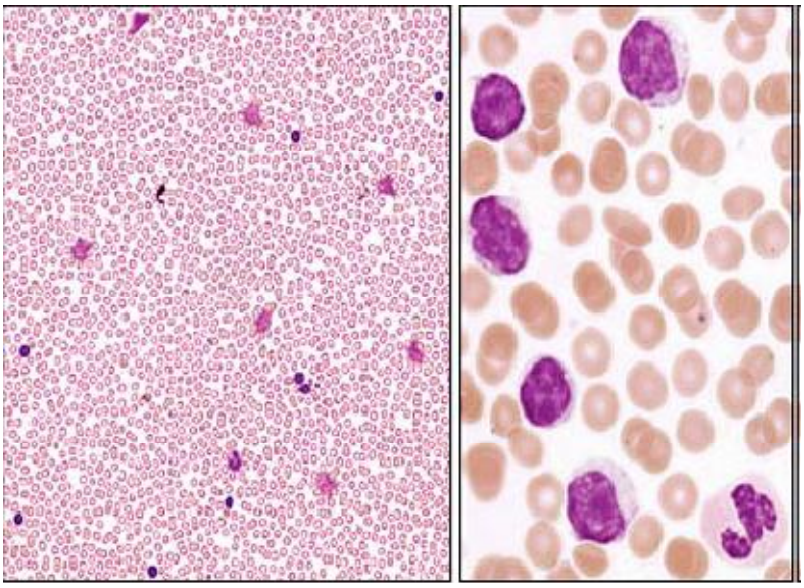

Figure 1.1CLL in blood smear(Tkachuk,2007)

Prognostic markers:

Cytogenetic*:*

The four most common chromosomeabnormalities are deletion of 13q14, trisomy 12,deletions at llq23 and structural abnormalities of 17p involving the p53 gene.These abnormalitiescarry prognostic significance. The13q14 deletion prevents expression of microRNAswhich control expression of proteins relevant to theCLL lifespan (p. 137). Expression of a novel gene CLLU.l is substantially greater in unmutated thanmutated cases(hoffbrand *etal*,2006).

1.2.1.1.8Staging

Two staging systems are used for CLL: the Rai or modified Rai(Table 2) And the Binetsystem(Table 3). The modified Rai system is commonly

Table 1.2 the Rai and modified Rai staging(Kern, 2002).

Rai Staging		Modified Rai Staging	
Stage 0:	lymphocytosis only (blood Low risk Rai stage 0 and marrow	Low risk	Rai stage 0
Stage I:	Lymphocytosis plus enlarged Nodes		
Stage II:	Lymphocytosis plus spleen and/or liver, ± nodes enlarged	Intermediate risk	Stages I and II
Stage III:	Lymphocytosis plus anemia (Hgb <11 g/dL), ± above		
Stage IV:	Lymphocytosis plus throm-bocytopenia (<100 _ 109/L) ± above	High risk	Stages III and IV

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

Stage	≥3 Lymphoid Sites	Hemoglobin <10 g/dL +/or Platelets <100,000/_L **A _**
A	_	_
B	+	_
C	_\ +	+

A = lowest stage; B = intermediate; C = highest.

_ = present; _= absent; _/_= present or absent.

The management of these cancers is adjusted to theirnatural course. Cure can rarely be achieved, and the medianoverall survival in most series is 8-10 years. Prognosis relates toage (poorer when older) and particularly to the extent ofdisease judged in terms of bulk and effect of tumor.Theoutlook for chronic leukemia worsens with increasing extentof disease at presentation and cytopenias (Binet stage B and C). (Provan,2003).

1.2.1.1.9Treatment:

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

Patients never receive treatment;Treatment isgiven for troublesome organomegaly, hemolyticepisodes and bone marrow suppression.

The lymphocytecount alone is not a good guide to treatment.Usually, patients in Binet stage C will needtreatment as will some in stage B (hoffbrand *etal,*2006).

1.2.1.1.10Supportive care:

The main objective of treatment for CLL is to prolong survivalwith 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 benignupper respiratory tract infections and antiviral measures toprevent the spread of herpetic infections are as important as specific treatment for the disease. Patients with repeated infectionsand very low serum immunoglobulin levels may benefit from courses of intravenous gamma - globulin injections,

Erythropoietin is now an accepted treatment for anemiaassociated with cancer therapy (Hoffbrand *etal,*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 *etal*, 2004).

Glutathione S-transferase M1 (GST M1) is anenzyme encoded by the *GST M1* gene on chromosome band1p13. 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)(YamKau Poon *etal,*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 alsobelieved to play a crucial role in the protection of DNAfrom oxidative damage. The genes encoding the muclass of enzymes are organized in a gene cluster on chromosome 1p13.3 and are known to be highlypolymorphic.These genetic variations can change an individual's susceptibility to carcinogens and toxins aswell as affect the toxicity and efficacy of certain drugs.GSTM1 products catalyze the conjugation of glutathioneto oxide derivatives of polycyclic aromatic hydrocarbons.Polymorphisms inthe 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 demonstratedgreat concordance (> 95%) between the genotype andPhenotype (Syed *etal*,2010).

1.3Previous studies:

Aprevious study done by (Tsabouri *etal*, 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 *etal*,2004).

Another study wasdone to examinewhether polymorphic variation in GSTs Confers susceptibility to chroniclymphocytic leukemia (CLL), GSTM1,GSTT1, andGSTP1 genotypes, They found that the frequency of both GSTM1and GSTT1nullgenotypes and the GSTP1-Ile allele washigher in cases than in controls and There wasevidence of a trend in increasing risk withthe number of putative "high-risk" alleles ofthe GST family carried (P =0.04). The risk ofCLL 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 heritableGST status may influence the risk of developingCLL (Yuille *et al*, 2002).

Other study 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 *etal*,2010) was designed to assess the GSTM1 gene variant in patients with coronary artery disease (CAD) they that There was no significant association of GSTM1 gene (null) polymorphism (_^{2} =

3.72, P = 0.053, OR = 1.17, 95% CI = 0.95 – 3.07) with CAD, (Syed *etal*,2010).

Chapter two

Rationale and objectives

2.1Rationale:

Chronic lymphocytic leukemia has increase prevalence in Sudan according toradiation and isoype center records , and many studies showed association betweenGSTs gene polymorphismspecially (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 gab regarding this polymorphism and its association with Sudanese CLL patients attended to radiation and isoype center Khartoum.

2.2 objectives

2.2.1 General objectives:

To study the association between GSTM1 null gene polymorphism and newely 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 withchronic lymphoblastic leukemia.

3.4 Exclusion Criteria

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

3.5 Ethical Consideration

Approvalwas 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 -20ºC 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 bySaturated 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 65c^{o},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 6minutes,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 5minutes, the supernatant was poured off without disturbing the precipitate, and then washed with 600 µl 70% ethanol (Rehydration),t he 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.

3.9.3Determination 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

Primer Name	**Primer Sequence**	**Product Size bp**	
		GSTM1	**GSTM1 Null**
GSTM 1Forward	5'-GAACTCCCTGAAAAGCTAAAGC-3'	215	Absence
GSTM 1 Reverse	5'-GTTGG-GCTCAAATATACGGTGG-3'		

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

Table 3.2 PCR mixture content:-

Reagent	Volume
Distilled water	13 µl
Forward primer	1µl
Reverse primer	1µl

DNA	5 µl
Total volume	20 µl

The PCR protocol as follow :-

Table 3.3 The PCR protocol

Profile	Temperature	Time duration	Number ofCycles
Initial Denaturation	94º C	5 minutes	1
Denaturation	95º C	1 minutes	35
Annealing	62 ºC	1 minutes	
Extension	72º C	1 minutes	
Final Extension	72º C	10 minutes	1

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.

Chapter four

The results

4.1 Demographic data:

Study included 70 samples 40 of them werecases and 30 of them were control. The results showed that 28/40 (70%) of the samples were males and 12/40 (30%) were females

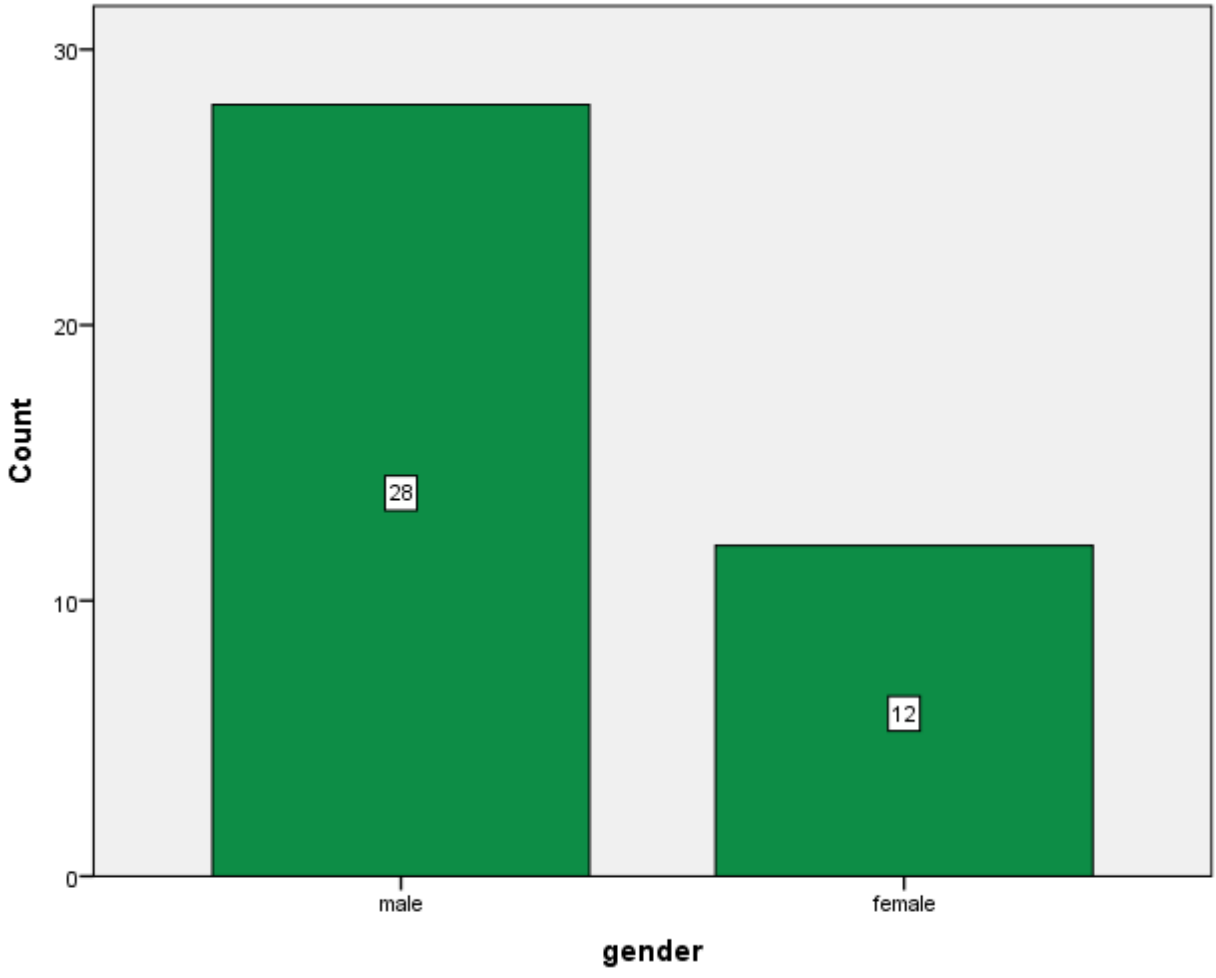

Figure (4.1) Gender distribution among the samples

The results showed that 6/40(15%) of the cases were GSTM1 null genotype and 34/40 (85%) of them were normal GSTM1genotype

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

			GSTM1 Normal genotype	GSTM1 Null genotype
Case and Control	Case	Count	34	6
		Percent	85%	15%
	Control	Count	13	17
		Percent	43.4%	56.6

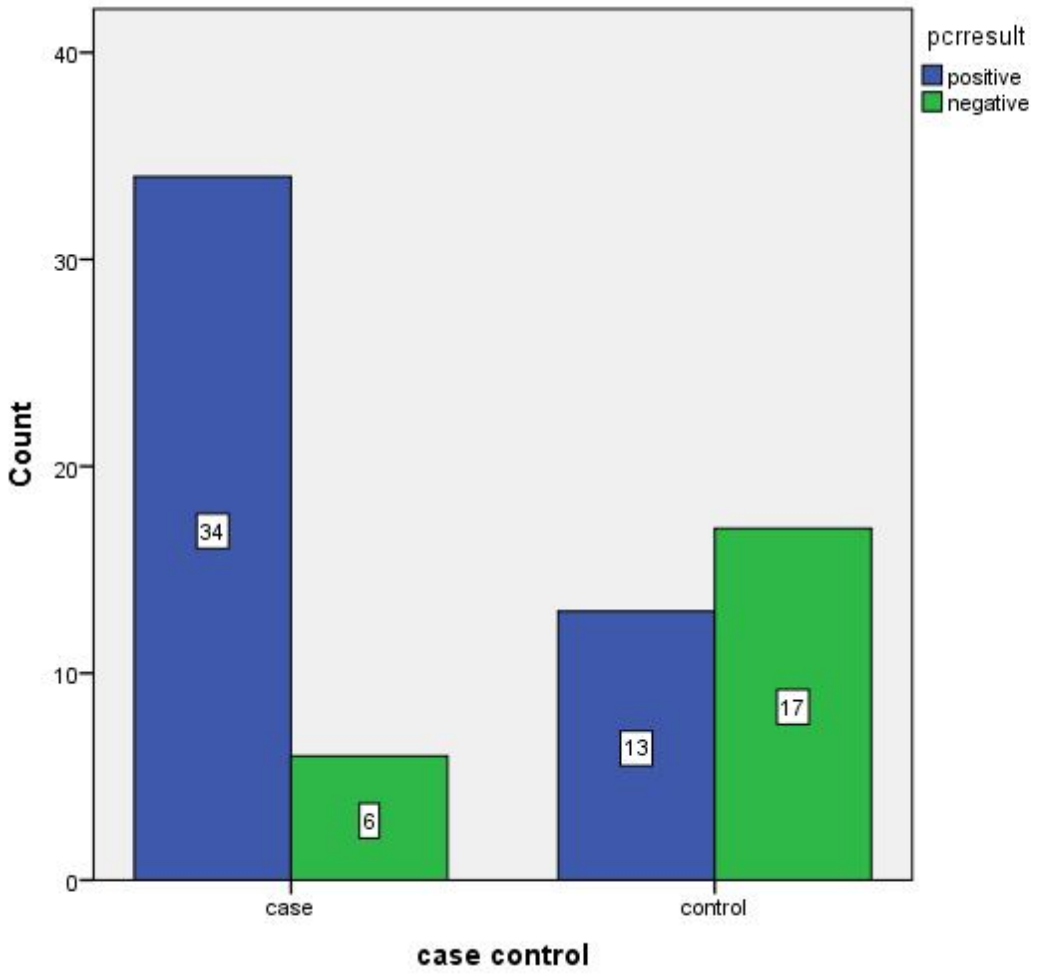

Figure 4.2 GSTM1 genotype among case and control subjects

4.2Comparisons 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

Hematological parameters	GSTM1 genotype	Mean	Std. Deviation	P value
TWBCs count	Normal	71.388	±7.9125	0.767
	Null genotype	63.850	±50.7287	
MCV	Normal	90.24	±9.046	0.224
	Null genotype	85.17	±10.629	
MCHC	Normal	30.89	±1.998	0.790
	Null genotype	30.67	±1.211	
Hb	Normal	10.265	±2.5311	0.989
	Null genotype	10.250	±1.8641	
MCH	Normal	28.37	±3.344	0.111
	Negative	26.00	±2.828	
Lymphocyte%	Normal	77.88	±9.716	0.198
	Null genotype	72.50	±5.648	
PCV	Normal	34.80	±9.804	0.451
	Null genotype	31.67	±4.676	
Neutrophil%	Normal	17.12	±7.244	0.066
	Null genotype	23.00	±5.404	
TRBCs count	Normal	3.6941	±1.00301	0.839
	Null genotype	3.7833	±.85186	
Blast%	Normal	.12	±.686	0.680
	Null genotype	.00	±.000	
Platelets	Normal	169.44	±64.193	0.369
	Null genotype	202.67	±157.073	
Eosinophil%	Normal	1.15	±1.417	0.764
	Null genotype	1.33	±1.211	
Monocyte%	Normal	4.06	±3.892	0.587
	Null genotype	3.17	±1.722	

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

Gender	GSTM1		Total	*p.value*
	Null genotype	Normal		
Male	5 (12.5%)	23 (57.5%)	28 (70%)	o.440
Female	1 (2.5%)	11 (27.5%)	12 (30%)	
Total	6 (15%)	34 (85%)	40 (100%)	

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 thr result showed that the GSTM1 null genotype was not risk factors for CLL (OR =0.3137, P=0.0293) and this findings disagreedwith 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 findingsdisagreed withstudy 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 wasstatistically 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 statisticallyinsignificant (*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 monocytepercentage 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.v*alue =0.440) and this findings is in agreement with previous study done by yaya Kassogue *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 momocyte percentage. And statistically insignificant lower TWBC count, plateletscount, neutrophil percentage and eosinophil percentage.
- GSTM1 null genotypeis notcorrelatedwith 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 variantsofCLL in each patient.

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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 1liter 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:

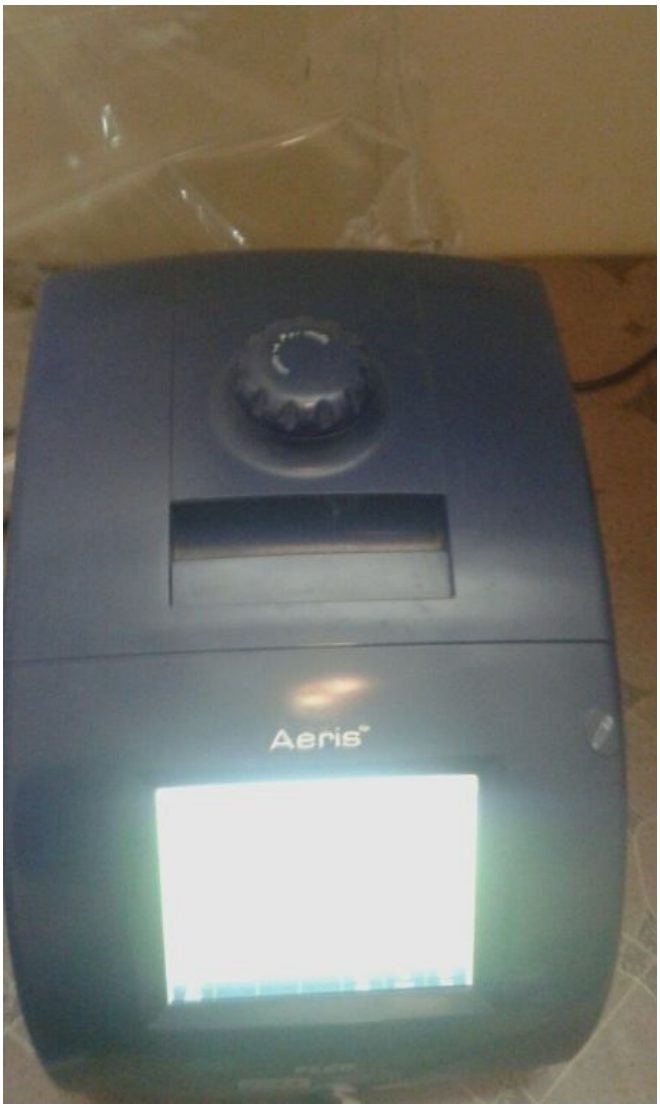
2.42 Tris base, 0.57 ml acetic acid, 50µl EDTA (0.01 M), and 100 ml distilled water.

6 M NaCl:

35 gm of NaCl added to 1 Liter of distilled water

Appendix 3: Images

PCR machine = ESCO



Gel electrophoresisdocumentation system : **Biometra , Germany**

Gel electrophoresis : analytik jena ,Germany .

PCR result detected by gel electrophoriesis