Chapter One
Introduction and Literature Review

1.1 Introduction
Leukemia is a disease resulting from the neoplastic proliferation of hemopoietic or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which form a clone of leukemic cells. Often there is a series of genetic alterations rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of tumors suppressor genes. The cell in which the leukemic transformation occurs may be a lymphoid precursor, a myeloid precursor or a pluripotent stem cell capable of differentiating into both myeloid and lymphoid cells. Genetic alterations leading to leukaemic transformation often result from major alterations in the chromosomes of a cell, which can be detected by microscopic examination of cells in mitosis (Bain, 2003).

GSTs are crucially involved in phase II metabolism, catalyzing the conjugation of soluble glutathione with reactive intermediates produced during the bioactivation of procarcinogens and detoxification of carcinogens. The GST isoenzymes expressed in human tissues comprise the alpha, mu, pi, theta, kappa, sigma, zeta and omega gene families. The glutathione-S-transferase (GST) genes are crucially involved in the de-toxification of a variety of exogenous carcinogens. The play a critical role by protecting against the reactive oxygen species ROS caused by the break-down of the peroxidased lipids and they are capable of oxidizing DNA and generating damage. It is believed that the polymorphisms in the
GST genes may play a role in the susceptibility to leukemogenesis (Cuven et al., 2015).
1.2 Literature Review
Acute leukemia is a clonal (that is, derived from a single cell) malignant disorder affecting all age groups. It is characterized by the accumulation of immature blast cells in the bone marrow, which replace normal marrow tissue, including haemopoietic precursor cells. This results in bone marrow failure, reflected by peripheral blood cytopenias and circulating blast cells. Infiltration of various organs is also a feature of some forms of leukemia. In most cases the a etiology is not obvious, but internal and external factors associated with damage to DNA can predispose to acute leukemia (Provan, 2003).

1.2.1 Acute Lymphoblastic Leukemia
Acute lymphoblastic leukemia's (ALLs) are a group of hematological neoplasias defined by cytomorphology, cytochemistry, immunological markers, and more recently, molecular markers (Munker et al., 2007).

1.2.1.1 Incidence
Acute lymphoblastic leukemia is primarily a disease of childhood and adolescence, accounting for 25% of childhood cancers and up to 75% of childhood leukemia. The peak incidence of ALL in children is between 2 and 5 years of age. Although ALL is rare in adults, risk increases with age; most adult patients are older than 50 years of age (Keohane et al., 2016).

1.2.1.2 Clinical Manifestation
The signs and symptoms of ALL result from the infiltration of the bone marrow and other organs by malignant blasts. Consequently, anemia, neutropenia, and thrombocytopenia. The signs of anemia are pale skin and mucous membranes, and easy fatigability; signs of neutropenia are infections, especially abscesses and pneumonias.
Thrombocytopenia is indicated by gum bleeding, petechial bleeding, sometimes retinal bleeding, and easy bruising. Hematomas are less frequent. Children often have bone and joint pain, and sometimes a painful enlargement of the spleen (Munker et al., 2007).

1.2.1.3 Classification of Leukemia
The main classification is into four types: acute and chronic leukemia's, which are further subdivided into lymphoid or myeloid (Hoffbrand et al., 2006).

Acute leukemia is subdivided into (a) acute lymphoblastic leukemia (ALL), in which the abnormal proliferation is in the lymphoid progenitor cell and (b) acute myeloid leukemia (AML), which involves the myeloid lineages. The distinction between the two leukemia's is based on morphological, cytochemical, immunological and cytogenetic differences and is of paramount importance as the treatment and prognosis are usually different (Provan, 2003).

1.2.1.3.1 The French-American-British classification of Acute Lymphoblastic Leukemia
The French-American-British (FAB) group distinguishes three categories of ALL according to morphological criteria. With the exception of the type L3, which correlates with the immunological type of B-ALL and which can be considered as a leukemic form of Burkett's lymphoma. The FAB types of ALL, Type L1 is more frequent in children with ALL, whereas L2 is more frequent in adults. The most frequent form of ALL is designated as Common-ALL (in adults 50–60% of all patients with ALL, in the pediatric age group more than 70%). The second most frequent form of ALL bears markers of T-cell lineage (T-ALL, 10–30% of all patients). A
very immature form of B-lineage ALL is designated as early pre-B-ALL, whereas B-ALL has a more mature immunophenotype with the expression of surface Ig (Munker et al., 2007).

1.2.1.3.1.1 ALL of L1 Subtype

In L1 ALL small cells, up to twice the diameter of a red cell, predominate. They have a high nucleocytoplasmic ratio. The nucleus is regular in shape with only occasional clefting or indentation, the chromatin pattern is fairly homogeneous (although smaller cells may show a greater degree of chromatin condensation) and the nucleoli, if visible at all, are small and inconspicuous. The scanty cytoplasm is slightly to moderately basophilic, rarely intensely basophilic, and in some cases shows a variable degree of vacuolation. In a minority of cases there are small numbers of azurophilic granules (Bain, 2003).

1.2.1.3.1.2 ALL of L2 Subtype

In L2 ALL the blasts are larger and more heterogeneous. The nucleocytoplasmic ratio is variable from cell to cell but the cytoplasm, which shows a variable degree of basophilia, may be moderately abundant. The nuclei are irregular in shape with clefting, folding and indentation being common, and with heterogeneity also of the chromatin pattern. Nucleoli are usually present and may be large. A variable degree of cytoplasmic vacuolation may be present, and in a minority of cases there are small numbers of azurophilic, but peroxidase-negative, granules (Bain, 2003).

1.2.1.3.1.3 ALL of L3 Subtype

In L3 ALL the blast cells are large but homogeneous. The nucleocytoplasmic ratio is lower than in L1 ALL. The nucleus is
regular in shape, varying from round to somewhat oval. The chromatin pattern is uniformly stippled or homogeneous, with one or more prominent, sometimes vesicular, nucleoli. In contrast to L1 and L2 ALL, in which mitotic figures are uncommon, the mitotic index is high and many apoptotic cells are seen. The cytoplasm is strongly basophilic with variable but prominent vacuolation (Bain, 2003).

1.2.1.3.2 The WHO Classification of Acute Lymphoblastic Leukemia

The most important alterations to the FAB classification made by the WHO group is that there is a clear separation of precursor- B and precursor-T lymphoblastic leukemia/lymphoma from cases with the immunophenotype of a mature lymphoid cell, whereas cases with a leukemic presentation and those with a lymphomatous presentation are grouped together. The practical implication is that most cases categorized in the FAB classification as L3 ALL are reassigned to the category of Burkett's lymphoma since they express surface membrane immunoglobulin, do not usually express TdT, and have the same translocations that are found in Burkett's lymphoma with a lymphomatous presentation. This reassignment is of some clinical relevance since it has been recognized for some time that these patients do much better with alternative intensive chemotherapeutic regimens rather than with chemotherapy that would be appropriate for ALL (Hoffbrand et al., 2005).

1.2.1.4 Laboratory Findings

Haematological investigations may reveal a normochromic, normocytic anaemia with thrombocytopenia in most cases. The total white cell count may be decreased, normal or increased to 200
x 10⁹/L or more. Blood film examination typically shows a variable numbers of blast cells. The bone marrow is hypercellular with >20%leukaemic blasts. The blast cells are characterized by morphology, immunochemical tests and cytogenetic analysis. Lumbar puncture for cerebrospinal fluid examination should be performed and may show that the spinal fluid has an increased pressure and contains leukaemic cells. Biochemical tests may reveal a raised serum uric acid, serum lactate dehydrogenase or, less commonly, hypercalcaemia. Liver and renal function tests are performed as a baseline before treatment begins (Hoffbrand et al., 2006).

1.2.1.4.1 Morphology and Cytochemistry

Morphological analysis of leukaemic cells in smears stained with Romanowsky stain distinguishes three subtypes (L1, L2 and L3) as classified by the French–American–British (FAB) cooperative group. Myeloperoxidase, Sudan black B and specific esterase are specific for myeloblasts and α-naphthyl butyrate (or acetate) esterase is specific for monoblasts, while periodic acid–Schiff reagent reacts positively in over 70% of ALL cases. Because of the subjective nature of distinction between L1 and L2 subtypes, and the poor correlation of these subtypes with immunological and genetic features, morphological classification system has not been useful in the clinical management of ALL. Contemporary classification of ALL relies on immunophenotyping, cytogenetics and molecular analyses (Hoffbrand et al., 2005).

1.2.1.4.2 Immunophenotyping

Immunophenotyping is now usually performed largely by flow cytometry using anticoagulated whole blood or bone marrow samples in which red cells have been selectively lysed. Antibodies that are directly labelled with fluorochromes are generally used
since this permits two-colour or three-colour analysis, by which co-expression of antigens can be evaluated. Forward light scatter (proportional to cell size) and sideways light scatter (determined by cell structure, including granularity) can be analysed and related to antigen expression. Techniques for detection of cytoplasmic and nuclear antigen expression are essential, the former for detecting the earliest expression of lineage-related antigens, such as CD3, CD13 and CD22 and for the detection of cytoplasmic antigens (myeloperoxidase) or cytoplasmic epitopes (CD79a), and the latter for detection of the nuclear expression of terminal deoxynucleotidyl transferase (TdT) (Hoffbrand et al., 2005).

1.2.1.4.3 Cytogenetic
Provides important prognostic information in both childhood and adult ALL. Abnormalities are detected in up to 85%. The major abnormalities are clonal translocations: t(9;22), t(4;11), t(8;14), t(1;19) or t(10;14). If no structural abnormalities are present, the abnormalities can be classified by the modal chromosome number: <46 (hypodiploid); 46 with other structural abnormalities (pseudodiploid); 47–50 (hyperdiploid); >50 (hyper-hyperdiploid). t(9;22)(q34;q11) produces the Philadelphia chromosome found in 5% of children and 25% of adults with ALL and is a very strong adverse prognostic factor in both. t(8;14) is associated with B-cell ALL (L3 morphology) and occurs in 5% of cases (dysregulates the myc proto-oncogene), t(1;19) is associated with B-cell precursor ALL; t(4;11) occurs in 80% of infants with ALL and 6% of adults (Provan et al., 2004).

1.2.1.5 Prognosis
The prognosis of childhood ALL has improved dramatically. Over 95% of children achieve a complete response, and over 80% of
children have long term disease-free survival and are presumed cured. Patients with favorable prognostic factors can be treated less aggressively, whereas patients with adverse prognostic factors may be treated more aggressively from the time of diagnosis. Nearly all adults with ALL are considered high risk. Prognostic factors of ALL are summarized in Table (1.1) (Kern, 2002).

**Table (1.1) Prognostic factors in ALL**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Favorable</th>
<th>Unfavorable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2 to 10 years</td>
<td>Below 2 years or above 10 years</td>
</tr>
<tr>
<td>WBCs count</td>
<td>Low WBC count at diagnosis</td>
<td>WBC &gt;50.000/µL</td>
</tr>
<tr>
<td>Phenotype</td>
<td>Precursor B cell</td>
<td>Precursor T cell, Mature B cell</td>
</tr>
<tr>
<td>Chromosome number or DNA Index</td>
<td>Hyperdiploidy, DNA Index&gt;1.16</td>
<td>Pseudodiploidy, Hypodiploidy, Near tetraploidy</td>
</tr>
<tr>
<td>Chromosome abnormalities</td>
<td>t(12;21), Trisomy 4 and trisomy 10</td>
<td>c-MYC alterations{t(8;14):t(2;8):t(8;22)}, MLL alteration(11q23),t(9;22) t(1;19)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian</td>
<td>African American, Hispanic</td>
</tr>
<tr>
<td>Time to complete remission</td>
<td>Short(&lt;7-14 days)</td>
<td>Prolonged time to remission or failure to achieve complete remission</td>
</tr>
</tbody>
</table>
1.2.2 Glutathione S-Transferase Theta
Glutathione S-transferases (GSTs) are a family of cytosolic enzymes that play an important role in the detoxification of various exogenous and endogenous reactive species. Individuals who have homozygous deletions in the GSTM1, GSTTI, or GSTP1 genes have reduced enzyme function. The absence of these enzymes may potentially increase cancer susceptibility because of a decreased ability to detoxify carcinogens such as benzo[α] pyrene-7, 8-diol epoxide, the activated form of benzo[α] pyrene. Previous studies indicated that genetic variants of GSTM1, GSTTI, and GSTP1 are associated with an increased risk of developing various cancers (Zi et al., 2014).

1.2.2.1 Classification
Human glutathione S-transferases are divided into eight distinct classes as alpha, kappa, mu, omega, pi, sigma, theta, and zeta based on amino acid sequence similarity and antibody cross-reactivity. The mu class of GSTs, encoded by the GSTM1 gene, is found on the chromosome 1p13.3. The theta class of GSTs, encoded by the GSTT1 gene, is located on the chromosome 22q11.23. An increased frequency of GSTM1 and GSTT1 null genotypes has been associated with several types of malignancies, including stomach cancer, lung cancer, bladder cancer, prostate cancer, cervical cancer, and acute leukemia (Tang et al., 2013).

1.2.2.2 Function
GST isoenzymes serve a variety of functions involved in carcinogen detoxication and intracellular transport of a wide spectrum of substance. GST enzymes have been considered to play a major part in phase II of drug-metabolism where they contribute to cell survival by detoxication of foreign compounds. GSTs
catalyse the reduction of peroxide-containing compounds that may otherwise be toxic to the cell. GSTs also serve an important role in the isomerisation of many biologically important molecules. The transferases can catalyse cis-transisomerisation reactions or the movement of a double bond within a polycyclic molecule (Sherratt and Hayes, 2001).
1.3 Previous Studies

A previous study done by (Guvan et al., 2015) studied the role of glutathione S-transferase M1, T1 and P1 gene polymorphisms in childhood acute lymphoblastic leukemia susceptibility in Turkish population and they found that there is no difference in the prevalence of the GSTM1 and GSTT1 null genotypes between the childhood ALL patients and the controls (\( P.value = 0.94 \)) (Cuven et al., 2015).

Study done by (Tang et al., 2013) performed an extensive meta-analysis on 26 published case-control studies to assess the strength of association between childhood acute leukemia risk and polymorphisms of GSTM1 and GSTT1, a stratification analysis showed that the risk of GSTM1 polymorphism are associated with childhood acute leukemia in group of Asians (OR = 1.94; 95%CI), Blacks (OR = 1.76; 95%CI,) With respect to GSTT1 polymorphism, significant association with childhood acute leukemia risk was only found in subgroup of Asian(Tang et al., 2013).

Another study done to examine the association between GSTT1 null polymorphism and Polycythaemia Vera in Sudanese patient and they found that the GSTT1 null polymorphism was detected in 23% of cases (17% males and 5% females) and in 22% of control subjects. And the association between GSTT1 null and polycythaemia vera was not statistically significance (\( P.value =0.64 \)) (Mohammed et al., 2015).
Chapter Two

Rationale and Objectives

2.1 Rationale

Acute lymphoblastic leukemia has increasing prevalence in Sudan based on National Cancer Institute records; there is many studies revealed association of acute lymphoblastic leukemia with certain genetic abnormality.

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 GSTT1 null polymorphism and various types of cancers. To the best of our knowledge, there are no published reports about the association between GSTT1 null polymorphism and ALL patients in Gezira State, so this study may fill the gap regarding this polymorphism and its association with Sudanese ALL patients attended National Cancer Institute at Wad Madani.
2.2 Objectives

2.2.1 General Objective
To study the association between GSTT1 null genotype and childhood acute lymphoblastic leukemia in Gezira State, Sudan.

2.2.2 Specific Objectives
- To determine the frequency of GSTT1 null genotype among Sudanese patients with childhood acute lymphoblastic leukemia.
- To examine the GSTT1 null polymorphism as risk factor for childhood acute lymphoblastic leukemia.
- To correlate the presence of this polymorphism with patient's hematological parameter, age, gender, family history of ALL or other malignancy
Chapter Three
Materials and Methods

3.1 Study Design
It was prospective case control study conducted in National Cancer Institute, Gezira state in the period from February to April 2016

3.2 Study Population
Forty acute lymphoblastic leukemia patients referred to National Cancer Institute as well as forty apparently healthy volunteers were enrolled as a control group.

3.3 Inclusion Criteria
Patients with acute lymphoblastic leukemia age range up to 15 years

3.4 Exclusion Criteria
No exclusion criteria

3.5 Ethical Consideration
Verbal consent of the selected parents to the study was taken after being informed with all detailed objectives of the study and its health risk and benefit in future

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

3.7 Data Presentation
The data were presented in tables and figures

3.8 Sampling
Non-probability sampling method was used (only who accepted study tests) were involved.

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

3.10 Methodology

3.10.1 Hematological parameters and Blast percentage
The results of hematological parameters and blast percentage at diagnosis were taken from a patient file from National Cancer Institute

3.10.2 DNA Extraction by salting out method

3.10.2.1 Principle Steps
Red blood cells was hemolysed by alkaline solution (red cell lyses buffer) then the membranes were digested by solution containing detergent (white cells lyses buffer) then protein was precipitated out by saturated NaCl and centrifugation finally DNA was precipitated by absolute ethanol then washed by 70% ethanol and eluted in 50 μl TE buffer.

3.10.2.2 Procedure
300 μl of blood sample was placed in 1.5 Ependorf tubes, 1000 μl RCLB was added to the tube, mixed well, centrifuged at 2500 rpm for 10 minutes, supernatant was discarded and the pellet (WBCs) washed again with 1000μl of RCLB (repeated until clear pellet is obtained). Add 300μl of WCLB and 10μl of 10% SDS to the clear white pellets. The mixture was incubated for 1 hour at room temperature. 100μl of 6M NaCl was added to precipitate the protein and mixed well by vortex. 200μl of ice cold chloroform were added to tube and centrifuged at 12000 rpm for 6 minutes. The aqueous 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 12000 rpm for 5 minutes. The supernatant was poured off without disturbing the precipitate and then washed with 600µl 70% ethanol. The tube content was left to air dry. The pellets were resuspended in 50µl TE buffer and leaved to dissolve overnight.

3.10.3 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

3.10.4 DNA Storage
DNA was transferred into 1ml Ependrof tube and preserved at -20ºC until PCR was performed

3.10.5 Molecular Analysis
3.10.5.1 Detection of GSTTI null genotype
All patients with ALL were screened for the presence of GSTTI null genotype using Allele specific PCR, the primers sequence used were as follow:-

<table>
<thead>
<tr>
<th>Table (3.1) Oligonucleotides Sequences of primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer</td>
</tr>
<tr>
<td>GSTT1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Beta globin</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
PCR mixture of 20µ was prepared using premix master mix tube
(maxime PCR premix kit {i-Tag\textsuperscript{TM}}) for each sample, with positive
and negative control in sterile Ependrof tub as follow :-

**Table (3.2) PCR mixture:**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double D.W</td>
<td>14µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1µl</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1µl</td>
</tr>
<tr>
<td>Template DNA</td>
<td>4µl</td>
</tr>
<tr>
<td>Total reaction volume</td>
<td>20µl</td>
</tr>
</tbody>
</table>

Optimized cycling protocol for PCR analysis of GSTT 1 on the
TECHNE (TC-312) as follow:

**Table (3.3) 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>40</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>

**3.10.5.2 Demonstration of PCR product**

Five µl of the PCR product (ready to load) was electrophoresed on
2% agarose gel, stained with ethedium bromide, 1X TBE buffer
was used as a running buffer .The voltage applied to the gel was
100 volt with time duration of 15 minutes. 50 bp DNA ladder was used as molecular weight marker with each patch of samples. Finally, PCR product was demonstrated by gel documentation system SYNGENE

3.11 Interpretation of result:
The GSTT1 normal genotype will give band (480 bp) while GSTT1 null give no band. Beta globin primer was used as positive control for all negative GSTT1null genotype and give band 500 bp size

3.12 Data Analysis
Data of this research was analyzed using the statistical package for the social sciences (SPSS) version 11.5 (independent test t-test for compare patient's age and hematological parameter in null and normal GSTT1 genotype, chi square test for correlate GSTT1 null polymorphism with gender and family history of ALL, regression for examine GSTT1 null polymorphism as risk factor).
Chapter Four
The Results

Demographic data:
Study included 80 samples 40 of there were cases and 40 of them were control. The results showed that 26/40 (65%) of the samples were males and 14/40 (35%) were females.

Figure (4.1) Gender distribution among the samples
The results showed that 6/40 (15%) of patients have family history of ALL and 34/40 (85%) have no family history.

Figure (4.2) frequency of family history of disease among case sample
The results showed that 33/40 (82.5%) of the cases were GSTT1 null genotype and 7/40 (17.5%) of them were normal GSTT1 genotype.

![Figure (4.3) GSTT1 genotype among patients](image1)

The result showed that 6/40 (15%) of the control were GSTT1 null and 34/40 (85%) are normal GSTT1 genotype.

![Figure (4.4) GSTT1 genotype among control subjects](image2)
4.1 Comparisons of hematological findings in GSTT1 null group and normal GSTT1 group

Mean of blast percentage was not statistically significantly higher \((p.\text{value}=0.14)\) in patients with normal genotype (mean ± SD: 75.14 ± 17.68) compared to those with null genotype (mean ± SD: 59.85 ± 25.42).

Mean of platelets counts was not statistically significant lower \((p.\text{value}=0.93)\) in patients with normal genotype (mean ±SD: 61.00 ± 87.88) compared to those with null genotype (mean ± SD: 64.06± 51.82).

Mean of TWBCs counts was not statistically significantly lower \((p.\text{value}=0.71)\) in patients with normal genotype (mean ±SD: 44.94 ± 33.44) compared to those with null genotype (mean ±SD: 58.35 ± 92.00).

Mean of Hb level was statistically significantly lower \((p.\text{value}=0.04)\) in patients with normal genotype (mean±SD: 4.89 ± 2.64) compared to those with null genotype (mean ±SD: 7.14 ± 2.52).

Table (4.1) the mean of hematological findings among cases

<table>
<thead>
<tr>
<th>Variables</th>
<th>Null GSTT1 (Mean ± SD)</th>
<th>Normal GSTT1 (Mean ± SD)</th>
<th>(P.\text{value})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts</td>
<td>59.85 ±25.42</td>
<td>75.14±17.68</td>
<td>0.14</td>
</tr>
<tr>
<td>Platelets</td>
<td>64.06± 51.82</td>
<td>61.00 ± 87.88</td>
<td>0.93</td>
</tr>
<tr>
<td>TWBCs</td>
<td>58.35 ± 92.00</td>
<td>44.94 ± 33.44</td>
<td>0.71</td>
</tr>
<tr>
<td>Hb</td>
<td>7.14 ± 2.52</td>
<td>4.89 ± 2.64</td>
<td>0.04</td>
</tr>
</tbody>
</table>
4.2 Comparison of age in GSTT1 null group and normal GSTT1 group

The mean of age was not statistically significant higher \((p.value=0.64)\) in patients with normal genotype \((mean±SD: 8.29 ± 3.23)\) compared to those with null genotype \((mean ±SD: 7.27 ± 3.56)\).

Table (4.2) the mean of age among the case

<table>
<thead>
<tr>
<th>Variable</th>
<th>Null GSTT1 (Mean ± SD)</th>
<th>Normal GSTT1 (Mean ± SD)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>7.27 ± 3.56</td>
<td>8.29 ± 3.23</td>
<td>0.64</td>
</tr>
</tbody>
</table>

4.3 Correlation between gender and GSTT1 polymorphism

The results showed that no statistically significant correlation between gender and GSTT1 polymorphism \((p.value=0.63)\).

Table (4.3) Correlation between gender and GSTT1 polymorphism

<table>
<thead>
<tr>
<th>Gender</th>
<th>GSTT1</th>
<th>Total</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Null</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>55%</td>
<td>10%</td>
<td>65%</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>27.5%</td>
<td>7.5%</td>
<td>35%</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>82.5%</td>
<td>17.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

4.4 Correlation between family history of ALL and GSTT1 polymorphism

The results showed that no statistically significant correlation between family history and the GSTT1 polymorphism \((p.value=0.22)\)
Table (4.4) Correlation between family history and GSTT1 polymorphism

<table>
<thead>
<tr>
<th>Previous history</th>
<th>GSTT1</th>
<th>Total</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Null</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>15%</td>
<td>0%</td>
<td>15%</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>67.5%</td>
<td>17.5%</td>
<td>85%</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>82.5%</td>
<td>17.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

4.5 Correlation of ALL and GSTT1 null polymorphism

The result showed that GSTT1 null genotype was a risk factor for ALL and there is a statistically significant correlation between GSTT1 null polymorphism and childhood ALL (OR: 26.71, 95%CI, P=0.00)
5.1 Discussion

Homozygotes for null alleles (deletion) of GSTM1 and GSTT1 have absent activity of the respective enzyme (Tang et al., 2013). The GSTs are involved in the metabolism of many environmental carcinogens, drugs and other xenobiotics. The polymorphisms result in a lack of enzymatic activity leading to a reduced detoxification role for GSTs. Thus the polymorphisms in the GST genes may be the factors contributing to the differences in leukemia and susceptibility to other cancer types. Several previous studies focused on the possible association between the polymorphism of GSTM1 and GSTT1 genes and the risk of childhood ALL development but the result are variable (Rollinson et al., 2000; Cong et al., 2014).

This case control study was conducted to determine the frequency of GSTT1 null genotype among Sudanese patients with childhood ALL and to examine the association between GSTT1 polymorphism and risk of developing childhood ALL.

In this study we found that the GSTT1 null genotype was risk factors for ALL (OR = 26.71, 95% CI, \( P\text{.value} =0.00 \)) and this findings is in agreement with previous study done by Tang et al who performed a extensive meta-analysis on 26 published case-control studies which reported significant association between childhood acute lymphocytic leukemia risk, association was only found in a subgroup of Asian populations (OR = 1.94; 95%CI).

Our finding is disagree with reports of Mohammed et al who reported that there were no significant association between GSTT1
and Polycythemia vera \( (P.value = 0.64) \), also disagree with reports of Guvan et al who reported no significant association between GSTT1 and childhood ALL in a Turkish population \( (P.value = 0.94) \).

The mean of blasts percentage in GSTT1 null cases was insignificantly lower than in normal GSTT1 \( (P.value = 0.14) \). The mean of TWBCs and platelets was higher among patients with GSTT1 null genotype than those with normal GSTT1 genotype and the difference was statistically insignificant. The mean of Hb was higher among patients with GSTT1 null genotype than those with GSTT1 normal genotype and the difference was statistically significant \( (p.value = 0.04) \). The mean of age in GSTT1 null cases insignificantly lower than in normal GSTT1 cases \( (P.value = 0.64) \).

There is insignificant association between GSTT1 polymorphism and gender \( (P.value = 0.63) \) and this findings is in agreement with previous study done by Kassogue et al who reported that there were no significant association between GSTT1 and chronic myeloid leukemia in Morocco \( (p.value = 0.13) \).

There is insignificant association between GSTT1 null polymorphism and family history of ALL \( (P.value = 0.22) \).
5.2 Conclusions

GSTT1 null genotype is a risk factor for ALL and there is statistically significant association between GSTT1 null polymorphism and ALL.

GSTT1 null genotype is associated with statistically insignificant higher platelets counts, and white blood cell counts. And statistically insignificant lower blasts percentage.

GSTT1 null genotype is associated with statistically significant increase in Hb concentration. With no correlation with age, gender and family history of ALL.
5.3 Recommendations

- Another study should be conducted including information on the subtype of childhood ALL is demanded to clarify the relationship between the GSTT1 polymorphisms and subtypes of childhood ALL.

- Another study should be conducted with larger sample size to confirm the association of GSTT1 null polymorphism with ALL.
References


Appendixes

Appendix 1:
Questionnaire

ID: ...........................................................................
Age: ...........................................................................
Gender: .................................................................
Do you have history of ALL in family: Yes( )       No( )
Hematological parameters:
HB:..............................
TWBCs:.....................
Platelets:....................
Blast:.......................
GSTT1 genotype:         Null( )                  Present( )
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

TECHNE

(TC 312)
Gel electrophoresis
SYNGENE