

بسم الله الرحمن الرحيم



Sudan University of Science and Technology

Collage of Graduate Studies

Association between Glutathione S Transferase Theta1 Null Polymorphism in Sudanese Patients with Acute Lymphoid Leukemia

العلاقة بين غياب جلوتاثيون اس ترانسفيريز ثيتا ١ و المرضى السودانيين المصابين بابيضاض الدم الليمفاوي الحاد

A Thesis Submitted for Partial Fulfillment of the Requirement for the Master Degree in Medical Laboratory Science in Hematology and Immunohematology

Submitted by:

Marwa Hamed Hassab Elgawi

B.Sc, Hematology and Immunohematology, Sudan University of Science and Technology

Higher diploma, Hematology and Immunohematology, Sudan University of Science and Technology

Supervisor:

Dr.Ibrahim Khider Ibrahim

Ph.D (Hematology), Assistant Prof (AL-Neelain University)

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الايه

قال تعالى:

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(إِنَّمَا يَخُشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ)

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Dedication

To...

My parents and family...

Respected teachers...

And friends

Acknowledgement

I would like to thank and extend utmost gratitude to my supervisor Dr. Ibrahim Khidir Ibrahim who always offered support and ideas to success the project.

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Abstract

Glutathione is a mother of antioxidants in the body, as presence of these tri-peptide molecules concludes the body cells from oxidative stress the absence of them increase this chain reaction leading to cell dysfunction, aging and early death.

This study was carried out in Khartoum state in radio isotope center-Khartoum and flowcytometry center during period of time from February to December 2015, to determine the frequency of null glutathione S transferase theta 1 (GSTT1) null genotype among patients with acute lymphoid leukemia. From 40 ALL patients and 30 healthy individual; 2.5 ml of venous blood samples was collected, the DNA extracted from the blood samples and polymerized in PCR machine by using GSTT1 primer, by using gel electrophoresis the results showed that the GSTT1 was null in 17% (5/30) of control group and 53% off ALL patients (21/40); and present GSTT1 in control group was 83% (25/30) and 47% (19/40) in ALL patients.

The study shows that there is a significant association between GSTT1 null genotype and ALL (OR: 5.5, p.value:0.002).

المستخلص

الجلوتاثيون هو مضاد الاكسدة الالهم في الجسم، كما وجود هذا الجزئ ثلاثي البيبتيدات في الجسم يخلص خلاياه من ظاهرة الضغط الاكسجيني، غيابه يزيد من هذه السلسله التفاعليه مؤديا الى خلل في وظائف الخلية، شيخوخة و موت الخلية المبكر.

اجريت هذه الدراسة في ولاية الخرطوم في مستشفى الخرطوم للعلاج بالاشعة والطب النووي ومركز الفلوسايتوميتر في الفترة من فبراير حتى ديسمبر ٢٠١٥، لتحديد نسبة تكرار غياب بروتين الجلوتاثيون اس ترانسفيريز ثيتا في المرضى المصابين بابيضاض الدم الليمفاوي الحاد. تم اختيار ٤٠ مريض بابيضاض الدم الليمفاوي الحاد و ٣٠ شخص كمجموعة ضبط. اخذت من كل فرد ٢,٥ مليليتر من الدم الوريدي، تم استخلاص الحمض النووي لعينات الدم واجراء تفاعل سلسلي بلمري باستخدام مبتدئ جلوتاثيون اس ترانسفيريز ثيتا لكل من المجموعتين. باستخدام تقنية الفصل البروتيني اوضحت النتائج غياب بروتين الجلوتاثيون اس ترانسفيريز ثيتا في مجموعة الضبط بنسبة ١٦,٧٪ وفي مجموعة المرضى ٥٢,٥٪؛ بينما كانت نسبة وجود بروتين الجلوتاثيون اس ترانسفيريز ثيتا في مجموعة الضبط ٨٣,٣٪ وفي مجموعة المرضى ٤٧,٥٪.

الدراسة اثبتت وجود علاقة بين غياب بروتين الجلوتاثيون اس ترانسفيريز ثيتا ١ ومرض ابيضاض الدم الليمفاوي الحاد. (OR: 5.5, p.value: 0.002)

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List of Abbreviation

ACML	Atypical chronic myeloid leukemia
ALL	Acute lymphoid leukemia
AML	Acute myeloid leukemia
ATLL	Adult T cell leukemia/lymphoma
BMT	Bone marrow transplant
BP	base pair
CBC	Complete blood count
CD	Cluster of differentiation
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia (CMML)
CNS	Central nervous system
DNA	deoxyriboneuclic acid
EBV	Epstein - Barr virus
EDTA	Ethylene di amine tetra acetic acid
FAB	French, American, British
FISH	Fluorescent insitu hybridization
GST	Glutathione S transferease
GSTM1	Glutathione S transferease mu 1
GSTT1	Glutathione S transferease theta 1
HLF	Hepatic leukemia factor
HTLV-1	Human T lymphotropic virus type I

ITP	Immune thrombocytopenic purpura
JMML	Juvenile myelomonocytic leukemia
LDH	Lactate dehydrogenase
m-BCR	Minor break point cluster region
MDS	Meylodyblastic syndrome
MPAL	Mixed phenotype acute leukemia
MPN	Myeloproliferative neoplasm
MPO	Myeloperoxidase
mRNA	Messenger ribonucleic acid
NSE	Nonspecific esterase
PAS	Periodic acid-schiff
PCR	Polymerase chain reaction
Ph	Philadelphia chromosome
RCLB	Red cells lyses buffer
RT-PCR	Real time- polymerase chain reaction
SBB	Sudan black B
TBE	Tris base edta
TCR	T cell receptor
WBC	White blood cells
WCLB	White cells lyses buffer
WHO	World health organization

Chapter One

Introduction and Literature Review

Introduction and literature review

1.1 Introduction:

Leukaemias are a very heterogeneous group of diseases, which differ from each other in etiology, pathogenesis, prognosis and responsiveness to treatment. Accurate diagnosis and classification are necessary for the identification of specific biological entities and underpin scientific advances in this field. The detailed characterization of hematological neoplasms is also essential for the optimal management of individual patients. Many systems for the classification of leukemia have been proposed. Between 1976 and 1999, a collaborative group of French, American and British hematologists (the FAB group) proposed a number of classifications, which became widely accepted throughout the world. In 2001, a quarter of a century after the first FAB proposals, a World Health Organization (WHO) expert group proposed an updated system for the classification of leukemia and lymphoma incorporating clinical features, hematological and histological features, immunophenotyping and the results of cytogenetic and, to a lesser extent, molecular genetic analysis. In 2008 a further updating of the WHO classification incorporated new knowledge and gave a greater importance to molecular genetic features (Barbara, 2010).

Acute leukaemias are malignant clonal disorders originating in hematopoietic stem cells characterized by the proliferation of poorly-differentiated blast (immature) cells in the bone marrow and a rapidly progressive fatal course if untreated (survival <6 months without treatment). Acute leukaemias primarily originate in the bone marrow. Proliferating leukemic blasts replace normal bone marrow cells and subsequently enter into the peripheral blood. They are clonal disorders that arise from malignant transformation of a single hematopoietic progenitor cell followed by proliferation and accumulation of abnormal clone. There is an arrest in the differentiation of immature cells into functionally mature cells. Acute leukaemias comprise about 50% of all cases of leukaemias (Counter V, *et al*, 2004).

The GSTs represent a set of xenobiotic detoxifying enzymes with a known series of polymorphic mutations that affect function. GST deficiency, rather than high GST activity, has been associated with an increased risk of cancer, although deficiency of GSTT1 and GSTM1 could enhance chemotherapeutic efficacy in some patients. Conflicting results have been

reported on the association between GSTM1 and GSTT1 genotypes and outcome as well as on the association between the risk of relapse in childhood ALL and GSTs polymorphisms (Counter , *et al*, 2004).

1.2 Literature review:

1.2.1 Leukemia

Leukemia is caused by the mutation of the bone marrow pluripotent or most primitive stem cells. This neoplastic expansion results in abnormal, leukemic cells and impaired production of normal red blood cells, neutrophils, and platelets. As the mutant cell line takes hold and normal hematopoiesis is inhibited, the leukemic cells spill into the peripheral blood and invade the reticuloendothelial tissue, specifically the spleen, liver, lymph nodes, and, at times, central nervous system. The leukemic stem cells have atypical growth and maturation capability. The mutant clone may demonstrate unique morphologic, cytogenetic, and immunophenotypic features that can be used to aid in the classification of the particular type of leukemia. Many of the leukemias have similar clinical features, but regardless of the subtype, the disease is fatal if left untreated.

The initial evaluation of leukemia is initially made by:

Noting the onset of symptoms, analyzing the complete blood count (CBC) results, observing the type of cell that predominates (cell lineage) and assessing the maturity of cells that predominate

Because leukemia is a disease of the bone marrow that causes normal bone marrow cell production to be crowded out as the abnormal, neoplastic cells take over, the CBC results will commonly show a decreased red cell count or anemia, as well as a decrease in platelets or thrombocytopenia. The level of anemia and thrombocytopenia tends to be more severe in acute leukemia. Leukocytosis is a hallmark feature of chronic leukemia, and because the spleen also becomes a site of extramedullary (outside of the bone marrow) hematopoiesis, prominent hepatosplenomegaly is most often associated with chronic leukemia. The type of cell that predominates in the peripheral blood and the bone marrow is defined according to cell lineages either myeloid or lymphoid. The myeloid stem cell gives birth to granulocytes, monocytes, megakaryocytes, and erythrocytes. Therefore, as will be described in the various sections of this chapter, the myeloid leukemias can involve proliferation of any stage of these four cell lines. By contrast, the lymphoid stem cell gives rise solely to lymphocytic lineage cells (Betty, 2007).

Cell maturity can be used to separate the initial diagnosis between acute and chronic leukemias. When blasts or other immature cells predominate, the leukemia is classified as acute, versus the predominance of more mature cell types being associated with chronic leukemia. The onset of acute versus chronic leukemia is distinctly different. Acute leukemia has a quick onset, whereas chronic leukemia has a slow, insidious course and may even be discovered on routine physical examination. Age is another factor that is often consistent in the different leukemic variants. Although acute leukemia may occur at any age, chronic leukemia is usually a disease seen in adults. To summarize, using both the cell lineage and the maturity of cells that predominate, leukemias can be categorized into four broad groups: acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelocytic leukemias and chronic lymphocytic leukemia (Betty, 2007).

1.2.2 Classification of leukemia

The purpose of any pathological classification is to bring together cases that have fundamental similarities and that are likely to share features of causation, pathogenesis and natural history. Making an accurate diagnosis of a haematological neoplasm is crucial for selection of the most appropriate treatment. Since there are many dozens, if not hundreds, of different types of leukaemia it is essential to have a classification that an individual case can be related to. Identification of homogeneous groups of biologically similar cases is important as it permits an improved understanding of the leukaemic process and ultimately benefits individual patients. Since such diagnostic categories or subgroups may differ from each other in the cell lineage affected, natural history, optimal choice of treatment and prognosis with and without treatment, their recognition permits the development of a selective evidence-based therapeutic approach with a resultant overall improvement in outcome. Identifying valid diagnostic categories also increases the likelihood of causative factors and pathogenetic mechanisms being recognized.

The development of the French–American– British (FAB) classification of acute leukaemia , and subsequently of other leukaemias and related conditions, by a collaborating group of French, American and British haematologists was a major advance in leukaemia classification, permitting uniform diagnosis and classification of these diseases over three decades. The FAB classification was based on morphology supplemented by cytochemistry and to some extent by immunophenotyping. Over the last decade the FAB classification has been increasingly

supplemented or replaced by the World Health Organization (WHO) Classification of Tumors of Haematopoietic and Lymphoid Tissues. The WHO classification is based on morphology (either cytology or histology) but also makes extensive use of immunophenotyping and of cytogenetic and molecular genetic analysis. The FAB classification continues to be of value for the preliminary morphological assessment of a case, since a careful morphological assessment indicates which supplementary tests are indicated and provides a context in which such tests can be interpreted. The FAB classification also remains in use in circumstances where immunophenotypic and genetic analysis is not readily available and in this circumstance it is important that cytochemistry is not neglected. However, since a precise diagnosis is important for choice of treatment it is desirable that even resource-poor countries should try to establish those diagnostic methods that are essential for optimal patient management and outcome. For clarity, it is important that FAB designations (which have a precise, carefully defined meaning) are not applied to WHO categories for which the diagnostic criteria differ. Publications, particularly those relating to acute leukaemia and the myelodysplastic syndromes (MDS), should state which classification is being used and should adhere strictly to the criteria of the relevant classification.

1.2.2.1 The nature and classification of acute leukaemia

Acute leukaemia comprises a heterogeneous group of conditions that differ in aetiology, pathogenesis, molecular mechanisms and prognosis. The heterogeneity is reduced if cases of acute leukaemia are divided into acute myeloid leukaemia (AML) (in North America previously designated 'acute non- lymphoblastic leukaemia'), acute lymphoblastic leukaemia (ALL) and mixed phenotype acute leukaemia (MPAL); even then, however, considerable heterogeneity remains within each of the groups. Although the best criteria for categorizing a case of acute leukaemia as myeloid or lymphoid may be disputed, the importance of such categorization is beyond doubt. Not only does the natural history differ but the best current modes of treatment are still sufficiently different for an incorrect categorization to adversely affect prognosis. Assigning patients to subtypes of AML or ALL is becoming increasingly important as the benefits of more targeted treatment are identified. Similarly, the suspected poor prognosis of MPAL suggests that the identification of such cases may lead to a different therapeutic approach and an improved outcome. Cases of acute leukaemia can be classified on the basis of morphology, cytochemistry,

immunophenotype, cytogenetic abnormality or molecular genetic abnormality, or by combinations of these characteristics. Patients may be assigned to the same or different subgroups depending on the characteristics studied and the criteria selected for separating subgroups. All classifications necessarily have an element of arbitrariness, particularly since they need to incorporate cut-off points for continuous variables such as the percentage of cells falling into a defined morphological category, positivity for a certain cytochemical reaction, or the presence of a certain immunological marker. An ideal classification of acute leukaemia must be biologically relevant. If it is to be useful to the clinical haematologist, as well as to the research scientist, it should also be readily reproducible and easily and widely applicable. Rapid categorization should be possible so that therapeutic decisions can be based on the classification. The classification should be widely acceptable and should change as little as possible over time so that valid comparisons can be made between different groups of patients. Ideal classifications of acute leukaemia do not yet exist, although many have been proposed.

1.2.2.2 The nature and classification of the myelodysplastic syndromes

The myelodysplastic syndromes are a group of myeloid neoplasms that are closely related to AML and in some cases precede it. Like AML, they result from mutation of a multipotent or, occasionally, a pluripotent haemopoietic stem cell. They differ in that haemopoiesis is ineffective, i.e. there is a normocellular or hypercellular bone marrow but despite this there is peripheral cytopenia as a result of an acquired intrinsic defect in myeloid maturation; there is an increased rate of death of precursor cells in the bone marrow (by a process known as programmed cell death or apoptosis) leading to a failure of production of adequate numbers of normal mature cells. MDS is also characterized by morphologically abnormal maturation, referred to as dysplasia. However, it should be noted that dysplasia is not specific for MDS nor even for a myeloid neoplasm. MDS evolves into AML as a result of further mutations that interfere with myeloid maturation leading to a progressive accumulation of blast cells. Not only may MDS evolve into AML, but patients presenting with apparently de novo AML may have associated dysplastic features. AML evolving from MDS and AML with associated dysplasia are likely to be closely related conditions. MDS is very heterogeneous, in some patients persisting unchanged for many years and in others leading to death from acute leukaemia or from the complications of bone marrow failure in a relatively short period of time. An adequate

classification of MDS must therefore be directed at recognizing categories of disease that differ in prognosis or that indicate a particular, sometimes relatively specific, and choice of treatment.

1.2.2.3 The nature and classification of chronic myeloid leukaemias

The chronic myeloid leukaemias can result from a mutation either in a multipotent myeloid stem cell or in a pluripotent lymphoid–myeloid stem cell.

In contrast to the majority of cases of AML, they are characterized by an increased peripheral blood count of mature granulocytes. Usually neutrophils predominate but often there is also an increase in eosinophils and basophils; less often the dominant cell is the eosinophil. Monocytes may also be increased. When the leukaemic clone derives from a pluripotent stem cell, the lymphoid component may be apparent before the myeloid component, simultaneously or subsequently. Irrespective of the timing of the appearance of the lymphoid component, the lymphoid cells are immature and their appearance represents evolution of the disease, known as acute transformation. The chronic myeloid leukaemias are classified partly on morphological criteria, which in the past were supplemented by cytochemistry (a neutrophil alkaline phosphatase score). However, when a specific cytogenetic or molecular genetic abnormality has been found to characterize a subtype of chronic myeloid leukaemia it becomes of considerable importance to incorporate this into any scheme of classification. A crucial distinction is between chronic myeloid leukaemias with and without a translocation between chromosomes 9 and 22 that leads to the formation of an abbreviated chromosome 22 known as the Philadelphia (Ph) chromosome. Chronic myeloid leukaemia with t(9;22) (q34;q11.2) is variously referred to as ‘chronic granulocytic leukaemia’, ‘chronic myelogenous leukaemia’, ‘chronic myelogenous leukaemia, BCR- ABL1 positive’ and ‘chronic myeloidleukaemia’. The chronic myeloid leukaemias are similar in nature to myeloproliferative neoplasms (MPN) such as polycythaemia Vera, essential thrombocythaemia and the conceptually more complex ‘idiopathic’ or ‘primary’ myelofibrosis. In these conditions differentiation is not predominantly to granulocytic cells but to erythrocytes in polycythaemia vera, to platelets in essential thrombocythaemia, and to all myeloid lineages in primary myelofibrosis. The distinguishing features of myelofibrosis are extramedullary haemopoiesis and myelofibrosis, which are not actually either ‘primary’ or ‘idiopathic’ but are reactive to the myeloid neoplasm. These other MPN can undergo clonal evolution, one result of which can be Ph-negative chronic myeloid leukaemia.

1.2.2.4 The nature and classification of the myelodysplastic/myeloproliferative neoplasms

The chronic myeloid leukaemias and other MPN are characterized by effective proliferation of myeloid cells and increased numbers of end cells; whereas the MDS are characterized by ineffective proliferation, morphological dysplasia and inadequate numbers of end cells of one or more lineages. However, conditions exist in which there is effective proliferation of cells of one lineage and ineffective proliferation of cells of another lineage. If a condition meets the criteria for both MDS and MPN it is classified as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN). If these overlap syndromes also have a high white blood cell count (WBC) they can legitimately be regarded as a form of (Ph-negative) chronic myeloid leukaemia. Juvenile myelomonocytic leukaemia (JMML), atypical chronic myeloid leukaemia (aCML) and chronic myelomonocytic leukaemia (CMML) are subtypes of MDS/MPN.

1.2.2.5 The nature and classification of lymphoid neoplasms

Lymphoid neoplasms can be categorized in two ways, according to the immaturity of the cell or according to the presence of absence of 'leukaemia' as a key feature of a type of disease. A lymphoid leukaemia is a neoplasm in which the predominant manifestations are in the blood and bone marrow, whereas the term 'lymphoma' refers to a disease characterized by a neoplastic proliferation of cells of lymphoid origin in organs and tissues such as lymph nodes, spleen, thymus and skin. Lymphoid neoplasms may be characterized by lymphoblasts, cells that are cytologically and immunophenotypically immature. If lymphoblasts are present in the bone marrow, with or without overspill into the blood, the condition is usually referred to as 'acute lymphoblastic leukaemia' (ALL). Primary infiltration of other lymphoid organs or tissues by lymphoblasts is referred to as 'lymphoblastic lymphoma'. In either case the lymphoblasts can be of either B lineage or T lineage, although ALL is more often of B lineage and lymphoblastic lymphoma more often of T. In the 2008 WHO classification, lymphoid precursor neoplasms are designated 'B lymphoblastic leukaemia/ lymphoma' and 'T lymphoblastic leukaemia/ lymphoma'. With regard to lymphoid neoplasms in which the neoplastic cells are immunophenotypically mature, a given condition is regarded as 'leukaemia' or 'lymphoma' according to the usual manifestations of the disease. However, again there is overlap. Thus the

most common leukaemia of mature lymphoid cells, chronic lymphocytic leukaemia, has a tissue counterpart designated 'small lymphocytic lymphoma' in which the peripheral blood lymphocyte count is not elevated. A rare subtype of mature T-cell neoplasm is designated adult T-cell leukaemia/ lymphoma because about 90% of patients present with leukaemia and about 10% with lymphoma without circulating neoplastic cells. Conditions that are predominantly lymphomas can also have a leukaemic phase when there is extensive disease. This is often the case with mantle cell lymphoma and sometimes with follicular lymphoma. It should be noted that leukaemia and lymphomas of immunophenotypically mature lymphocytes do not necessarily have cells that resemble normal mature lymphocytes cytologically. The neoplastic cells may be very large and appear very abnormal. They are also not necessarily clinically indolent; some, such as Burkitt lymphoma, are as clinically aggressive as acute leukaemia.

1.2.2.6 The FAB classification of acute leukaemia

The FAB classification of acute leukaemia was first published in 1976 and was subsequently expanded, modified and clarified. It deals with both diagnosis and classification.

Initially, acute lymphoblastic leukemia was largely a diagnosis of exclusion. Although some cases had characteristic cytological features, others were categorized as 'lymphoid' only because they did not show any definite cytological or cytochemical evidence of myeloid differentiation. With the availability of a wide range of monoclonal antibodies directed at

Antigens expressed on lymphoid cells, the diagnosis of ALL is now based on positive criteria. It is sufficient at this stage to say that ALL is classified broadly as B lineage and T lineage. B-lineage ALL includes a small minority of cases with the immunophenotypic features of mature B cells (regarded as non-Hodgkin lymphoma rather than as ALL in the WHO classification) and a large majority of cases with the immunophenotype of B-cell precursors. The latter group includes a major subset designated common ALL, expressing a surface membrane antigen known as the common ALL antigen or CD10. The FAB group have assigned ALL to three cytological categories: L1, L2 and L3. (Barbara, 2010)

1.2.3 Diagnosing acute leukemia

The diagnosis of acute leukaemia usually starts from a clinical suspicion. It is uncommon for this diagnosis to be incidental, resulting from the performance of a blood count for a quite different reason. Clinical features leading to suspicion of acute leukaemia include pallor, fever or other signs of infection, pharyngitis, petechiae and other haemorrhagic manifestations, bone pain, hepatomegaly, splenomegaly, lymphadenopathy, gum hypertrophy and skin infiltration. A suspicion of acute leukaemia generally leads to a blood count and film being performed and, if this shows a relevant abnormality, to a bone marrow aspiration. The diagnosis then rests on an assessment of the peripheral blood and bone marrow. Radiological features can also be of value, with a mediastinal mass being strongly suggestive of T-lineage ALL. The peripheral blood in AML usually shows leukocytosis, anaemia and thrombocytopenia. The leukocytosis reflects the presence of circulating blast cells, while the number of neutrophils is usually reduced and few cells of intermediate stages of maturation are seen (hiatus leukaemicus). In some patients the total WBC is normal or low and, in the latter group, circulating blast cells may be infrequent or even absent. In a minority of patients, there are increased eosinophils and, considerably less often, increased basophils. There may be evidence of dysplastic maturation such as poikilocytosis and macrocytosis, hypolobated or agranular neutrophils or hypogranular/agranular or giant platelets. The peripheral blood film in ALL may show leukocytosis resulting from the presence of considerable numbers of circulating blast cells, but many patients have a normal total leucocyte count and blast cells may be infrequent or even absent. There is usually anaemia, neutropenia or thrombocytopenia but sometimes the neutrophil count, platelet count or even both may be normal and occasionally the platelet count is actually increased. In contrast to AML, the myeloid cells do not show any dysplastic features. A minority of patients have a reactive eosinophilia. The FAB classification requires that peripheral blood and bone marrow films be examined and that differential counts be performed on both. In the case of the bone marrow, a 500-cell differential count is required. Acute leukaemia is diagnosed if one of the following three features is present: 1 At least 30% of the total nucleated cells in the bone marrow are blast cells; or 2 The bone marrow shows erythroid predominance (erythroblasts $\geq 50\%$ of total nucleated cells) and at least 30% of non-erythroid cells are blast cells (lymphocytes, plasma cells and macrophages also being excluded from the differential count of non-erythroid cells); or 3 The

characteristic morphological features of acute promyelocytic leukaemia are present. Cases of ALL will be diagnosed on the first criterion since erythroid hyperplasia does not occur in this condition, but the diagnosis of all cases of AML requires application also of the second and third criteria. The bone marrow in acute leukaemia is usually hypercellular, or at least normocellular, but this is not necessarily so since some cases meet the above criteria when the bone marrow is hypocellular (Barbara, 2010).

1.2.3.1 Distinguishing between acute myeloid and acute lymphoblastic leukaemias

The diagnosis of acute leukaemia using FAB criteria requires that bone marrow blast cells (type I plus type II) constitute at least 30% either of total nucleated cells or of non-erythroid cells. The further classification of acute leukaemia as AML or ALL is of critical importance. When the FAB classification was first proposed, tests to confirm the nature of lymphoblasts were not widely available. The group therefore defined as AML cases in which at least 3% of the blasts gave positive reactions for myeloperoxidase (MPO) or with Sudan black B (SBB). Cases that appeared to be non-myeloid were classed as 'lymphoblastic'. The existence of cases of AML in which fewer than 3% of blasts gave cytochemical reactions appropriate for myeloblasts or monoblasts was not established at this stage, and no such category was provided in the initial FAB classification. In the 1980s and 1990s the wider availability and application of immunological markers for B- and T-lineage lymphoblasts, supplemented by ultra-structural cytochemistry and the application of molecular biological techniques to demonstrate rearrangements of immunoglobulin and T-cell receptor genes, demonstrated that the majority of cases previously classified as 'lymphoblastic' were genuinely lymphoblastic but that a minority were myeloblastic with the blast cells showing only minimal evidence of myeloid differentiation. These latter cases were designated M0 AML. It should be noted that SBB is more sensitive than MPO in the detection of myeloid differentiation and more cases will be categorized as M1 rather than M0 if it is used. Correct assignment of patients to the categories of AML and ALL is very important for prognosis and choice of therapy. Appropriate tests to make this distinction must therefore be employed. Despite the advances in immunophenotyping, cytochemical reactions remain useful in the diagnosis of AML. Cytochemical demonstration of MPO activity can also give prognostic information, since a higher percentage of MPO-positive blasts is strongly

associated with a better prognosis. The FAB group recommended the use of MPO, SBB and non-specific esterase (NSE) stains. If cytochemical reactions for myeloid cells are negative, a presumptive diagnosis of ALL should be confirmed by immunophenotyping. When immunophenotyping is available the acid phosphatase reaction and the periodic acid–Schiff (PAS) reaction (the latter identifying a variety of carbohydrates including glycogen) are no longer indicated for the diagnosis of ALL. When cytochemical reactions indicative of myeloid differentiation and immunophenotyping for lymphoid antigens are both negative, immunophenotyping to demonstrate myeloid antigens and thus identify cases of M0 AML is necessary; the panel of antibodies used for characterizing suspected acute leukaemia normally includes antibodies directed at both lymphoid and myeloid antigens so that the one procedure will identify both M0 AML and ALL. It should be noted that when individuals with an inherited MPO deficiency develop AML, leukaemic cells will give negative reactions for both MPO and SBB (Barbara, 2010).

1.2.4 Acute lymphoid leukemia

Acute lymphocytic leukemia (ALL), also called acute lymphoblastic leukemia, is a cancer that starts from the early version of white blood cells called lymphocytes in the bone marrow (the soft inner part of the bones, where new blood cells are made).

Leukemia cells usually invade the blood fairly quickly. They can then spread to other parts of the body, including the lymph nodes, liver, spleen, central nervous system (brain and spinal cord), and testicles (in males). Other types of cancer also can start in these organs and then spread to the bone marrow, but these cancers are not leukemia.

The term “acute” means that the leukemia can progress quickly, and if not treated, would probably be fatal within a few months. Lymphocytic means it develops from early (immature) forms of lymphocytes, a type of white blood cell. This is different from acute myeloid leukemia (AML), which develops in other blood cell types found in the bone marrow.

Other types of cancer that start in lymphocytes are known as lymphomas (non-Hodgkin lymphoma or Hodgkin disease). The main difference between these types of cancers is that

leukemias like ALL mainly affects the bone marrow and the blood, and may spread to other places, while lymphomas mainly affect the lymph nodes or other organs but may involve the bone marrow. Sometimes cancerous lymphocytes are found in both the bone marrow and lymph nodes when the cancer is first diagnosed, which can make it hard to tell if the cancer is leukemia or lymphoma. If more than 25% of the bone marrow is replaced by cancerous lymphocytes, the disease is usually considered leukemia. The size of lymph nodes is also important. The bigger they are, the more likely the disease will be considered a lymphoma. For more information on lymphomas, see our documents Non-Hodgkin Lymphoma and Hodgkin Disease.

1.2.5 Cytogenetic of acute lymphoid leukemia

Clonal chromosomal abnormalities are found in up to 80% of patients with ALL. They are closely related to the biology of the disease and indicate the genes involved in leukaemogenesis. Cytogenetic classification is based on the number of chromosomes and structural changes, which are important in both childhood and adult ALL to distinguish good- from high-risk patients. Cytogenetic studies in ALL are particularly difficult owing to the frequent low mitotic index of the abnormal blasts and the notoriously poor chromosome morphology. This can be largely overcome by using FISH or RT-PCR to screen for the significant chromosomal abnormalities.

1.2.5.1 B-precursor ALL

t(9;22)(q34;q11) Of significance is the Ph chromosome, which is associated with a particularly poor outcome in ALL. This abnormality occurs more frequently in adults (in whom it accounts for up to 20% cases, increasing exponentially with age) than in children (among whom it is found in 2–3% cases). The Ph translocation, t(9;22)(q34;q11), in ALL is cytogenetically indistinguishable from that found in CML. In the majority of cases of ALL, the breakpoint in BCR occurs between exons 1 and 2 (e1 and e2) in the minor breakpoint cluster region (m-BCR) and exons 1 and 2 of ABL(e1a2). This generates a 7-kb mRNA giving rise to a p190 protein product. In some cases, the breakpoint is in M-BCR, resulting in the production of the p210 protein product as found in CML. Both result in raised tyrosine kinase activity, which induces the increased growth of the clone. As described for CML, FISH provides an accurate method of detection of this abnormality in poor-quality metaphases. The use of RT-PCR for the detection of mRNA in Ph-positive cases can be used to monitor the clone in remission with much greater

sensitivity than that achieved by cytogenetic analysis. The two molecular variants of Ph-positive ALL do not apparently correlate with either different clinical features or with prognosis.

t(1;19)(q23;p13) The translocation t(1;19)(q23;p13) fuses the E2A gene at 19p13 with the PBX1 gene at 1q23 to form the E2A/PBX1 fusion gene. The translocation is strongly associated with a pre-B immunophenotype and it occurs in two forms, a balanced translocation, t(1;19), and an unbalanced form, der(19)t(1;19) (in which the reciprocal product, der(1)t(1;19), is lost and the normal chromosome 1 is duplicated). Clinically, patients with the two forms appear identical, but the unbalanced form is associated with a significantly better prognosis than the balanced form. FISH probes directed to E2A reliably detect balanced and unbalanced forms of t(1;19) and the other rare abnormalities involving E2A.

t(17;19)(q21;p13) The translocation t(17;19)(q21;p13) is a variant of t(1;19) resulting in the fusion of E2A with the hepatic leukaemia factor (HLF) gene on chromosome 17. Although limited numbers of patients have been reported, the prognosis is poor.

MLL rearrangements

The translocation t(4;11)(q21;q23) is the most frequently occurring MLL rearrangement in ALL, in which MLL fuses with AF4 located to chromosome band 4q21. This translocation identifies a particularly well-defined subgroup with a number of associated high-risk features, including white blood cell count $> 50 \times 10^9/L$ (frequently $> 100 \times 10^9/L$) and a pre-B immunophenotype with some expression of myeloid features. It is most common in infants with ALL and in adults it is found more frequently in patients over the age of 40 years. The translocation occurs rarely between the ages of 2 and 10 years. A poor prognosis is associated with infants and adults. MLL is involved in a large number of translocations with a range of partner genes, many of which have been molecularly identified. A dual color FISH probe is available which reliably detects all MLL rearrangements.

t (12;21)(p13;q22)

This translocation occurs in up to 25% of children and 3% of adults with B-lineage ALL. In spite of this high incidence, it was discovered much later than the other ALL associated abnormalities. The reason was that it is invisible by G-banded chromosomal analysis and was brought to light

by chance chromosome painting in 1994. The translocation results in the fusion of the ETV6 gene on chromosome 12 with AML1 on chromosome 21. The ETV6–AML1 fusion can be reliably detected by dual-color FISH using specific probes and by RT-PCR. Initial reports associated this translocation with a good prognosis. This has been reviewed as a number of patients have been shown to experience a late relapse. Deletions of the homologous ETV6 allele have been reported in the majority of cases, which is believed to be a significant initiating event in the development of leukaemia in patients with t(12;21) (Hoffbrand, *et al*, 2005).

Ploidy

Ploidy classification embraces a heterogeneous group of numerical chromosomal changes, with an important role in prognosis. In particular, the high hyperdiploid group (which includes patients with 51–65 chromosomes) has been consistently shown to have the best outcome compared with any other chromosomal group in ALL. Chromosomal gains are non-random, with chromosomes 4, 6, 10, 14, 18, 17, 21 and X most frequently gained. The good prognosis has been linked to the gains of chromosomes 4, 10, 17 and 18. Interphase FISH using chromosome-specific centromere probes can be successfully used to look for high hyperdiploid clones in bone marrow samples which fail to achieve a cytogenetic result. Structural chromosomal changes accompany chromosomal gain in more than 50% of high hyperdiploid cases. It appears that structural change does not compromise the excellent prognosis of this group. It is of particular interest that high hyperdiploidy is the most frequent abnormal finding in children and a rare finding in adults. It is important to exclude the presence of one of the poor-risk translocations in a high hyperdiploid clone before designating the patient as good risk. In contrast to high hyperdiploidy, near-haploidy (23–29 chromosomes) and low hypodiploidy (30–39 chromosomes), both rare findings in ALL, are associated with an extremely poor prognosis. The chromosomes gained to the haploid chromosome set reflect the same additional chromosomes as seen in the high hyperdiploid patients. These abnormal clones often have an accompanying cell population with an exact doubling of their chromosome number. Although this resembles a classic good-risk high hyperdiploid karyotype, it confers no change to the dismal prognosis of these patients. Near-triploidy and near-tetraploidy are rare in childhood ALL, but in adults they account for approximately 5% of patients, in whom they are associated with T-lineage ALL.

The overall incidence of cases T-ALL classified by immunophenotyping is approximately 10% in childhood and 18% in adult ALL. The main chromosomal changes are shown in the chromosomal abnormality detection rate by cytogenetic analysis is extremely low. It has been reported that ~20% of cases have translocations mapping to the chromosomal regions encoding one of the four T-cell receptor (TCR) genes, alpha/delta (TCR α / δ), beta (TCR β) and gamma (TCR γ) at 14q11, 7q32 or 7p15 respectively. These translocations juxtapose a TCR promoter/enhancer element to one of many transcription factors located at or near the breakpoints of the partner chromosomes. Molecular studies have indicated that the incidence of these rearrangements is much higher than reported by cytogenetics alone. Similarly, molecular techniques have demonstrated hemi- and homozygous loss of genes located at 9p21-p22 in up to 80% of childhood cases of T-ALL, many without cytogenetic evidence of deletion of 9p. The genes of interest are two related tumour-suppressor genes, MTS1 and MTS2 otherwise known as p16 (INK4a) and p15 (INK4b). These genes encode proteins that act as inhibitors of cyclin D-dependent serine-threonine kinases, which are necessary for the transition from G1 to S phase of the cell cycle. Further, it has been demonstrated that deletions of TAL1 and the cryptic translocation t(5;14)(q35;q32), involving transcriptional activation of HOX11L2, occur in 45% of T-ALL. Also found, but not exclusive to T-ALL, are abnormalities of the long arm of chromosome 6 (6q), in approximately 12% patients, A poor outcome. (Hoffbrand, *et al*, 2005).

1.2.5.2 Mature B-cell ALL

More than 80% of mature B-cell ALL (ALL L3) have one or other of three translocations consistently found in Burkitt's lymphoma: t(8;14)(q24;q32) (approximately 80%), t(2;8)(p12;q24) (6%) or t(8;22)(q24;q11) (14%). Chromosomes 14, 2 and 22 code for the immunoglobulin heavy-chain (IgH), and light-chain kappa (Ig κ) and lambda (Ig λ) genes respectively. The oncogene c-MYC is located at chromosome band 8q24. As a result of each translocation, the coding regions for c-MYC are brought into close proximity with one of the Ig genes. The recombination of Ig genes with c-MYC upregulates c-MYC expression, resulting in uncontrolled expansion of a malignant B-cell clone. Although the translocation appears identical in African Burkitt's lymphoma (which occurs endemically in equatorial Africa and is associated with the presence of the Epstein-Barr virus (EBV) in more than 90% cases) and in sporadic cases (found in Europe and North America, in the absence of viral infection), a difference is seen

at the molecular level. In sporadic cases, breakpoints occur either within or 5' of c-MYC and in the switch region of IgH, c-MYC is structurally altered as a result of this translocation. Most endemic African Burkitt's lymphomas involve the IgH JH region, c-MYC is not rearranged and breakpoints are scattered widely over a 300-kb region upstream of the gene (Hoffbrand, *et al*, 2005).

1.2.5.3 T-lineage ALL

The overall incidence of cases T-ALL classified by immunophenotyping is approximately 10% in childhood and 18% in adult ALL. The main chromosomal changes are shown in Table 30.2. The chromosomal abnormality detection rate by cytogenetic analysis is extremely low. It has been reported that ~20% of cases have translocations mapping to the chromosomal regions encoding one of the four T-cell receptor (TCR) genes, alpha/delta (TCR α / δ), beta (TCR β) and gamma (TCR γ) at 14q11, 7q32 or 7p15 respectively. These translocations juxtapose a TCR promoter/enhancer element to one of many transcription factors located at or near the breakpoints of the partner chromosomes. Molecular studies have indicated that the incidence of these rearrangements is much higher than reported by cytogenetic alone. Similarly, molecular techniques have demonstrated hemi- and homozygous loss of genes located at 9p21-p22 in up to 80% of childhood cases of T-ALL, many without cytogenetic evidence of deletion of 9p. The genes of interest are two related tumor-suppressor genes, MTS1 and MTS2 otherwise known as p16 (INK4a) and p15 (INK4b). These genes encode proteins that act as inhibitors of cyclin D-dependent serine-threonine kinases, which are necessary for the transition from G1 to S phase of the cell cycle. Further, it has been demonstrated that deletions of TAL1 and the cryptic translocation t(5;14)(q35;q32), involving transcriptional activation of HOX11L2, occur in 45% of T-ALL. Also found, but not exclusive to T-ALL, are abnormalities of the long arm of chromosome 6 (6q), in approximately 12% patients (Hoffbrand, *et al*, 2005).

1.2.6 Pathophysiology and Classification

Acute lymphoblastic leukemia represents a clonal proliferation of immature lymphocyte precursors. The cells may be B-cell precursors (~80 to 85% of cases) or T-cell precursors (~15 to 20% of cases). In rare cases, the lineage is unclear. The FAB classification divides ALL into three groups (L1, L2, and L3) based strictly on morphology. The L1 versus L2 FAB types do not

correspond to phenotype (i.e., precursor B cell versus precursor T cell) or to cytogenetic abnormalities and have limited prognostic significance. L1 has a slightly better prognosis than L2, but this may be largely due to age. The L3 type consists of mature B cells (not precursors) and corresponds to blood involvement by Burkitt's lymphoma.

1.2.6.1 FAB Classification of ALL

L1: Most common type in childhood, monomorphic, small to intermediate-sized blasts that have round nuclei, scant cytoplasm (high nucleus to cytoplasm [n-c] ratio), homogeneous nuclear chromatin, and inconspicuous nucleoli.

L2: Most common type in adults, Larger, more variable cells with more abundant cytoplasm (lower n-c ratio), irregular nuclear contours, and more prominent nucleoli.

L3: Rare (~1–3% of ALL). The cells are characterized by deeply basophilic (blue) cytoplasm with prominent clear cytoplasmic vacuoles containing lipid.

The new WHO classification differentiates subtypes based on phenotype (precursor B cell versus precursor T cell) and cytogenetic abnormalities. The FAB L1 and L2 designations are not used, and L3 is considered to be a leukemic phase of Burkitt's lymphoma (William, 2002).

Epidemiology

Acute lymphoblastic leukemia is the most common malignancy in childhood and represents ~85% of childhood acute leukemias. Acute lymphoblastic leukemia also occurs in adults but is uncommon (~15% of adult acute leukemias). The highest incidence of ALL is between 1 and 5 years of age. There is a slight male predominance overall. There is a marked increase in risk of ALL in children with trisomy 21 (Down syndrome). Other inherited anomalies predisposing to ALL include Bloom syndrome, Fanconi's anemia, and ataxia-telangiectasia. There is also an increased risk following exposure to ionizing radiation. However, in the majority of cases, there is no known predisposing factor. There has been much discussion recently about an association between living near high-voltage electric power lines and ALL. Although this question remains unsettled, most evidence at present does not support such an association.

1.2.6.2 WHO Classification of ALL

Precursor B-cell ALL: Cytogenetic subgroups (with oncogenes involved): t(9;22)(q34;q11); BCR/ABL (the Philadelphia chromosome) t(v;11q23); MLL rearranged (MLL = myeloid-lymphoid leukemia gene) t(1;19)(q23;p13); E2A/PBX1 t(12;21)(p12;q22); TEL/AML1

Precursor T-cell ALL

Burkitt-cell leukemia (Hoffbrand, *et al*, 2005).

1.2.7 ALL in childhood

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Remarkable progress has been achieved in its treatment such that the 5-year event-free survival rates in the most successful clinical trials now range from 75% to 83%.

1.2.7.1 Etiology and pathogenesis

The causes of the vast majority of cases remain to be clarified but likely involve an interaction between the host inherited susceptibility, environment, haemopoietic development and chance.

Primary genetic abnormalities and cooperative mutations

Acquired genetic changes are considered to be central to the development of leukaemia. These changes affect the number (ploidy) and/or the structure of chromosomes, such as translocations, inversions, deletions, point mutations, and amplifications. Transformation of haemopoietic stem cells requires subversion of the controls of normal proliferation, a block in differentiation, resistance to death signals (apoptosis) and enhanced self-renewal. The primary mechanisms of leukaemia induction include aberrant activation of proto-oncogenes (e.g. MYC, TAL1, LYL1, LMO2 and HOX11) and generation of fusion genes encoding active kinases (e.g. BCR-ABL) or transcription factors (e.g. TEL-AML1, E2A-PBX1 and MLL linked to one of many fusion partners). Current multistep models of carcinogenesis predict that the oncogenic events triggered by chromosomal rearrangements are not sufficient by themselves to induce overt leukaemia. The spectrum of secondary mutations involved in the development of leukaemia and their role in the clinical heterogeneity of specific leukaemia subtypes remain to be clarified.

These mutations involve both genetic and epigenetic changes in key growth regulatory pathways, including those controlled by the tumour suppressors RB (the retinoblastoma protein and related family members, p130 and p107) and p53. RB plays a critical role in controlling cell cycle entry. The hypophosphorylated RB inhibits the ability of the E2F family of transcription factors to induce the transcription of genes necessary for S-phase entry. Mitogenic signals induce the formation of active cyclin D-dependent kinases, which, together with cyclin E–Cdk2, phosphorylate RB and abolish its growth-inhibitory functions. The activity of cyclin D-dependent kinases is in turn regulated by the INK4 proteins (p16INK4a, p15INK4b, p18INK4c and p19INK4d), which specifically inhibit the activity of cyclin D-dependent kinases and prevent the phosphorylation of RB. Inactivating mutations or deletions of RB are rare in ALL. By contrast, functional silencing of p16INK4a and p15INK4b occurs in almost all cases of childhood T-cell ALL and in a small proportion of B-lineage ALLs. Although the loss of p16INK4a and p15INK4b expression may play a role in the development of leukaemia, it has no independent prognostic significance. The p53 transcription factor function is a sensor of aberrant cellular proliferation, DNA damage and hypoxia, and its activation results in either cell cycle arrest or apoptosis. The activity of p53 is negatively regulated by HDM2 (the human homologue of mouse Mdm2), which directly binds to p53 and induces its degradation. The p14ARF tumour suppressor in turn binds HDM2 to antagonize its ability to negatively regulate p53. Silencing of p14ARF is a frequent event in ALL, and overexpression of HDM2 or silencing of the p53 transcriptional target p21CIP1 is observed in approximately 50% of ALL cases. Importantly, p16INK4a and p14ARF are encoded by alternative reading frames in the same genetic locus. The high frequency of homozygous deletions that eliminate the expression of both gene products suggests that alterations of both the RB and p53 pathways collaborate in the pathogenesis of ALL. However, it is likely that additional key regulators will be identified as targets for genetic or epigenetic alterations in ALL.

Prenatal origin of some leukaemias

The retrospective identification of leukaemia-specific fusion genes (e.g. MLL–AF4, TEL–AML1) in the neonatal blood spots and the development of concordant leukaemia in identical twins indicate that some leukaemias have a prenatal origin. ALL with the t(4; 11)/MLL–AF4 has a high concordance rate in identical twins (nearly 100%) and a very short latency period (a few

weeks to a few months), suggesting that this fusion alone is either leukaemogenic or requires only a small number of cooperative mutations to cause leukaemia. By contrast, the concordance rate in twins with the TEL–AML1 fusion or T-cell phenotype is lower, and the postnatal latency period is longer, suggesting that additional postnatal events are required for leukaemic transformation. This concept is supported by the identification of rare cells expressing TEL–AML1 fusion transcripts in approximately 1% of cord blood samples from newborns, a frequency 100-fold higher than the incidence of ALL defined by this fusion transcript. However, clearly, not all childhood cases develop in utero. For example, the t(1;19)/E2A–PBX1 ALL appears to have a post-natal origin in most cases.

Environmental factors and host pharmacogenetics

The MLL gene, located on chromosome 11q23, is frequently involved in infant leukaemias and in therapy-related acute myeloid leukaemia (AML) after treatment with topoisomerase II inhibitor. It is possible that transplacental fetal exposure to topoisomerase II inhibitors, such as flavonoids in food and drink, quinolone, benzene metabolites, catechins, podophyllin resin, and even estrogens, is a critical event in the generation of leukaemias with MLL rearrangements. A case–control study disclosed significant associations between in utero exposure to DNA-damaging drugs, herb medicines or pesticides and the development of infant leukaemias with MLL rearrangements. Conceivably, a reduced ability of fetuses or their mothers to detoxify these agents could underlie an enhanced susceptibility to ALL. Genetic polymorphisms of carcinogen-detoxifying enzyme have been variously associated with the development of leukaemia. For example, deficiency of glutathione S-transferases (GST-M1 and GST-T1), enzymes that detoxify electrophilic metabolites by catalyzing their conjugation to glutathione, is associated with infant leukaemias without MLL rearrangement and with ALL in black children. Polymorphisms of another enzyme, reduced nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase, which converts benzoquinones to less toxic hydroxyl metabolites, has been associated with the development of infant and childhood ALL. Cytochrome P-450 CYP1A1*2A genotype has also been linked to an increased risk of childhood ALL. Recent studies suggest that folate pathways may play a role in the susceptibility to ALL, and that folate supplement may reduce the risk, observations warranting additional studies for confirmation. Although environmental factors such as chemical mutagens have been implicated in the aetiologies of leukaemia, solid evidence

is lacking. Previous studies have largely excluded residential exposure to magnetic fields as a major instigating factor in leukaemogenesis. Two hypotheses have suggested that abnormal response to common infections plays an important role in the development of childhood ALL. Because high socioeconomic status and social isolation are associated with an increased risk of childhood B-precursor ALL, one hypothesis proposes that many such cases, especially those diagnosed between 2 and 5 years of age, are the consequence of a delayed exposure to common infections to a time when there is increased lymphoid cell proliferation and hence increased chance of leukaemogenic genetic mutations. The other hypothesis suggests that transiently increased rates of leukemia, sometimes in clear geographic clusters, are due to population mixing, resulting in infection in previously unexposed and hence susceptible individuals.

1.2.7.2 Clinical presentation

The presenting symptoms and signs are quite variable. Most patients have an acute onset, while in others the initial signs and symptoms appear insidiously. Fever occurs in approximately 50–60% of patients. In at least two-thirds of these patients, fever is due to leukaemia and will resolve within 72 h after the start of induction therapy. Fatigability and malaise are common. Over one-third of the patients may present with a limp, bone pain, arthralgia, or refusal to walk due to leukaemic infiltration of the periosteum, bone or joint or to expansion of marrow by leukaemic cells. Children with prominent bone pain sometimes have nearly normal blood counts, which can lead to a delay in diagnosis. Many patients have manifestations of bleeding, especially epistaxis or oozing from gum. Occasionally, patients present with life-threatening infection or bleeding. Less common symptoms and signs include headache, vomiting, respiratory distress and anuria. Rarely, ALL is detected during routine examination. Physical examination often reveals pallor, petechiae and ecchymoses in the skin or mucous membranes, and bone tenderness. Liver, spleen and lymph nodes are the most common sites of extramedullary involvement and are enlarged in more than half the patients. Facial and/or abducens nerve palsies are occasionally seen. Ocular involvement can manifest as leukaemic infiltration of the orbit, optic nerve, retina or anterior chamber of the eye (hypopyon). Epidural spinal cord compression is a rare but serious presenting finding and requires immediate treatment to prevent permanent sequelae. Overt testicular involvement occurs in only 2% of patients, mostly infants or adolescents with T-cell ALL. Less common presenting features include subcutaneous nodules (leukaemia cutis), enlarged salivary

glands and priapism. Finally, in some patients, infiltration of the tonsils, adenoids, appendix or mesenteric lymph nodes leads to a surgical intervention before leukaemia is diagnosed. Patients with cerebellar ataxia who develop a lymphoid neoplasm should be suspected of having ataxia telangiectasia. Increased serum alpha-fetoprotein is a useful aid in the diagnosis. Recognition of ataxia telangiectasia is important because these patients have excessive and sometimes fatal complications from treatment with irradiation (leukoencephalopathy) and cyclophosphamide (haemorrhagic cystitis) (Hoffbrand, *et al*, 2005).

1.2.7.3 Laboratory findings

Anemia, abnormal leucocyte and differential counts, and thrombocytopenia are usually present at diagnosis. The initial leucocyte counts range from 0.1 to $1500 \times 10^9/L$ (median $12 \times 10^9/L$) and are increased ($> 10 \times 10^9/L$) in slightly over one-half of patients. Hyperleucocytosis ($> 100 \times 10^9/L$) occurs in 10–15% of patients. Profound neutropenia ($< 0.5 \times 10^9/L$) occurs in 40% of patients, rendering them at high risk of infection. The majority of circulating leucocytes are lymphocytes or lymphoblasts. In patients with low initial counts ($< 2 \times 10^9/L$), often there are no circulating lymphoblasts in blood smears. Hypereosinophilia, generally reactive, may be present at diagnosis. Thrombocytopenia (median count $50 \times 10^9/L$) is usually present at diagnosis and severe haemorrhage is uncommon, even when platelet counts are as low as $20 \times 10^9/L$, provided that infection and fever are absent. Coagulopathy, usually mild, may occur in T-cell ALL and is only rarely associated with severe haemorrhage. More than 75% of patients present with anaemia; haemoglobin is commonly < 8 g/dL. Anaemia or thrombocytopenia is often mild (or even absent) in patients with T-cell ALL. Rarely, pancytopenia followed by a period of spontaneous haemopoietic recovery may precede the diagnosis of ALL.

1.2.8 ALL in adult

Classification of blast cell phenotype in adult ALL requires morphological and cytochemical evaluation, immunophenotyping, cytogenetic and molecular genetic analysis. Morphology remains the method by which acute leukaemia is initially detected and, together with cytochemical reactions, is the major aid in distinguishing between ALL and acute myeloid leukaemia (AML). For more precise subclassification of ALL into B or T lineages, immunological techniques must be used to detect lineage-specific antigens as well as surface or

intracytoplasmic molecules. Cytogenetic analysis is still a prerequisite for diagnosis of ALL because it has prognostic value, but molecular genetic techniques for identification of particular subsets of ALL (e.g. BCR–ABL-positive ALL) are of increasing importance. Molecular markers, particularly rearrangements of T-cell receptor (TCR) and immunoglobulin heavy-chain (IgH) genes are presently used to evaluate therapeutic efficacy in individual patients by detection of MRD (Hoffbrand, *et al*, 2005).

1.2.8.1 Morphology

The cytological features of leukaemic blast cells in ALL and their division into L1 to L3 according to the French–American– British (FAB) classification are discussed; the distribution of L1 and L2 subtypes is of minor relevance for prediction of outcome in ALL. The subtype L3, observed in approximately 5% of adult ALL patients, should, however, be identified because it is indicative for mature B-cell ALL, which is subject to different treatment. The diagnosis should be confirmed by surface marker analysis (Hoffbrand, *et al*, 2005).

1.2.8.2 Clinical relevance of cytogenetic and molecular genetic analysis in adult ALL

Cytogenetic abnormalities are independent prognostic variables for predicting the outcome of adult ALL. In three multicentre studies, clonal chromosomal aberrations could be detected in approximately 62–85% of adult ALL patients. The Ph chromosome t(9;22)(q34;q11) results from a translocation involving the breakpoint cluster region of the BCR gene on chromosome 22 and the ABL gene on chromosome 9. PCR analyses revealed an incidence of 20–30% BCR–ABL+ ALL in adult compared with 3% in childhood ALL patients. One-third of adult ALL patients with a Ph chromosome show M-BCR rearrangements (resulting in a 210-kDa protein), similar to patients with chronic myeloid leukaemia (CML), whereas two-thirds have m-BCR rearrangements (resulting in a 190-kDa protein). It is noteworthy that BCR–ABL is more frequently detected than the corresponding chromosome abnormality t(9;22) because of occasional difficulties in obtaining adequate material for cytogenetic analysis. The most frequent form of 11q23 abnormalities in ALL is t(4;11)(q21;q23). The translocation is frequently detected in infant leukaemia and in patients with the pro-B ALL subtype (CD10 negative). The overall incidence in adults is approximately 5%. The role of cytogenetic analysis in adult ALL has to be re-evaluated critically. The most frequent cytogenetic aberrations and those with the largest

prognostic impact can also be detected by the corresponding molecular genetic abnormalities, as mentioned above. These techniques are more reliable and have a greater sensitivity, e.g. a detection level of more than 10^{-6} for BCR–ABL. They are, therefore, more useful for initial detection of the aberrations and for follow-up analysis of minimal residual disease. In addition, the observed incidence of the majority of cytogenetic aberrations is very low and therefore a correlation to clinical outcome and even more therapeutic consequences are limited. Nevertheless, cytogenetic analysis is still recommended as a routine diagnostic method in ALL.

1.2.8.3 Clinical manifestation

Most adult ALL patients initially present with clinical symptoms resulting from bone marrow failure. Physical findings such as pallor, tachycardia, weakness and fatigue are due to anaemia; petechiae or other haemorrhagic manifestations are attributable to thrombocytopenia; infectious complications are due to neutropenia. Clinical signs of leukaemia related directly to infiltration of typical organs with leukaemic blasts, such as lymphadenopathy, splenomegaly, and hepatomegaly, are present in most patients but are infrequently the problems for which the patient first seeks medical advice. One-third had infection or fever at presentation, and one-third presented with haemorrhagic episodes. Weight loss was observed only occasionally. Approximately one-half of the patients presented at diagnosis with lymphadenopathy, splenomegaly and hepatomegaly, and hilar lymph node enlargement or a thymic mass (detected on chest radiographs or computerized tomography scans) in approximately 14% of patients. Most patients (85%) with mediastinal masses had T-cell ALL. Massive thymic enlargement can cause dyspnoea, especially when associated with pleural effusions. Although 7% of ALL patients at presentation had central nervous system (CNS) involvement (as demonstrated by leukaemic blast cells in the cerebrospinal fluid), only 4% of these initially had CNS symptoms such as headache, vomiting, lethargy, nuchal rigidity and cranial or peripheral nerve dysfunction. Virtually any organ can be infiltrated by ALL blast cells, and approximately one-tenth of the patients had such organ involvement but with a wide variation between subtypes. Most often a pleural effusion was observed, and this occurred almost exclusively in those patients with mediastinal enlargement and T-cell ALL. Some of those patients also had a pericardial effusion. Bone or joint pain was rarely observed compared with childhood ALL; bone lesions could be found in only 1% of cases. Initial involvement of the testis was very rare (< 1%). Leukaemic infiltration of

retina, skin, tonsils, lung, or kidney was observed only occasionally, particularly in mature B-cell ALL and to a lesser extent in T-cell ALL, all of them associated with a poorer outcome.

1.2.8.4 Laboratory evaluation

The peripheral blood cell value shows differences between the subtypes of adult ALL. Overall, the leucocyte count was elevated in 59% of the cohort, 14% had normal counts and 27% had leucopenia. In 92% of the patients, leukaemic blast cells were seen in the blood film. Thus, 'aleukaemic' leukaemias account for only a small proportion of cases of adult ALL. With automated blood counting, the diagnosis may be missed in patients with normal or decreased white blood cell (WBC) counts and with low or zero blast cells in peripheral blood. In any case, but also for this reason, microscopic examination of blood films in people suspected of having acute leukaemia is an absolute requirement. An elevated blood count $> 100 \times 10^9/L$ was observed in 16% of the patients, and occasionally WBC counts $> 500 \times 10^9/L$ occurred. In general, a high WBC count is found more frequently in T-cell ALL patients compared with those with B-lineage ALL. Neutrophils $< 500 \times 10^6/L$ were seen overall in 23% of the patients. Severe neutropenia at diagnosis is observed more often in B-precursor ALL (28%). Thrombocytopenia $< 25 \times 10^9/L$ occurred in one-third of adult ALL patients, corresponding roughly with the symptoms of infection and bleeding present at diagnosis. Anaemia at diagnosis is observed in most adult ALL patients, but only in a small proportion is it severe with haemoglobin < 8 g/dL. Bone marrow aspiration or biopsy is mandatory for diagnosis of ALL. In $< 15\%$ of patients, the bone marrow cannot be aspirated and a biopsy must be performed. Dry taps are due to densely packed blast cells, fibrosis, or inadequate technique; the first two resolve after therapy. Most patients have $> 50\%$, or even $> 90\%$, of blast cells in the bone marrow. In $< 3\%$ of cases, the blast cells constitute $< 50\%$ of the nucleated marrow cells. A lumbar puncture should be done to determine whether the CNS is involved. If there is a risk of bleeding as a result of a very low platelet count, or of blast cell contamination due to a high leukaemic blast content in the peripheral blood, lumbar puncture should be postponed. When the leucocyte count in the spinal fluid is low or the morphological detection of blasts is inconclusive, demonstration of an immunologically defined blast cell population can confirm a diagnosis of CNS involvement. The most frequent metabolic abnormality is an increased serum uric acid level in approximately one-half of the patients; hypercalcaemia is rare. Serum lactate dehydrogenase (LDH) is often elevated

as a result of cell destruction in patients with large tumors mass. In a small proportion of patients, the initial fibrinogen level was <1 g/L. Disseminated intravascular coagulation in ALL was rarely observed at diagnosis (Hoffbrand, *et al*, 2005).

1.2.9 Clinical Features

Signs and symptoms of ALL usually relate to bone marrow infiltration and suppression of normal hematopoiesis. Common signs and symptoms may include the following:

- Pallor and fatigue
- Petechiae or other bleeding signs
- Fever: Fever is present in approximately half of patients. This may be due to infection, but, in many cases, no infection can be identified.
- Bone or joint pain: Bone or joint pain is common, probably due to expansion of the medullary cavity by the malignant cells. The initial symptom in young children may be limping or reluctance to walk.
- Hepatosplenomegaly and lymphadenopathy
- CNS involvement: ALL can involve the central nervous system (leukemic meningitis). Leukemic meningitis is uncommon at original diagnosis, but the spinal fluid may be a site of relapse after treatment. The blood-brain barrier decreases penetration of chemotherapy into the cerebrospinal fluid, providing a “pharmacologic sanctuary” for the leukemic cells.

The testes in males may also be a site of relapse. Testicular involvement presents as painless enlargement of the testes. Testicular relapse is usually followed by bone marrow relapse within a short time, unless systemic treatment is given. A large mediastinal mass may be present in precursor T-cell ALL. Respiratory distress due to compression of the trachea may be the presenting complaint. Hyperleukocytosis with leukostasis is less common in ALL than AML but may occur.

1.2.10 Laboratory finding

Anemia and thrombocytopenia are almost always present. The white cell count is variable: it may be high, normal, or occasionally decreased. Blasts are usually present on blood smear but

may be absent or hard to find in up to 5% of cases (a leukemic leukemia). Serum uric acid and lactic dehydrogenase may be increased.

1.2.10.1 Bone Marrow examination

The bone marrow in ALL usually shows a monomorphic population of blasts, with marked decrease in normal hematopoietic precursors of all types. “Block” PAS reactivity may be present, although this occurs in less than half of ALL cases. Reactivity for myeloperoxidase, Sudan black B, and specific and nonspecific esterases are absent.

1.2.10.2 Immunophenotyping

Immunophenotyping is usually performed by flow cytometry on either blood or a bone marrow aspirate.

- Precursor B-cell ALL characteristically expresses B-cell-associated antigens such as CD19 and CD20. Most cases express CD10 (the common acute lymphoblastic leukemia antigen or cALLA); CD34 (human progenitor cell antigen) is frequently expressed; cell surface immunoglobulin expression is absent, but there may be mu (μ) heavy chain present in the cytoplasm. Terminal deoxynucleotidyl transferase (TdT, a nuclear marker) is often present.
- Precursor T-cell ALL characteristically expresses T-cell antigens such as CD2, CD5, and CD7. CD1a may be present; CD4 and CD8 (the T helper and T suppressor subset antigens) are characteristically either both absent or both expressed. CD3 may be present in the cytoplasm but is absent from the cell surface. Terminal deoxynucleotidyl transferase is usually present.
- Mature B-cell ALL (Burkitt-cell leukemia) is characterized by the presence of cell surface immunoglobulin, with light chain restriction (either kappa or lambda light chain, but not a normal mixture of both) and B-cell antigens such as CD19 and CD20. CD10 (cALLA) may be present or absent.

Expression of myeloid antigens (CD13, CD15, CD33, and others) occurs in 30 to 50% of cases of ALL, more often in children than adults. In most cases, there is only a single myeloid antigen expressed. Myeloid antigen expression does not appear to have any independent prognostic significance in children but may be an adverse prognostic factor in adults

1.2.10.3 Cytogenetic

Cytogenetic analysis has become critical for prediction of outcome and selection of therapy in ALL. Chromosomal alterations are present in $\geq 75\%$ of cases. There may be abnormalities in chromosome number or structural alterations in chromosomes, usually reciprocal translocations.

1.2.10.4 Molecular Diagnostic Tests

The presence of clonal rearrangements of the lymphocyte antigen receptor genes (immunoglobulin genes for B cells, the T-cell receptor genes for T cells) can be used as evidence of lymphocyte malignancy and can also be used to suggest lineage. Molecular tests can also be used to detect chromosomal abnormalities not detected on standard cytogenetics.

1.2.10.5 Differential Diagnosis

The differential diagnosis of ALL includes the following:

- **Reactive lymphocytosis:** Reactive lymphocytosis is often seen with viral infections such as infectious mononucleosis (Epstein-Barr virus), viral hepatitis, cytomegalovirus and others. The lymphocytes usually appear variable, with abundant cytoplasm. Pertussis (whooping cough) may be associated with a striking lymphocytosis; the lymphocytes usually look small and mature, with prominent nuclear folds (“buttock cells”). The hemoglobin and platelet count are usually normal in patients with reactive lymphocytosis, and there may be symptoms related to the primary illness.
- **Immune thrombocytopenic purpura (ITP):** ITP presents with petechia or other signs of bleeding, mimicking a common presentation of acute leukemia. In ITP, the hemoglobin and white blood count are usually normal and the patients otherwise appear healthy.
- **Aplastic anemia:** Aplastic anemia presents with anemia, thrombocytopenia, and leukopenia and clinically may resemble a leukemic leukemia. Hepatosplenomegaly is usually absent. The diagnosis is based upon a hypocellular bone marrow without a predominance of lymphoblast.
- **Chronic lymphocytic leukemia (CLL):** CLL is characterized by a pre- dominance of small mature-appearing lymphocytes instead of blasts. Flow cytometry of CLL demonstrates a mature B-cell phenotype rather than the immature phenotype seen in ALL. Chronic lymphocytic

leukemia is characteristically a disease of adults, whereas ALL is more common in children (William, 2002).

1.2.10.6 Recurring Translocations in ALL

Trans-Genes location Frequency Involved Significance t(12;21) 25% of pediatric TEL/AML1 Favorable outcome; usually precursor B-cell missed on standard cytogenetic ALL analysis and must be detected by molecular methods t(9;22) $\leq 5\%$ of pediatric BCR/ABL Highly unfavorable outcome; (Philadelphia ALL; ~30% of often associated with myeloid chromosome) adult ALL antigen expression

t(1;19) ~5% of pediatric E2A/PBX1 Previously associated with ALL; precursor unfavorable outcome; less B-cell phenotype significant with aggressive therapy

t(v;11q23) 4–8% of MLL; numerous Common in infant ALL (<1 year); pediatric ALL partners highly unfavorable outcome; often CD10–, CD15+ phenotype

t(8;14); t(2;8); <5% of pediatric c-MYC Associated with Burkitt-cell t(8;22) ALL oncogene; leukemia; formerly highly immunoglobulin unfavorable outcome, less genes significant with aggressive therapy

- Acute myeloid leukemia: AML, particularly minimally differentiated AML (M0 and M1 in the FAB classification), can be morphologically indistinguishable from ALL. Cytochemical stains and immunophenotyping usually permit distinction.

1.2.11 Treatment

Treatment of ALL is usually separated into three phases: remission induction, intensification (consolidation), and continuation (maintenance). Treatment includes several drugs that have different mechanisms of action. The total duration of therapy is 2 to 3 years. Treatment of the central nervous system (CNS) is an essential part of therapy, even in the absence of overt CNS involvement, to prevent CNS relapse.

- Induction: Typical induction regimens include a corticosteroid (prednisone or dexamethasone) and vincristine, plus L-asparaginase (in children) or an anthracycline (in children with high-risk

disease and in adults). This phase lasts approximately 4 to 6 weeks and is designed to reduce the leukemic burden to clinically undetectable levels (i.e., induce a complete remission [CR]).

- **Intensification (consolidation):** Intensification regimens can include higher doses of the drugs used to induce remission or a combination of different drugs. Examples of consolidation regimens include (1) methotrexate with or without 6-mercaptopurine, (2) high-dose L-asparaginase, (3) an epipodophyllotoxin such as VP16 with cytosine arabinoside (cytarabine; ara-C), or (4) a combination of vincristine, dexamethasone, L-asparaginase, doxorubicin, and thioguanine with or without cyclophosphamide. This phase typically involves repeated cycles of therapy over approximately 6 months (longer in high-risk patients).
- **Continuation (maintenance):** This phase typically includes weekly methotrexate (orally or by intramuscular injection) and daily oral 6-mercaptopurine. This phase typically lasts approximately 2 to 21/2 years.
- **CNS therapy:** Prophylactic CNS therapy is required in order to prevent CNS relapse. Intrathecal chemotherapy with methotrexate or cytosine arabinoside is used, together with high doses of drugs that cross the blood-brain barrier such as dexamethasone, methotrexate, or cytosine arabinoside. Craniospinal irradiation is used in patients with CNS involvement at diagnosis and in some patients considered at high risk for CNS relapse.

1.2.11.1 Complications of Treatment

Complications of therapy can be divided into immediate and long-term complications.

- **Immediate complications:** Immediate complications include nausea, vomiting, and alopecia. Infection due to granulocytopenia and hemorrhage due to thrombocytopenia may also occur.
- **Long-term complications:** The majority of children treated for ALL have few or no major long-term effects. Avascular necrosis of the femoral heads may occur due to corticosteroids. Methotrexate therapy may result in leukoencephalopathy and impaired intellectual performance. Short stature and impaired intellectual performance may occur in patients given craniospinal irradiation; the growth impairment appears to be at least partially related to endocrine dysfunction and can often be improved with growth hormone. A rare complication linked to craniospinal irradiation is the development of brain tumors, occurring at a median of 10

years after therapy. These are characteristically aggressive gliomas, and survival is usually short. Another uncommon complication is the development of acute myeloid leukemia, which is linked to chemotherapy with alkylating agents and topoisomerase II inhibitors (epipodophyllotoxins and anthracyclines). The AML often passes through a brief myelodysplastic phase; it tends to be poorly responsive to therapy, and survival tends to be brief.

1.2.12 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. The prognosis in adults is less optimistic; less than 40% of adults are cured. Efforts have been made to stratify treatment based on prognosis: 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. The initial risk stratification in children is based on age and WBC count, and then readjusted after cytogenetic results are available. An anthracycline is added to the induction regimen in children considered at high risk, and the intensity of therapy during the consolidation phase is increased. Patients at particularly high risk (such as those with a Philadelphia chromosome) may be considered for allogeneic bone marrow transplant (BMT) in first CR.

Special Types of Acute Lymphoblastic Leukemia

Some types of ALL deserve special mention:

- **ALL in infants (<1 year old):** ALL in infancy is associated with translocations involving the mixed-lineage leukemia (MLL) gene on chromosome 11q23 [most often t(4;11)], a very high white cell count, and a highly adverse outcome. The immunophenotype frequently shows expression of CD15 and lack of CD10 (cALLA).
- **Philadelphia chromosome-positive ALL:** The Philadelphia chromosome [t(9;22); bcr/abl rearrangement] is present in ~20 to 30% of adult ALL cases but <5% of pediatric cases. The cells usually have a precursor B-cell phenotype, but there is often expression of myeloid antigens such

as CD13, CD15, or CD33. Response to therapy is poor; the complete response rate is lower, and remissions tend to be brief.

- Precursor T-cell ALL: Precursor T-cell ALL has a predisposition to occur in young or adolescent males. The white count is often very high ($>100,000/L$), and CNS involvement is common. Patients often have a mediastinal mass, probably reflecting origin in the thymus, and may present with respiratory symptoms due to tracheal compression or pleural effusions. Lineage ALL was thought to have a worse prognosis than precursor B-cell ALL, but with aggressive therapy, the differences have decreased.

1.2.12.1 Prognostic Factors in ALL

Favorable and unfavorable factors

.Age 2 to 10 years Below 2 years or above 10 years WBC count Low WBC count at WBC $>50,000/L$ diagnosis

.Phenotype Precursor B cell Precursor T cell Mature B cell

.Chromosome Hyperdiploidy Pseudodiploidy number or DNA DNA Index >1.16 Hypodiploidy Index Near tetraploidy

.Chromosome t(12;21) c-MYC alterations [t(8;14); abnormalities Trisomy 4 and trisomy 10 t(2;8); t(8;22)]

.MLL alterations (11q23), t(9;22) (Philadelphia chromosome), t(1;19)

.Sex Female Male

.Ethnicity Caucasian African American, Hispanic

.Time to complete Short ($<7-14$ days) Prolonged time to remission or remission failure to achieve complete remission

Prognostic significance of these factors may vary with therapy

Type may be a favorable prognostic factor in adult ALL (at least in young males). • Burkitt-cell leukemia: Burkitt-cell leukemia (ALL-L3 in the FAB classification) is currently considered to be

a leukemic phase of Burkitt's lymphoma. It is associated with translocations involving the c-MYC oncogene and one of the immunoglobulin genes, most often t(8;14) (c-MYC and the immunoglobulin heavy chain gene). Patients often have bulky mass lesions, most frequently in the abdomen. This type of ALL was formerly considered to have a very poor outcome, but survival in children is significantly improved with use of intensive chemotherapy regimens designed for Burkitt's lymphoma. The outcome for adults with Burkitt-cell leukemia remains poor (William, 2002).

1.2.13 Gene Expression Analysis

Analysis of gene expression, using microarrays, in ALL is bringing a new understanding of the disease process.

Chromosomal translocations target genes that appear to be transcription factors or kinases. One such kinase is ABL. The t(9;22) translocation results in the fusion of the gene BCR to ABL, and results in the activation of the ABL tyrosine kinase. Imatinibmesylate is a small tyrosine kinase inhibitor that is active against the tyrosine kinase subclass III family, which includes ABL, c-KIT, PDGF, and ARG. Imatinib occupies the kinase pocket, prevents access to ATP, and therefore prevents phosphorylation of substrate. Clinically when used as a single agent, it results in sustained remission in Ph⁺ chronic myeloid leukaemia (CML) in chronic phase and short remissions in CML in blast phase as well as in Ph⁺ ALL patients. In the latter category, drug resistance is largely due to mutations in the BCR-ABL fusion gene or gene amplification occurring in a highly proliferative cell population. This is the first time that therapy has been designed to target specifically the expression of an abnormal gene and it is remarkable that "switching off" the expression of the gene product stops proliferation and induces apoptosis and in some instances loss of the malignant clone.

. The Host Polymorphism Until now we have been discussing the molecular advances in understanding the biology of the disease and the advances in monitoring therapeutic response. However, host-related factors such as pharmacodynamics and pharmacogenomics can strongly affect treatment effectiveness and influence clinical outcomes in individual patients. A diversity of genes encode for drug-metabolizing enzymes, drug transporters, or drug receptors.

Polymorphisms in these genes can affect the kinetics and dynamics of numerous drugs and result in interindividual variations in drug response, toxicity, and clinical outcome. The most well-known of this is the polymorphism of the thiopurine methyltransferase enzyme. This enzyme methylates thiopurines, preventing the incorporation of metabolites into the nucleus. Patients who have a homozygous or heterozygous deficiency of the enzyme tend to have a better therapeutic response because of the higher levels of thiopurine metabolites achieved. However, they also have increased hematopoietic and hepatic toxicities, and homozygous-deficient patients may even have a fatal outcome if treated with standard doses of thiopurines. These children also have an increased incidence of developing therapy-related acute myeloid leukemia and irradiation-induced brain tumors as a consequence of antimetabolite therapy. Glutathione S-transferase (GST) detoxifies a wide range of anticancer drugs by catalyzing their conjugation to glutathione, and the absence of both alleles in the GSTM1, GSTT1, and the GSTP1 Val105/Val105 genotypes are associated with a lower rate of relapse. Similarly polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) enzyme may result in increased toxicity and sensitivity to methotrexate. In addition to these enzymes, polymorphisms in the cytochrome P450 (CY P450) enzyme systems, which detoxify many drugs, may lead to unwanted drug interactions (Udomsinprasert, *et al*, 2005).

1.3 Glutathione S transferase

Glutathione S-transferases (GSTs), previously known as ligandins, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. The GST family consists of three superfamilies—the cytosolic, mitochondrial, and microsomal—also known as MAPEG—proteins. (Udomsinprasert, *et al*, 2005, Sheehan, *et al*, 2001, Allocati, *et al*, 2009) Members of the GST superfamily are extremely diverse in amino acid sequence, and a large fraction of the sequences deposited in public databases are of unknown function (Atkinson, 2009).

1.3.1 Classification

Protein sequence and structure are important additional classification criteria for the three super families (cytosolic, mitochondrial, and MAPEG) of GSTs: while classes from the cytosolic

superfamily of GSTs possess more than 40% sequence homology, those from other classes may have less than 25%. Cytosolic GSTs are divided into 13 classes based upon their structure: alpha, beta, delta, epsilon, zeta, theta, mu, nu, pi, sigma, tau, phi, and omega. Mitochondrial F GSTs are in class kappa. The MAPEG superfamily of microsomal GSTs consists of subgroups designated I-IV, between which amino acid sequences share less than 20% identity. Human cytosolic GSTs belong to the alpha, zeta, theta, mu, pi, sigma, and omega classes, while six isozymes belonging to classes I, II, and IV of the MAPEG superfamily are known to exist. (Oakley, 2011, Eaton, 1999, Josephy, 2010).

GST Class

Homo sapiens GST Class Members (22)

.Alpha: GSTA1, GSTA2, GSTA3, GSTA4, GSTA5

.Delta

.Kappa: GSTK1

.Mu: GSTM1, GSTM1L (RNAi), GSTM2, GSTM3, GSTM4, GSTM5

.Omega: GSTO1, GSTO2

.Pi: GSTP1

.Theta: GSTT1, GSTT2, GSTT4

.Zeta: GSTZ1 (aka GSTZ1 MAAI-Maleylacetoacetate isomerase)

.Microsomal: MGST1, MGST2, MGST3

1.3.2 Function

The activity of GSTs is dependent upon a steady supply of GSH from the synthetic enzymes gamma-glutamylcysteine synthetase and glutathione synthetase, as well as the action of specific transporters to remove conjugates of GSH from the cell. The primary role of GSTs is to detoxify xenobiotics by catalyzing the nucleophilic attack by GSH on electrophilic carbon, sulfur, or nitrogen atoms of said nonpolar xenobiotic substrates, thereby preventing their interaction with crucial cellular proteins and nucleic acids.(Josephy,2010, Hayes, *et al*,2005) Specifically, the function of GSTs in this role is twofold: to bind both the substrate at the enzyme's hydrophobic

H-site and GSH at the adjacent, hydrophilic G-site, which together form the active site of the enzyme; and subsequently to activate the thiol group of GSH, enabling the nucleophilic attack upon the substrate (Eaton and Bammler, 1999). The glutathione molecule binds in a cleft between N and C-terminal domains - the catalytically important residues are proposed to reside in the N-terminal domain (Nishida, *et al*, 1998). Both subunits of the GST dimer, whether hetero- or homodimeric in nature, contain a single non substrate binding site, as well as a GSH-binding site. In heterodimeric GST complexes such as those formed by the cytosolic mu and alpha classes, however, the cleft between the two subunits is home to an additional high-affinity non substrate xenobiotic binding site, which may account for the enzymes' ability to form heterodimers. (Hayes, *et al*, 2005, Vargo, 2001) GSTs are in class kappa. The MAPEG superfamily of microsomal GSTs consists of subgroups designated I-IV, between which amino acid sequences share less than 20% identity. Human cytosolic GSTs belong to the alpha, zeta, theta, mu, pi, sigma, and omega classes, while six isozymes belonging to classes I, II, and IV of the MAPEG superfamily are known to exist. (Oakley, 2011, Eaton, 1999, Josephy, 2010)

1.3.3 Implications in cancer development

There is a growing body of evidence supporting the role of GST, particularly GSTP, in cancer development and chemotherapeutic resistance. The link between GSTP and cancer is most obvious in the overexpression of GSTP in many cancers, but it is also supported by the fact that the transformed phenotype of tumor cells is associated with aberrantly regulated kinase signaling pathways and cellular addiction to overexpressed proteins. That most anti-cancer drugs are poor substrates for GSTP indicates that the role of elevated GSTP in many tumor cell lines is not to detoxify the compounds, but must have another purpose; this hypothesis is also given credence by the common finding of GSTP overexpression in tumor cell lines that are not drug resistant (Tew, *et al*, 2011).

1.3.4 GST polymorphism

GST constitute multifunctional enzymes that are coded by at least eight distinct loci: α (GSTA); μ (GSTM); θ (GSTT); π (GSTP); σ (GSTS); κ (GSTK); ω (GSTO); and ζ (GSTZ), each one composed of one or more homodimeric or heterodimeric isoforms. These enzymes are involved in the conjugation reactions between glutathione (GSH) and variety of potentially toxic and

carcinogenic compounds. Additionally, GSTs display peroxidase activity and this can protect against oxidative damage the deficiency in the activity of this enzyme can be derived from the inherited GSTs polymorphism; GSTT1 (22q11.23), GSTM1 (1q13.3) and GSTP1 (11q13) (Mo, *et al*, 2009, Bessa, *et al*, 2009).

1.4 Previous studies

There is study done by Mhemet, *et al*, they study the GSTT1 polymorphism in turkish population, they found that the association between GSTT1 null polymorphism and ALL is not statistically significant (p.value =0.7).

In other study of Ahajany,*et al*, in iranian people the GSTT1 null polymorphism show insignificant correlation with ALL according to gender and age (gender p. value = 1.0, age p. value = 0.8).

1.5 Rationale

Acute lymphoid leukemia is a major health problem in Sudan; direct causes of ALL are idiopathic although there are factors increase the individual chances to develop leukemia such as chemicals, physical agent radioactive and others.

As a role of GSTT1 in detoxification and antioxidant of cells, and the reported findings regarding its role in development of different cancers, detection of GSTT1 null genotype among ALL patients using molecular techniques widely used to find out the association between GSTT1 and ALL .

Study of GSTT1 in ALL patients in Sudan, well help to understand the correlation between ALL and GSTT1 null genotype for the development of new strategies on ALL treatment and boost ALL control programs in Sudan.

1.6 Objectives

1.6.1 General objectives

To determine the frequency and association of GSTT1 null genotype with ALL patient's

1.6.2 Specific objectives

.To determine the frequency of GSTT1 null genotype among ALL patient's using allele specific PCR.

.To examines the role of GSTT1 null polymorphisms as a risk factor for ALL.

.To correlate between GSTT1 null genotype and patient's age and gender.

Chapter Two

Materials and Methods

Chapter Two

Materials and methods

2.1 Study design

This is a case control study was done during the period from February 2015 to December 2015, to determine the frequency of GSTT1 Null genotype in Sudanese patients with ALL.

2.2 Study area

This study was done in Khartoum city in leukemic patient from Radio Isotope Center and flowcytometer center, 40 blood samples were collected from patients diagnosed as acute lymphoblastic leukemia, and 30 blood samples were collected from healthy individuals as control group.

2.3 Sample collection

Venous blood collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol, the blood was added to the EDTA anticoagulant and gently mixed.

2.4 Materials

2.4.1 DNA extraction

Salting out protocol

- . RCLB.
- . WCLB.
- . TE buffer.
- . 6 M NaCl.
- . proteinase K

. chloroform

. ethanol

2.4.2 Agarose gel electrophoresis

. Gel casting tray

. Agarose

. TBE buffer (10 ml TBE + 90 ml water)

. Ethidium bromide

.Loading dye

2.4.3 DNA polymerization

. PCR machine

. DNA product

. Master Mix

. Primer (forward and reverse)

2.4.4 Other materials

* Centrifuge

* Incubator

* Eppendorf tubes

* Automatic pipettes

* Electrophoresis machine

* Vortex

2.5 Methods

2.5.1 DNA extraction

300 µl of well mixed blood was added in 1.5 Eppendorf tube, 1000 µl of RCLB was added to the tube, and mixed well, and centrifuged at 2500 rpm for 10 minutes, the supernatant was discarded and the pellet (WBC) washed again with 1000 µl of RCLB (repeated 2 to 3 times until clear pellet were obtained), 300 µl of WCLB and 10 µl of proteinase K were added to the clear white pellet, the mixture was incubated for 1 hour at the incubator, 100 µl of 6M NaCl was added to precipitate the protein and mixed well by vortexing, 200 µl of ice cold chloroform were added to the tube and centrifuged at full speed (18000 rpm) for 6 minutes, the aqueous phase was transferred carefully to clean Eppendorf tube to which double volume of cold absolute ethanol was added to precipitate the DNA, the tube then centrifuged at 14000 rpm for 5 minutes, the supernatant was poured off without disturbing the precipitate, and washed with 600 µl of 70% ethanol, the tube content was centrifuged at 6000 rpm for 5 minutes, the ethanol Discarded and the tube was left to dry, the pellet were re-suspending in 100 µl TE buffer and leaved to dissolve overnight.

2.5.2 DNA check by Agarose gel

Agarose gel was prepared by 2gm weighted agarose powder dissolved in 100 ml TBE buffer, which prepared by adding 10 ml TBE to 90 ml water, the mixture were boiled, after cooling 2 µl of ethidium bromide and the gel were poured in the casting tray, the combs were placed on the tray with agarose gel, after the gel polymerized the combs were removed, the running buffer were added and the samples were loaded.

Detection of GSTT1 / null genotype

All patients with ALL were screened for the presence of GSTT1 null genotype using allele specific PCR. The primers sequence used were as follow:-

Table 2.1: Oligonucleotides Sequences for GSTT1

Primer direction	Sequence	Product size(bp)
Forward primer	5'TTC CTT ACT GGT CCT CAC ATC TC3'	480
Reverse primer	5'TCA CCG GAT CAG GCC AGCA3'	

PCR mixture of 20 μ l was prepared using premix master mix tubes (MaximeTMPCR premix Kit{i-Taq}) for each sample, with positive and negative controls in sterile eppindroff tube as follow:-

Reagents	Volume
Double D.W	14 μ l
forward primer	1 μ l
Reverse primer	1 μ l
Template DNA	4 μ l
Total reaction volume	20 μ l

Table 2.2: Optimized cycling protocol for PCR analysis of GSTT1 on the PCR machine TECHN (TC412) as follow:

Profile	Temperature	Time duration	Number of Cycles
Initial Denaturation	94°C	10 minutes	1
Denaturation	95°C	1 minutes	45
Annealing	62°C	1 minutes	
Extension	72°C	1 minutes	
Final extension	72°C	10 minutes	1

2.5.3 Demonstration of PCR product

Four µl of the PCR product (ready to load) was electrophoresed on 2% agarose gel, and was 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 45 minutes. 100 bp DNA ladder was used as molecular weight marker with each patch of samples .Finally, PCR product was demonstrated by gel documentation system "SYNGENE", GSTT1 band appear at 480 bp. (figure 2.1)

2.6 Data analysis

The collected data for control group and case group proceeds for analysis using chi square test for the correlation of GSTT1 with study group and gender, independent t-test for correlation with Age and crosstab for the OD ratio by SPSS computerized program, and the results presented in form of tables and figures.

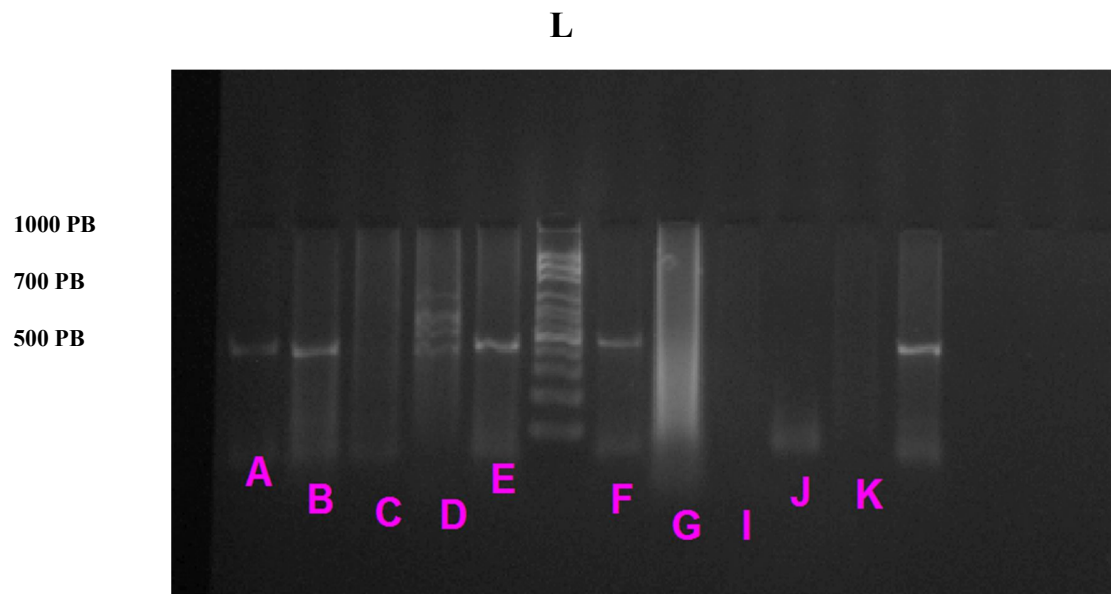


Figure (2.1): Band of GSTT1 in ALL patient's DNA using 100 pb ladder as marker (GSTT1 band in 480pb)

Chapter Three

The Results

Chapter Three

3.0 Results

This study was done among 30 healthy people and 40 patients with acute lymphocytic leukemia in Khartoum state to determine null GSTT1 genotype in both study groups.

Among study group GSTT1 was null in 53% of ALL patients (21/40) and 17% in control group (5/30), while it was present in 47% of ALL patients (19/40) and 83% in control group (25/30).

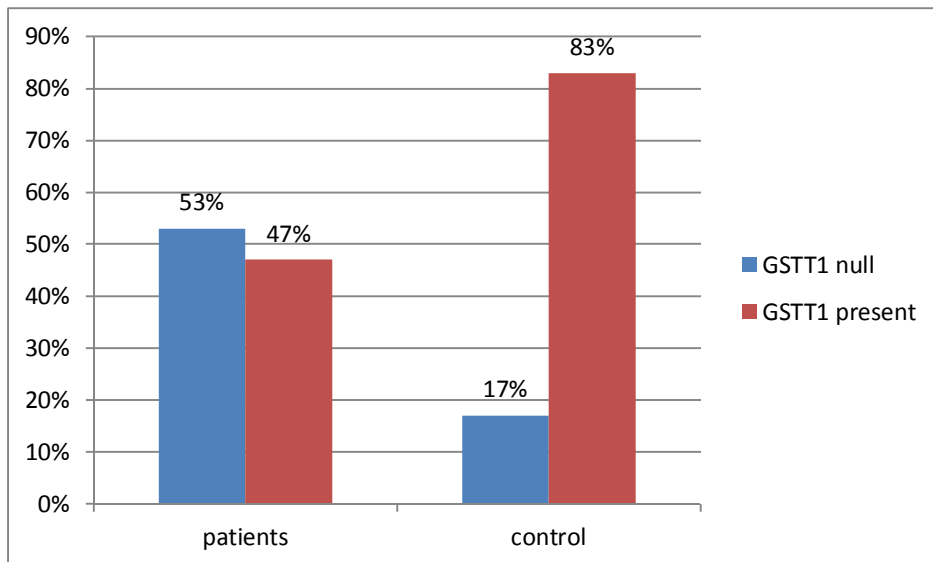


Figure 3.1: Distribution of GSTT1 genotypes among study group

In ALL patients GSTT1 was null in 38% of males and 13% of females, and presented in 33% of males and 16% of females

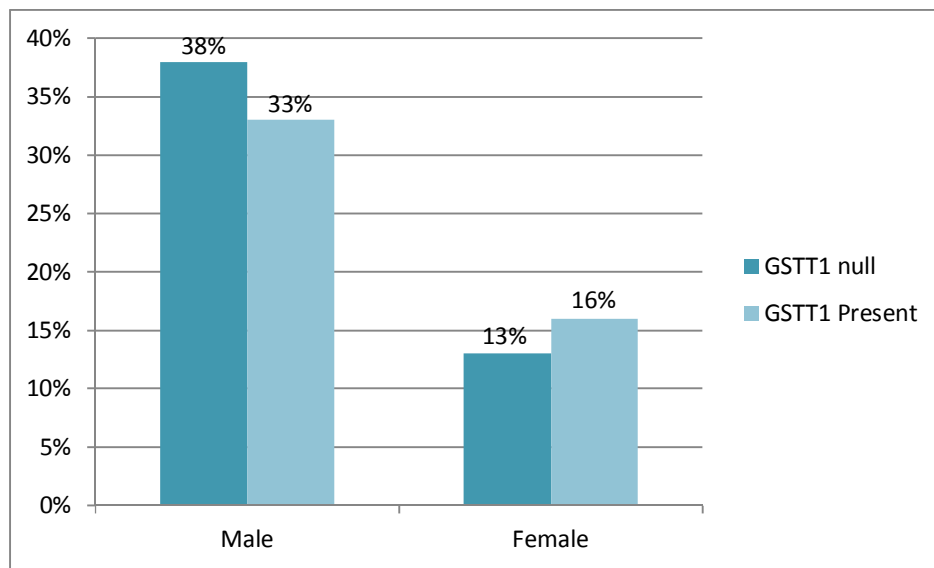


Figure 3.2: Distribution of GSTT1 in case group according to gender

In control group GSTT1 was null in 4% of males and 13% of females, and presented in 63% of males and 20% of females

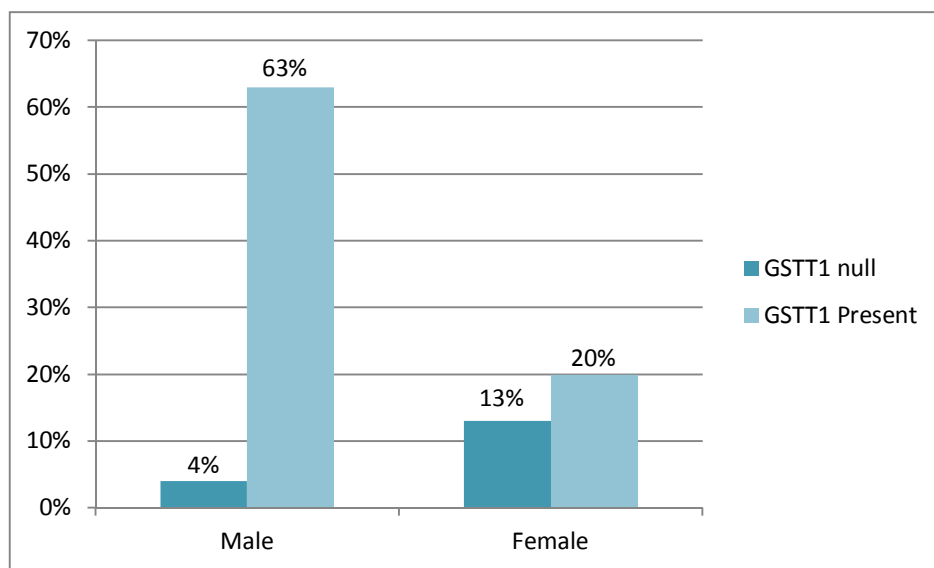


Figure 3.3: Distribution of GSTT1 in control group according to gender

. The results showed insignificant association of GSTT1 with age (p.value: 1.02)

Table (3.1): GSTT1 in study group related to age

Age	GSTT1	N	Mean	SD	P.value
	Present	44	14.25	6.8	1.02
	Null	26	12.77	5.2	

. The results showed insignificant association of GSTT1 with gender (p.value: 0.33), 16 male and 10 female of study group was null GSTT1 and presented in 32 male and 12 female

Table (3.2): GSTT1 in study group related to gender

GSTT1	Gender		Total	P.value
	Male	female		
Null	16	10	26	0.33
Present	32	12	44	
Total	48	22	70	

. The results showed significant association of GSTT1 with study groups (p.value: 0.002), in total of 30 healthy individuals only 5 was with null GSTT1 and the rest of 25 was presented with normal genotype, while in total of 40 ALL patients 21 was null genotype and the rest of 19 patients was presented with normal genotype

Table (3.3): GSTT1 gene related to samples

GSTT1	Samples		Total	P.value
	control	case		
Null	5	21	26	0.002
Present	25	19	44	
Total	30	40	70	

. GSTT1 null genotype showed significant association related to samples (p.value: 0.002, OR: 5.5).

Chapter Four

Discussion Conclusion and Recommendations

Discussion, conclusion and recommendation

4.1 Discussion

One of the most serious health problems that with idiopathic causes; cancer with its different types are one of these illnesses which all offers concern to treat the complication not the disease itself, that's make it more difficult to control and expect the progression among different patients.

Leukemia or blood cancer is one of these major health problems, myeloid or lymphoid leukemia both are unknown cause, despite there are a factors may increase risk to develop these disease such as chemicals, radioactive, genetic factors and others.

As a role of GSTT1 in detoxification and anti-oxidation this study led to find the correlation between null GSTT1 genotype and ALL patients in Sudanese people.

By using PCR to determine presence or absence of GSTT1 gene in ALL patient in compare with healthy individuals as control, our results showed that GSTT1 null genotype was significantly associated with ALL patient (OR: 5.5, 95% CI: 1.76 , P.value:0.002); the result showed different result from study in Turkish population which showed no association between GSTT1 null genotype and ALL Turkish patient(Mehmet, *et.al*, 2015), the differences between study populations may yield these differences because of different genetic structure. other studies investigated the association between GSTT1 gene and risk of relapse in childhood ALL cases were relapsed patients and controls were successfully treated the null GSTT1 showed significantly association with relapsed ALL patients (Martin, *et.al*, 2012); the prevalence of GSTT1 null genotype in ALL patients in our study was 38% in males and 13% in females, in control group GSTT1 null was detected in 4% of males and 13% of females.

According to gender and age, the result showed that GSTT1 null genotype had insignificant correlation to ALL (gender p.value:0.33) (Age p.value: 1.02), this agreed with study in Iranian people with insignificant association the p.value was <0.05 (gender: 1.0 and Age: 0.8) (Ahajany, 2011).

Our findings suggest the presence of association between GSTT1 null genotype and increase chance to develop ALL.

4.2 Conclusion

Our study showed that there is association of GSTT1 null genotype with ALL, with no significantly association with the gender or ages of patients.

4.3 Recommendations

- . Increase sample size and further studies are needed to elucidate the pathological basis of the observation.
- . More Studies are recommended to observe GSTT1 effect on prognosis of ALL patients and their response to chemotherapy.
- . Study the GSTT1 null polymorphism for separate types of ALL.

Chapter five

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Appendices

Appendix I

DNA extraction

Salting out protocol

.RCLB: 8.3 gm of NH_4CL , 1 gm KHCO_3 , 1.8 gm 5% EDTA and 1 liter of distilled water.

.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 μ EDTA (0.01M), and 100 ml distilled water.

.6 M Nacl: 35 gm of Nacl added to 1 liter of distilled water

Appendix II

Questionnaire

Sudan University of Science and Technology

Collage of Graduate Studies

Determination the frequency of glutathione S transferase thta1 null polymorphism among Sudanese patients with acute lymphoid leukemia

Questionnaire

. No.of patien:

.Gender:

- Male

- Female

Age:

.Type of leukemia:

. GSTT1:

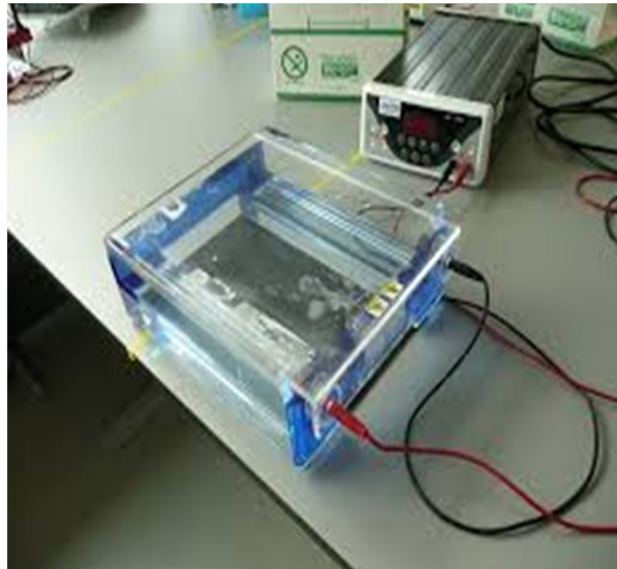
-Null

-Present

Appendix III



PCR (TC-412)



Gel electrophoresis



Gel imaging system (syngene)