Introduction

Acute Lymphoblastic Leukemia (ALL) is a malignant disorder characterized by a clonal expansion of lymphoid progenitor cells arrested at different differentiation steps, whose progressive accumulation causes bone marrow involvement with more than 20% blast cells at diagnosis (Schultz et al., 2007). The causes of ALL remain largely unknown, although environmental, immunodeficiency and genetic factors were found to play an important role (Pui, 2006).

The intensity of treatment required for favorable outcome varies substantially among subsets of ALL patients. Risk-based treatment assignment is utilized for patients with ALL so that those patients who have a very good outcome with modest therapy can be spared more intensive and toxic treatment, while a more aggressive, thus more toxic, therapeutic approach can be provided for patients who have a lower probability of long-term survival (Strefford et al., 2007).

The loss or gain of chromosomes to produce a numerical chromosomal deviation from multiples of the haploid chromosomal complement is called

aneuploidy, and has been classified as one of the crucial prognostic factors affecting the outcome of the disease (Le et al., 2006). This abnormality results from abnormal chromosomal distribution during meiosis I or II or during mitosis. Non-disjunction in meiosis I and II can be differentiated, while abnormal chromosomal distribution during mitosis leads to an aberration in only a proportion of the cells (chromosomal mosaicism) (Harrison and Foroni, 2002).

Cells with more than 46 chromosomes are called hyperdiploid, whereas the presence of fewer than 46 chromosomes is called hypodiploidy (Chang et al., 2006). Up to one third of children presenting with ALL have a hyperdiploid karyotype. Significantly, hyperdiploidy has been shown to be an independent risk factor. Children with hyperdiploid ALL respond well to standard chemotherapy regimens and have consistently shown a superior outcome when compared with their non-hyperdiploid counterparts (Paulsson et al., 2006). In contrast, hypodiploidy in ALL can be considered as a significant adverse risk factor despite treatment with contemporary intensive therapies (Sunil et al., 2006).

Characterization of the genetic changes has yielded a wealth of information on the mechanism of

leukemogenesis. These findings have also allowed the development of sensitive therapy protocols directed against specific genetic defects. It is not known whether malignant transformation precedes or results from aneuploidy. However, aneuploid genome would permit the loss of recessive genes that might result in loss of regulatory control of lymphoid growth and differentiation or over expression of proto-oncogenes, leading to malignant transformation. This abnormality is usually associated with a short complete remission and poor prognosis (Swansbury, 2003).

Aim of the Work

- Identification of all types of aneuploidy in acute lymphoblastic leukemia.
- Illustration of prognostic impact of aneuploidy on patients' outcome.

Acute Lymphoblastic Leukemia

Acute Lymphoblastic Leukemia (ALL) is a neoplastic disease that results from multistep somatic mutations in a single lymphoid progenitor cell at one of several discrete stages of development **(Pui, 2006)**. ALL is considered the most common cancer in children and is among the most curable of the pediatric malignancies **(Alison et al., 2000)**.

Incidence of ALL

ALL is the most common malignancy diagnosed in patients younger than 15 years, accounting for 23 percent of all cancers and 76 percent of all leukemias in this age group. Only 20 percent of adult acute leukemias are ALL. Age-specific incidence patterns are characterized by a peak between the ages of 2 and 4 years, followed by falling rates during later childhood, adolescence, and young adulthood. Incidence rises again in the sixth decade and reaches a second, smaller peak in the elderly **(Pui, 2006)**.

For unexplained reasons, the incidence of ALL is substantially higher for white children than for black children, with a nearly 3-fold higher incidence at 2 to 3 years for white children compared to black children.

The incidence of ALL appears to be highest in Hispanic children (43 per million) (Xie et al., 2003).

Etiology of ALL

The causes of ALL remain largely unknown, although environmental, immunodeficiency and genetics factors were found to play an important role (Pui, 2006).

I. Environmental factors:

Many chemicals can damage DNA and cause disruption of genetic information. Some chemicals are carcinogenic i.e. react directly with DNA, forming covalent bonds, to cause a mutation in the base pair sequence, e.g. benzyl chloride. Others are pro-carcinogenic i.e., inactive by themselves but may be metabolized to carcinogens by the action of particular enzymes e.g. aromatic hydrocarbon (Luxton, 2000).

Exposure to ionizing radiation and electromagnetic fields yields to DNA damage directly either by breaking the strands or by forming pyrimidine dimers. Moreover, radiation interacts with other molecules within the cell, often water, causing ionization and the formation of highly reactive free radicals (Belson et al., 2007). However, Sarkodee-Adoo et al. (2003) noticed that the majority of leukemias

occurring after exposure to radiation are AML rather than ALL.

Oncogenic viruses are able to transform cells into tumor cells from which a cancer can subsequently develop. This is accompanied either by insertion of the viral genome into host DNA at particular sites causing activation and expression of the gene or gene modification (retroviruses) or by direct expression of viral oncogenes in the host cell (DNA viruses) **(Luxton, 2000)**. Adult T-cell leukemia virus type-1 (HTLV-I) and Epstein Barr virus (EBV) have been associated with a form of ALL **(Belson M et al., 2007)**.

II. Immunodeficiency:

HLA class I molecules serve the essential immunological function of presenting antigen to CD8+ T lymphocytes. Tumor cells may present tumor-specific antigen to T cells via these molecules, but many tumors show loss or down-regulation of HLA class I expression and this may serve as an immune escape mechanism **(Mc Evoy et al., 2003)**.

Moreover, abnormally low serum immuno-globulin levels have been observed in 30% of newly diagnosed acute leukemia patients. It is unclear whether such abnormalities precede the development

of leukemia or are a consequence of the disease (Broder et al., 1998).

III. Genetic factors:

Only a minority (5%) of cases are associated with inherited, predisposing genetic syndromes. Children with Down syndrome have a 10 to 30 times greater risk of leukemia; acute megakaryoblastic leukemia predominates in patients younger than 3 years, and ALL is predominant in older age groups. In cases of Down syndrome, B cell precursor ALL is more likely, with leukemic cells lacking both favorable and adverse genetic abnormalities (Pui, 2006).

Patients with ataxia-telangiectasia have a 70 times greater risk of leukemia and a 250 times greater risk of lymphoma, particularly of the T cell phenotype (Pui, 2006).

Pathophysiology

In ALL, a lymphoid precursor cell becomes genetically altered and subsequently undergoes deregulated proliferation and clonal expansion. In most cases, the pathophysiology of transformed lymphoid cells reflects the altered expression of genes whose products contribute to the normal development of B-cells and T-cells. It has been long thought that

leukemic blasts represent the clonal expansion of hematopoietic progenitors blocked in differentiation at discrete stages of development. Recent data challenge this theory and suggest that leukemia arises from the stem cell that acquires features of differentiated cells. Nevertheless, leukemic blasts provide large uniform populations for molecular and functional analyses (Kathleen et al., 2003).

ALL generally is thought to arise in the bone marrow, but leukemic blasts may present systemically at the time of presentation in the bone marrow, thymus, liver, spleen, lymph nodes, testes and central nervous system (Clark et al., 2003).

Molecular Pathogenesis

The pathway for activating cell growth and division has several stages. The proto-oncogenes encode the proteins taking part in this scheme (Fig. 1). Not surprisingly, mutations that result in hyperactivation of any of the components involved in this can turn proto-oncogenes into oncogenes (David Clark and Lonnie Russell, 2004).

Corruption of this signaling pathway occurs as a result of damage of chromosome, moving a gene next to another thereby producing an abnormal product

(translocation), a point mutation of the gene giving rise to abnormal or truncated product and a gene being duplicated many times giving an over expressed product **(Luxton, 2000)** (Table 1).

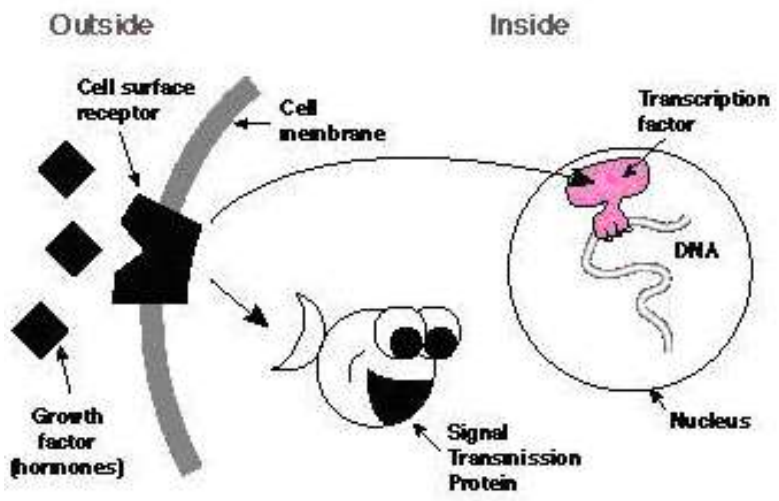

Fig. (1): Normal role of proto-oncogenes **(David Clark and Lonnie Russell, 2004)**.

Other genes within the cell are responsible for halting cell proliferation and allowing repair to damaged DNA. These genes are known as tumor suppressor genes (TSG) (anti-oncogenes). Loss of these genes leads to cells progressing through the proliferation cycle without the necessary brakes responsible for either repair or apoptosis of damaged DNA **(Luxton, 2000)**. About 50 tumor suppressor genes have been described e.g., p53 **(Wojcik et al., 2005)**, retinoblastoma **(Sakamoto et al., 2004)** and

$p16^{INK4a}$ gene **(Bertin et al., 2003)**. TSG can be either recessive to be oncogenic i.e. both genes need to be damaged, or in some cases, only one of the alleles needs to be damaged for the oncogenic effect **(Luxton, 2000)**.

Table (1): Mechanisms of oncogenesis associated with some oncogenes and tumor suppressor genes

Mutated proteins lead to:

- Enhanced binding, stimulating the growth signal (v-SIS, RAS, MYC).
- Reduced binding, preventing the stop signal for the cell cycle (p53)
- Reduced enzyme activity, allowing continual stimulation of signaling pathway (RAS)
- Loss of control domains (ErbB, SRC).

Over expression of gene product resulting in:

- Over stimulation of the receptor signal (c-SIS, NEU)
- Over stimulation of the second messenger (MYC, RAF)
- Expression during the wrong phase of the cell cycle (MYC)

Blocking of binding sites by:

- Competitive blocking (SRC by v-CRK)
- Non-competitive blocking (p53 by E6 or MDM2)

(Luxton, 2000)

Thus, oncogenes drive the cell to replicate and the loss of tumor suppressor genes allow a damaged cell to continue to replicate without the normal repair mechanisms operating **(Bram and McManus, 2000)**.

Classification of ALL

Acute lymphoblastic leukemia is a heterogeneous group of disorders comprising subtypes. The differentiation of these types depends on the morphological classification on one hand, which is usually done with the French-American-British (FAB) system and on the immunophenotype & cytogenetic findings on the other hand, on which the WHO classification depends **(Harris et al., 2000)**.

I. French-American-British (FAB) Classification of ALL:

This classification proposed criteria for classification of ALL into three subtypes based on blast cytology as seen by Romanowsky stained BM smears (Table 2). The FAB classification relies on morphology, dividing blasts into L1, L2 and L3 by their appearance. Its major advantage is its ease of use. The cytological criteria are well defined. They do not require high technology and can be applied in most laboratories throughout the world. The major disadvantage is their modest clinical relevance and they do not adequately define biological and treatment groups **(Sharma et al., 2004)**.

Special Morphological Variants of ALL:

More morphological types of ALL have been noticed. These types have special morphological variations. These variants were illustrated by **Rina in 2004** and summarized in (Table 3).

Table (2): FAB classification of ALL **(Mihaela et al., 2002)**

Histological Type	Characterization
L1	• 80-88% of cases • Small cells usually twice the size of normal small lymphocytes, sparse cytoplasm and high N/C ratio. • Round nuclei with slight nuclear indentations and convoluted nuclear outlines.
L2	• 8-18% of cases • Larger than L1 with considerable heterogenecity with nuclear irregularity. • Lower N/C ratio compared to L1. • Nucleoli prominent varying from 1-4 • Cytoplasm variably basophilic with occasional vacuoles
L3	• 1-3% of cases • Similar features to small non-cleaved cell lymphoma of Burkitt and non-Burkitt type. • Round to oval nuclei with nuclear irregularities. • Stippled and homogeneous chromatin, 2-4 nucleoli. • Deeply basophilic abundant cytoplasm with cytoplasmic-oil-red positive vacuoles.

N/C: Nuclear cytoplasmic ratio

Table (3): Variants of ALL

Type of ALL	Characterization
ALL with vacuoles	• Cytoplasmic vacuolation is not a feature to B-ALL • More prominent in L1/L2. • The vacuoles are periodic acid Schiff (PAS).
ALL with cytoplasmic granules	• 4.5-7% of cases. • Azurophilic granules staining weakly pink or orange. • Usually B-cell precursor phenotype. • Has been associated with Down syndrome. • May be associated with poor prognosis in ALL-L2 cases.
Aplastic presentation of ALL	• Rare cases of ALL presenting with pancytopenia and hypoplastic bone marrow. This is followed within few weeks or months by overt leukemia.
Bone marrow necrosis ALL	• May present with extensive bone marrow necrosis. • Overt leukemia may be present with repeated bone marrow biopsy.
ALL with eosinophilia	• Has been reported with L1 and L2. • No correlation with immunophenotype. • Associated with t(5; 14) translocation. • Should be distinguished from myelomonocytic leukemia with eosinophilia (FAB- M4 EO).
Hand-mirror cells	• Blasts have a cytoplasmic projection resembling hand-mirrors. • No association with prognosis, immunophenotype or cytogenetics.

(Rina, 2004)

II. Immunologic classification of ALL:

Panels of monoclonal antibodies allow distinction of the origin (T or B) and the degree of maturation of leukemic clone **(Darrell and Kenneth, 2000)**. Blast cells characteristics of ALL subtypes are shown in **Table 4**.

Table (4): Immunological types of ALL **(Darrell and Kenneth, 2000)**

Subtype	Common Phenotype	Potentially Associated Genetic Abnormalities
B-precursor ALL	DR, CD19, CD20(-/+), CD24, CD10, CD34, TdT	t(12;21) in 20-25%
		t(9;22)
		11q23 rearrangements
Pre-B ALL	DR, CD19, CD20 (+/-), CD24, CD10, CD34(-), cIgM, TdT (+/-)	t(1;19)
B-ALL	DR, CD19, CD20, CD22, CD24, CD10(+/-), CD34(-), TdT(-), SIg	t(8;14), t(2;8), t(8;22)
T-ALL	DR(-/+), CD1, CD2, cCd3, CD5, CD7, dual CD4/CD8, CD10(+/-), CD34(-/+), CD45 weak, TdT	15-25% have t(1;14)

Abbreviations: +/-, variable, more often positive; -/+, variable, more often negative; (-), negative; DR, HLA-DR; SIg, surface Ig; cIg, cytoplasmic Ig.

Asynchronous/ Aberrant Antigen Expression and Mixed Phenotypic Acute Leukemia:

Many cases of precursor B-cell ALL express combination of early & late antigens not observed in

normal lymphocyte development. This type of asynchronous antigen expression is also found in T-cell ALL. In some cases the lymphoblast aberrantly express one or more myeloid-associated antigen in addition to lymphoid antigen or, less commonly, combinations of both B and T-cell associated antigens are present. In the past these cases were often referred to as mixed lineage or biphenotypic leukemias. However, as more monoclonal antibodies have become available for assessment of acute leukemias by flow cytometry, the finding of aberrant antigen expression in ALL is recognized as a very common phenomenon **(Frater et al., 2003)**.

Presently most cases of ALL that express myeloid antigens are designated simply as ALL with aberrant myeloid antigen expression. Recent studies have mostly shown no independent prognostic significance associated with myeloid antigen expression. The rare cases that express multiple and major antigen of both lymphoid and myeloid lineages may still be referred to as biphenotypic or mixed phenotype acute leukemias. These can be of two types, mixed lineage leukemia (MLL) in which the leukemic blasts co-express multiple and major antigens of both lymphoid & myeloid lineage or bilineal leukemias in